

Effects of stretch receptor ablation on the optomotor control of lift in the hawkmoth *Manduca sexta*

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Summary

In insects, fast sensory feedback from specialized mechanoreceptors is integrated with guidance cues descending from the visual system to control flight behavior. A proprioceptive sensory organ found in both locusts and moths, the wing hinge stretch receptor, has been extensively studied in locusts for its powerful influence on the activity of flight muscle motoneurons and interneurons. The stretch receptor fires a high-frequency burst of action potentials near the top of each wingstroke and encodes kinematic variables such as amplitude and timing. Here, I describe the effects of stretch receptor ablation on the visual control of lift during flight in the hawkmoth *Manduca sexta*. Using a combination of extracellular muscle recordings, force and position measurements and high-speed video recording, I tracked power muscle activity, net vertical flight force (lift),

abdomen deflection and wing kinematics in response to image motions of varying velocity during tethered flight in a wind tunnel. As a result of bilateral ablation of the wing hinge stretch receptors, visually evoked lift decreased to nearly one-third of that exhibited by intact animals. The phase and frequency of indirect power muscle action potentials and the patterns of abdominal deflection were unaffected; however, wingstroke amplitude was clearly reduced after ablation. Collectively, these results suggest that stretch receptor feedback is integrated with descending visual cues to control wing kinematics and the resultant aerodynamic force production during flight.

Key words: insect, flight, mechanosensory, proprioception, optomotor, hawkmoth, *Manduca sexta*.

Introduction

Many of the behaviors exhibited by flying insects are mediated visually. For example, insects rely on complex visual processing (i) to maintain their orientation with respect to the retinal panorama (Collett et al., 1993), (ii) to infer range to foraging sites (Lehrer et al., 1988; Srinivasan, 1993), (iii) to pursue prey or conspecifics (Frye and Olberg, 1995; Land, 1992) and (iv) to avoid collision with obstacles (Gabbiani et al., 1999; Rind and Simmons, 1998; Robertson and Johnson, 1993). These behaviors require extremely rapid and precise sensorimotor control of wing motions and the resultant aerodynamic forces. The magnitude of forces produced by the wings of insects such as flies and moths varies widely within each wingstroke cycle (Dickinson et al., 1999; Ellington, 1995) illustrating the importance of controlling wing kinematics on a cycle-by-cycle basis. The time course and magnitude of these 'unsteady' aerodynamic forces are determined by wing kinematics such as the velocity, amplitude and timing of rotation at each stroke reversal (Sane and Dickinson, 2001). The sensorimotor circuitry controlling these wing kinematics utilizes extremely rapid phasic feedback signals from specialized mechanoreceptors such as campaniform sensilla on the wingblade, which encode time-varying strains and forces

(Dickinson, 1990), and stretch receptors in the wing hinge, which encode wingstroke kinematics and forces (Frye, 2001; Möhl, 1985a) within each wingstroke cycle.

Locust flight has been a model system for understanding the role of proprioceptive feedback – including feedback from the stretch receptor – in modulating the motor patterns that control wing motions [(Pearson and Ramirez, 1990; Reye and Pearson, 1988); for a review, see Burrows (Burrows, 1996)]. During tethered flight, the locust stretch receptor fires a burst of action potentials near the top of each wingstroke. The burst contains information about the position of the wing at each dorsal stroke reversal (Möhl, 1985a). It has been suggested that stretch receptor feedback is used to counter external perturbations of wing motions as well as to correct for asymmetric output of the flight pattern generator (Möhl, 1985b).

The hawkmoth *Manduca sexta* is a sophisticated flyer able to achieve speeds of up to 5 m s^{-1} (Willmott and Ellington, 1997a) and maneuver in virtually any direction. This nocturnal forager hovers at individual flowers set against a spatially complex and dim visual background while maintaining stability in often turbulent winds. While less extensively studied than flies and locusts, hawkmoths are becoming

increasingly important to the study of the aerodynamics (Willmott and Ellington, 1997b), musculoskeletal mechanics (Daniel and Tu, 1999), motor patterning (Kammer, 1968; Wendler et al., 1993), olfactory sensitivity (Willis and Arbas, 1998) and visual processing (Farina et al., 1995; Kern and Varju, 1998) associated with the control of flight in insects. Yack (Yack, 1992) discovered a stretch receptor at the base of the hindwing of several species of atympanate moths including *Manduca sexta*. The stretch receptor is tonically active when the wings are at rest; it responds to slow, experimentally controlled sinusoidal wing elevation with a matched modulation in discharge frequency, and faster oscillation evokes a phasic burst of spikes centered near the top of each excursion. Here, I address the importance of feedback from this specialized wing proprioceptor in the control of optomotor stabilization in the hawkmoth *Manduca sexta*.

Materials and methods

Animals and preparation

The data for this paper were collected from male and female *Manduca sexta* within 2–4 days of adult eclosion. The moths were reared on artificial diet and maintained under a 17h:7h L:D photoperiod at 26°C. A tether was fashioned from a rigid stainless-steel tube 37 mm long, 1.5 mm in diameter and pinched at one end to form a wedge. The wedge was glued between the metathoracic coxae with cyanoacrylate adhesive. Moths were tethered ventrally to preserve, as much as possible, the natural mechanical deformations of the thorax associated with wing motions. Moths were prepared within an hour of subjective night and allowed to recover and photoadapt to dark conditions for at least 1 h. They were considered ready to use when the pupil was fully expanded (determined qualitatively using episcopic illumination). Experiments generally lasted 30–40 min, after which the moth was gently removed from the tether and weighed. The tethering preparation did not seem to harm the moths because they were able to fly after being removed from the tether.

