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Control of moth flight posture is mediated by wing mechanosensory feedback

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ABSTRACT

Flying insects rapidly stabilize after perturbations using both visual and mechanosensory inputs for active control. Insect halteres are mechanosensory organs that encode inertial forces to aid rapid course correction during flight but serve no aerodynamic role and are specific to two orders of insects (Diptera and Strepsiptera). Aside from the literature on halteres and recent work on the antennae of the hawkmoth *Manduca sexta*, it is unclear how other flying insects use mechanosensory information to control body dynamics. The mechanosensory structures found on the halteres, campaniform sensilla, are also present on wings, suggesting that the wings can encode information about flight dynamics. We show that the neurons innervating these sensilla on the forewings of *M. sexta* exhibit spike-timing precision comparable to that seen in previous reports of campaniform sensilla, including haltere neurons. In addition, by attaching magnets to the wings of moths and subjecting these animals to a simulated pitch stimulus via a rotating magnetic field during tethered flight, we elicited the same vertical abdominal flexion reflex these animals exhibit in response to visual or inertial pitch stimuli. Our results indicate that, in addition to their role as actuators during locomotion, insect wings serve as sensors that initiate reflexes that control body dynamics.

KEY WORDS: Flight control, *Manduca sexta*, Wings, Abdominal deflection, Sensorimotor processing

INTRODUCTION

Flying insects require mechanosensory information to reject environmental perturbations and execute maneuvers. While visual input is crucial for maintaining stability (Dyhr et al., 2013), the transduction mechanism of insect visual systems results in processing speeds that may be too slow to account for the rapid behaviors observed in free flight (Land and Collett, 1974; Theobald et al., 2010). Thus, insects combine visual input with extremely fast and precise mechanoreception (Bender and Dickinson, 2006; Fayyazuddin and Dickinson, 1996; Fox and Daniel, 2008; Hinterwirth and Daniel, 2010; Huston and Krapp, 2009; Pix et al., 1993; Sherman and Dickinson, 2004) to navigate through complex, unpredictable environments.

True flies (Diptera) and twisted-wing insects (Strepsiptera) show convergent evolution of an elegant solution for sensing body rotations in the mechanosensory organs known as halteres. Evolutionarily derived from wings, halteres are paired, club-shaped

structures located next to the wings that oscillate during flight; they use spatially distributed mechanosensors (campaniform sensilla) to precisely detect linear or rotational accelerations, triggering correctional flight maneuvers (Derham, 1711; Dickinson, 1999; Fayyazuddin and Dickinson, 1996; Fox and Daniel, 2008; Fraenkel and Pringle, 1938; Nalbach and Hengstenberg, 1994; Pringle, 1948; Sherman and Dickinson, 2003). During rotational maneuvers or instabilities during flight, halteres experience an inertial force that is orthogonal to the plane of oscillation (the Coriolis force), the detection of which mediates these compensatory reflexes (Dickinson, 1999; Nalbach and Hengstenberg, 1994; Pringle, 1948; Sherman and Dickinson, 2003). Yet while halteres are actuated structures, they serve no known aerodynamic function and are only found in these two insect orders (Pix et al., 1993).

Recently, the antennae of *Manduca sexta* (Linnaeus 1763) have been implicated in inertial sensing, with the mechanoreceptors at the base of each antenna mediating abdominal reflexes to mechanical pitch stimuli (Hinterwirth and Daniel, 2010; Sane et al., 2007). During this reflex, moths vertically flex the abdomen in response to an inertial pitch stimulus, adjusting the center of lift relative to the center of mass (Dyhr et al., 2013). Moreover, removal of the antennae both eliminates the inertial reflex and compromises overall flight performance (Hinterwirth and Daniel, 2010; Sane et al., 2007). But aside from these two examples of insects using inertial sensing to aid flight control [and the head of dragonflies as a detector of roll (Mittelstaedt, 1950)], it remains a long-standing question as to how most flying insects collect mechanosensory information to control body dynamics.

Although experimental evidence implicates the antennae of *M. sexta* as inertial sensors, it does not preclude other structures from serving similar roles. The halteres are evolutionarily derived from wings and the two possess the same sensory structures, campaniform sensilla. Perhaps then the evolution of gyroscopic sensing could have predated the development of the halteres. Alternatively, the campaniform sensilla embedded in the wings could detect other forces that the wings experience, including aerodynamic and inertial-elastic forces, or the mechanical state of the wing after collision events or damage. Early work in the desert locust *Schistocerca gregaria* demonstrated that ablation of the campaniform sensilla on the hindwings has important behavioral consequences, disrupting the control of twist in the forewings and disabling the animal's ability to produce lift or thrust (Gettrup, 1965; Gettrup, 1966; Gettrup and Wilson, 1964). Later experiments examining how the campaniform sensilla of the hindwing respond to wing twisting found them most responsive to strain magnitude during wing supination, providing input to motor neurons that control the flight muscles (Elson, 1987). Similar results showing the importance of feedback from wing campaniform sensilla regulating the timing of flight muscle contraction have been found in flies (Heide, 1979; Heide, 1983).

While previous research highlights the importance of wing proprioceptive input during flight, the wings of modern insects may

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retain the ability to initiate reflexes that control body dynamics in a manner similar to the visual or inertial sensory systems (Dyhr et al., 2013; Hengstenberg, 1988; Hinterwirth and Daniel, 2010; Pix et al., 1993). To explore this issue, we combined anatomical, electrophysiological and behavioral studies and determined that the wings of *M. sexta* can indeed function as sensors that induce steering reflexes, suggesting that the capacity to use wings as sensory structures may be common to most flying insects.

