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Visual Input to the Efferent Control System of a Fly's "Gyroscope"

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Dipterous insects (the true flies) have a sophisticated pair of equilibrium organs called halteres that evolved from hind wings. The halteres are sensitive to Coriolis forces that result from angular rotations of the body and mediate corrective reflexes during flight. Like the aerodynamically functional fore wings, the halteres beat during flight and are equipped with their own set of control muscles. It is shown that motoneurons innervating muscles of the haltere receive strong excitatory input from directionally sensitive visual interneurons. Visually guided flight maneuvers of flies may be mediated in part by efferent modulation of hard-wired equilibrium reflexes.

Flies are among the most maneuverable of all flying animals and generate elaborate flight behaviors under visual control (*1*). For example, a male housefly initiates a corrective tracking maneuver within 30 ms of detecting a deviation in the flight trajectory of a female that it is chasing (*2*). Flies have several unique specializations that enable them to detect and respond to moving targets with such rapidity. These specializations include a visual system with a flicker fusion rate of 300 Hz (*3*) and wings capable of achieving an aerodynamic performance that is two to three times greater than that generated by conventional steady-state mechanisms (*4*). Perhaps the most remarkable specialization of the flight system of flies is the evolutionary transformation of the hind wings into equilibrium organs called halteres, tiny club-shaped organs that beat antiphase to the wings during flight (*5*). Although the halteres have lost their aerodynamic role through evolution, the sensory fields at their base have hypertrophied relative to their homologs at the base of the wings (*6*). In the blow fly *Calliphora vicina*, the haltere is equipped with about 335 strain-sensitive campaniform sensilla organized in five distinct fields on the haltere base (*7*). Sensory cells innervating a subpopulation of these sensilla encode Coriolis forces that result from the cross product of the haltere's linear velocity with the angular velocity of the fly's body around the yaw, pitch, or roll axes (*8*). Through their strong connections with steering motoneurons of the wing, the haltere afferents mediate stabilizing flight control reflexes (*9*, *10*). With their halteres removed, flies are unstable and quickly crash to the ground (*11*).

In many animals, efferent regulation modulates the sensitivity of sensory systems. In mammals, mechanical feedback mediated through efferent control of the outer hair cells within the cochlea is responsible for the sharp tuning of the primary auditory receptors (*12*). In vertebrate muscle spindles, fusimotor efferents can adjust the length of intrafusial fibers to set the sensitivity of the spindle sensory afferents (*13*). Similarly, in

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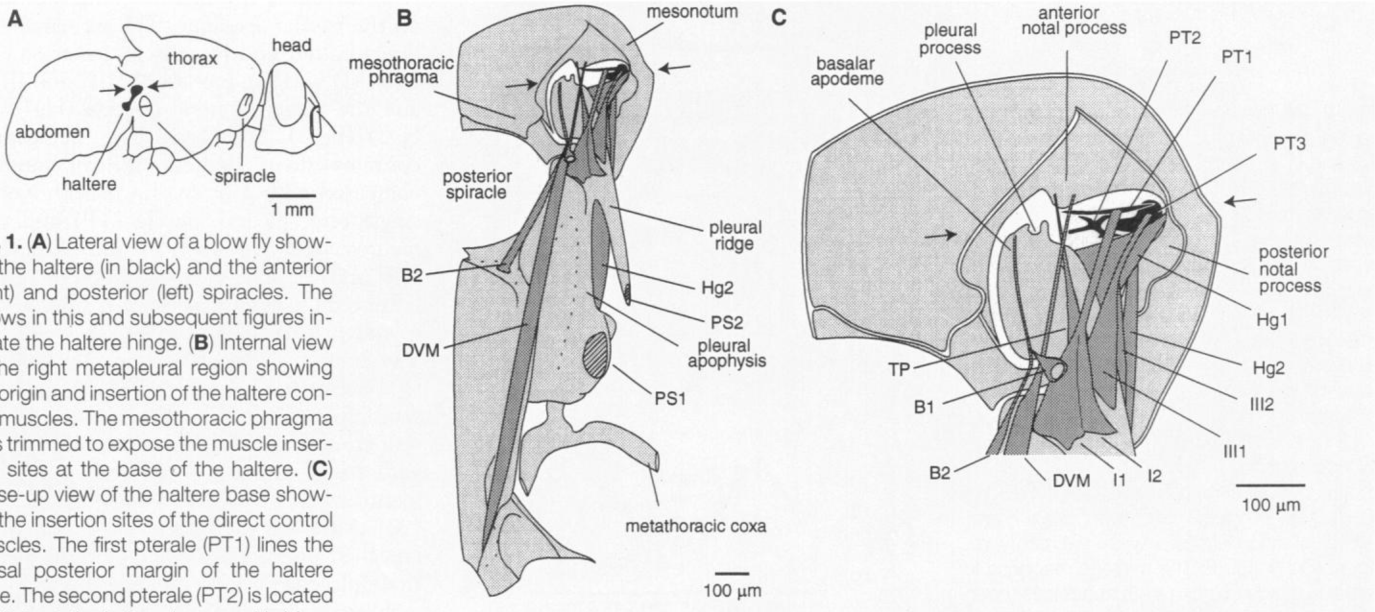

