

Context-dependent olfactory enhancement of optomotor flight control in *Drosophila*

Dawnis M. Chow* and Mark A. Frye

Department of Physiological Science, University of California, Los Angeles, CA 90095-1606, USA

*Author for correspondence (e-mail: dmchow@ucla.edu)

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SUMMARY

Sensing and following the chemical plume of food odors is a fundamental challenge faced by many organisms. For flying insects, the task is complicated by wind that distorts the plume and buffets the fly. To maintain an upwind heading, and thus stabilize their orientation in a plume, insects such as flies and moths make use of strong context-specific visual equilibrium reflexes. For example, flying straight requires the regulation of image rotation across the eye, whereas minimizing side-slip and avoiding a collision require regulation of image expansion. In flies, visual rotation stabilizes plume tracking, but rotation and expansion optomotor responses are controlled by separate visual pathways. Are olfactory signals integrated with optomotor responses in a manner dependent upon visual context? We addressed this question by investigating the effect of an attractive food odor on active optomotor flight control. Odorant caused flies both to increase aerodynamic power output and to steer straighter. However, when challenged with wide-field optic flow, odor resulted in enhanced amplitude rotation responses but reduced amplitude expansion responses. For both visual conditions, flies tracked motion signals more closely in odor, an indication of increased saliency. These results suggest a simple search algorithm by which olfactory signals improve the salience of visual stimuli and modify optomotor control in a context-dependent manner, thereby enabling an animal to fly straight up a plume and approach odiferous objects.

Key words: olfaction, vision, motor control, multisensory, integration, sensory function.

INTRODUCTION

Locating the source of an odor plume poses challenging problems. Not only must organisms identify the signature of an attractive chemical odorant, they must also orient and navigate towards its source while compensating for discontinuities in the signal caused by turbulence, convection and the movement of the animal itself (David et al., 1982; Fares et al., 1980; Vickers, 2000; Vickers et al., 2001). Furthermore, unlike visual or acoustic stimuli, odors contain no spatial information. Animals such as moths and flies meet these challenges by integrating more-reliable visual information to stabilize their wind-borne heading and plume position (Gilbert and Keunen, 2008).

Flying creates two general types of optic-flow over the retina: wide-field image rotation generated during steering maneuvers, and translation generated during straight flight. Insects turn syn-directionally with a rotating panorama to minimize the drift of the visual world and thereby correct unintended deviations in course (Götz, 1968; Götz, 1975; Hassenstein and Reichardt, 1956; Robert, 1988). By contrast, lateral visual expansion generated while approaching stationary objects triggers rapid evasive turns called body-saccades – sharp high-velocity turns thought to be functionally analogous to gaze-stabilizing eye movements in humans (Tammero and Dickinson, 2002b). Rotational stability and expansion-triggered saccades are thought to be necessary for straight flight and collision avoidance, respectively (Collett, 1993; Tammero and Dickinson, 2002b). The spatial, temporal and contrast-response properties associated with each type of flow-field suggest that separate premotor control circuits mediate expansion and rotation optomotor flight behaviors (Duistermars et al., 2007a), and the expansion control system is further subdivided into parallel pathways for landing and avoidance of collisions (Tammero and Dickinson, 2002a).

Despite the sophisticated role of vision in flight control, the visual resolution of compound eyes is rather poor. Over a century ago, vision scientists imagined that such eyes “...[give] a picture about as good as if executed in rather coarse wool-work and viewed at a distance of a foot” (Mallock, 1894). The spatial resolution of the fly visual system is three orders of magnitude worse than that of humans (Land and Nilsson, 2002) and thus presents a problem for the flying animal: fruit flies cannot visually discriminate food sources at any appreciable distance.

An odor plume can be fragmented, but it is identifiable at a distance and triggers robust motor responses. Behavioral studies with *Drosophila* have disclosed immediate bilateral increases in wing-beat frequency (WBF) and wing-beat amplitude (Σ WBA) upon exposure to attractive odorant, and a dependence of response kinetics on visual feedback (Frye and Dickinson, 2004; Budick, 2007). In free flight and tethered flight, these visually independent changes in wing kinematics are integrated with visual feedback to enable a fly to navigate to an odor source (Duistermars and Frye, 2008; Frye et al., 2003). As rotational signals are used to stabilize flight heading, and expansion cues mediate collision avoidance and landing, we posit that olfactory signals have a context-dependent influence on these optomotor behaviors. Here, we report that attractive odorant enhanced the ability of a fly to control visually its heading by increasing sensitivity to rotational motion but reduced sensitivity to expansion signals. Finally, flies showed better tracking of experimentally imposed image motion in the presence of olfactory cues, suggesting an olfactory-mediated increase in visual salience.

MATERIALS AND METHODS

Animals and preparation

For all experiments, adult female *Drosophila melanogaster* (Meigen) 3–6 days post-eclosion were starved for 4–6 h on water to provoke

strong reactions to food odors. Flies were reared in a laboratory colony on a standard diet and were maintained on a 16h:8h light:dark cycle. UV-curing glue was used to tether cold-anesthetized flies to a fine steel wire. Subsequently, flies were allowed 1 h for recovery. Therefore, the total elapsed time without food was approximately 5 h. The average response to appetitive odor during flight includes increases in both WBF and Σ WBA (Budick and Dickinson, 2006; Frye and Dickinson, 2004), but with considerable inter-individual variation characteristic of olfactory behavioral experiments. Here, we sought to examine the influence of odor on optomotor behaviors, and thus the crucial prerequisite is active odor responses. Therefore, data from individual flies were software selected for the experimental set if, in response to odor, WBF increased by half a standard deviation during a 5-s test odor pulse at the beginning of each experiment, and samples were thereafter excluded only if they stopped flying during the trial. 57% of the animals that successfully completed a trial (167 out of 293 individuals) increased WBF during the test pulse and were included in the data set.