RESULTS

Manduca sexta forewings possess campaniform sensilla

Manduca sexta forewings are richly equipped with campaniform sensilla dorsally and ventrally (Fig. 1), with concentrations of sensilla at the wing base. On the dorsal surface, five groups of campaniform sensilla are found at the base of the radial wing vein (Fig. 1B); on the ventral surface, two to three groups are found at the base of the ventral subcostal wing vein (Fig. 1C). Distally along the wing, approximately 45 campaniform sensilla can be found on the dorsal and ventral surface (Fig. 1D, blue circles). In all, nearly 250 campaniform sensilla are found on each *M. sexta* forewing, comparing favorably with the number found on the wings (between 120 and 154 campaniforms) and halteres (338 campaniforms) of the blowfly *Calliphora vicina* (Gnatzy et al., 1987), and close to four times the number found on the hindwing of the locust *Schistocerca gregaria* (Gettrup, 1965).

Manduca sexta forewing campaniform sensilla exhibit high spike-timing precision

A general feature of campaniform sensilla afferents (Chapman et al., 1979; Dickinson, 1990a; Dickinson, 1990b; Fox and Daniel, 2008; Fox et al., 2010), which is also thought to be important to flies' sensitivity to rapid inertial perturbations (Dickinson, 1999; Sherman and Dickinson, 2003), is the ability to precisely encode mechanical stimuli across a broad range of frequencies. We therefore performed intracellular recordings from the forewing nerve of *M. sexta* while mechanically stimulating the wing with band-limited Gaussian noise (10–200 Hz) with a motorized lever arm to confirm such abilities for *M. sexta* campaniform afferents (Fig. 2A–C). There are numerous ways to describe how stimulus input is transformed into neuronal output. These methods include calculating the coherence between the stimulus and response (Borst and Theunissen, 1999), the spike-triggered average (Dickinson, 1990a), or looking at higher-order features of the spike-triggered average (Fox et al., 2010). However, we sought a concise, quantitative metric that would allow us to compare *M. sexta* campaniform afferents with previous reports of other insects. We thus calculated the standard deviation of spike arrival time (i.e. jitter) at specific stimulus events as a measure of timing precision (Mainen and Sejnowski, 1995). The jitter value for half of the spiking events across all animals was less than 0.60 ms (median jitter=0.52 ms, mean ± 1 s.d.=1.02±1.04 ms; Fig. 2D,E). This jitter value is nearly 10 times smaller than the period of the highest frequency component of the stimulus and close to 80 times smaller than the *M. sexta* wingbeat period of 40 ms, indicating that the neurons innervating the wing campaniforms were capable of precisely encoding mechanical stimuli across a broad range of frequencies.

Perturbation of the wings during tethered flight induces compensatory reflexes

To determine whether mechanosensory input from only the wings could contribute to flight control, we attached small (13 mg) rare-earth magnets on the wings of tethered, flying *M. sexta* (Fig. 1D, black circles) and placed the moths inside of a rotating magnetic field (strength: 3.8 mT; approximate torque: 650 nN m; see Materials and methods) produced by a pair of Helmholtz coils (Fig. 3A). To minimize visual input from the rotating coils, we conducted all experiments in near-dark conditions. We stimulated moths about the animals' pitch axis with a stimulus that is nearly 10 times larger than when *M. sexta* are subject to whole-body mechanical pitch rotations
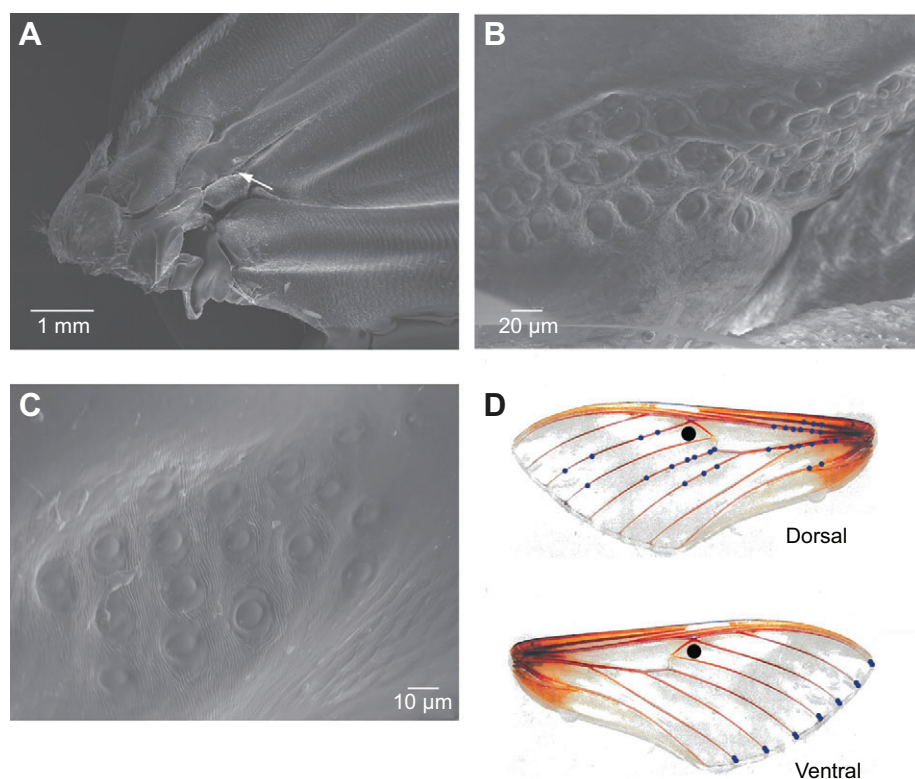