Open-loop flight arena and experimental design

A force beam was constructed to measure lift and thrust simultaneously during tethered flight. The beam consisted of a 70 mm × 12 mm × 1.5 mm brass bar mounted horizontally, with the vertical tether bolted to one end (Fig. 1 inset). A 2 mm × 2 mm first-surfaced mirror was affixed to the end of the horizontal bar, and vertical displacements during flight were tracked by reflecting a helium–neon laser off the mirror onto an optical sensor (UDT Sensors Inc.). The voltage output of the sensor circuitry was linearly proportional to a composite moment applied

to the tip of the vertical tether (i.e. this sensor responded to both vertical and horizontal forces).

Horizontal displacements of the tether were independently tracked with a light-emitting diode (LED) and optical sensor (UDT Sensors Inc.). This LED/detector pair was rigidly fixed to the horizontal brass bar and tracked only horizontal displacements (Fig. 1 inset, sensor mounted just below the moth). Forces were calibrated by loading the beams with static weights. Thrust is defined as net horizontal force, and lift is net vertical force minus the weight of the animal. The thrust signal was subtracted from the composite force signal to determine pure lift. Moths were tethered as close as possible to the center of mass to minimize crosstalk between the lift and thrust channels resulting from pitching moments.

The resonant frequency of the lift (thrust) force beam was 175 Hz (700 Hz), and wingbeat frequency did not exceed 28 Hz. For the lift beam, displacements did not exceed

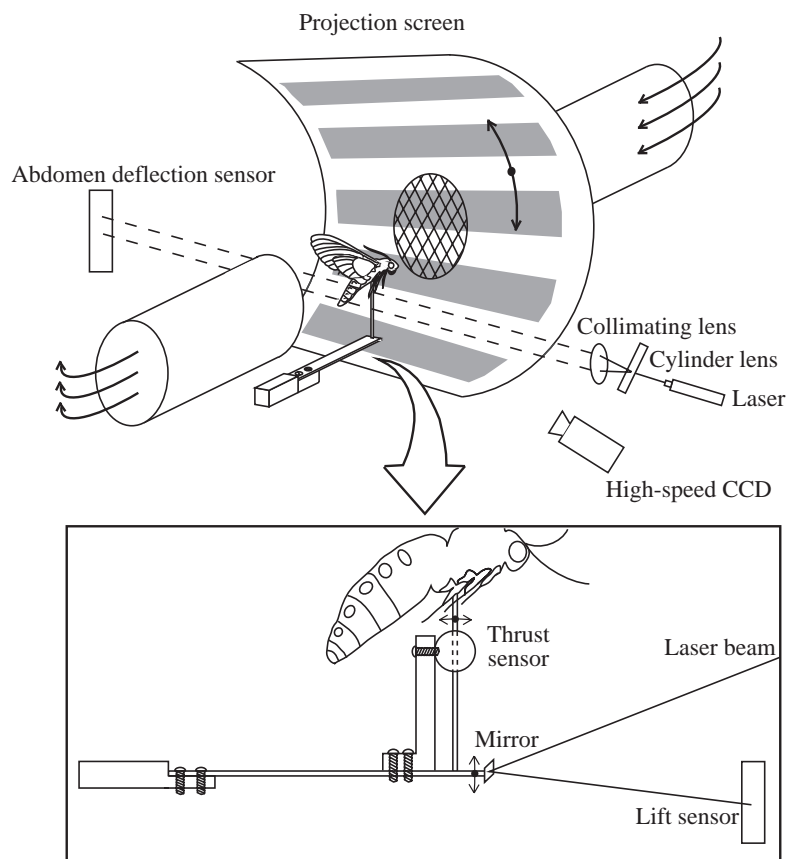


Fig. 1. The arena for tethered flight. The moth was tethered behind a translucent screen subtending 180° at the eye. A pattern of horizontal stripes was projected onto the screen and moved vertically by a computer-controlled servomotor (not shown). A laser system (inset) tracked up–down displacement of the tether and was calibrated to net vertical force (lift); an LED/photodetector pair tracked fore–aft displacement and was calibrated to horizontal force (thrust). A laser beam focused into a two-dimensional ‘sheet’ of light was cast across the abdomen onto an optoelectronic sensor to track angular deflections of the abdomen. A CCD camera was oriented orthogonal to the longitudinal axis of the moth and recorded wing motion at 500 frames s⁻¹.

0.05 mm, and sensor output was linear over this range with a slope of 0.048 N V^{-1} . Displacement of the thrust beam did not exceed 0.025 mm, and sensor output was linear over this range with a slope of 1.76 N V^{-1} . No attempt was made to dampen high-frequency oscillations exerted by the beating wings, so temporal phase offsets in the force recordings were negligible. This study presents lift data only. In several taxa of flies, lift responses are mediated primarily by motion detectors oriented within the frontal half of the visual field (Götz and Wehrhahn, 1984) where the stimulus was presented to moths in this study.

The force beam/tether assembly was mounted within a closed-circuit, open-throat wind tunnel (Fig. 1). The tunnel was constructed from air-duct hose 11 cm in diameter and powered by a fan mounted within the hose behind the moth. At the mouth of the wind tunnel, a section of honeycomb material (aircraft flooring) 4 cm thick with holes 5 mm in diameter served as an airflow straightener. It was painted white and mounted flush within a cylindrical projection screen, which subtended 180° at the eye of the moth and was placed 9 cm in front of the moth's head (Fig. 1). According to Willmott and Ellington (Willmott and Ellington, 1997b), *Manduca sexta* typically maintain a body angle of 25° during free flight at 1.5 m s^{-1} . Therefore, windspeed was set at 1.5 m s^{-1} and moths were tethered accordingly at 25° from their long axis.