Flight arena and olfactory delivery

We used an electronic LED flight simulator equipped with a video camera to present visual cues, track wing motions and detect landing responses. The arena was outfitted with a mass-flow-regulated odor-delivery system. The LED arena geometry, visual display technology, camera system and odor control have been described previously (Duistermars and Frye, 2008; Frye and Dickinson, 2004; Reiser and Dickinson, 2008). Briefly, visual stimuli were computer controlled on 48 individual 8×8 LED panel arrays arranged in a cylinder. A fly was tethered in the center of the cylinder, and a diode cast a shadow of the beating wings onto an optical sensor, which encoded amplitude and frequency for each individual wing stroke (Fig. 1A). These values were digitized at 500 Hz (National Instruments data acquisition PCI card, Austin, TX, USA) and stored on a computer using Matlab (Mathworks, Natick, MA, USA).

An odor port and vacuum produced a smooth and continuous odor plume verified visually using vapor from a small piece of dry ice (Fig. 1A). The odor port consisted of a 'double-barrel' pair of 20-gauge stainless-steel hypodermic tubes (Small Parts, Miramar, FL, USA), epoxy-sealed within a 200 μ l pipette tip to create a single odor plume. Teflon tubing connected the ends of the hypodermic tubes with polystyrene tubes (5 ml) containing either water or the attractive odorant. Room air was delivered from a mass-flow controller (Sable Systems, Las Vegas, NV, USA) at 40 ml min⁻¹ by means of a computer-controlled solenoid valve (Fig. 1A).

Experimental protocol

We examined visuo-olfactory integration in *Drosophila melanogaster* with a two-part approach. First, the left-minus-right wing-beat amplitude (Δ WBA) signal was coupled to the movement of the digital display, allowing flies active 'closed-loop' control over the visual panorama in a manner consistent with free flight. Flies were challenged with 'open-loop' stimuli, over which they had no control. Open-loop test trials were interspersed with closed-loop inter-test segments to ensure that the animals remained actively engaged in optomotor control. Second, we combined the two approaches in a 'biased closed-loop' experiment. Under closed-loop control, a periodic signal was added such that the flies had to compensate actively for the imposed bias by steering (varying Δ WBA) to stabilize image motion (Frye and Dickinson, 2004; Tammero et al., 2004).

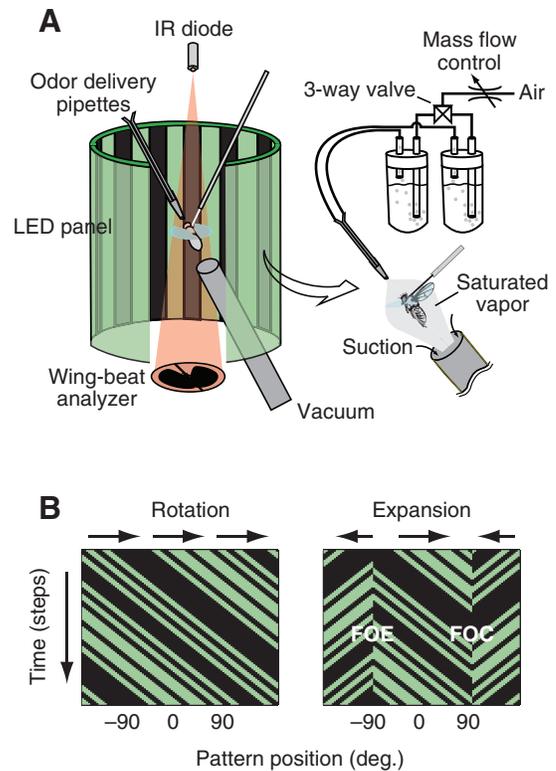


Fig. 1. Visual and olfactory stimuli were delivered to a fly tethered within a computer-controlled flight-simulator modified with odor delivery. (A) The tethered fly was suspended in the center of a cylindrical array of LEDs. Mass-flow-regulated water or apple-cider vinegar vapor was delivered to the antennae and removed continuously with a gentle vacuum to produce a continuous plume. (B) Flies were presented with wide-field patterns of rotation and expansion stimuli. Space-time plots indicate the movement in time of one representative horizontal row of the visual display. FOE, focus of expansion; FOC, focus of contraction.

We primarily used two types of experimental visual stimuli: wide-field (panoramic) rotating and expanding motion. Expansion simulates a lateral body translation, with the focus of expansion at 90° to the left of the fly and the focus of contraction at 90° to the right of the fly, whereas rotational motion simulates nontranslatory turning about the vertical axis. Each wide-field pattern was generated using random checkerboards of nonuniform spatial wavelength. Several studies show that multisensory enhancement is greater when some of the component unimodal stimuli are weak (Guo and Guo, 2005; Meredith and Stein, 1986). Thus, we used patterns of varying contrast in the closed-loop experiments in order to assess responses to different stimulus strengths [for calculation and calibration of contrast, see Reiser and Dickinson (Reiser and Dickinson, 2008)]. In addition to wide-field patterns, we also presented several other visual stimuli, including a dark bar on a bright background, 15° in width and 120° tall (subtended at the eye), which simulates a desired perch and thus was actively fixated in the frontal field of view (Maimon et al., 2008). A flickering display rapidly reversed spatial contrast and thus formed a no-motion control stimulus.