Fig. 1. The forewings of *Manduca sexta* contain campaniform sensilla. (A) Patches of campaniform sensilla are found on the dorsal surface of the radius near the wing hinge. (B) Scanning electron micrograph from a different wing (location as indicated in A) shows that each group is densely packed with campaniform sensilla. (C) Ventral patches are arrayed in precise patterns, but are less dense than those on the dorsal surface. (D) Locations of distal campaniform sensilla on the dorsal and ventral surfaces of a male forewing (blue circles). Black circles indicate the position of the magnets during wing perturbation experiments. The *M. sexta* forewing is ~5 cm long.
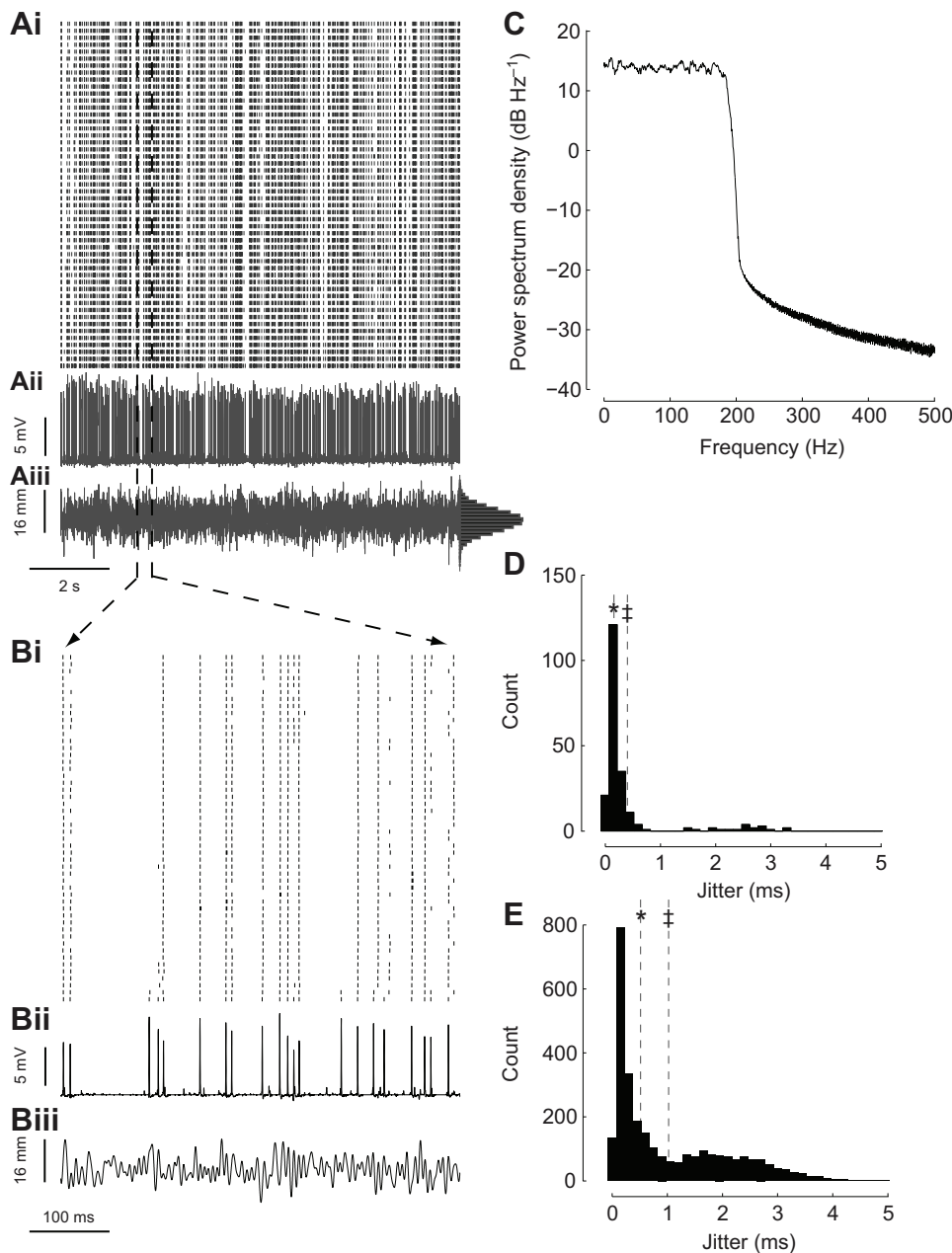
Fig. 2. Temporal precision of campaniform sensilla afferent neurons. (Ai) Raster plot showing timing of spikes in response to repeated presentations of a 10 s Gaussian mechanical stimulus applied to the wing tip. (Aii) Intracellular voltage recorded from a neuron during the first stimulus presentation. (Aiii) Stimulus waveform, with histogram of amplitude distributions at right. (Bi–Biii) Expanded 500 ms view of data shown in A. (C) Power spectra of stimulus, showing band limit of 10–200 Hz. (D) Histogram of jitter for 213 spiking events from same neuron as shown in A. Median jitter value: 0.16 ms (*); mean value ± 1 s.d.: 0.40±0.70 ms (‡). (E) Histogram of jitter for 2886 spiking events from 15 neurons in seven animals. Median jitter value: 0.52 ms (*); mean value ± 1 s.d.: 1.02±1.04 ms (‡).

at the same frequency and amplitude (Hinterwirth and Daniel, 2010), using three 10 s trials of a 20 deg (peak-to-peak), 3 Hz sinusoid rotation of the coils.

To determine the efficacy of the wing mechanical perturbation in eliciting the abdominal reflex, we measured moths' abdominal angle, θ_a, as a function of time and compared it with the angle of the Helmholtz coils, θ_c (Fig. 3B).

First we tested *M. sexta*'s visual response to the rotating coils in the absence of a magnetic field (no power to the coils). Even in low-light conditions, *M. sexta* with magnets on their wings showed moderate abdominal flexion in response to the visual signal of rotating Helmholtz coils (Fig. 3C, black line). A Fourier transform of both θ_c and θ_a reveals a sharp peak at the driving frequency of 3 Hz of the pitch stimulus produced by the rotating coils (Fig. 3D). We used the real and imaginary components of the Fourier transforms to determine the gain and phase of the abdominal response. In 12 trials of four moths, the mean gain was 0.121±0.092 with a phase of −49.76±29.17 deg (mean ± circular s.d.).