A striped pattern was printed on acetate and fixed to a Plexiglas cylinder housing an incandescent lamp. The lamp was positioned above and slightly behind the moth. The moth's head was shielded from direct illumination by a narrow strip of aluminum positioned just below the lamp assembly. This arrangement produced an image on the projection screen that varied sinusoidally in brightness contrast along the vertical dimension with a spatial frequency of $0.025 \text{ cycles degree}^{-1}$ and was virtually free of angular distortion. A computer-controlled servomotor (not shown in Fig. 1) rotated the projection image with high precision such that image motion always started from exactly the same position on the screen referenced with respect to the bottom of the screen (90° below the moth's visual horizon).

Moths respond to motion of this large-field pattern by modulating flight forces and abdominal deflection in register with the moving image. Abdominal deflections were tracked optically. Focusing a laser beam sequentially through a cylinder lens and a collimating lens created a two-dimensional 'sheet' of light, which was focused orthogonally to the longitudinal axis of the abdomen. The abdomen cast a shadow on an optical sensor (10 cm linear position sensor, UDT Technologies) the voltage output of which was linearly proportional to the angle of the abdomen in its dorsal-ventral plane of motion.

The moths in this study did not receive visual feedback correctly matched to their attempts to reduce image slip. This 'open-loop' design was necessary to maintain control over image dynamics on the eye. The moths often did not exert average lift equivalent to body weight, which is likely to be an inherent liability of the open-loop experimental condition. Under closed-loop conditions, flies exert compensatory

visually evoked flight forces twice those exhibited under open-loop conditions (Heisenberg and Wolf, 1993).

This experiment was designed to test the ability of *Manduca sexta* to modulate flight behavior in response to large-field image motion after stretch receptor ablation. Therefore, responses to 14 different image velocities were compared for ablated versus intact or sham conditions. To control for transient responses to the onset of high-contrast image motion, the striped pattern was moved continuously throughout the experiment. 'Test' sequences at different velocities were embedded within 'adaptation' sequences at a fixed velocity. During an adaptation sequence, the striped pattern oscillated up-and-down at 160° s^{-1} for 3 s. This was followed immediately by a 3 s test sequence comprising continuous upward motion at a velocity selected at random by the computer; this regime (adaptation, test, adaptation) was repeated continuously for up to six blocks of test trials.

Stretch receptor ablation, power muscle electromyographic activity and high-speed videography

From its origin in the subalar membrane of the metathoracic wing hinge (Yack, 1992), the stretch receptor axon alone projects through a short segment of peripheral nerve before

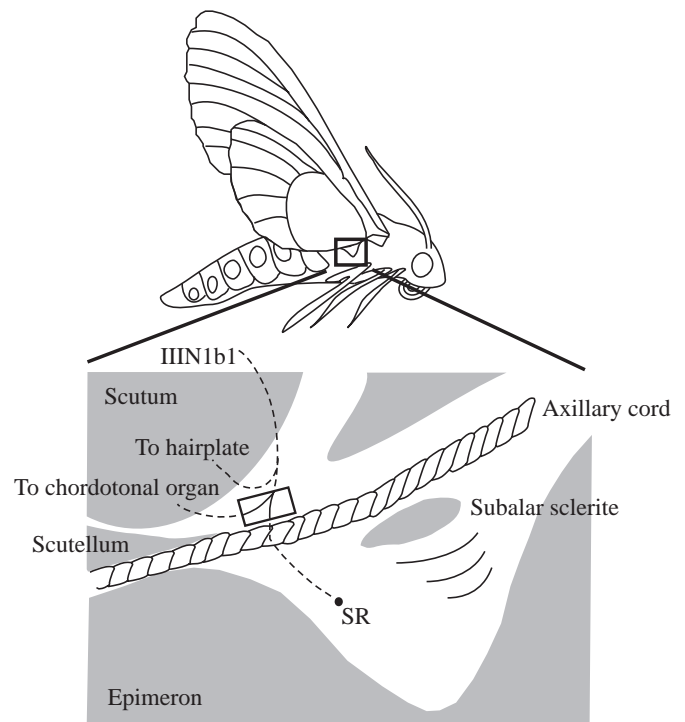


Fig. 2. Surgical ablation of the metathoracic wing hinge stretch receptor (SR). The stretch receptor sensory terminals are embedded within the subalar membrane between the metathoracic epimeron and subalar sclerite. The stretch receptor axon initially passes through a nerve composed of only its axon. A tiny incision was made in the soft cuticle (represented by the box) just dorsal to the axillary cord, and the stretch receptor was ablated by cutting its peripheral nerve. Nerve IIN1b1 contains the axons from a hair plate on the scutum, a chordotonal organ and the stretch receptor.

entering nerve IIN1b1 (Fig. 2). After making a small incision (approximately 1 mm×1 mm) in the soft cuticle at the base of the scutum (box, Fig. 2), the stretch-receptor-specific branch of IIN1b1 was visually identified and cut. For surgical sham (control) experiments, the stretch receptor nerve branch was surgically exposed but not cut. Visualization of stretch receptor nerves occasionally required the aid of a 0.02% solution of a vital dye (Janus Green B; Sigma Chemical Company) applied to the dissection site and washed away with fresh saline after 60 s. Janus Green temporarily and selectively stains nerve tissue in *Manduca sexta* (Miller, 1979).