The first open-loop experiment consisted of an actively controlled small-field stripe interspersed with wide-field open-loop expansion or rotation. Contrast was set to an intermediate value (67%) for the wide-field patterns. The expansion stimulus is an

approximation of a translational flow field, but angular velocity is constant across the azimuth in order to maintain maximal similarity to the rotational stimulus. Thus, the expansion and rotation stimuli were identical, except that, for expansion, the direction of motion across the rear field of view was reversed. To examine landing responses, flies were presented with a 7.5° dark square that expanded to 120° within 0.48 s, giving a rate of expansion of 250 deg. s^{-1} . The trajectory of object expansion followed a constant velocity to maintain similarity to the expanding wide-field stimulus and therefore did not represent the accelerating trajectory of a true looming stimulus. Nevertheless, a prior study showed that constant-velocity expansion elicits strong landing responses (Tammero and Dickinson, 2002a), and the influence of spatio-temporal variations in visual expansion on saccades has been examined by Bender and Dickinson (Bender and Dickinson, 2006). The landing stimulus was presented at -120° , -60° , 0° , 60° and 120° azimuthal position, where 0° corresponded to the fixed heading of the fly. For the biased closed-loop experiment, the flies were presented with either a wide-field expansion or rotational pattern under closed-loop control with an added frequency-modulated bias signal. The stimulus waveform was constructed from sine waves of fixed amplitude and varying in frequency (1, 2, 4 and 8 Hz, each lasting 4, 4, 1.5 and 1 s, respectively). Therefore, the patterns were swept back and forth along a sinusoid that increased in frequency, then decreased in frequency along the same steps.

Undiluted commercial apple-cider vinegar served as the appetitive odorant. Air was either bubbled through aqueous vinegar or passed over a piece of filter paper saturated with vinegar. The two methods revealed no detectable response differences. Aliquots of vinegar were stored frozen; a new aliquot was used for each experiment. The odor stimulus was interspersed with the water vapor control such that the single plume port released a continuous mass-flow regulated stream of vapor onto the antennae. Odor was typically switched on at least two seconds before the onset of any visual stimuli and off at the end of each stimulus presentation. For each fly, each visual stimulus condition was presented before adding odor to prevent carry-over effects of odor on vision-only treatments. After each visual stimulus in open-loop experiments, flies were allowed at least 5 s of closed-loop control of the small-field stripe without the odor. Experiments were conducted in a random-block repeated-measures design, such that each fly received each stimulus in random order and only once.

Visual control signals, including azimuthal pattern-position, WBF, Σ WBA and Δ WBA, along with the odor control sync signals, were digitized at $500 \text{ samples s}^{-1}$ and stored on a computer using standard data-acquisition hardware. Visual stimuli, odor delivery, data acquisition and video acquisition were controlled using custom Matlab scripts.

Data analysis

Means were taken for each trial of duration 10 s in the closed-loop experiment, and then these were grouped by odor treatment. Δ WBA responses were processed with a fifth-order zero-phase 200 Hz low-pass digital Butterworth filter. To derive a mean value for each individual fly, five Δ WBA responses were averaged, and then the maximum response was taken from the first 0.3 s of the response mean. Subsequently, the response means (R) from each fly were normalized against the maximum response mean (R_{max}) for that fly, giving R/R_{max} . Statistical significance was assessed using a paired t -test on the normalized data. To derive a value for each open-loop visual stimulus, the maximum response within the first 0.3 s of each

individual Δ WBA response was normalized against the maximum individual response for that fly across all treatments. Statistical significance was assessed using two-factor repeated measures ANOVA.

In order to calculate the peak-to-peak amplitude of the Δ WBA response at each frequency epoch, a sine wave was fitted to the Δ WBA trace of each fly using an optimization algorithm. Sine waves were fitted using a sum-of-squares minimization routine. Once a fit was defined, peak-to-peak amplitude was found from the maximum and minimum fly responses within 100 samples (0.2 s) of the corresponding minimum and maximum values of the fit. r -squared goodness of fit was calculated with the following equation: $r^2 = 1 - \Sigma(\text{observed } \Delta\text{WBA} - \text{fit})^2 / \Sigma(\text{observed } \Delta\text{WBA} - \text{mean } \Delta\text{WBA})^2$. Data analysis was conducted with custom-scripts written in Matlab.

Digitized pictures during the landing experiment were acquired at 30 Hz. A custom algorithm detected the increase in average image brightness caused by lateral extension of the legs during the landing reflex. Digital filtering of the image eliminated variance in luminance caused by the beating wings, and subtraction of the first frame enhanced the image contrast. Analysis performed by hand on three sample flies indicated a zero false-positive rate and a false-negative rate of 7.14%. The algorithm generally only detected responses involving all six legs, although sometimes very brief responses involving only one or two legs were seen, which were not included. The landing probability was defined as the percentage of landing responses detected by the algorithm out of all stimulus presentations at that position for all flies.

