

A comparison of visual and haltere-mediated feedback in the control of body saccades in *Drosophila melanogaster*

John A. Bender* and Michael H. Dickinson

Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA

*Author for correspondence (e-mail: jrbender@caltech.edu)

Accepted 4 October 2006

Summary

The flight trajectories of fruit flies consist of straight flight segments interspersed with rapid turns called body saccades. Although the saccades are stereotyped, it is not known whether their brief time course is due to a feed-forward (predetermined) motor program or due to feedback from sensory systems that are reflexively activated by the rapid rotation. Two sensory modalities, the visual system and the mechanosensory halteres, are likely sources of such feedback because they are sensitive to angular velocities within the range experienced during saccades. Utilizing a magnetic tether in which flies are fixed in space but free to rotate about their yaw axis, we systematically manipulated the feedback from the visual and haltere systems to test their role in determining the time course of body saccades. We found that altering visual feedback had no significant effect on the dynamics

of saccades, whereas increasing and decreasing the amount of haltere-mediated feedback decreased and increased saccade amplitude, respectively. In other experiments, we altered the aerodynamic surface of the wings such that the flies had to actively modify their wing-stroke kinematics to maintain straight flight on the magnetic tether. Flies exhibit such modification, but the control is compromised in the dark, indicating that the visual system does provide feedback for flight stability at lower angular velocities, to which the haltere system is less sensitive. Cutting the wing surface disrupted the time course of the saccades, indicating that although flies employ sensory feedback to modulate saccade dynamics, it is not precise or fast enough to compensate for large changes in wing efficacy.

Key words: *Drosophila*, saccade, vision, haltere, feedback.

Introduction

Once initiated, simple behaviors may either follow a predetermined motor program or utilize sensory feedback to adjust for changes in external or internal conditions. Whether the nervous system uses a feed-forward or feedback strategy is likely to depend on many factors, such as the speed of the behavior and the likelihood that system dynamics can be accurately predicted. One behavior for which one might expect that feed-forward control is sufficient is in the saccadic eye movements of primates (Yarbus, 1967). These behaviors are so rapid that the position of the target is unlikely to change much during the course of motion. Further, because the dynamics of a fluid-filled sphere are relatively simple – compared, say, to a multijoint limb (Fisk and Goodale, 1985) – it may be reasonable to predict the actuation required to move the eyeball to the desired orientation without the use of sensors. However, there is evidence that even for such mechanically simple behaviors as eye saccades, feedback can nevertheless play a role (Jürgens et al., 1981; Soetedjo et al., 2002). Because of the slow speed of visual processing relative to the duration of the saccade, this feedback arises from other, faster sensory modalities (Jürgens et al., 1981).

Saccadic eye movements are not limited to primates, but are

rather ubiquitous behaviors among animals with image-forming visual systems. They have been observed across three phyla, even in animals in which the eyes cannot be moved independently of the head (Land, 1999). The degree to which these homologous behaviors employ feedback and, if so, whether such feedback arises from the visual system itself or from other modalities is not known. In the fruit fly, *Drosophila melanogaster*, flight is characterized by sequences of straight flight interspersed with rapid turns called ‘body’ saccades (after Land and Collett, 1974), which are rapid, stereotyped turns during which the fly changes directions by about 90° in 70 ms (Tammero and Dickinson, 2002a). Flies in free flight must generate torque to start rotating and countertorque to stop (Fry et al., 2003). However, rigidly tethered flies generate torque for approximately 500 ms, much longer than the duration of a free flight saccade, and never generate countertorque (Heisenberg and Wolf, 1979). This suggests that reafferent feedback does play a role in structuring the saccade motor program, once a saccade has begun.

Visual expansion can trigger saccades (Tammero and Dickinson, 2002a; Tammero and Dickinson, 2002b; Bender and Dickinson, 2006), but the time course of saccades in

tethered flight is very long even when flies are allowed to rotate their own visual environment (Heisenberg and Wolf, 1979; Tammero and Dickinson, 2002b), immediately suggesting that the visual system provides little feedback to the saccade motor program, once a turn is initiated. This lack of influence is surprising, given that open-loop rotation of large-field visual patterns elicits strong syndirectional turning responses (after Götz, 1964). However, flies also have specialized mechanosensory organs called halteres that are capable of providing feedback during saccades. The halteres are evolutionarily modified hindwings that beat antiphase with the wings and function as gyroscopes (Pringle, 1948). The haltere system is linearly sensitive to angular velocity (Dickinson, 1999) and possesses frequency response properties that are complementary to the low-pass characteristics of the visual system (Sherman and Dickinson, 2003). The halteres also allow feedback into the flight control circuitry to occur much more rapidly than vision does, as the main pathways operate through mixed chemical/electrical synapses (Trimarchi and Murphey, 1997; Fayyazuddin and Dickinson, 1999). The lack of haltere-mediated feedback to rigidly tethered animals is a likely explanation for the elongated time course of saccades relative to free flight. We recently developed a preparation that allows flies to rotate freely about one axis, thus providing them feedback from the halteres and other mechanosensory systems (Bender and Dickinson, 2006). Flies tethered in this way perform saccades with a time course more closely resembling that observed in free flight, suggesting that sensory feedback, possibly from the halteres, plays some role in determining saccade dynamics. However appealing, this hypothesis is unproven and the relative contribution of each modality is still uncertain.

In the present study, we tethered *Drosophila melanogaster* to steel pins and placed them in a magnetic field such that they were free to rotate about their functional yaw axis. We observed the flies with a digital camera and used an electronic panorama to manipulate the visual feedback received by flies during saccades, in combination with alterations of haltere feedback and wing aerodynamics. The results suggest that haltere-mediated feedback is mainly responsible for terminating saccades, but the visual system does play an important role in maintaining straight flight.

Materials and methods

Flies

We based our analysis on the behavioral performance of 45 3- to 5-day-old female fruit flies, *Drosophila melanogaster* Miegen. Our flies were descended from a laboratory culture originating with 200 wild-caught females. We prepared the animals for each experiment as previously described (Bender and Dickinson, 2006). Briefly, we anesthetized the flies by cooling them to 4°C on a Peltier stage and then attached a 50 µm diameter stainless steel pin (nominally 0.1 mm minutien, Fine Science Tools, North Vancouver, BC, Canada)

to the notum using UV-activated cement (Duro, Düsseldorf, Germany).

To add weight to the halteres in some experiments, we applied a small amount of UV-activated cement to the endknob, roughly doubling its volume. For haltere ablation experiments, we removed the left haltere just above its base by pulling gently on the stalk with fine forceps. This method left intact the df2 campaniform sensillum and the large chordotonal organ, which are thought to be largely responsible for the response to rotations about the yaw axis (Pringle, 1948; Fayyazuddin and Dickinson, 1996). Almost all of these flies were capable of remaining airborne in free flight, if allowed.