Extracellular activity in the dorsolongitudinal (DLM) and dorsoventral (DVM) muscles was recorded with paired

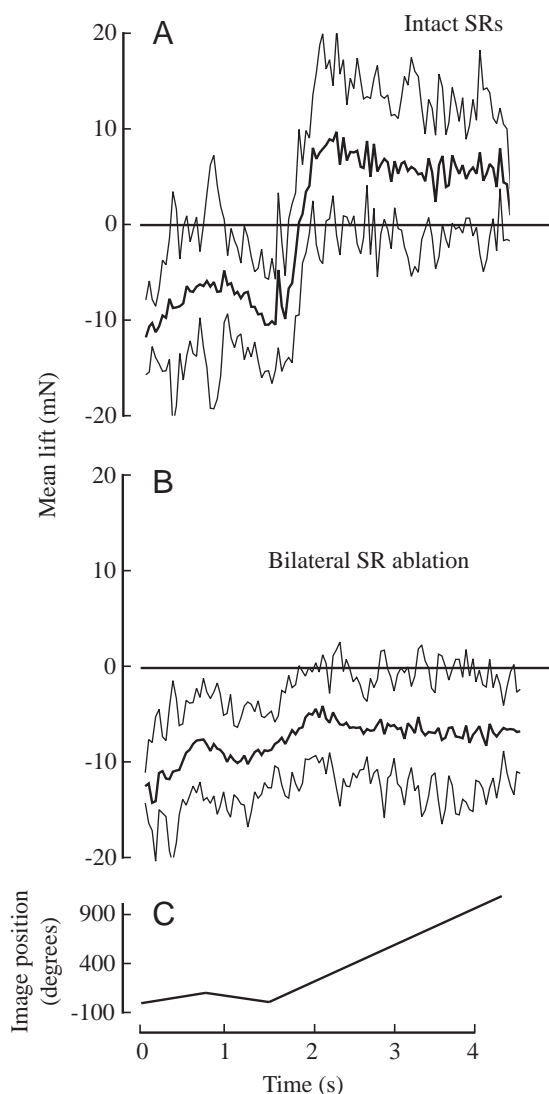


Fig. 3. Lift evoked by the hawkmoth in response to large-field image motion. Lift is defined as net vertical force; zero lift corresponds to mean body weight. The image was oscillated up and down in steps of constant velocity (C). Resultant lift responses, averaged across animals, are plotted for intact moths (A) ($N=9$) and for moths with stretch receptors (SRs) ablated (B) ($N=10$). Thick lines plot mean force, and thin lines plot \pm s.d.

nichrome wires insulated except at the tip (0.0254 mm diameter; AM Systems). The electrode wires were inserted through pin holes punched in the sclerotized cuticle at muscle insertion sites and fixed in place with a droplet of a molten mixture of beeswax and rosin. Signals were a.c.-coupled and differentially amplified (AM Systems; model 1800).

A high-speed infrared-sensitive CCD camera (monochromatic Redlake Motion Scope) was focused orthogonally to the longitudinal axis of the moth to track the path of the right wing at 500 frames s^{-1} (Fig. 1). The wingstroke was illuminated with fiberoptic microscopy lamps (Fiber-Lite) fitted with collimating lenses and long-pass infrared filters. Hawkmoths are relatively insensitive to infrared light, but the optical force sensors are extremely sensitive to the infrared flux of the video illumination system, so flight forces and video sequences could not be recorded simultaneously. A spot of opaque white ink (White-Out) was applied to the ipsilateral wing tip to enhance contrast in the video images. Two-dimensional projections of the wingtip path were digitized using mouse-click macros written in NIH Image (v. 1.62). Lift, thrust, abdomen angle, amplified DLM and DVM activity and the position of the projected image were digitized at 10 kHz with a LabVIEW data-acquisition system and stored on computer.

Values in the text are presented as means \pm s.d.

Results

Effects of stretch receptor ablation on visually elicited lift generation

As the striped pattern is oscillated up and down in velocity steps of $160^\circ s^{-1}$ (the adaptation sequences), lift rises and falls along roughly a triangular wave trajectory (Fig. 3A, first 1.5 s). After the onset of a high-velocity test sequence ($400^\circ s^{-1}$), lift

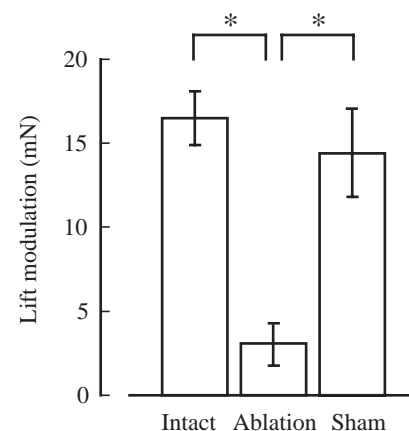


Fig. 4. For one test velocity ($400^\circ s^{-1}$), responses to a sham ablation were tested. Lift modulation corresponds to the amount of lift evoked in response to a step in image speed (see Materials and methods). The surgical sham had no effect on mean lift modulation, while stretch receptor ablation reduced lift modulation to approximately 30% of the intact response (Student's t -test, $*P<0.05$, $N=9$ intact, $N=10$ ablated and $N=5$ sham). Values are means \pm s.d.

rises to a plateau (Fig. 3A, last 2.5 s). Animals with bilaterally ablated metathoracic stretch receptors show a qualitatively similar pattern of force production, while the magnitude of