RESULTS

Attractive odor straightens the flight path

Minimizing rotational retinal slip results in a straight flight trajectory, which could help stabilize a fly within an odor plume. Thus, we first examined the impact of an attractive odorant, apple-cider vinegar, on active visual stabilization. Flies were given active (closed-loop) control of the visual scene for four different visual conditions: (i) wide-field rotation, (ii) wide-field expansion, (iii) a small-field vertical stripe 15° wide and (iv) a no-motion flicker stimulus. Stimuli were presented to each fly for 20 s in a random-block design, with the attractive odorant activated during the final 10 s of each trial. Flown under closed-loop conditions, flies generally maintain near-zero average velocity of a rotating wide-field pattern. In addition, when presented with a single vertical bar, they fixate the position of the bar to the frontal field of view (Maimon et al., 2008).

Δ WBA is a quantitative proxy for yaw torque, and thus the variability in this signal is a direct measure of steering activity. Upon odor exposure, Δ WBA variance decreased in wide-field expansion and rotation. Data collected using low-contrast and high-contrast wide-field stimuli were qualitatively similar and thus were pooled (Fig. 2A, paired t -test, expansion $P < 0.001$, rotation $P < 0.05$). Upon exposure to odor, there were no significant changes in variance during the presentation of flicker or during object tracking, indicating that the odor-mediated decrease in Δ WBA variance was dependent on the context of active visual control (Fig. 2A). These results suggest that, upon encountering an attractive odor, flies use wide-field cues, but not small-field cues, to stabilize their flight heading better (Fig. 2B, paired t -test, expansion $P < 0.001$, rotation, $P < 0.001$).

An important caveat is that, during flight, increased aerodynamic power output decreases steering capacity (Lehmann and Dickinson, 2001). The odor-evoked increases in total wing-

beat amplitude (Σ WBA) and WBF are reflected in increased aerodynamic power output as the cubed product of WBF and Σ WBA is proportional to total aerodynamic power output (Lehmann and Dickinson, 1998; Lehmann and Dickinson, 1997). Therefore, the odor-mediated changes in power output could effectively limit Δ WBA variance. To test this hypothesis, we plotted Δ WBA against $(\Sigma$ WBA \times WBF)³, a proxy for aerodynamic power output (Duistermars et al., 2007b) and confirmed that Δ WBA variance indeed tends towards zero at high power output (Fig. 2C). However, over the full range of power values, the distribution of points associated with odor clusters more tightly around zero than that of responses without odor (Fig. 2C), and thus the steering variance during odor presentation is lower than for the no-odor control (Fig. 2C, inset). This result confirms two separate motor responses to attractive odors: a bilateral increase in Σ WBA and WBF that results in increased aerodynamic power output, and an increase in active visual stabilization that results in a straight flight heading.

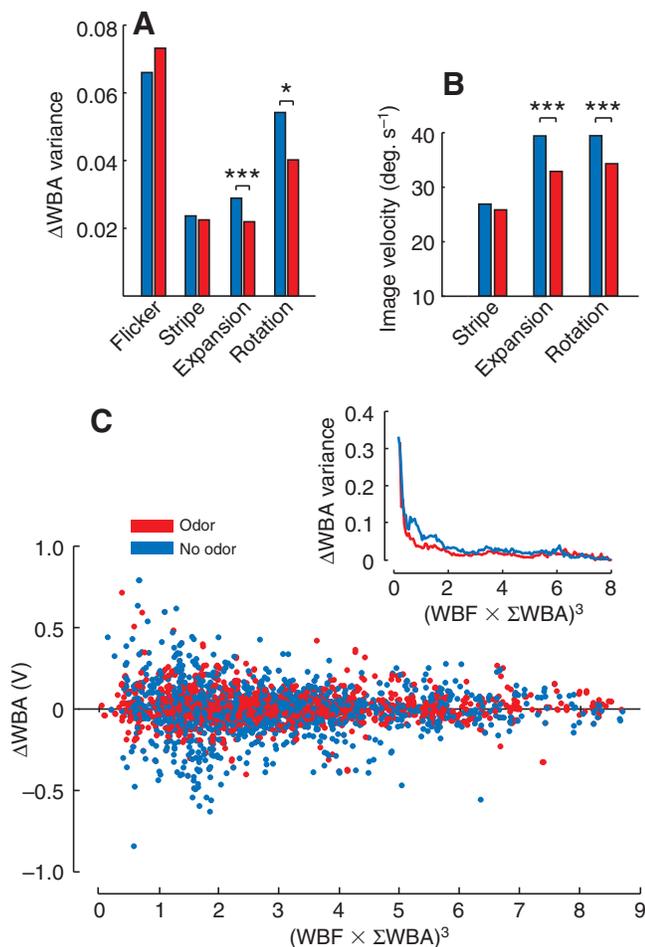


Fig. 2. Attractive odorant decreases steering variance and increases visual stability under closed-loop control. (A) Flies showed a decrease in mean Δ WBA variance under the influence of odor (expansion $P < 0.001$, rotation $P < 0.05$, paired t -test) and (B) a corresponding decrease in mean image velocity ($P < 0.001$, paired t -test), indicating increased visual stabilization. (Flicker, $N = 64$; SF stripe, $N = 64$; WF expansion, $N = 100$; WF rotation, $N = 106$). ($*P < 0.05$, $***P < 0.001$). (C) Δ WBA responses were binned and sorted by increasing $(\Sigma\text{WBA} \times \text{WBF})^3$. The inset shows the variance calculated from Δ WBA responses plotted against power.