Fig. 1. (**A**) Lateral view of a blow fly showing the haltere (in black) and the anterior (right) and posterior (left) spiracles. The arrows in this and subsequent figures indicate the haltere hinge. (**B**) Internal view of the right metapleural region showing the origin and insertion of the haltere control muscles. The mesothoracic phragma was trimmed to expose the muscle insertion sites at the base of the haltere. (**C**) Close-up view of the haltere base showing the insertion sites of the direct control muscles. The first pterale (PT1) lines the dorsal posterior margin of the haltere base. The second pterale (PT2) is located medially at the haltere base and bridges the dorsal and ventral margin of the haltere's articulation with the metathorax. The third pterale (PT3) forms the posterior corner of the haltere base. The largest sclerite, the basalare, is fused to the pleural process and sits just anterior and ventral to the haltere. These four sclerites are surrounded anteriorly and dorsally by the anterior notal process, posteriorly by the posterior notal process, and ventrally by the pleural process.

decapod crustaceans, centrifugal control of muscle tension is used to regulate the sensitivity and operating range of stretch receptors (*14*). In this report, we provide anatomical and physiological evidence that the halteres of flies are also equipped with an efferent control system composed of the serial homologs of the steering muscles of the wing. By recording from motor units within identified haltere control muscles, we show that this efferent system is under the direct control of motion-sensitive visual interneurons. Thus, the haltere control muscles provide a means by which visual information can modify mechanosensory feedback.

Table 1. Comparison of muscles associated with wing (mesothorax) and haltere (metathorax).

Type	Origin	Insertion	Mesothorax (*34*)	Metathorax
Power muscle	Notum	Notum	DLM1 to DLM6	None
Power muscle	Notum	Ventral pleuron	DVM1 to DVM7	DVM
Control muscle				
Direct	Thoracic phragma	Basalare	B1	B1
Direct	Pleuron	Basalare	B2	B2
Direct	Notum	Basalare	B3	None
Direct	Pleural apophysis	Pterale I	I1, I2	I1, I2
Direct	Pleural apophysis	Pterale III	III1 to III4	III1, III2
Direct	Pleural apophysis	Posterior notal process	Hg1 to Hg4	Hg1, Hg2
Indirect	Pleural apophysis	Sternum	PS1, PS2	PS1, PS2
Indirect	Pleural apophysis	Notum	TP1, TP2	TP