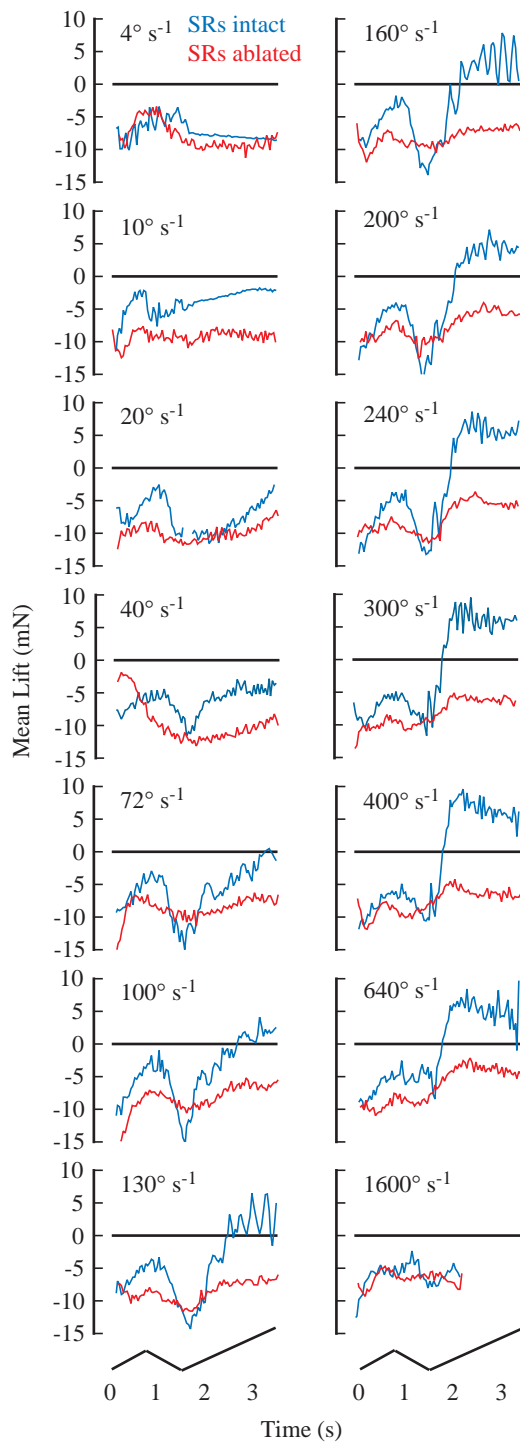


Fig. 5. Lift in response to large-field image motion for test sequences of varying velocity (indicated numerically in each plot). Mean lift is plotted for the adaptation cycle preceding the test sequence. Image position is illustrated schematically at the bottom of the figure as a time reference. Data for intact moths are plotted in blue ($N=9$) and for stretch-receptor-ablated moths in red ($N=10$).

evoked lift is greatly diminished (Fig. 3). I define a measure of the strength of lift responses (lift modulation) as the difference between the lift at the onset of the test sequence and the maximum lift during the trial. Intact moths ($N=9$) show average lift modulation of 16.5 ± 1.6 mN compared with 3.1 ± 1 mN for the ablated animals ($N=10$) and 14.4 ± 2.6 mN for a surgical sham ($N=5$) condition (Fig. 4). Stretch receptor ablation, but not the surgical sham, results in a significant decrease in lift modulation ($P=0.031$, Student's t -test).

Fig. 5 shows lift responses to a series of test velocities ranging from 4 to 1600°s^{-1} for moths with stretch receptors intact (blue) and stretch receptors ablated (red). At both the lowest and highest velocities tested ($4\text{--}20^\circ \text{s}^{-1}$ and 1600°s^{-1}), lift responses are small in magnitude, while at mid-range velocities ($40\text{--}640^\circ \text{s}^{-1}$) lift rises quickly and plateaus within approximately 1.5 s. Stretch receptor ablation results in lower-magnitude lift in response to large-field image motion (Fig. 5). Mechanical stops in the image-rotating servomotor limited the duration of the 1600°s^{-1} test trial to 1 s.

Stretch receptor ablation had little, if any, influence on the velocity-tuning of lift modulation. For both the intact and ablated groups of moths, lift modulation rises with increasing image speed, peaks between 200 and 300°s^{-1} and falls at greater speeds (Fig. 6). Intact animals were able to exert a mean lift modulation of 16.5 ± 1.6 mN ($N=9$) in response to the optimal velocity tested (240°s^{-1}), while the stretch-receptor-ablated group showed a maximum of 4.7 ± 0.54 mN ($N=9$). Stretch-receptor-ablated animals showed a marked decrease in lift modulation, especially at the mid-range image speeds of $100\text{--}700^\circ \text{s}^{-1}$.

Effects of stretch receptor ablation on abdominal deflection, power muscle activity and wing kinematics

During tethered flight, moths pitch their abdomen up and

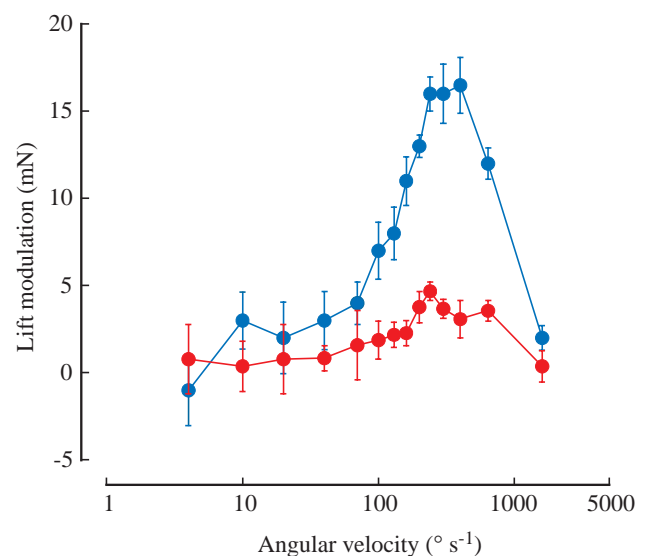


Fig. 6. Mean lift modulation *versus* angular velocity (image speed). Data from intact moths are plotted in blue ($N=9$) and from stretch-receptor-ablated moths in red ($N=9$). Values are means \pm s.d.

down in register with large-field image motion. At high test velocities, the abdomen pitches dorsally in response to upward image motion until a plateau is reached, presumably reflecting a morphological limit to the excursion (Fig. 7). Ablation of the stretch receptors had virtually no effect on the magnitude or time course of abdominal motion during flight (Fig. 7), suggesting that stretch receptor feedback is not distributed to all motoneurons involved in optomotor responses during flight.