In further experiments, we clipped off portions of the wing to reduce the aerodynamic surface. In some flies, we cut away the posterior half of the right wing. In others, we removed the distal third of the left wing. Data from the latter group were collected at a camera speed of 101 frames s⁻¹, and those flies did not receive the experimental visual feedback detailed below. Some of the wing-clipped flies could remain airborne in free flight at least temporarily, but many could not. Flies that did not maintain flight for 10 min while tethered in our arena were excluded from analysis.

An additional set of flies were tethered and observed in near-total darkness at a camera speed of 101 frames s⁻¹. Whereas the room lights were turned off during all recordings, for these sessions we also covered the entire arena and camera with thick, black fabric to remove as many external visual cues as possible. Under these conditions, we measured a luminance of <0.1 lux in the visible spectrum. As previous experiments have shown, flight duration and performance decrease in the absence of closed-loop visual feedback (Heisenberg and Wolf, 1988; Dickinson, 1999). Therefore, under dark conditions we included data from flies that flew continuously for 5 min or more. As an internal control and to reduce dark adaptation (Bernhard and Ottoson, 1960), we alternated 1 min periods of darkness with 1 min periods during which the arena displayed a stationary pattern.

Flight arena and calibration

As detailed in an earlier study (Bender and Dickinson, 2006), we placed the fly and pin in the magnetic field of two vertically aligned rare earth (NdFeB) magnets (K&J Magnetics, Jamison, PA, USA). This configuration allowed the fly to rotate freely about the long axis of the pin – its functional yaw axis (Fig. 1B), an approach first attempted by Heisenberg and Wolf using a long, flexible filament (Heisenberg and Wolf, 1979). We recorded the fly's orientation around this axis by illuminating it from beneath with an array of 940 nm light-emitting diodes (LEDs) and fixing a mirror below the fly to reflect its image to an infrared-sensitive digital camera (A602f, Basler, Ahrensburg, Germany). The average frame rate of the camera was 564 frames s⁻¹, with the exceptions noted above, and the shutter speed was 1.7 ms for all experiments. We tuned the camera's image threshold for each fly before each experiment and saved the fly's orientation in each frame for later analysis. The fly and lighting sat within a cylindrical

arena composed of 32×64 green LEDs (Fig. 1A), each subtending approximately 5.6° of azimuth. The individual LEDs were refreshed at 800 Hz, and the pattern displayed on them was updated at about 220 Hz by the associated control board (M. B. Reiser and M. H. Dickinson, manuscript submitted for publication). The control board was under the command of a PC that analyzed the incoming camera images and updated the visual stimulus accordingly. The 1.8 ms interval between frames, plus a measured average latency of 3.5 ms between the camera and the PC (including image analysis), plus the 4.5 ms pattern update rate, totaled an average maximum delay of about 10 ms between a fly's behavior and our ability to alter the visual display in response to it. This represents roughly two wing strokes or 10–20% of the duration of a free-flight saccade.

At the start of each experiment, we first subjected the fly to

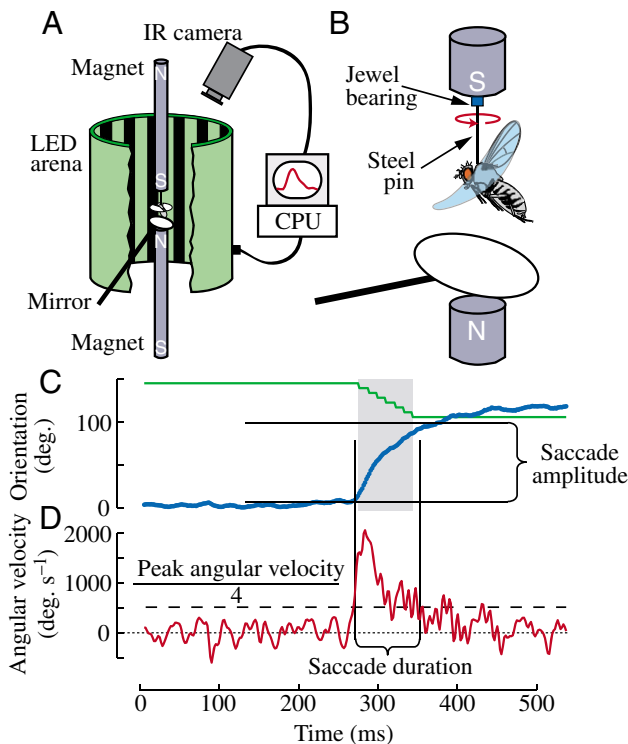


Fig. 1. Experimental design. (A) Fly orientation was determined at 564 Hz by a near-IR camera and custom software, which was used to modify the visual stimulation presented on a 32×64 cylindrical array of LEDs. N, magnetic north; S, south. (B) Flies were glued to a steel pin, which was placed in a magnetic field such that they could rotate only about their functional yaw axis. (C) Manipulation of visual feedback during saccades. When the realtime software detected the initiation of a saccade, the striped visual stimulus was rotated by 40° in 80 ms (gray box). The blue trace shows the fly's observed orientation; the green trace shows the angular position of the striped pattern. (D) *Post hoc* calculation of saccade dynamics. Saccade duration was the time during which the fly's angular velocity exceeded one-quarter of its maximum value during the saccade. Saccade amplitude was the difference between the median of the orientation measurements taken in the 50 ms immediately before and after the saccade.

a calibration that made it possible to unambiguously determine the fly's heading with respect to the camera. This calibration period consisted of 1 min of rotation elicited using a stimulus in which poles of expansion and contraction, set apart by 180° , were rotated around the fly (Bender and Dickinson, 2006). Under these conditions, flies robustly fixate the pole of contraction (Tammero et al., 2004).

Saccades and visual feedback manipulation

Following calibration, the LEDs of the arena displayed a horizontal square wave pattern with a fundamental spatial frequency of 22.5° , which we will term the 'background' stimulus. When presented with open-loop rotation of this pattern, flies responded with syndirectional turning. The stripe width (half the spatial frequency of the square wave pattern) was chosen from open-loop experiments to maximize the flies' responses (Fig. 2A). The observed value agrees with the theoretical prediction that the strongest motion response should arise when the stripe width (proportional to temporal frequency for a given angular velocity) is twice the interommatidial distance of 5° (Götz, 1964; Egelhaaf et al., 1989), and also matches recent findings using a similar electronic display (Sherman and Dickinson, 2003). On top of this background, we displayed a 'foreground' stimulus consisting of a dark, vertical