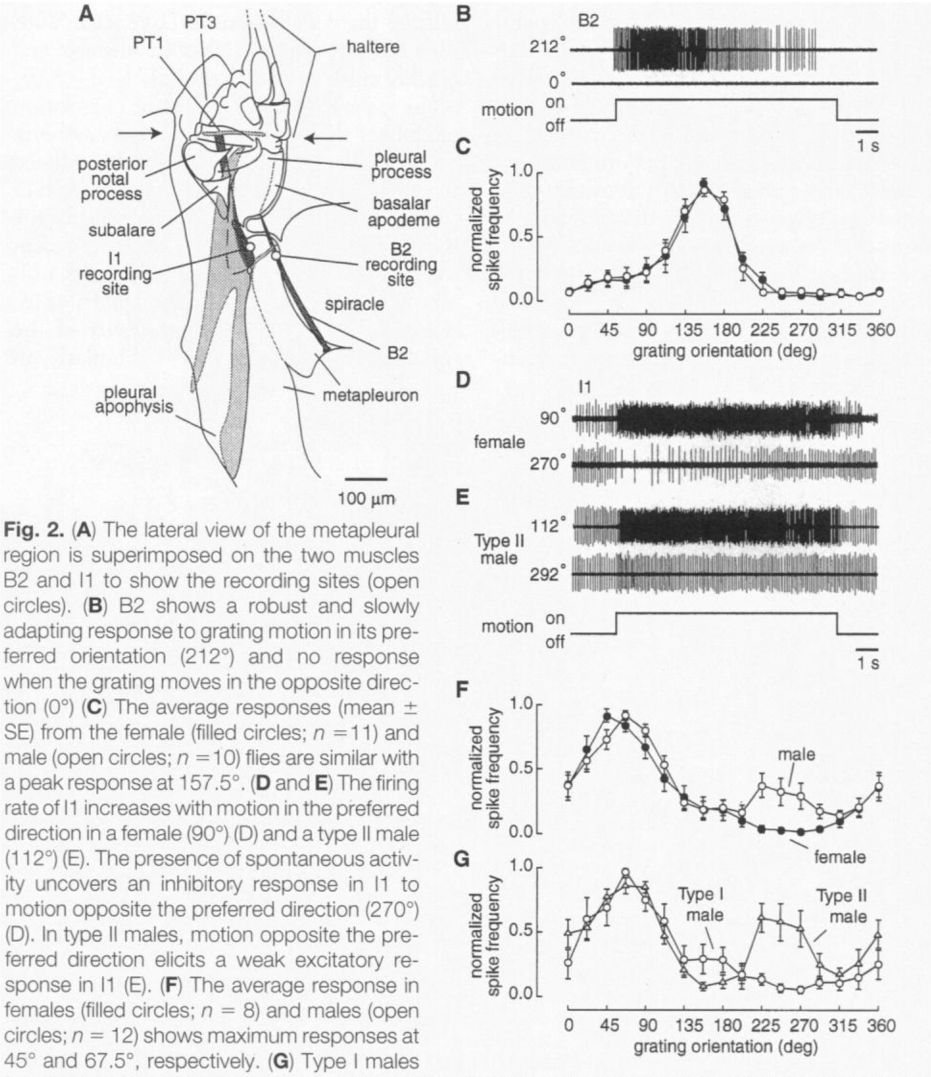

Fig. 2. (**A**) The lateral view of the metapleural region is superimposed on the two muscles B2 and I1 to show the recording sites (open circles). (**B**) B2 shows a robust and slowly adapting response to grating motion in its preferred orientation (212°) and no response when the grating moves in the opposite direction (0°) (**C**) The average responses (mean ± SE) from the female (filled circles; $n = 11$) and male (open circles; $n = 10$) flies are similar with a peak response at 157.5°. (**D** and **E**) The firing rate of I1 increases with motion in the preferred direction in a female (90°) (D) and a type II male (112°) (E). The presence of spontaneous activity uncovers an inhibitory response in I1 to motion opposite the preferred direction (270°) (D). In type II males, motion opposite the preferred direction elicits a weak excitatory response in I1 (E). (**F**) The average response in females (filled circles; $n = 8$) and males (open circles; $n = 12$) shows maximum responses at 45° and 67.5°, respectively. (**G**) Type I males (open circles; $n = 6$) have average responses similar to those of the females, whereas type II males (open triangles; $n = 6$) exhibit a second response peak at 225°.