Bilateral ablation of the hindwing stretch receptors had no significant effect on the frequency or phase of activation of the power muscles during flight. A representative sample of recordings from the DLM and DVM before and after stretch receptor ablation in one moth is shown in Fig. 8. Prior to stretch receptor ablation, the mean frequency of the DLM depressors was 19.8 ± 0.9 Hz, after ablation it was 19.6 ± 1.6 Hz ($P=0.73$, $N=5$, paired Student's *t*-test). The mean phase of antagonistic DVM elevator activity within the cycle defined by DLM activation was $31 \pm 5\%$ before stretch receptor ablation and $28 \pm 2\%$ after ablation ($P=0.2$, $N=5$, paired Student's *t*-test). These results suggest that stretch receptor feedback is not strongly targeted to the two muscles, which provide the vast majority of mechanical power to the wingstroke.

The modulation in lift during large-field image motion probably results from characteristic changes in wingstroke amplitude. Total wingstroke amplitude increases and decreases during up-and-down image motion. However, amplitude is not modulated symmetrically during the upstroke and downstroke. Instead, the total wing excursion of the upstroke is fixed while the excursion of the downstroke is varied (Fig. 9, compare blue traces for the sham condition). I quantified the magnitude of this change in downstroke excursion by measuring the 'half-amplitude' of each wingstroke. Half-amplitude was defined as the difference between the position of the wing at the end of the downstroke and the position the wing would occupy if it were extended directly laterally towards the camera and orthogonal to the longitudinal axis of the moth. Normally, moths respond to the up-and-down visual stimulus by increasing and decreasing half-amplitude (Fig. 9). As a result of stretch receptor ablation, modulation in half-amplitude is greatly diminished. For one moth (Fig. 9), stretch receptor ablation reduced the wingstroke half-amplitude by 35% (Student's *t*-test, $P<0.001$). Also, the entire stroke plane appears to be shifted caudally, and the envelope comprising fore-aft wing motions appears to 'open' as a result of stretch receptor ablation. While this finding was consistent for three individuals (trajectories averaged across individuals, Fig. 9, bottom), the sample size is too low for a rigorous statistical analysis. Also, because *Manduca sexta* wings demonstrate significant spanwise bending during flight, the

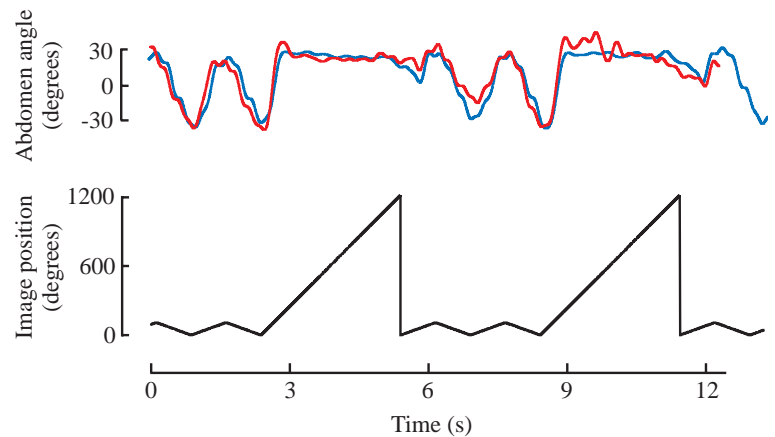


Fig. 7. Abdominal deflections in response to large-field image motion. Averaged responses are plotted for intact (blue, $N=3$) and stretch-receptor-ablated moths (red, $N=3$).

two-dimensional projections of the wingtip path in Fig. 9 cannot be transformed into the spherical coordinate system of natural wing motions. Therefore, these videography results must be viewed with caution until more rigorous three-dimensional analyses and statistical tests can be performed. Nevertheless, these results strongly suggest that stretch receptor ablation does indeed affect wing amplitude and wingstroke trajectory.

Discussion

Here, I have shown the effects of selectively ablating a single pair of mechanosensory neurons on the optomotor control of flight forces. After bilateral ablation of the hindwing stretch receptors, visually elicited lift production falls by nearly 70% (Fig. 4, Fig. 5, Fig. 6). Ablation had no influence on visually elicited abdominal deflections (Fig. 7), suggesting that stretch receptor feedback is not distributed to the entire flight control network. The frequency and phase of activation of the

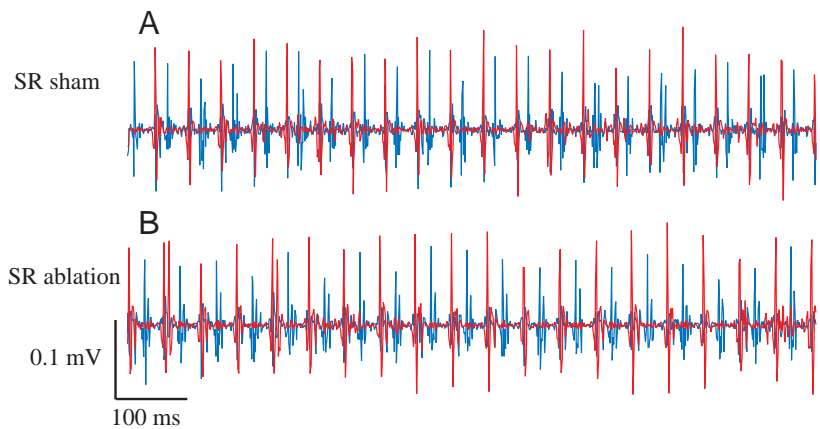


Fig. 8. Extracellular recordings of dorsolongitudinal (red) and dorsoventral (blue) muscle activity during tethered flight after a surgical sham (A) and after bilateral stretch receptor ablation (B). Stretch receptor (SR) ablation did not significantly influence the frequency or phase of activation in these muscles (see Results).