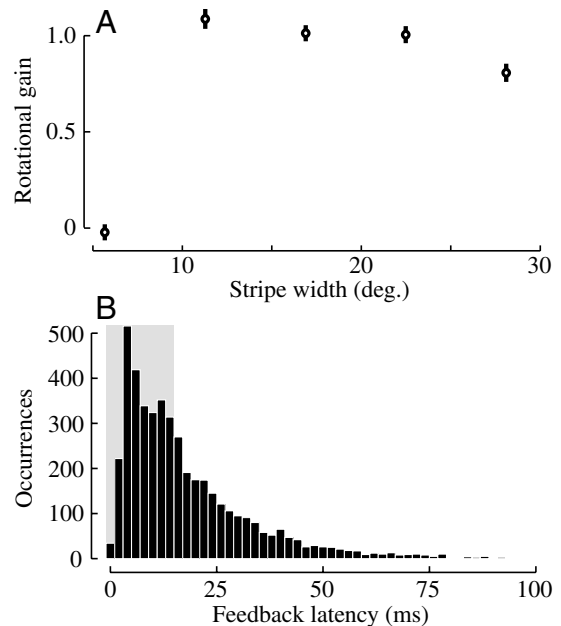


Fig. 2. (A) The rotational gain (classic optomotor response) of flies in a rotating drum featuring vertical stripes. Gain was calculated as the mean angular velocity of the fly divided by the angular velocity of the pattern during a single trial. Values are means \pm s.e.m. across all trials, where each trial consisted of 10 s of rotation with a given stripe width. $N=4$ flies, $n=[123, 123, 123, 125, 130]$ trials. Stripe widths tested correspond to 1–5 pixels in our visual arena. (B) Error between real-time and *post hoc* saccade detection timing. Only events during which the real-time software detected a saccade within 15 ms after its true initiation time as calculated *post hoc* (gray box) were included for further analysis.

stripe, 45° wide, which was controlled independently of the background pattern.

Each experimental trial was conducted during a spontaneous saccade made by the fly. The trial type was selected randomly *ad hoc*, with the constraints that each trial begin at least 1 s after the previous trial and that two consecutive trials be of different types. To determine in real time when a saccade was made, we approximated the fly's angular velocity by averaging its instantaneous velocity between each unique pair drawn from three consecutive camera images (e.g. the average of the instantaneous velocity measured between frames 1 and 2, frames 2 and 3, and frames 1 and 3; a total of 3.5 ms of flight). The instantaneous angular velocity between two frames was calculated by measuring the difference between the fly's orientation in each frame and dividing by the elapsed time between those two frames. When this estimate exceeded 650 deg. s⁻¹, our software automatically initiated a rotation of either the foreground, background, both, or neither by 40° in 80 ms at a constant angular velocity of 500 deg. s⁻¹. These values were chosen because they corresponded roughly to rotations generated by the fly's own motion during a typical saccade in this preparation (Bender and Dickinson, 2006). The visual rotation was either with or against the direction of the saccade, and the foreground could be rotated in the opposite direction from the background. Our real-time velocity thresholding technique resulted in many more false positives than false negatives; however, only trials during which the fly both performed a saccade (as determined *post hoc*; see below) and met the real-time saccade criterion were used in further analysis. The discrepancies between the timing of the two (real-time and *post hoc*) thresholds is shown in Fig. 2B and can be seen for a single trial in Fig. 1C as the difference between the beginning of the 'saccade duration' period and the gray box indicating the time course of the experimental feedback. Trials where the real-time threshold was reached before or more than 15 ms after the *post hoc* threshold were discarded, leaving only saccades with a distribution of total detection latencies ranging from 10 to 25 ms, including delays due to both the hardware and software. By way of comparison, the latency of vision-to-motor responses in a flying housefly is 30 ms (Land and Collett, 1974).

After the experiments were over, we identified and quantified saccadic flight sequences as in our prior analysis (Bender and Dickinson, 2006). We filtered the orientation data (140 Hz, low-pass) and applied the central difference formula to estimate angular velocity. We used the *post hoc* velocity threshold of 350 deg. s⁻¹ calculated during our previous study to separate saccadic events from straight flight and slow turning. We defined the duration of each saccade as the time during which the fly's angular velocity exceeded one-quarter of its maximum value for that event. We then quantified the amplitude of each saccade as the difference between the fly's median orientations across two 50 ms windows: one window before and one after the period defining saccade duration (Fig. 1C,D). Only saccades with amplitudes between 15° and 150° were analyzed.

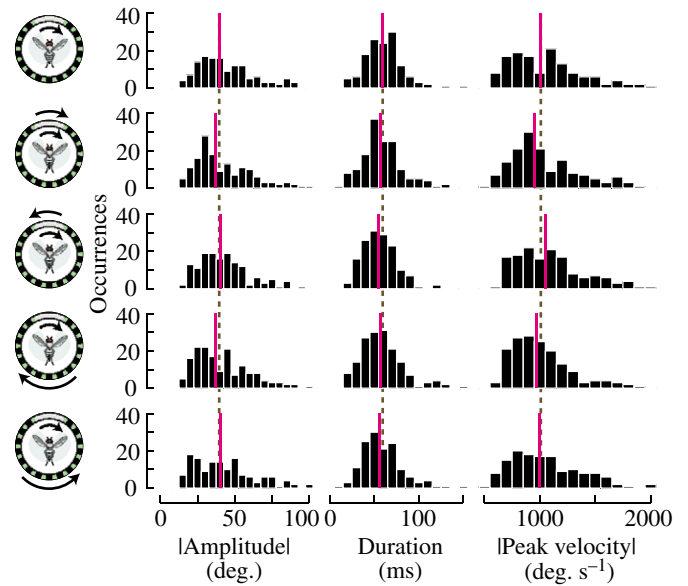


Fig. 3. Artificial visual rotation during saccades does not affect saccade dynamics (amplitude, duration or peak angular velocity). Flies performed spontaneous saccades in a visual panorama displaying a 45° dark stripe in the foreground (represented here in light gray for clarity) over a background of thin vertical stripes with a spatial frequency of 22.5°. When a fly began a saccade, either the foreground or background was rotated by a predetermined amount. Top to bottom: no visual rotation (control, $n=134$ saccades); foreground rotated with fly's turn ($n=151$); foreground rotated against fly's turn ($n=148$); background rotated with ($n=163$); background rotated against ($n=142$). Most of the distributions (here and in other figures) are neither normal nor log-normal (Shapiro–Wilk test, $W<0.05$); therefore, pink bars show the median value. None of the distributions here differed significantly from the control (Kruskal–Wallis nonparametric one-way ANOVA with Bonferroni correction for multiple comparisons, $P>0.05$). $N=14$ flies.

Results

Effects of visual feedback during saccades

When a fly performed a saccade, we tested one of eight different visual feedback conditions. The thin, background stripe pattern or the prominent, foreground stripe was rotated by 40° in 80 ms, either in the same or the opposite direction as the turn the fly was making. The results of these four trial types are shown in Fig. 3, compared with the control condition (top row) in which the visual arena remained unchanged. We calculated the effects of our visual feedback manipulations on the amplitude, duration and peak velocity of saccades. We also tested the possible independent combinations of foreground and background movement directions (data not shown). None of these trial types resulted in a response that was statistically different from the control (Kruskal–Wallis one-way nonparametric ANOVA with Bonferroni correction for multiple comparisons, $P>0.05$).