Visual-context-dependent influence of olfactory input on flight control

In order to test the hypothesis that odor differentially influences rotation and expansion optomotor responses, we tracked wing steering kinematics in response to open-loop presentations of expansion and rotation optic flow in the presence and absence of an attractive odor. Aversive odors are irrelevant to active tracking behavior and thus were not investigated. Flies were presented with a sequence of five visual stimuli of duration 1 s in which the visual panorama rotated clockwise or expanded from left to right, interspersed with segments of duration 1 s in which the fly had active closed-loop control of a vertical stripe (ensuring that the animal was actively engaged in visual flight control throughout the experiment). Flies responded strongly to both treatments with an increase in Δ WBA, representing a rightward turn. An example raw data trace is shown in Fig. 3A. The effect of odor is subtle, yet clearly evident as an increase in Δ WBA for rotation and a decrease for expansion (Fig. 3A,B). There was no difference in Σ WBA arising from

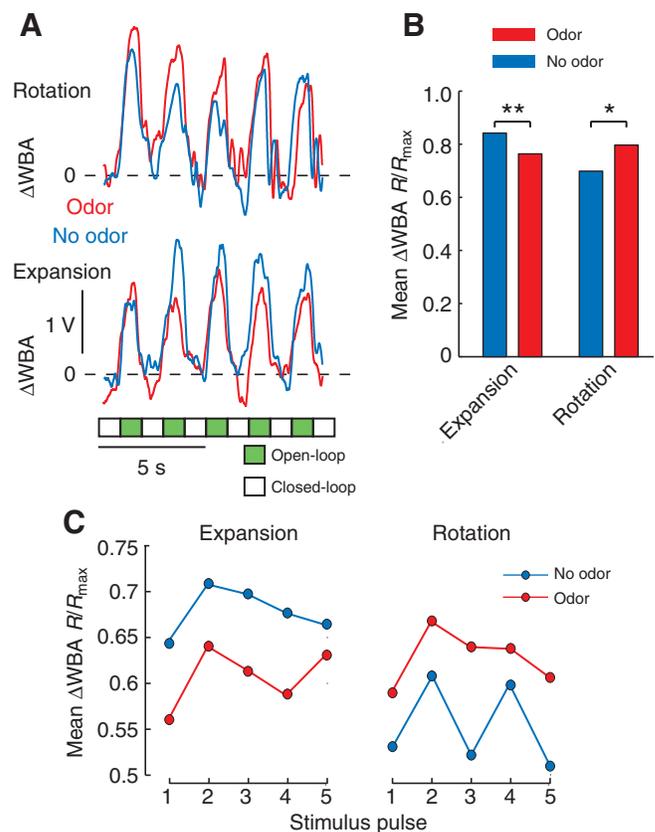


Fig. 3. Rotational optomotor responses are enhanced and expansion-avoidance optomotor responses are suppressed under the influence of attractive odor. (A) Example raw Δ WBA responses evoked by five rotation and expansion stimuli either in the presence of odor (red) or without odor (blue). (B) Odor increased the mean amplitude of rotational optomotor responses but decreased the mean expansion amplitude ($N = 46$, $*P < 0.05$, $**P < 0.01$, paired t -test). (C) This is the same as (B) but separated by each one-second visual-stimulus presentation. There was no significant time-course effect (two-factor repeated measures ANOVA, odor $P < 0.05$, time $P > 0.05$). Note: *within-subjects* design eliminates the need for error bars. R/R_{max} is the normalized response magnitude (see text for details).

olfactory treatment in expansion, although Σ WBA decreased significantly in odor for rotation (data not shown). Odor-mediated suppression of mean expansion responses and enhancement of mean rotation responses were independent of the time course of stimulus presentation (two-factor repeated measures ANOVA, odor $P < 0.05$, time $P > 0.05$). Although we designed the expansion and rotation stimuli to produce steering responses of similar magnitude, we note here that the rotation responses are nevertheless smaller than the expansion responses, which is consistent with previous findings (Tammero et al., 2004; Duistermars et al., 2007a; Duistermars et al., 2007b).

Odor interacts with the expansion optomotor pathway 'upstream' of the landing pathway

Because it decreased sensitivity to expanding cues (Fig. 3), we hypothesized that odor might facilitate landing by increasing visual tolerance of a stimulus expanding on the retina. In response to a small square expanding at 250 deg. s^{-1} , flies rapidly extend their legs in a stereotypical landing reflex (Fig. 4A) (Borst, 1990; Tammero and Dickinson, 2002a). We presented landing stimuli in random order at several positions around the arena. The probability of eliciting a landing reflex (see Materials and methods) increases as the stimulus is presented progressively towards the front of the fly (Tammero and Dickinson, 2002a). At each azimuthal stimulus position, except at that of the heading of the fly, odor slightly decreased landing response probability (Fig. 4B, two-factor repeated measures ANOVA $P < 0.01$).