To exclude the possibility that *M. sexta* were sensitive to the magnetic field produced by the Helmholtz coils, we performed an additional behavioral assay in which we removed the magnets from the wings and observed moths' responses to rotating and powered Helmholtz coils. *Manduca sexta* responses to the rotating coils in the presence of a magnetic field without placing magnets on the wings were not significantly different from moths with magnets in the unpowered coils (gain: 0.115±0.101; phase: −50.53±15.88 deg; *N*=5 trials, 3 moths; two-sample *t*-test gain: *P*=0.902). We thus pooled these data for comparison with wing perturbation experiments.

To experimentally test the hypothesis that the wings of *M. sexta* serve as both actuators and sensors to control body dynamics, perturbation of only these structures during flight should result in a compensatory abdominal reflex. Thus we performed a third experimental treatment in which we attached magnets to the wings of moths experiencing tethered flight in rotating, powered Helmholtz coils. In nine trials of three moths, *M. sexta* tested with
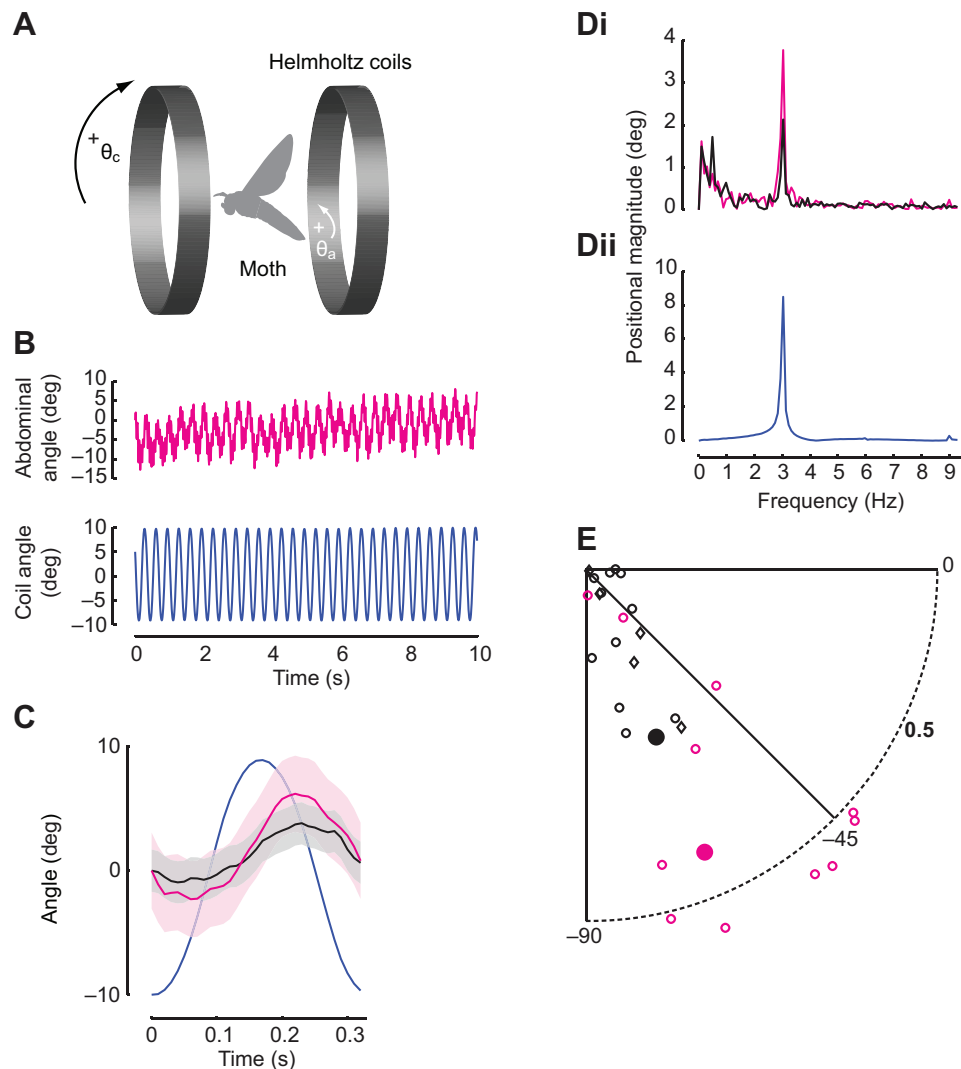
Fig. 3. Moths respond to visual motion of Helmholtz coils in the absence of a magnetic field, whereas magnetic perturbation of the wings results in large abdominal responses. (A) Each moth was ventrally tethered at the thorax and placed between a pair of Helmholtz coils mounted to a stepper motor. Motion of the coils relative to the page is clockwise/counterclockwise. Figure is not to scale. θ_c, Angular position of the coils (stimulus); θ_a, angular position of the abdomen (response). (B) Example response (top, magenta) and stimulus (bottom, blue) during perturbation of the wingstroke. (C) Cycle averages of θ_c (blue line) and θ_a in response to the visual signal of the rotating Helmholtz coils (black) and in response to perturbation of the wingstroke (magenta, same trial shown in B). Shaded areas indicate ±s.d. This moth had magnets on its wings in both trials. (Di) Discrete Fourier transform (DFT) of the abdominal responses in both conditions shows a peak at the stimulus frequency, showing a gain of 0.258 and a phase of −67.0 deg in response to visual stimulation only (black), and a gain of 0.444 and a phase of −64.7 deg in response to visual and mechanical stimulation (magenta). Note that the signal shown in B is non-stationary, which manifests as a large mean offset in the DFT. (Dii) DFT of the motion of the Helmholtz coils. (E) Polar plot of the gains and phases of abdominal responses. Distance from the origin indicates the gain of the response, and the angle indicates phase. Black circles: moths with magnets on their wings, subject to rotating Helmholtz coils (no magnetic field present; N=17 trials, 4 moths). Black diamonds: moths subject to a rotating magnetic field without magnets on their wings (N=5 trials, 3 moths). Magenta circles: moths with magnets on their wings subject to a rotating magnetic field (N=12 trials, 4 moths). Solid circles indicate trials shown in C. Moths subject to wing perturbations exhibit significantly greater mean abdominal response gain than moths with magnets on their wings, but no magnetic field (P<0.05; repeated-measures ANOVA).