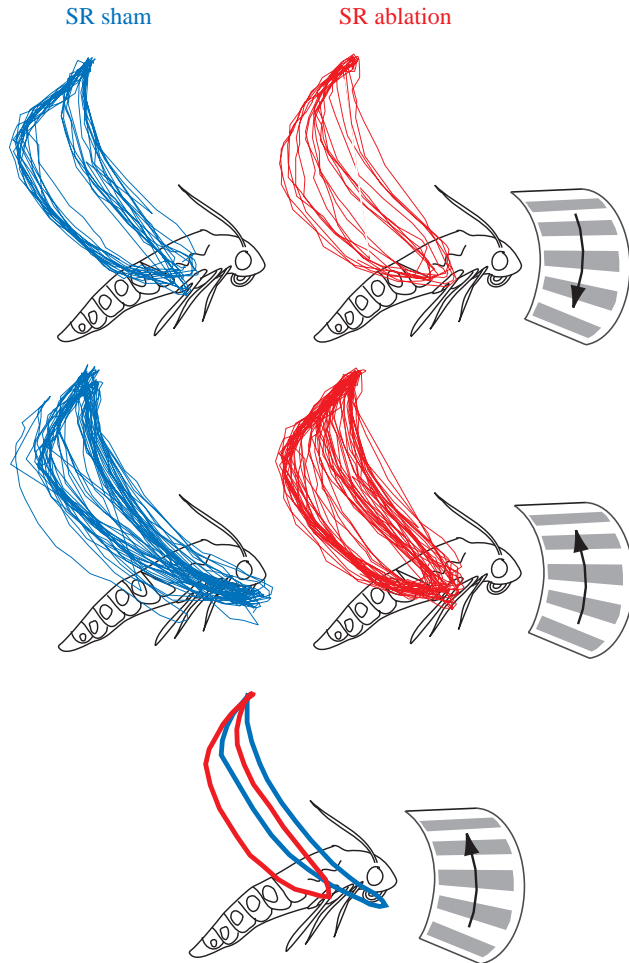


Fig. 9. Wingtip projection during upward and downward image motion at 160°s^{-1} . The two-dimensional projection of the wingpath is plotted for a single individual before (blue) and after (red) stretch receptor ablation and for downward (top row) and upward (middle row) image motion. The average wingpath evoked by upward image motion is shown in the bottom diagram ($N=3$). The data were digitized from video images recorded at 500frames s^{-1} .

large indirect power-producing muscles are unaffected by ablation (Fig. 8); however, ablation does result in a significant reduction in wingstroke amplitude (Fig. 9). Surgical ablations are problematic in that the experimental treatment cannot be reversed, so these results must be interpreted with appropriate caution. Nevertheless, the results from this experimental approach have contributed broadly to our understanding of the behavioral function of mechanosensory feedback [e.g. the function of the haltere organs in flies (Dickinson, 1999); the function of the tegulae in locusts (Fischer and Eckhard, 1999)].

Sensory physiology and targets of stretch receptor feedback within the flight control circuit

This paper and its companion (Frye, 2001) focus only on the metathoracic stretch receptor described by Yack (Yack, 1992). Virtually nothing is known about the mesothoracic stretch receptor, and it may differ in its response to wing motion as

well as in its synaptic interactions with the flight rhythm generator. However, unlike locusts, whose meso- and metathoracic wing pairs beat out of phase and differentially control steering and power production (Pearson and Wolf, 1987), the hindwings and forewings of hawkmoths are morphologically coupled and beat together during the entire stroke cycle (Willmott and Ellington, 1997a). Therefore, motions of the coupled wings are encoded by the hindwing stretch receptor (Frye, 2001; Yack and Fullard, 1993).

In several ways, the stretch receptor activity patterns in *Manduca sexta* are similar to those of the well-studied locust stretch receptor. In both species, the stretch receptor fires a high-frequency burst of spikes near the top of each wingstroke and its activity encodes several aspects of wing motion. Stretch receptors discharge tonically in response to experimental wing elevation in moths (Yack and Fullard, 1993) and locusts (Heukamp, 1983). During tethered flight, the number of spikes within each burst is proportional to wingstroke amplitude. Although it has not been explicitly measured, the onset of the stretch receptor burst in locusts may be tightly phase-locked with respect to wing motion [see fig. 10 in Möhl (Möhl, 1985a)], as is the case in the hawkmoth (Frye, 2001).

In the locust, stretch receptor feedback exhibits a widespread influence on flight motor patterns originating within the central nervous system, particularly on those controlling wing depression. In a reduced preparation in which the flight motor rhythm is elicited by wind on the head, motor oscillation frequency (assayed by basalar muscle potentials) increases immediately during stretch receptor stimulation (Reye and Pearson, 1988). This control of muscle activity emerges from well-studied synaptic interactions between the stretch receptor afferents and flight motoneurons. Stretch receptors form fast excitatory, often monosynaptic, connections with motoneurons of an indirect depressor (dorsolongitudinal muscle), as well as two direct depressors (basalar and subalar muscles); they form polysynaptic inhibitory connections with indirect elevator (dorsoventral muscle) motoneurons (Burrows, 1975). The influence of the stretch receptor is powerfully phasic such that stretch receptor spikes occurring near the onset of a depressor spike can reset and entrain the flight rhythm, while stretch receptor activity midway between depressor spikes has no effect (Pearson et al., 1983). During flight in semi-intact preparations, stretch receptor activity reduces the rate of depolarization in DVM motoneurons (Wolf and Pearson, 1988), while reducing the degree of hyperpolarization and advancing the depolarization of basalar motoneurons (Pearson and Ramirez, 1990). In locusts, therefore, stretch receptor feedback influences the activity of indirect power-producing muscles as well as the activity of smaller muscles inserting directly on the wing hinge.