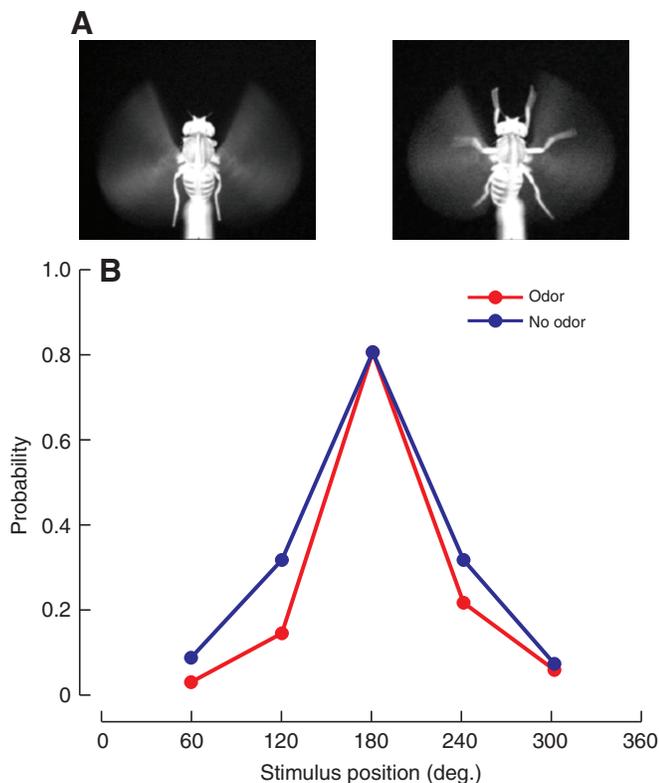


Fig. 4. Attractive odorant influences the probability of a reflexive landing response. (A) Image sequence depicts the leg extension of a typical landing response evoked by a rapidly expanding visual object. (B) The probability of landing was decreased by attractive odorant at all azimuthal positions except directly in front of the fly ($N=14$, $P < 0.01$, two-factor repeated measures ANOVA).

The interaction of olfactory-mediated flight control and heading stability

An increase in sensitivity to open-loop rotational motion (Fig. 3) is consistent with the increased flight stability observed when the fly has active control over retinal image motion (Fig. 2). However, how does reduced sensitivity to open-loop expansion allow the animal to fly straighter while actively controlling an expanding panorama (Fig. 3)? It would seem reasonable to posit that, under fully closed-loop conditions, the performance should be 'sloppier' with an expanding panorama because the fly tolerates more image expansion before exhibiting steering maneuvers. However, for all combinations of spatial, temporal and contrast properties yet tested, wide-field expansion evokes stronger steering responses than equivalent wide-field rotation (Duistermars et al., 2007a; Duistermars et al., 2007b; Tammero et al., 2004). Without a deeper understanding of the maximum operational gain that is possible, it is very difficult to predict what the influence of a *change* in gain might be. For a system operating under nearly saturated conditions, a decrease in gain could damp feedback oscillations and improve stability. By contrast, if the system is operating below maximum gain, then an increase could push the system closer to oscillation instability. Similarly, the temporal delay between visual motion detection and resultant corrections from the motor circuits greatly impact the influence of varying the stimulus conditions.

To resolve the apparent inconsistency between the open-loop and closed-loop conditions presented, we presented the fly with a combination of both. Thus, we imposed a bias on the ability of the fly to control the visual panorama actively and then tested how odor influences optomotor sensitivity. Under these conditions, if the fly were to ignore the bias signal completely, then the wing kinematics would be independent of the bias signal, and the visual panorama would follow the motion trajectory defined by the bias. Conversely, if the fly fully compensated for the bias, then the wing kinematics of the animal would follow the bias, and the visual display would move in a manner indistinguishable from the normal closed-loop conditions. (Frye and Dickinson, 2004; Tammero et al., 2004). We used a sinusoidally varying frequency waveform that ranged from 1–8 Hz. An example trace is shown in Fig. 5A. Under biased closed-loop conditions, flies attempted to correct for the sinusoidal stimulus by varying their Δ WBA sinusoidally in phase with the stimulus velocity (Fig. 5B).

As with the open-loop experiments, the amplitude of Δ WBA was reduced in expansion and increased in rotation (Fig. 5B). To test this difference quantitatively, we fitted a sine function to the Δ WBA response of each frequency epoch for each fly. Wide-field expansion generally showed a lower average peak-to-peak amplitude response in odor (except at 1 Hz), whereas the average amplitude was increased for rotation at all frequencies (Fig. 5C, two-factor repeated measures ANOVA, expansion $P < 0.05$, rotation $P < 0.001$). Consistent with the previous results, the effect of expansion suppression, although statistically significant, was subtler than that of rotation enhancement.