To address the issue of visual control in a more extreme setting, we observed several flies in a visual setting alternating between a static display and total darkness

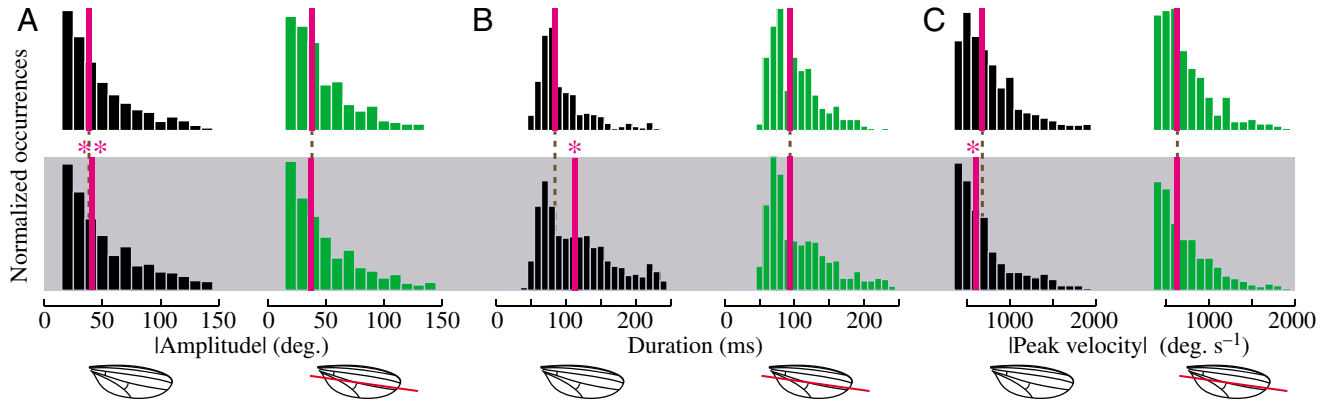


Fig. 4. Vision plays only a minor role in modulating saccade dynamics, independent of wing aerodynamics. Top row: lights on; bottom row: lights off. (A) Saccade absolute amplitude, (B) duration, (C) peak absolute velocity. $N=7$ intact flies ($n=726$ light, $n=629$ dark saccades), $N=3$ clipped ($n=308$ light, $n=586$ dark saccades). Statistical analysis was as in Fig. 3 (* $P<0.001$; ** $P<0.02$).

(Fig. 4). These suggested significant differences ($P<0.05$) in the duration, amplitude and peak angular velocity of saccades performed in the dark compared with saccades performed in the light. However, the P -values calculated by ANOVA statistics directly depend on the number of degrees of freedom in the model, with increasing degrees of freedom tending to decrease P -values. Therefore, because of the relatively large number of saccades analyzed in these experiments compared to our other analyses, the threshold for statistical significance must be more stringent. Taking all these factors into consideration, we interpret these results to indicate that saccade dynamics are mostly independent of the visual environment, with the possible exception of saccade duration (Fig. 4).

Effects of haltere-mediated feedback

In order to assess the contribution of the halteres to saccade dynamics, we made two types of haltere manipulations. We increased the mass of the haltere endknobs in some flies and ablated the left haltere in others. The rotation-sensitive haltere mechanosensory transduction pathway depends on the deflection of the haltere out of its stroke plane by Coriolis forces (Nalbach, 1993), which are a function of the endknobs' inertia (Fig. 5B). Thus, since the Coriolis forces scale with mass, increasing the mass of the endknobs should act to increase the gain of the haltere feedback. On the other hand, unilateral haltere ablation should decrease the total haltere-mediated feedback. In the absence of any manipulation of visual feedback, we found that weighting the halteres significantly decreased the median amplitude and peak velocity of saccades (Fig. 5C, second row) compared to control ($P<0.001$) and also tended to decrease saccade duration, but not significantly. Ablating one haltere statistically increased saccade amplitude, duration and peak angular velocity, an effect that is laterally symmetric, in the sense that an ablation of the left haltere equally influences saccades to the left and right (amplitude shown in Fig. 8A; $P>0.05$). The results of these experiments suggest that feedback from the halteres plays

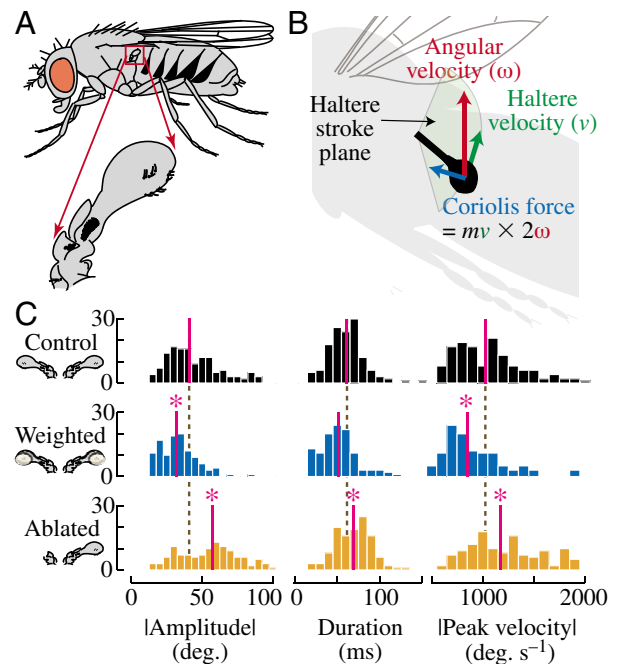


Fig. 5. Modifying haltere feedback changes saccade dynamics. (A) The halteres. (B) Angular rotations of the fly's body cause the halteres to be deflected out of their stroke plane by Coriolis forces. In our preparation, these forces are proportional to the fly's angular velocity about its yaw axis (ω) and the halteres' mass (m) and velocity (v). The deflections caused by the Coriolis forces are sensed by haltere mechanoreceptors. (C) Changing the amount of haltere feedback affects saccade dynamics. Top to bottom: control (same data as top row of Fig. 3, $N=14$ flies, $n=134$ saccades); haltere feedback increased by adding mass to the haltere endknobs ($N=6$, $n=113$); haltere feedback decreased by ablating left haltere ($N=5$, $n=121$). Statistical analysis was as in Fig. 3 (* $P<0.001$).

a role in terminating the saccade motor pattern, and may be integrated centrally rather than *via* simple ipsilateral projections. This is plausible, given what is known about the

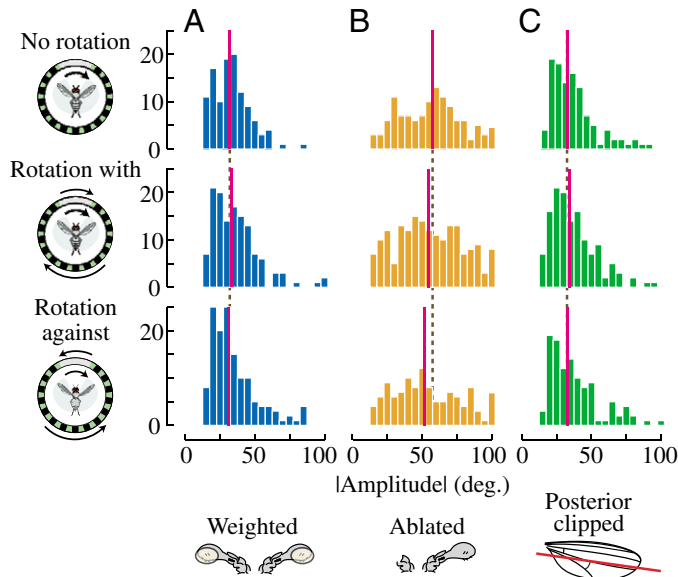


Fig. 6. No crossmodal effects were observed of visual feedback and haltere-mediated feedback, or visual feedback and aerodynamic modification, during saccades. Top to bottom: no visual rotation (control); foreground with/background with; foreground against/background against. (A) Halteres weighted ($N=6$ flies, top to bottom $n=[113, 130, 137]$ saccades); (B) left haltere ablated ($N=5$, $n=[121, 187, 121]$); (C) posterior of wing ablated ($N=5$, $n=[110, 136, 110]$). Statistical analysis was as in Fig. 3 ($P>0.05$).

projections of haltere afferent neurons (Chan and Dickinson, 1996).