The haltere is situated in the metathoracic segment behind the posterior spiracle (Fig. 1A). As with the wing, the haltere is attached to the thorax by a complex articulation consisting of four hard sclerites [basalare and pteralae (PT) 1 to 3; Fig. 1C] interconnected by more flexible membranous cuticle. Collectively, these cuticular elements make up a complex hinge that allows the stalk of the haltere to rotate over a 180° arc with respect to the thorax. The muscles of the haltere insert on various element within this hinge (Fig. 1, B and C), providing a means of controlling haltere kinematics during flight (*15*). The mesothoracic wing muscles of flies may be divided into three functionally, physiologically, and anatomically distinct classes: large indirect asynchronous muscles that provide the power to move the wings back and forth, small direct synchronous muscles that adjust kinematics during steering maneuvers, and small indirect synchronous muscles that are thought to regulate the tension of the thorax (*16*). Although greatly reduced in size, the small metathoracic segment contains members of all three flight muscle groups (Table 1).

The metathorax contains only a single pair of indirect asynchronous muscles compared with 13 pairs in the mesothorax. As first described by Pringle (*17*), activity in this single dorsal ventral muscle (DVM) is sufficient to oscillate the haltere back and forth within its stroke plane. Compared with the power muscles, the reduction in the number of direct control muscles associated with the haltere is less extreme, from 13 in the mesothorax to 8 in the metathorax, most of which originate on the metapleuron (Table 1). Two of the muscles (B1 and B2) insert through a common tendon on the basalar apodeme. The six remaining direct haltere control muscles insert on pterale I (I1 and I2), pterale III (III1 and III2), and the posterior notal process (Hg1 and Hg2) (Fig. 1, B and C). The metathorax contains three indirect control muscles compared with four in the mesothorax: a single tergo-pleural muscle (TP) and two pleurosternal muscles (PS1 and PS2) (Fig. 1, B and C). The gross morphology of the haltere muscles in *Calliphora* is similar, but not identical, to that previously described in a crane fly (*18*) and a horse fly (*19*).

To examine the effect of descending visual information on the activity of the haltere control muscles, we presented animals with visual gratings while recording from identified muscles with glass microelectrodes (*20*). Although the haltere muscles are tiny and their insertions overlap, by careful electrode placement, we were able to make unambiguous identification of two muscles (I1 and B2) (Fig. 2A) whose activities are modulated by descending visual input (*21*). Movement of a visual grating elicited a robust, slowly adapting increase in the firing rate of B2 (Fig. 2B). This response was directionally sensitive, with a peak response at 157.5° (Fig. 2C), corresponding to motion downward and slightly toward the ipsilateral side. In animals in which the background activity in B2 was high, we observed an inhibitory response to stripes moving opposite to the preferred direction. The response of B2 was identical in males (n = 10) and females (n = 11). The directional sensitivity of B2 is similar to that of lobula plate interneurons within the vertical system (*22*). Muscle I1 also responded to the motion of a visual grating (Fig. 2D), although its directional tuning is roughly orthogonal to that of B2, with a preferred direction of 45° corresponding to motion upward and toward the ipsilateral side (Fig. 2F). In most preparations, I1 fired spontaneously in the absence of visual motion. In females (n = 8), motion opposite to the preferred direction resulted in an inhibition of this background activity (Fig. 2D). The directional tuning of I1 in females was similar to that of the horizontal system visual interneurons in the lobula plate (*23*). The directional sensitivity of I1 in males was more complex, suggesting a peculiar sexual dimorphism (*24*).

Experiments on tethered animals have indicated that visual stimuli modulate the activity of wing-steering muscles during flight (*25, 26*). In the course of our experiments, we also examined the responses of identified wing control muscles to the same visual stimuli that activated the haltere control muscles. We found no evidence for threshold responses in any of the wing muscles to the presentation of visual stimuli in quiescent animals. This negative result cannot be explained by a general inability to record from the wing muscles, because we routinely recorded from the same muscles during steering responses in tethered flying animals. This failure of visual motion to elicit responses in the wing-steering muscles while generating robust responses in the haltere control muscles of the same animals has two possible explanations. First, the motoneurons of the wing control muscles might be neurally gated such that descending visual input does not elicit threshold events unless the animal is flying. Context-dependent gating of visual information is well characterized in the flight control system of locusts (*27*). However, if such a gating mechanism were in effect, it is peculiar that it should operate to uncouple the descending input from the wing motoneurons but not from serially homologous haltere motoneurons. Perhaps because of their small size, there are no deleterious consequences from maintaining the connections with haltere muscles, whereas the wing muscles are required for grooming and courtship behaviors when the animals are not flying. A second, nonexclusive interpretation is that the descending visual interneurons do indeed make stronger connections with haltere control motoneurons than with wing control motoneurons.