Olfactory enhanced salience of visual input

Although the differences are repeatable and significant, we were impressed by the rather subtle influence that odor has on optomotor steering kinematics. We reasoned that any influence of olfactory cues might arise from increased perceptual salience of visual input regardless of the specific visual context and that increased salience would mediate the differences between optomotor reactions, which might be subtle 'by design' so as not to compromise optomotor flight control. We found that, during

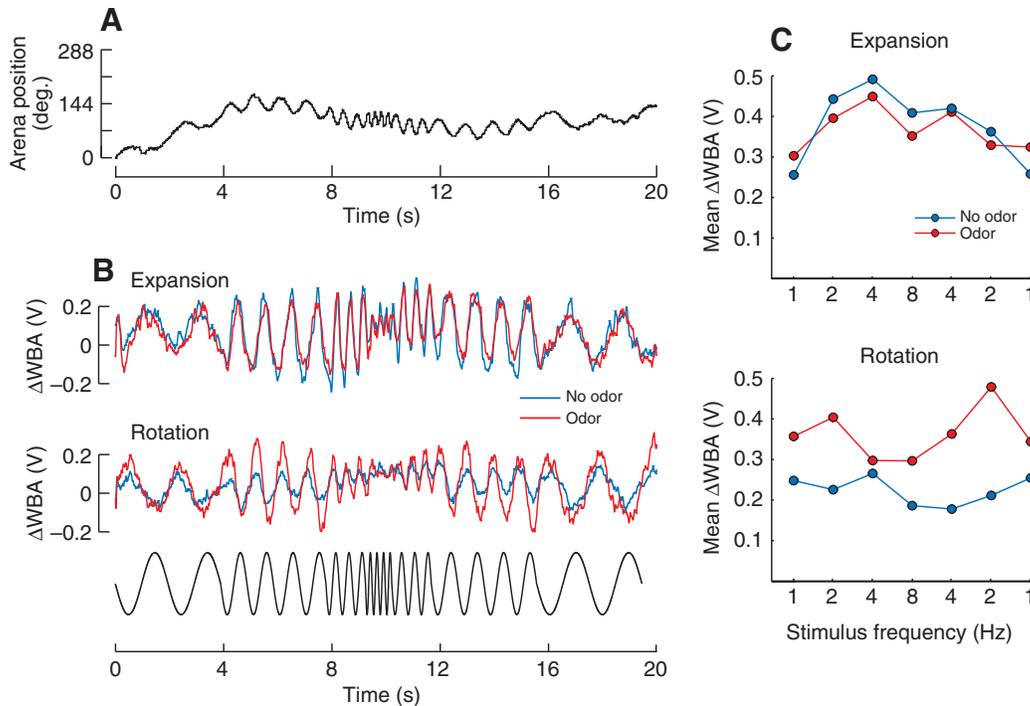


Fig. 5. The combination of enhanced rotation responses and suppressed expansion responses persists under conditions in which the fly has active control of image motion. (A) An example position trace shows the small remnant of a sinusoidal bias added to the control of the fly over the arena heading. (B) Top, mean Δ WBA responses with odor (red) and without odor (blue) ($N=43$). Bottom, the frequency-modulated stimulus position waveform of the imposed bias. (C) Mean peak-to-peak response amplitude for each consecutive stimulus epoch. The response amplitude was quantified and tested statistically using fitted sine functions. Δ WBA response amplitudes were significantly enhanced by odor at all frequencies for rotation (two-factor repeated measures ANOVA, $P<0.001$) but were only suppressed significantly between 2 Hz and 8 Hz for expansion (two-factor repeated measures ANOVA, $P<0.05$).

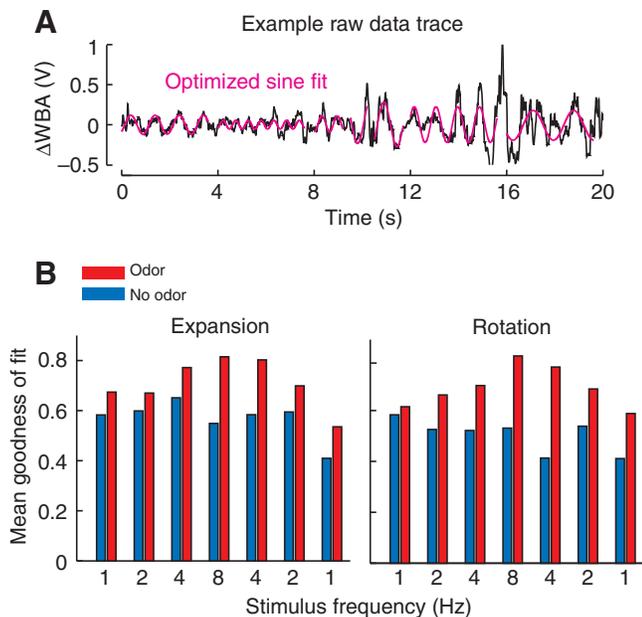


Fig. 6. Olfactory cues increase the saliency of visual stimuli. (A) The example optomotor response trace (black) and optimized sine-fit for each frequency epoch (pink). (B) Mean r -squared values for each stimulus epoch with and without odor.

both the expansion- and rotation-biased closed-loop treatments, the experimental trials with odor had higher average r -squared values than trials without odor when fit to sine functions (Fig. 6). This suggests that the flies are tracking the imposed visual bias more closely when exposed to odor, resulting in a more sinusoidal, less variable, Δ WBA waveform. This recapitulates our closed-loop findings (Fig. 2) and, taken together, implies that odor increases the saliency of visual signals.