To test whether modification of the haltere-mediated feedback unmasked some visual effect (for example, one might imagine that an animal with impaired halteres would rely more heavily on visual feedback), we also tested the responses of these flies to a subset of the visual feedback manipulations described above. During a saccade, we rotated the visual foreground and background together, either in the same or the opposite direction to the saccade. Neither of these conditions affected the saccade amplitude (Fig. 6A,B), duration, or peak velocity in animals with altered halteres ($P>0.05$, data not shown).

In some circumstances, manipulations of the halteres can cause them to adopt a different stroke frequency than that of the wings (Sellke, 1936; Pringle, 1948). Because the halteres provide sensory feedback to the wing control circuitry on a stroke-by-stroke basis even in the absence of body rotation (Heide, 1983; Fayyazuddin and Dickinson, 1999), the observed effects of haltere alteration might be due not to a modulation of rotation sensitivity but to a disruption of wingbeat-synchronous afferents on the haltere that are required for normal steering muscle function. To determine whether our flies had an altered haltere stroke frequency, we analyzed sequences of high-speed video ($6000 \text{ frames s}^{-1}$) for any indication of a phase shift relative to the wingbeats but detected no such change.

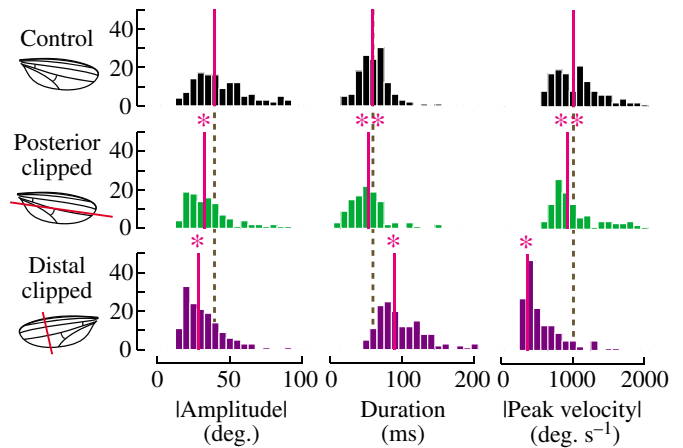


Fig. 7. Altering wing aerodynamics affects saccade dynamics. Top to bottom: control (same data as top row of Fig. 3; $N=14$ flies, $n=134$ saccades); posterior half of right wing removed ($N=5$, $n=110$); distal third of left wing removed ($N=5$, $n=1292$ for statistical purposes, although only 150 randomly selected saccades are plotted). Statistical analysis was as in Fig. 3 (* $P<0.001$; ** $P<0.02$).

Modification of aerodynamics

To probe the compensatory capacity of the equilibrium system, we forced flies to alter their wingstroke kinematics by clipping away parts of their wings. In one group of flies, we removed the posterior half of the right wing, and in another, we cut off the distal third of the left wing. We found that flies in which the posterior half of a wing had been ablated performed saccades with significantly smaller amplitude, shorter duration and lower peak angular velocities than control (Fig. 7), and these effects were independent of visual feedback (Fig. 6C). Removing the distal third of the wing also decreased saccade amplitudes, but it resulted in saccades with longer duration and much lower peak velocities (Fig. 7). We measured the saccade dynamics of posterior-clipped flies alternating between a lit and darkened visual environment, and found no significant differences in amplitude, duration, or peak velocity (Fig. 4).

We applied a detailed mathematical model of the forces produced during flapping flight (Dickson et al., 2006) to simulate the aerodynamics of flies with one wing surface altered to match our experiments. In this model, both of the wing alterations we made induced a significant yaw moment relative to flies with intact wings, assuming flies did not change their wing motion in response to the alteration in wing area. However, high-speed video analysis of real flies indicates that, when maintaining straight flight, the clipped-wing flies were continuously compensating by differentially altering wing flip timing in a manner similar to that seen during turns in intact flies (H. Sugiura and M. H. Dickinson, manuscript in preparation). These compensatory changes were particularly large in the distal-clipped flies, and more subtle in the posterior-clipped flies. There are limits to the compensation, however, such that when we inadvertently cut away too much

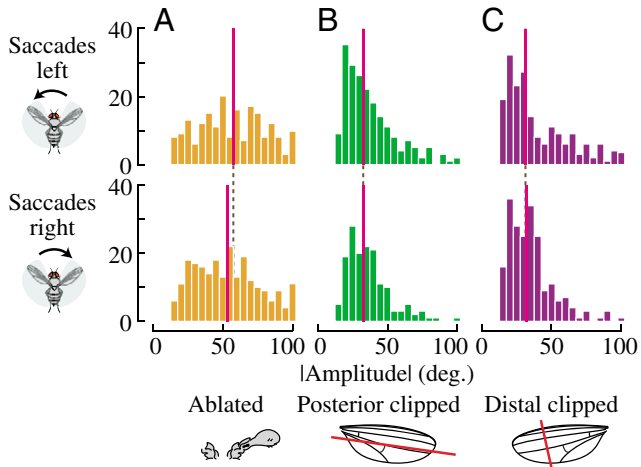


Fig. 8. Changes in saccade dynamics are laterally symmetric in asymmetrical preparations. Statistical analysis was as in Fig. 3, using leftward saccades as the control condition ($P>0.05$).

of the wing surface in some flies in each group (which we could not further analyze), they spun continuously like a propeller on our magnetic tether. This implies that our

manipulations did have effects on aerodynamic force production, in agreement with the model, and that flies must have been compensating in order to sustain straight flight. However, in clipped flies flying stably, we found no differences between saccades to the right and left in terms of amplitude (Fig. 8B,C), duration, or peak velocity (data not shown) ($P>0.05$). It is also worth noting that because wing motion is due in part to complex, but subtle, effects of translational and rotational inertia, some of the changes in wing motion observed in clipped flies may have been caused not by active compensation, but by passive effects as a result of the change in wing mass and shape.