magnets on their wings displayed strong abdominal reflexes in response to the rotating coils in the presence of a magnetic field (Fig. 3C, magenta line) and there is a strong 3 Hz component to θ_a (Fig. 3D). Each moth responded differently to both the visual stimulus of the rotating coils alone and the dynamic torque stimulus (Fig. 3E). Accordingly, we observed a significant effect of individual moths on the gain of the abdominal response in each experimental treatment (coils off: P<0.05; coils on: P<0.05; repeated-measures ANOVA; Fig. 3E). In spite of this variability between animals, a polar plot (Fig. 3E) shows that wing perturbation induced significantly stronger abdominal reflexes than the visual signal of

the rotating coils alone (gain: 0.354±0.164, phase: −67.31±10.30 deg; P<0.05; repeated-measures ANOVA; N=12 trials, 4 moths). We saw no effect of trial number or the interaction between trial number and experimental treatment (data not shown).

DISCUSSION

This research was motivated by the notion that wings, as the evolutionary precursors to the gyroscopic sensory organs of Diptera (halteres), could provide mechanosensory information that is involved in flight control. Moreover, sensory information would be encoded by campaniform sensilla, similar to what has been found in

previous studies of these sensory structures. Accordingly, we were able to show that the wings of *M. sexta* have campaniform sensilla distributed over the wing blade and in patches at the base of the wing. In addition, neural recordings indicate that these sensory cells are capable of rapidly and precisely encoding mechanical stimuli. Importantly, direct perturbations to the wings via a magnetic field rotating in the pitch axis causes compensatory reflexes similar to those elicited by both visual pitch stimuli and whole-body rotations (Hinterwirth and Daniel, 2010). The evolution of the haltere, with its reduced size and knob-like shape that generates negligible lift and drag forces during flight, allows true flies and strepsipterans to detect inertial forces independent of aerodynamic loading. In contrast, the wings of large insects such as *M. sexta* clearly generate aerodynamic forces and, as we show, also detect wing bending dynamics. Thus, wings could serve the dual role of both sensor and actuator. That is, by actuating the wing, a flying insect could detect information during about its body dynamics that would be otherwise unavailable without flapping, such as the Coriolis force associated with body rotations or different wing bending dynamics between the wings due to aerodynamic loading.

Temporally precise campaniform sensilla are embedded in *M. sexta* forewings

The inertial reflexes observed in flies, such as changes in wing kinematics, head position or abdominal position in response to imposed whole-body rotations (Dickinson, 1999; Hengstenberg, 1988; Nalbach and Hengstenberg, 1994), are largely attributed to the arrays of strain-sensitive campaniform sensilla found at the base of each haltere (Bender and Dickinson, 2006; Chan and Dickinson, 1996; Dickinson, 1999; Fayyazuddin and Dickinson, 1996; Fraenkel and Pringle, 1938; Gnatzy et al., 1987; Hengstenberg, 1988; Nalbach and Hengstenberg, 1994; Sherman and Dickinson, 2003; Sherman and Dickinson, 2004). These sensilla are also found on the wings of other insects [Diptera (Cole and Palka, 1982; Gnatzy et al., 1987); Lepidoptera (Ando et al., 2011; Dombrowski, 1991); Orthoptera (Gettrup, 1965; Gettrup, 1966); and Hemiptera (Zacwilichowski, 1931)], including *M. sexta* (Fig. 1).

A general feature of the neurons innervating these sensilla, in conjunction with the structural specialization of the campaniform sensilla dome (Fox and Daniel, 2008; Fox et al., 2010; Fraenkel and Pringle, 1938; Gnatzy et al., 1987; Pringle, 1948), is the sub-millisecond spike-timing precision of the afferents (Chapman et al., 1979; Dickinson, 1990a; Dickinson, 1990b; Fox and Daniel, 2008; Fox et al., 2010). Our recordings from afferent neurons innervating *M. sexta* wing campaniform sensilla show that these neurons exhibit a level of spike-timing precision similar to values observed in fly halteres (Fox and Daniel, 2008).

Halteres experience large inertial-elastic forces in their plane of oscillation (Nalbach, 1993). As a fly rotates in an axis orthogonal to that of the halteres, they experience a Coriolis force that is much smaller than the inertial in-plane forces (Nalbach, 1993). Previous work (Fox et al., 2010; Pringle, 1948) has suggested that detecting the small, out-of-plane motions that arise from the Coriolis force requires high temporal precision. Moreover, the importance of proprioceptive feedback from the campaniform sensilla on wings and halteres for regulating normal wingstroke kinematics has long been known (Fayyazuddin and Dickinson, 1999; Heide, 1979; Heide, 1983). Our electrophysiological results suggest that the forewing campaniform sensilla exhibit sufficient precision for both proper wingstroke maintenance as well as detecting rapid changes in body dynamics or wing bending.

Wing campaniforms provide sensory feedback to abdominal response

The above morphological and electrophysiological evidence suggests that wings could provide information relevant to flight control. However, confirmation of such a role would require demonstrating that perturbing only the wings, and leaving the rest of the animal undisturbed, mediates a reflex similar to the abdominal movements observed in response to a mechanical pitch stimulus (Hinterwirth and Daniel, 2010). Our preparation allowed us to provide the wings with just such a dynamic torque stimulus while leaving the rest of the animal unperturbed. While we were unable to determine the detailed effects of our preparation on wing kinematics or wing strain patterns, by comparing responses of moths in this system with the coils on or off we were able to clearly identify the responses due to a mechanical stimulus provided solely to the wings (Fig. 3).