As yet, we know virtually nothing about the central projections or synaptic interactions of the hawkmoth stretch receptor. In the moth, stretch receptor ablation does not impair the output of the entire flight control network, and several lines of evidence suggest that the targets of stretch receptor feedback differ between hawkmoths and locusts. Hawkmoths exhibit

characteristic pitching or ‘ruddering’ motions that are qualitatively similar to those exhibited by flies (Zanker, 1988) and locusts (Robertson and Johnson, 1993) and help the animal to control rotational moments during flight maneuvers. Stretch receptor ablation does not influence these deflections in abdomen angle (Fig. 7) in the moth, suggesting that feedback from this mechanoreceptor is not distributed throughout the flight control network. Neither the mean frequency nor the phase relationships of power muscle activation change after stretch receptor ablation, suggesting that hawkmoths lack the strong synaptic connections seen in locusts between the stretch receptors and DLM and DVM motoneurons.

Mechanisms and behavioral functions of mechanosensory integration

By encoding the timing of depression at the end of the downstroke, the proprioceptive tegulae of locusts (and perhaps also of moths, although there are no published data) function to keep the motor rhythm phase-locked with respect to wing motions. Even without experimental manipulation of external sensory cues, ablation of the tegulae sensory organs results in a decrease in lifting power during ascents in free flight (Fischer and Eckhard, 1999), as well as perturbations in wing kinematics and decreased lift production during tethered flight (Wolf, 1993). Feedback from the halteres of flies encodes gyroscopic forces elicited as the fly rotates in space. *Drosophila melanogaster* tethered in a flight arena in which they have closed-loop control of the visual panorama exhibit fast compensatory responses to experimental rotation about their inertial frame of reference. These rotational equilibrium reflexes are abolished by surgical ablation of the halteres (Dickinson, 1999), providing good evidence that haltere feedback functions to maintain torsional stability during flight.

Two proposed functions of feedback from the stretch receptor include compensating for rapid perturbations to wing kinematics, as might be expected during flight in turbulent wind, and compensating for subtle temporal imperfections in the flight motor rhythm (Möhl, 1985b). The evidence for these hypotheses is strong: stretch receptor neurons respond to widely varying wing motions during flight (Möhl, 1985a) and they form monosynaptic connections with flight motoneurons (Burrows, 1975). During afferent stimulation, the stretch receptors both entrain the flight rhythm (Reye and Pearson, 1988) and correct for asymmetries inherent in the motor pattern (Möhl, 1985b). So powerful is the influence of stretch receptors on motoneuron activity in locusts that they satisfy the criteria used to define elements of a locomotor pattern generator (Weeks, 1981). Therefore, the stretch receptors probably participate in virtually all aspects of flight control including the modulation of aerodynamic forces in response to visual cues.

The visual control of flight forces has been studied most extensively in the fly. Tethered flies modulate aerodynamic output, head angle and abdominal deflection in a combined attempt to minimize large-field motion on the retina (Collett et al., 1993). Collectively, these behaviors comprise the well-

studied ‘optomotor response’, which operates around all three axes of rotation (Blondeau and Heisenberg, 1982) and is thought to mediate flight stabilization by allowing an animal to counteract unintended deviations in course (Götz, 1972). Like flies, hawkmoths in tethered flight modulate lift (Fig. 3) as well as yaw torque (Dombrowski, 1991) in response to the direction and velocity of large-field image motion. During controlled large-field visual stimulation, freely flying moths (*Macroglossum stellatarum*) exhibit powerful optomotor control of rotation and translation while they hover at an artificial nectary (Kern and Varju, 1998), allowing them to compensate for drift or buffeting winds. The stretch receptor would respond to perturbations in wing kinematics resulting from a wind gust, but this study shows that this proprioceptor is involved in optomotor control of lift even in the absence of any external mechanical stimuli. Moths exhibit a 70% reduction in the magnitude of optomotor-mediated lift after stretch receptor ablation (Fig. 4, Fig. 5). The apparent ‘tuning’ of lift modulation with respect to image velocity (Fig. 6) emerges from processes inherent to the visual detection of motion (O’Carroll et al., 1997). Descending visual interneurons provide excitatory input to flight muscle motoneurons in *Manduca sexta* (Rind, 1983), and these cells probably receive input from velocity-tuned motion-sensitive interneurons in the lobula plate such as those described in *Macroglossum stellatarum* (Wicklein and Varju, 1999). Ablation affects the magnitude, not the velocity tuning, of optomotor responses, suggesting that stretch receptor feedback acts downstream of the motion detection pathway.

Exactly where in the hawkmoth the integration of visual and stretch receptor feedback occurs has yet to be determined, but it does not appear to be in the control of the indirect elevators and depressors powering the wingstroke (Fig. 8). However, stretch receptor ablation does result in significantly reduced wingstroke amplitude in response to upward image motion (Fig. 9). In flies, the mechanical power produced by the indirect depressors and elevators is transmitted to steering kinematics by tiny muscles inserting directly on the wing hinge sclerites (Dickinson and Tu, 1997). Within the stroke cycle, wing amplitude and trajectory are tightly correlated with the phase of steering muscle activation (Tu and Dickinson, 1996). Perhaps, in *Manduca sexta*, stretch receptor feedback is integrated in the optomotor control of muscles such as the pleurodorsals and subalars, which insert directly onto the wing hinge. These small muscles control wingstroke amplitude during steering maneuvers (Dombrowski, 1991; Rheuben and Kammer, 1987) and are active during optomotor responses in *Manduca sexta* (Rind, 1983). In addition, the pleurodorsal muscles receive excitatory input directly from the visual system. In quiescent *Manduca sexta*, these muscles exhibit strong spiking responses that are directionally tuned to large-field image motion (Wendler et al., 1993). The hypothesis that stretch receptor feedback is targeted to steering muscle control in the hawkmoth remains to be tested directly. The specific encoding properties of the *Manduca sexta* stretch receptors are the subject of a companion paper (Frye, 2001).

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