Vision does seem to play a major role in the ability of these flies to compensate for aerodynamic asymmetries in order to maintain a stable heading. Our data indicate that orientation is more variable and the average magnitude of a fly's angular velocity is larger in total darkness (Fig. 9). We observed only small differences in these metrics between intact and wing-clipped flies, compared to the differences between light and darkness. Together, our results indicate that visual feedback is of great importance during straight flight, but plays only a minor role in determining saccade dynamics.

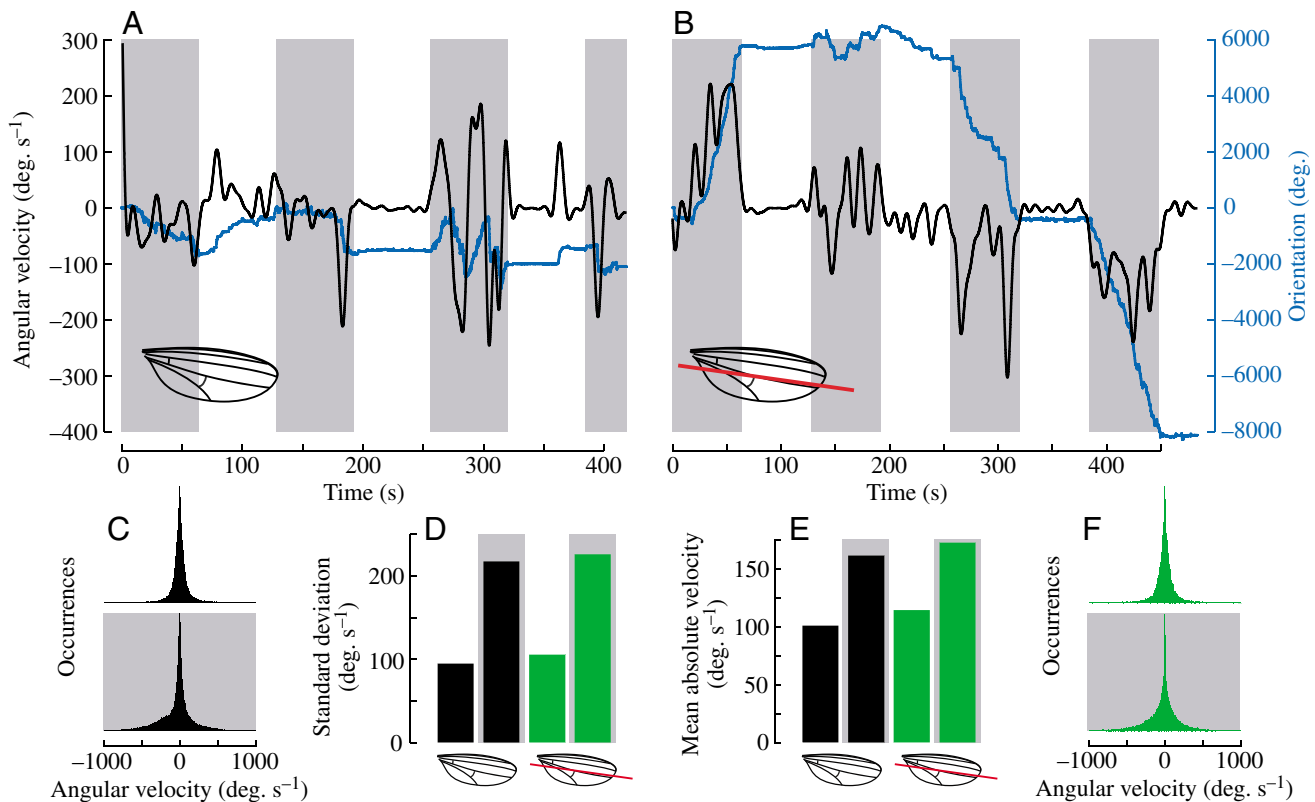


Fig. 9. Both intact and wing-clipped flies stabilize orientation using visual feedback. (A,B) Characteristic traces from the entire recording session of (A) an intact fly and (B) a fly in which the posterior half of the right wing was removed. Black: angular velocity, lowpass filtered at 0.1 Hz; blue: orientation. Gray boxes denote periods when the arena lights were turned off (1 min off, 1 min on). (C,F) Angular velocity histograms from all flies tested (C: intact; F: clipped). Top row: lights on; bottom row: lights off. (D) Standard deviations of the distributions in C and F. (E) Each bar shows the mean of the absolute values of all the velocity measurements taken in that condition. $N=7$ intact flies, $N=3$ clipped; $n=157024$ intact-light samples, $n=175648$ intact-dark, $n=59532$ clipped-light, $n=63265$ clipped-dark.

Discussion

Magnetically tethered fruit flies performing saccades show no measurable response to rotatory visual feedback (Fig. 3). However, manipulations that altered haltere sensitivity did affect the time course of saccades (Fig. 5), independent of visual feedback (Fig. 6). When the wing surfaces were asymmetrically altered, flies could manipulate wing motion to trim yaw torque and keep from spinning, but they exhibited different, laterally symmetric saccade dynamics (Figs 7 and 8). Collectively, these results suggest that the time course of the saccade is determined by a feed-forward motor program that is influenced, but not precisely structured, by mechanosensory feedback.

Haltere-mediated feedback and saccade termination

Our data indicate that increased haltere feedback leads to smaller saccades, presumably by premature triggering of the counterturn phase necessary to overcome the animal's inertia and stop rotation (Fry et al., 2003). This is consistent with the hypothesis that a major reason for the differences between the saccade behavior as observed in free and rigidly tethered flies is the lack of haltere feedback under tethered conditions (e.g. Heisenberg and Wolf, 1979; Bender and Dickinson, 2006). Electrophysiological studies in rigidly tethered flies suggested that saccades are caused by changes in steering muscle activity, including a burst of action potentials in the second basalar muscle (b2) and a phase advance of the first basalar muscle (b1) (Heide and Götz, 1996). However, these transient changes in activity last much longer than in the free flight or magnetically tethered cases, and probably represent feed-forward components of the underlying motor program that play out in the absence of haltere-mediated rotational feedback. Presumably, such feedback somehow terminates these changes and initiates others responsible for generating the counterturn that stops the turn.

It is difficult to make strong claims based only on our ablation experiments, because such manipulations might compromise the function of the haltere in other ways. In particular, ablation disrupts the feedback from all the mechanosensory organs on the haltere, only two of which (the df2 campaniform field and the chordotonal organ) are thought to encode Coriolis forces (Pringle, 1948; Fayyazuddin and Dickinson, 1996). Even in the absence of body rotation, the other campaniform fields provide wingbeat-synchronous input that is necessary for proper phase tuning of wing steering muscles (Heide, 1983; Fayyazuddin and Dickinson, 1996). In fact, flies with both halteres removed will not maintain flight on a magnetic tether for more than a few minutes at most, although they will fly for several minutes in total darkness; however, their saccades and general flight dynamics are extremely disturbed. Thus, removal of the phasically active campaniform inputs that do not encode Coriolis forces could cloud the interpretation of experiments addressing the haltere's role as an equilibrium organ. The experiments with weighted halteres are less ambiguous, because the manipulation is unlikely to severely alter the function of the phasically active,

Coriolis-insensitive campaniform fields. Indeed, our observation that increasing haltere mass decreased saccade size is difficult to explain except by the predicted increase in haltere sensitivity.