Studies on the role of inertial sensing in insect flight have typically looked at responses to whole-body rotations as well as the impact of sensor ablation (Bender and Dickinson, 2006; Dickinson, 1999; Hengstenberg, 1988; Hinterwirth and Daniel, 2010; Nalbach and Hengstenberg, 1994; Pix et al., 1993; Sane et al., 2007; Sherman and Dickinson, 2003; Sherman and Dickinson, 2004; Viollet and Zeil, 2013). The primary drawback of testing the effect of campaniform ablation with our experimental setup is the technical challenge of eliminating each of the nearly 250 campaniform sensilla per wing. These structures are micron-scale in size, and thus extremely difficult to ablate without potentially damaging the rest of the wing. Additionally, this experimental approach does not account for the contributions of other potential inertial sensors. Aside from technical limitations, inertial sensors such as the halteres are responsible not only for measuring body rotations, but also for the regulation of normal wing kinematics, similar to wing campaniform sensilla (Gettrup, 1966; Heide, 1983). Given the multiple roles of mechanosensory feedback in the regulation of flight locomotor patterns, interpreting the results of ablation experiments is rather difficult. Further, only a few experiments have looked for compensatory reflexes as a direct result of manipulating putative inertial sensors (Hinterwirth et al., 2012). To our knowledge, this is the first demonstration that the wing campaniform sensilla induce reflexes involved in the control of body dynamics, a sensory role predicted by Pringle (Pringle, 1948; Pringle, 1957). Our findings that wing pitch perturbations in *M. sexta* elicit an abdominal reflex are also consistent with the effect of virtual pitch rotations on the blowfly *Calliphora erythrocephala* (Nalbach and Hengstenberg, 1994) and the effect of yaw rotations on the strepsipteran *Xenos vesparum* (Pix et al., 1993).

The 3 Hz pitch stimulus from a rotating magnetic field drove wing perturbations that resulted in a much larger abdominal response (35-fold increase) than *M. sexta* subject to whole-body pitch rotations at the same driving frequency and amplitude (Hinterwirth and Daniel, 2010). This large difference in the strength of the abdominal responses observed in the present study compared with those reported by Hinterwirth and Daniel (Hinterwirth and Daniel, 2010) is likely due to our targeted perturbations subjecting the wings to torques an order of magnitude larger than would be experienced by whole-body rotations of the same amplitude and frequency, eliciting larger abdominal excursions. Previous work on the inertial sensing capacities of the halteres and antennae suggests that these structures act as high-pass filters, such that the stronger the rotational input, the stronger the behavioral response (Hinterwirth and Daniel, 2010; Sherman and Dickinson, 2003). Thus, the wing torques experienced during the whole-body rotations used by Hinterwirth and Daniel,

whether due to aerodynamic or inertial forces, might have been too low to excite the wing-mediated abdominal response. Our results provide strong evidence that forces acting on the wings that are likely small relative to the normal patterns of aerodynamic and inertial forces and moments produced are closely monitored by the nervous system and provide information relevant to control.

Using the wings as sensory structures in flight may introduce sensory noise from wing bending due to inertial-elastic effects that could limit the capacity for *M. sexta* to detect and respond to inertial stimuli (Combes and Daniel, 2003). It remains unclear whether the frequency bandwidth of the compensatory reflex in *M. sexta* is limited, at least compared with insects with halteres. However, *M. sexta* is much larger than both dipteran and strepsipteran species and, because of its greater inertia, may not require the sensory bandwidth (at higher frequencies) needed for smaller insects. Alternatively, mechanosensory information from the wings could be combined with inertial information from the antennae, a previously established gyroscopic sensor in *M. sexta*, potentially remedying any signal-to-noise issues. This interaction between mechanosensory sensing mechanisms may explain why *M. sexta* that had their antennae removed were unable to sustain stable flight (Sane et al., 2007). The antennae may act as the primary inertial sensor in *M. sexta*, with the wings providing additional information to control body dynamics. Further experiments that address the extent to which information from wing campaniform sensilla is used in conjunction with the antennae along with other modalities will provide a better understanding of the wings' sensory capacity and influence over body dynamics.

Can wings separate aerodynamic and inertial forces?

Though it is tempting to interpret our data as implicating the wings as sensors of rotational body dynamics similar to the halteres, the arrangement of campaniform sensilla distal to the wing base (Fig. 1D) suggests that the wing campaniform sensilla control abdominal reflexes arising from the detection of wing bending due to multiple different forces. During flapping, insect wings undergo large bending and torsion (Wootton, 1981; Wootton, 1992). Whereas aerodynamic and inertial forces are thought to contribute nearly equally to wing bending for flies (Ennos, 1988; Lehmann et al., 2011), a combination of computational and experimental techniques have suggested that wing bending in *M. sexta* may be due mostly to inertial-elastic, rather than aerodynamic, loading (Combes and Daniel, 2003; Daniel and Combes, 2002). Thus, the amount of bending an insect wing undergoes is likely a function of a combination of wing stiffness, flapping frequency, aerodynamic loading and inertial forces. Nonetheless, a model comparing aerodynamic, inertial-elastic and Coriolis forces in a flapping wing subject to rotations about different axes will allow testing of the plausibility of insect wings' inertial sensing capacity. Indeed, such a comparison of the forces acting on the halteres of *Calliphora* allowed Nalbach to conclude that whereas the primary inertial force is two orders of magnitude larger than the Coriolis force, the Coriolis force is the only force acting on the haltere that would allow a fly to determine the axis and sign of a rotational maneuver or perturbation (Nalbach, 1993). Thus, our findings that wing campaniform sensilla mediate changes in body posture and dynamics that are due to small disturbances in wing bending are very much in keeping with previous work on halteres.