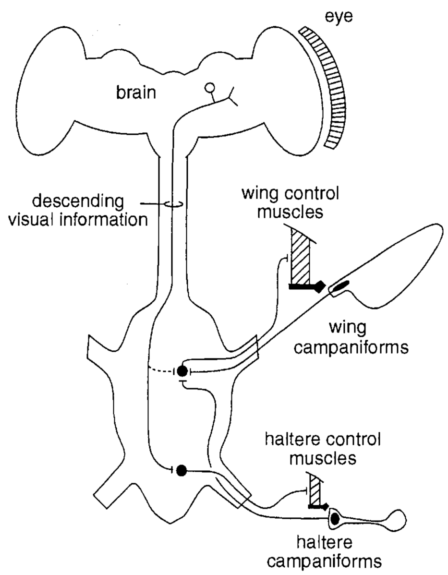

Fig. 3. Cartoon illustrating the control-loop hypothesis by which the visual system can actively manipulate the haltere equilibrium reflex loop. Motoneurons are indicated by heavy lines. The descending visual interneuron activates the haltere-steering muscles (bottom hatched box) to change haltere kinematics during flight. This change in haltere beating would in turn activate haltere campaniform neurons that feed forward to wing-steering motoneurons. By such a mechanism, visual interneurons could influence wing-steering muscles (top hatched box) without any direct connections (dotted line) with wing motoneurons.

The anatomical and physiological evidence presented here indicates that the halteres of flies are equipped with an efferent control system that is under the influence of descending visual interneurons. This pathway, together with the known connections between haltere afferents and neck motoneurons (*28*), might explain why optokinetic head turns in walking animals are absent after haltere ablation (*9*). During flight, the haltere efferent system might serve a number of functions. Extensive behavioral evidence suggests that the mechanosensory afferents at the base of the haltere act to initiate compensatory reflexes in flying animals (*8, 9, 28*). In at least some cases, these reflexes result from strong monosynaptic connections between haltere afferents and wing-steering motoneurons consisting of both chemical and electrical components (*29, 30*). The speed and accuracy of these reflexes are in large part responsible for the remarkable stability of flies, as evidenced by their instability after haltere ablation. One potential problem with equilibrium reflexes, however, is that they act to counteract voluntary behaviors. For example, the vestibular-ocular circuitry in vertebrates must ignore retinal slip when an individual actively turns its head (*31*). The haltere motor system provides two possible mechanisms by which the stability reflexes would not interfere with voluntary commands: reflex desensitization and control-loop manipulation. By changing kinematics or altering the mechanics of the haltere base, the control muscles could attenuate the sensitivity of the haltere afferents during voluntary flight maneuvers and thereby inhibit corrective responses. Alternatively, rather than disabling the haltere reflexes, the fly might actively manipulate the reflex loop to generate voluntary maneuvers (Fig. 3). By activating control muscles, the descending interneurons could alter haltere kinematics in a way that would mimic the changes that occur during externally imposed flight perturbations. The resulting compensatory activation of wing muscles in the absence of external perturbations would result in a change in the animal's flight trajectory.

At present, we favor the reflex-loop manipulation hypothesis over the reflex desensitization hypothesis for several reasons. First, this architecture could explain the apparently weak connections between descending visual input and the steering muscles of the wing. Visual input might influence wing kinematics during flight, not through direct connections with wing motoneurons, but rather through their input to the haltere muscle system. The resulting visually mediated changes in haltere kinematics would in turn influence wing kinematics through the connections between campaniform afferents and wing-steering motoneurons. Second, physiological evidence indicates that the steering muscles of the wing fire at precise times within the wingbeat cycle and that this phase tuning is due in part to the input of haltere afferents (*26*). This haltere-dependent phase tuning would be lost during voluntary steering maneuvers if the control muscles acted to desensitize the haltere afferents. Third, during any time in which they disabled or desensitized their equilibrium reflexes, flies would be especially susceptible to flight perturbations. With the control-loop strategy, the equilibrium reflexes would remain operational at all times so that the animal could compensate for flight perturbations that occurred during voluntary maneuvers. We attempted to test the control-loop hypothesis by recording from haltere muscles during steering responses in tethered flight. Unfortunately, we have not yet been able to unambiguously identify muscles spikes in I1 or B2 during flight because of the background activity in the nearby DVM, leg muscles, and afferents within the haltere sensory nerve. Similarly, it has proven difficult to electrically stimulate the haltere control muscles without also directly activating afferents within the adjacent haltere nerve.