Collectively, the results of our experimental manipulations support the hypothesis that saccade dynamics are tuned by the amount of rotational feedback provided by the halteres, but not the eyes. The minimal role of vision is further corroborated by the finding that saccade dynamics are fairly constant even in total darkness (Fig. 4). Confirmation of this working hypothesis will require electrophysiological records under conditions in which haltere feedback can be experimentally induced. In addition, genetic methods offer a potential means of selectively disturbing the input from the Coriolis-sensitive haltere fields. The gene *shaking-B²* is required for the function of a subset of gap junctions in the fly, including those between the halteres and wing motor neurons (Thomas and Wyman, 1984; Trimarchi and Murphey, 1997). Flies with a null allele of this gene flew poorly in our apparatus, preventing a direct comparison with our other results. However, this strain did appear to perform saccade-like behaviors and is worthy of future attention.

On the role of visual feedback during flight

It is known that flies can discriminate prominent foreground objects from the visual background (Reichardt and Poggio, 1979; Egelhaaf, 1985; Reichardt et al., 1989) and tend to fixate large, vertical stripes in the foreground (Götz, 1980; Götz, 1987). For this reason, we tested our flies with combinations of foreground and background rotations during saccades, and found that none of these manipulations had any significant effect. However, the *P*-values obtained when rotating the foreground stripe against the direction of the saccade were more nearly significant than those measured during the other rotation conditions (*P*=0.15 for duration; *P*=0.07 for velocity). Testing the figure-ground distinction was not a major goal of this analysis, however, and we do not have sufficient data to test for effects of the relative orientation of the fly and the large stripe.

Although vision appears have a potent role in maintaining overall flight stability (Fig. 9), our results do not support a role for visual feedback in terminating body saccades. This is noteworthy, given the large quantity of studies showing its importance in other flight behaviors (e.g. Reichardt, 1969; Collett and Land, 1975; Götz, 1975; Reichardt and Poggio, 1976; Wolf and Heisenberg, 1990; Tammero and Dickinson, 2002a; Egelhaaf et al., 2002; Higgins, 2004; Frye and Dickinson, 2004). However, most previous studies have focused on the role of vision as an equilibrium system to maintain straight flight or a detection system to initiate maneuvers, not its use during active turns. The strength of optomotor responses under open-loop conditions (Götz, 1964) raises questions about the influence of vision during self-generated motion. In particular, there must be a mechanism by which strong compensatory reflexes do not continually counteract voluntary turns. One possibility is that some form

of internal efference copy (Sperry, 1950; von Holst and Mittelstaedt, 1950) is used to counteract reafference generated by motion. Another possibility is that the visual system is simply too slow, such that an internally generated motion command results in substantial body rotation before optomotor reflexes can act to attenuate it.

The visual system has bandpass filter characteristics that largely suppress its response to rotations above 600 deg. s⁻¹ (Sherman and Dickinson, 2003; Hengstenberg, 1991); however, the majority of the saccades we observed in the magnetically tethered preparation have peak velocities below this value (Bender and Dickinson, 2006), and our experimental visual rotations were of a constant 500 deg. s⁻¹. Heisenberg and Wolf, working on rigidly tethered preparations, performed a set of experiments related to the ones we present here (Heisenberg and Wolf, 1979). They reported a response to rotations of a full-field visual pattern by 30° in 200 ms (150 deg. s⁻¹) in the same direction as the saccade, but due to technical limitations at that time, they show only the results of a few trials. They found no response to visual rotation opposite the direction of the saccade, either with single-stripe or checkerboard patterns, but did note a syndirectional turning response to an open-loop rotation of the visual field. Therefore, we analyzed the flies' responses to our 'false positive' trials, in which the visual rotation was initiated but a *post hoc* analysis did not indicate a saccade, to determine the open-loop responses to the visual rotation shown during saccades. Flies made small (2–3°) course corrections in response to these stimuli, which were comparable in magnitude to the statistically insignificant changes in saccade amplitude we measured when presenting the flies with the same rotating visual stimuli. These observations suggest that the visual feedback during saccades may be too brief to elicit a substantial response, but that ongoing feedback from the haltere system acts to terminate the saccade motor program. This also supports a previous study concluding that haltere-mediated and visual feedback are combined by the fly's flight control system as a weighted sum, with greater emphasis placed on the mechanosensory feedback (Sherman and Dickinson, 2004).

The authors gratefully acknowledge G. Card for assistance collecting high-speed video and Dr D. Altshuler for aid with statistical analysis. M. Reiser and Dr A. Straw also contributed to the development of the stimulating and recording systems. Prof. R. Wyman at Yale University graciously provided us with *shaking-B²* mutant flies for this study. This work was supported by award N00014-01-1-0676 from the Office of Naval Research, by the Institute for Collaborative Biotechnologies through grant DAAD19-03-D-0004 from the US Army Research Office, and by the Packard Foundation.

References

- Bender, J. A. and Dickinson, M. H. (2006). Visual stimulation of saccades in magnetically tethered *Drosophila*. *J. Exp. Biol.* **209**, 3170-3182.
- Bernhard, C. G. and Ottoson, D. (1960). Comparative studies on dark