In addition to the question of the relative strength of wing Coriolis forces during rotations, it remains unclear whether insect neural systems can disambiguate between the detection of these different sources of wing bending. Campaniform sensilla arrangement varies dramatically between insects (Cole and Palka, 1982; Dombrowski, 1991; Gettrup, 1966; Schäffner and Koch, 1987a; Schmidt and Smith, 1985; Zacwilichowski, 1931), and the arrangement of campaniform sensilla on the wing could reflect a bias during evolution toward the detection of aerodynamic, Coriolis or other forces relevant to behavior (Schäffner and Koch, 1987a; Schäffner and Koch, 1987b). Linking the spatial arrangement of campaniform sensilla to the encoding properties of the innervating neurons, as well as the wings' biomechanics, is crucial to the development of a full model of the wing's sensory role. Nevertheless, the results from our behavioral experiments suggest that wings themselves can indeed function not only as actuators during flight, but also as sensors to control body dynamics.

Much of the interest in halteres stems from their distinction as the only true gyroscopes in the animal kingdom, and this unique sensory function is unlikely to have evolved *de novo* in halteres (Pix et al., 1993; Pringle, 1948). Given that the halteres are evolutionarily derived from wings (hindwings in Diptera and forewings in Strepsiptera) and campaniform sensilla are found on both halteres and wings, behavioral reflexes mediated by aerodynamic or gyroscopic sensing might have evolved within wings and later been refined with the evolution of the halteres. Our results show that the wings of *M. sexta* provide sensory information that can be used to control body posture, and thus dynamics, during flight. Thus, insect wings may indeed serve as both sensors and actuators during flight. Future work examining the forces to which the wing and wing campaniform sensilla are most sensitive during rotations, along with how this information is combined with other sensory modalities, will allow a more comprehensive understanding of the wings' role in flight as a dual sensor and actuator.

MATERIALS AND METHODS
Animals
Manduca sexta were raised in the Department of Biology at the University of Washington, Seattle. For scanning electron micrographs (SEMs) and determining locations of distal wing campaniform sensilla, we used 16 male and eight female moths that were between 1 and 8 days post-eclosion. For electrophysiological experiments, we used three male and four female moths 1–5 days post-eclosion. For experiments using a simulated mechanical stimulus, we used four male moths 1–3 days post-eclosion.

Mapping campaniform sensilla/SEMs
We examined each moth to ensure that the wings had not sustained any significant damage. After identifying sex and day of eclosion, we excised a single forewing from one side after anesthetizing the animal by chilling it in a refrigerator for 15 min. We used wings from 14 male and seven female moths. We then removed the scales using damp Kimwipes and cotton swabs. We mounted wings between two microscope slides using glycerol, viewed them under a dissecting microscope, and recorded locations of the wing campaniforms on pictures of a *M. sexta* wing. For SEMs, after chilling and excising each wing sample (two male and one female), we removed and descaled the wing base and desiccated it overnight. We then mounted, sputter-coated in gold (Hummer V: Au Target, Technics, San Jose, CA, USA) and viewed each sample with a scanning electron microscope (JSM-840A, JEOL, Tokyo, Japan).

Electrophysiology and jitter analysis
We recorded intracellularly from the axons of afferent neurons innervating the campaniform sensilla on the dorsal and ventral surfaces of forewing nerve IINc1 (Nüesch, 1957). We pulled sharp intracellular electrodes from quartz capillary tubes using a model P2000 Micropipette Puller (Sutter Instruments Co., Novato, CA, USA). We filled electrodes with 3 mol l^{-1} KCl, with resultant resistances of between 10 and 30 MΩ. We monitored membrane potential using an Axoclamp-2B amplifier (Molecular Devices,

Sunnyvale, CA, USA), sampling membrane and stimulus voltage at 10 kHz, and recorded on a Windows platform using the MATLAB data acquisition toolbox. We stimulated the wing by attaching a model 322C length controller (Aurora Scientific, Aurora, ON, Canada) directly to the wingtip. We supplied pre-built stimulus voltages from a Windows computer to the servo-lever system. Stimuli consisted of between 10 and 50 repeats of Gaussian white noise mechanical stimulation band-limited to 10–200 Hz, with RMS amplitude of 3.8 mm at the wingtip, which is approximately an order of magnitude lower than the base-to-tip excursions experienced by the wing during a normal wingstroke (8.5 cm). A standard deviation of 3.8 mm was the maximum our setup allowed in order to maintain a linear voltage to wingtip distance relationship.

We identified single-spike events that were consistently elicited by repeated presentations of the stimulus with a modified version of the event identification protocol of Yen et al. (Yen et al., 2007). First, in order to avoid results due to including data in which the cell was adapting, we excluded initial repetitions of the stimulus where the firing rate averaged across the repetition differed by more than 20% from the average firing rate across all trials. We then binned the adapted responses to repeated trials of the stimulus into histograms at a 1 ms resolution, and set a threshold at 30 times the mean firing rate in order to define firing boundaries of events. We determined from the collections of all events in the data set the events that consisted of single spikes with no contaminating spikes in a 20 ms window around the event for at least 20% of the trials. We note that varying this exclusion between 10 and 90% of the trials did not greatly affect the jitter analysis. For each event, we extracted the timing of the spike on each trial, and calculated the jitter as the standard deviation of the event time across trials (Mainen and Sejnowski, 1995).