The haltere efferent control pathway is based on a neural architecture that may be common among insects and provides a parsimonious explanation for the evolution of halteres from aerodynamically functional hind wings. In locusts, hind wing mechanoreceptors make strong connections with fore wing motoneurons and function to maintain a correct phase relation between the metathoracic and mesothoracic wing pairs (*32*). Although the hind wings of flies have lost all aerodynamic function, they have retained and elaborated mechanoreceptors capable of entraining motoneurons of the fore wing. Wings, like halteres, are susceptible to Coriolis forces. However, without using a sophisticated multiplexing strategy, it would be difficult for the nervous system to distinguish between aerodynamic forces and Coriolis force acting on wings. The reduction of the halteres' aerodynamic role may have resulted from disruptive selection acting to specialize the fore wing as an aerodynamic organ and the hind wing as a Coriolis force-sensitive equilibrium organ. Although the haltere efferent system provides a sophisticated means of integrating visual and mechanosensory information, the underlying circuitry probably arose from a general pattern of segmentally repeated sensory-motor reflexes.

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20. We used low-resistance (2 to 5 Mohms) glass microelectrodes filled with insect saline (*29*) for extracellular recording. The visual stimuli were created on a Tektronix 608 oscilloscope (Tektronix, Beaverton, OR) by a Picasso CRT Image Synthesizer (Innisfree, Cambridge, UK). The stimulus consisted of a square wave grating of light and dark stripes with a spatial frequency of 21° moving at a velocity of 42° s^{-1} to produce a contrast frequency of 2 Hz. The animal was oriented with its longitudinal axis aligned perpendicular to the surface of the CRT screen. The motion stimuli were presented in 16 orientations from 0° to 360° in steps of 22.5°. An orientation of 0° corresponded to upward motion; an orientation of 90° corresponded to lateral motion toward the ipsilateral side. For each fly, the spike frequency was normalized to the maximum response. We recorded from a total of 29 individuals (13 females and 16 males). In 11 animals (6 females and 5 males), we recorded the activities of both B2 and I1 sequentially in the same individual.
21. As with the wing-steering muscles that were studied, haltere muscles B2 and I1 are innervated by a single motoneuron (*33*). Insect synchronous muscles fire one overshooting action potential in response to each spike in their presynaptic motoneuron. It is therefore possible to unambiguously monitor the threshold activity of the motoneurons through the spikes in their target muscles.
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A Marine Natural Product Inhibitor of Kinesin Motors

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Members of the kinesin superfamily of motor proteins are essential for mitotic and meiotic spindle organization, chromosome segregation, organelle and vesicle transport, and many other processes that require microtubule-based transport. A compound, adociasulfate-2, was isolated from a marine sponge, *Haliclona* (also known as *Adocia*) species, that inhibited kinesin activity by targeting its motor domain and mimicking the activity of the microtubule. Thus, the kinesin-microtubule interaction site could be a useful target for small molecule modulators, and adociasulfate-2 should serve as an archetype for specific inhibitors of kinesin functions.

Eukaryotic cells depend on dynamic microtubule (MT)-mediated events executed by members of the kinesin superfamily. Various kinesins are necessary for cell division (*1*) and for vesicle (*2*) and organelle (*3*) transport. More than 100 kinesin superfamily members are currently known. Different kinesins share a common 350–amino acid motor domain, which is necessary and sufficient for adenosine triphosphate (ATP)–dependent force generation (*4*), attached to a variety of cargo-binding or effector tail domains. The atomic structure of the motor domain (*5–8*) and the basic elements of the kinetic cycle (*9*) are now known. However,

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