- adaptation in the compound eyes of nocturnal and diurnal Lepidoptera. *J. Gen. Physiol.* **44**, 195-203.
- Chan, W. P. and Dickinson, M. H. (1996). Position-specific central projections of mechanosensory neurons on the haltere of the blow fly, *Calliphora vicina*. *J. Comp. Neurol.* **369**, 405-418.
- Collett, T. S. and Land, M. F. (1975). Visual control of flight behavior in the hoverfly, *Syrirta pipiens* L. *J. Comp. Physiol. A* **99**, 1-66.
- Dickinson, M. H. (1999). Haltere-mediated equilibrium reflexes of the fruit fly, *Drosophila melanogaster*. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **354**, 903-916.
- Dickson, W. B., Straw, A. D., Poelma, C. and Dickinson, M. H. (2006). An integrative model of insect flight control. In *44th AIAA Aerospace Sciences Meeting and Exhibit*. Reno, NV; USA.
- Egelhaaf, M. (1985). On the neuronal basis of figure-ground discrimination by relative motion in the visual system of the fly. *Biol. Cybern.* **52**, 123-140.
- Egelhaaf, M., Borst, A. and Reichardt, W. (1989). The nonlinear mechanism of direction selectivity in the fly motion detection system. *Naturwissenschaften* **76**, 32-35.
- Egelhaaf, M., Kern, R., Krapp, H. G., Kretzberg, J., Kurtz, R. and Warzecha, A. K. (2002). Neural encoding of behaviourally relevant visual-motion information in the fly. *Trends Neurosci.* **25**, 96-102.
- Fayyazuddin, A. and Dickinson, M. H. (1996). Haltere afferents provide direct, electrotonic input to a steering motor neuron in the blowfly, *Calliphora*. *J. Neurosci.* **16**, 5225-5232.
- Fayyazuddin, A. and Dickinson, M. H. (1999). Convergent mechanosensory input structures the firing phase of a steering motor neuron in the blowfly, *Calliphora*. *J. Neurophysiol.* **82**, 1916-1926.
- Fisk, J. D. and Goodale, M. A. (1985). The organization of eye and limb movements during unrestricted reaching to targets in contralateral and ipsilateral visual space. *Exp. Brain Res.* **60**, 159-178.
- Fry, S. N., Sayaman, R. and Dickinson, M. H. (2003). The aerodynamics of free-flight maneuvers in *Drosophila*. *Science* **300**, 495-498.
- Frye, M. A. and Dickinson, M. H. (2004). Motor output reflects the linear superposition of visual and olfactory inputs in *Drosophila*. *J. Exp. Biol.* **207**, 123-131.
- Götz, K. G. (1964). Optomotorische untersuchung des visuellen systems einiger augenmutanten der Fruchtfliege *Drosophila*. *Kybernetik* **2**, 77-92.
- Götz, K. G. (1975). The optomotor equilibrium of the *Drosophila* navigation system. *J. Comp. Physiol. A* **99**, 187-210.
- Götz, K. G. (1980). Visual guidance in *Drosophila*. In *Development and Neurobiology of Drosophila* (ed. O. Siddiqi, P. Babu, L. M. Hall and J. C. Hall), pp. 391-407. New York, London: Plenum Press.
- Götz, K. G. (1987). Course-control, metabolism and wing interference during ultralong tethered flight in *Drosophila melanogaster*. *J. Exp. Biol.* **128**, 35-46.
- Heide, G. (1983). Neural mechanisms of flight control in Diptera. In *Bionareport*. Vol. 2 (ed. W. Nachtigall), pp. 35-52. Stuttgart: Fischer.
- Heide, G. and Götz, K. G. (1996). Optomotor control of course and altitude in *Drosophila melanogaster* is correlated with distinct activities of at least three pairs of flight steering muscles. *J. Exp. Biol.* **199**, 1711-1726.
- Heisenberg, M. and Wolf, R. (1979). On the fine-structure of yaw torque in visual flight orientation of *Drosophila melanogaster*. *J. Comp. Physiol. A* **130**, 113-130.
- Heisenberg, M. and Wolf, R. (1988). Reafferent control of optomotor yaw torque in *Drosophila melanogaster*. *J. Comp. Physiol. A* **163**, 373-388.
- Hengstenberg, R. (1991). Stabilizing head movements in the blowfly *Calliphora*. *Zool. Jahrb. Abt. Allg. Zool. Physiol. Tiere* **95**, 297-304.
- Higgins, C. M. (2004). Nondirectional motion may underlie insect behavioral dependence on image speed. *Biol. Cybern.* **91**, 326-332.
- Jürgens, R., Becker, W. and Kornhuber, H. H. (1981). Natural and drug-induced variations of velocity and duration of human saccadic eye movements: evidence for a control of the neural pulse generator by local feedback. *Biol. Cybern.* **39**, 87-96.
- Land, M. F. (1999). Motion and vision: why animals move their eyes. *J. Comp. Physiol. A* **185**, 341-352.
- Land, M. F. and Collett, T. S. (1974). Chasing behavior of houseflies (*Fannia canicularis*) – description and analysis. *J. Comp. Physiol. A* **89**, 331-357.
- Nalbach, G. (1993). The halteres of the blowfly *Calliphora*. 1. Kinematics and dynamics. *J. Comp. Physiol. A* **173**, 293-300.
- Pringle, J. W. S. (1948). The gyroscopic mechanism of the halteres of Diptera. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **233**, 347-384.
- Reichardt, W. (1969). Movement perception in insects. In *Processing of Optical Data by Organisms and Machines* (ed. W. Reichardt), pp. 465-493. New York, London: Academic Press.

- Reichardt, W. and Poggio, T.** (1976). Visual control of orientation behavior in the fly. I. A quantitative analysis. *Q. Rev. Biophys.* **9**, 311-375.
- Reichardt, W. and Poggio, T.** (1979). Figure-ground discrimination by relative movement in the visual system of the fly. I. Experimental results. *Biol. Cybern.* **35**, 81-100.
- Reichardt, W., Egelhaaf, M. and Guo, A. K.** (1989). Processing of figure and background motion in the visual-system of the fly. *Biol. Cybern.* **61**, 327-345.
- Sellke, K.** (1936). Biological and morphological studies on damaging grassland craneflies (Tipulidae, Dipt). *Z. Wiss. Zool.* **148**, 465-555.
- Sherman, A. and Dickinson, M. H.** (2003). A comparison of visual and haltere-mediated equilibrium reflexes in the fruit fly *Drosophila melanogaster*. *J. Exp. Biol.* **206**, 295-302.
- Sherman, A. and Dickinson, M. H.** (2004). Summation of visual and mechanosensory feedback in *Drosophila* flight control. *J. Exp. Biol.* **207**, 133-142.
- Soetedjo, R., Kaneko, C. R. S. and Fuchs, A. F.** (2002). Evidence that the superior colliculus participates in the feedback control of saccadic eye movements. *J. Neurophysiol.* **87**, 679-695.
- Sperry, R. W.** (1950). Neural basis of the spontaneous optokinetic response produced by visual inversion. *J. Comp. Physiol. Psychol.* **43**, 482-489.
- Tammero, L. F. and Dickinson, M. H.** (2002a). The influence of visual landscape on the free flight behavior of the fruit fly *Drosophila melanogaster*. *J. Exp. Biol.* **205**, 327-343.
- Tammero, L. F. and Dickinson, M. H.** (2002b). Collision-avoidance and landing responses are mediated by separate pathways in the fruit fly, *Drosophila melanogaster*. *J. Exp. Biol.* **205**, 2785-2798.
- Tammero, L. F., Frye, M. A. and Dickinson, M. H.** (2004). Spatial organization of visuomotor reflexes in *Drosophila*. *J. Exp. Biol.* **207**, 113-122.
- Thomas, J. B. and Wyman, R. J.** (1984). Mutations altering synaptic connectivity between identified neurons in *Drosophila*. *J. Neurosci.* **4**, 530-538.
- Trimarchi, J. R. and Murphey, R. K.** (1997). The *shaking-B²* mutation disrupts electrical synapses in a flight circuit in adult *Drosophila*. *J. Neurosci.* **17**, 4700-4710.
- von Holst, E. and Mittelstaedt, H.** (1950). Das reafferenzprinzip – (wechselwirkungen zwischen zentralnervensystem und peripherie). *Naturwissenschaften* **37**, 464-476.
- Wolf, R. and Heisenberg, M.** (1990). Visual control of straight flight in *Drosophila melanogaster*. *J. Comp. Physiol. A* **167**, 269-283.
- Yarbus, A. L.** (1967). *Eye Movements and Vision*. New York: Plenum.