Helmholtz coils

We used a pair of Helmholtz coils (U8481500, American 3B Scientific, Atlanta, GA, USA) mounted to a stepper motor that has been described previously (Hinterwirth and Daniel, 2010) (Fig. 3A). The Helmholtz coils were 150 mm apart and each coil had a mean radius of 150 mm (inner radius: 287 mm, outer radius: 311 mm). We controlled the stepper motor through a custom Arduino script that generated a 20 deg (peak-to-peak), 3 Hz sinusoid about the pitch axis, which was converted into 0.2 deg steps by a micro-step controller (G201, GeckoDrive Inc., Tustin, CA, USA). To track the motion of the Helmholtz coils, we attached an infrared LED to the coils. We powered the coils with 5 A and 7.25 V, resulting in a constant field strength of approximately 3.8 mT.

Animal preparation for magnetic field wing perturbations

All behavioral experiments rely on the abdominal flexion that occurs in response to open-loop visual and mechanical pitch rotational stimuli (Dyhr et al., 2013; Hinterwirth and Daniel, 2010). We suspended each moth in the Helmholtz coils by attaching them via a ventral tether to a stable platform in between the coils. To do so, we chilled each moth in a refrigerator for 20 min and glued a ventral tether between the mesothoracic and metathoracic segments. We dark-adapted each moth for 20 min before beginning trials. We conducted all experiments in near-total darkness and illuminated moths with infrared LEDs. We recorded three trials per treatment per moth with a high-speed camera (100 frames s⁻¹, 400 μs shutter, Phantom Miro, Vision Research, NJ, USA). We conducted analyses only on trials that consisted of at least 10 s of continuous flight.

In the first set of experiments, we tested the ability of *M. sexta* to detect magnetosensory information as seen in monarch butterflies (*Danaus plexippus*) (Merlin et al., 2009) by powering the coils and rotating them around tethered flying moths. After these controls, we again chilled moths and placed a pair of small rare-earth magnets (mass: 13 mg, diameter: 1.6 mm, height: 0.8 mm; Magcraft NSN0591) between the first and second medial veins of each forewing, with the wing in between the attracted magnets (Fig. 1D, black circles). For comparison, the mass of a typical *M. sexta* wing is 42 mg. We mounted the magnets distal to the wing base, and ~2.5 mm from the wing's leading edge. We positioned the magnets to ensure that the polarity was the same for both wings and for all animals. We allowed moths to dark-adapt after 20 min, and ran trials in both the presence and absence of the magnetic field.

Analysis of perturbation data

We digitized videos of moths placed in the Helmholtz coils with DLTdv5 (Hedrick, 2008), using the penultimate abdomen segment, tether and LED as points of interest. Using a custom MATLAB script (The MathWorks, Natick, MA, USA), we computed the angular position of the coils (stimulus), θ_c, and the abdomen (response), θ_a, following the convention of Dyhr et al. (Dyhr et al., 2013) (Fig. 3A). We subtracted the mean of θ_a to remove linear trends from the data, and then performed discrete Fourier transforms of the stimulus and response to calculate the gain and phase of the abdominal reflex at the driving frequency of 3 Hz. The gain of the response, $G(f)$, is defined as the frequency-dependent ratio of the response amplitude to the stimulus amplitude. The phase of the response, $\theta(f)$, is defined as the difference in time lag normalized to the period length. We implemented repeated-measures ANOVAs and two-sample *t*-tests in MATLAB on the calculated gain values.

Calibration of wing perturbation stimulus

To obtain an estimate of the stimulus applied to the wings by the Helmholtz coils, we measured the torsional stiffness of the *M. sexta* wing as well as the amount of induced torsion to the wings caused by the magnets when the Helmholtz coils rotated. To measure torsional stiffness, we collected seven 1–3 day post-eclosion males and anesthetized each animal by chilling them in a refrigerator for 15 min. We then excised the left forewing of each animal. We clamped the wing to a micromanipulator, lowering it onto an insect pin that we placed on a scale (Mettler PL200, Hightstown, NJ, USA). We lowered each wing such that the first cubital vein was lightly touching the insect pin at the start of the measurement. We then measured the moment arm from the first cubital vein to the wing's point of attachment at the micromanipulator. We recorded vertical displacement of the wing and the mass applied to the pin. We recorded eight measurements per wing; we first lowered the wing onto the pin by 200 μm, then by 100 μm increments for each subsequent measurement. After each trial, we measured the length from the leading edge of the wing to the first cubital vein. From this length and the vertical displacement, we calculated the angle to which the wing had been twisted, and calculated the torque on the wing by multiplying the measured mass applied to the pin by the acceleration due to gravity, and the measured moment arm. We performed a linear regression on each measurement of torque versus angle to obtain the torsional stiffness for each wing. From these measurements, we obtained a torsional stiffness of 2.17±0.98 μN m deg⁻¹ (supplementary material Fig. S1). To estimate the torsion applied to the wings by the rotating magnetic field, we attached magnets to the excised wings of five 1- to 3-day-old post-eclosion moths. We used three males and two females. We taped wings to a tether and rotated the powered Helmholtz coils for 10 s around the wings in two different trials: at 0 and 45 deg with respect to horizontal. We recorded each trial at the same frame rate and shutter speed as the behavioral experiments. We observed a maximum deflection of ±0.3 deg at 3 Hz of the trailing edge of the wings, suggesting that a still wing experiences approximately a 650 nN m torque. This amount of torque is ~10 times larger than the expected torque for a plate with the same moment of inertia as a *M. sexta* wing rotating 20 deg (peak-to-peak), at 3 Hz. It is important to note that this torque estimate does not take into account wing motion relative to that of the Helmholtz coils, but is probably much smaller than the torques associated with normal *M. sexta* wingbeat kinematics.

Competing interests
The authors declare no competing financial interests.

Author contributions
B.H.D., Z.N.A. and T.L.D. designed the experiments. B.H.D. obtained the images shown in Fig. 1, carried out the experiments and analyzed the data shown in Fig. 3. Z.N.A. carried out the experiments and analyzed the data shown in Fig. 2. B.H.D., Z.N.A. and T.L.D. wrote the paper.

Supplementary material

Supplementary material available online at
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