DISCUSSION

In this series of experiments, we investigated the impact of an attractive odorant on the control of visually mediated flight-equilibrium reflexes. Under real-time closed-loop feedback conditions, we found that attractive odorant increased the ability of flies to stabilize image motion (Fig. 2). Flies did not show significant variation in the ability to fixate a small-field object, suggesting that cross-modal integration is specific to the wide-field optomotor systems (Fig. 2). When we interrupted the active visual course control of the fly and rotated or expanded the world briefly, we found separable odor influences. The steering response magnitude increased for rotational stimuli and decreased for expansion stimuli (Figs 3 and 5). Rotation optomotor enhancement and expansion optomotor suppression were further supported by experiments using biased closed-loop feedback control in which odor reduced the amplitude of expansion compensation but enhanced rotation compensation (Fig. 5). Furthermore, we provide evidence that olfactory input selectively enhances the saliency of wide-field rotation and expansion visual cues (Fig. 6).

Ethological implications for plume tracking behavior

Male moths display upwind anemotaxis during flight, interspersed with cross-wind casting to track pheromone plumes emitted by female conspecifics (Preiss and Kramer, 1986; Vickers, 2000). Recently, *Drosophila melanogaster* has been shown to use a similar plume-tracking strategy (Budick and Dickinson, 2006). As flying insects cannot differentiate self-motion from wind cues using mechanosensory input alone, reliance on visual side-slip cues has been shown to play an important role in anemotaxis (Kennedy and Marsh, 1974; Vickers, 2000). An odor-activated increase in rotational optomotor gain might help maintain a straight heading in a plume, whereas enhanced tracking of wide-field expansion cues might facilitate optomotor anemotaxis. Concomitantly, suppressed expansion avoidance enables closer approach to smelly objects. Therefore, context-dependent olfactory-enhanced optomotor reflexes might underlie the visual dependence of airborne odor localization across taxa.

Opto-olfactory control systems

The spatial and temporal dynamics of visually mediated flight behavior indicate that the optomotor reactions to wide-field rotation and expansion are mediated by separate neural pathways (Duistermars et al., 2007a; Duistermars et al., 2007b). Similarly, expansion-triggered behaviors are further subdivided into collision avoidance and landing and are probably mediated by separate neural circuits (Tammero and Dickinson, 2002a). The latter analysis posits a three-way local circuit of elementary motion detectors comprising frontal and lateral inputs such that collision-avoidance pools from the lateral arrays, and landing pools from the frontal array (Tammero and Dickinson, 2002a). This tripartite model forms a conceptual framework for the olfactory results presented here such that olfactory cues both amplify rotational optomotor responses and selectively diminish the sensitivity of the peripheral collision-avoidance circuits. This idea is supported by two lines of evidence. First, olfactory stimuli suppress both collision-avoidance turns evoked by lateral expansion (Figs 3 and 5) and landing responses to lateral expansion (Fig. 4B). Second, the probability of landing responses is not influenced by frontal expansion (Fig. 4B). Similar olfactory-mediated enhancement of visual circuits is observed within descending pre-motor neurons in moths, which are visually specific yet exhibit amplified spiking activity when the moth is exposed to pheromone (Olberg and Willis, 1990).

Olfactory-mediated increase in visual salience

It was intriguing to find that optomotor responses more closely reflect an imposed visual stimulus under the influence of odor, by contrast to no odor, in which the steering responses are significantly less dependent on the visual stimulus. Under closed-loop feedback conditions, the mean goodness of fit between a fitted sine function and the resultant wing kinematics of the fly during flight improves under both rotation and expansion at all tested frequencies when the fly was presented with attractive odor (Fig. 6). This suggests to us that odor enhances the salience of ambient visual cues. Casually speaking, flies seem to be 'paying attention' to the visual panorama within an odor plume more so than within the water vapor control. These results are consistent with other findings in *Drosophila*, such as a frequency-specific increase in brain activity attributed to olfactory-enhanced salience of a visual object (van Swinderen and Greenspan, 2003), and olfactory-enhanced memory retrieval associated with a weakly detectable visual stimulus (Guo and Guo, 2005); however, to our knowledge, this is the first behavioral evidence that the perceptual salience of visual stimuli is enhanced

by olfactory input specifically to improve active optomotor control during flight.

An adjustable cross-modal search algorithm

During free-flight, visual feedback is required for odor localization, but the behavioral effects are subtle and highly variable. Simulations confirm that small modifications to flight heading and collision cues are at least sufficient to enable odor localization when the search algorithm is iterated over hundreds of saccades, as occurs in free flight (Frye et al., 2003; Reynolds and Frye, 2007). A subtle effect of odor on the flight-control algorithm might be preferable, however, as a robust influence could potentially leave the animal vulnerable to course perturbations imposed by gusts of wind and pursuit from predators. By contrast, small, yet consistent, modifications to flight behavior take advantage of sensory reflexes to bias merely the heading of the animal towards the odor source without compromising adaptive reflexes.

Animals seeking widely dispersed chemical signals search actively even when they are not actually experiencing an odor plume. The improved sine fits for both expansion and rotation suggest that odor might tip the balance from sensory-independent exploratory search behavior towards a sensory-dependent active tracking algorithm. It therefore stands to reason that the course correction to rotation and expansion are implemented as a result of increased visual salience. As a working hypothesis, we suggest that odor triggers rotational optomotor responses that enhance straight flight; simultaneously, expansion responses are suppressed to enable an animal to approach more closely an appropriately smelly visual feature. Odor-enhanced visual salience tips the balance from variable sensory-independent exploratory behavior towards more-stereotyped sensory-dependent local search behavior to steer a fly to its goal. The modulation of optomotor control by olfactory input represents a relatively simple, yet robust, mechanism by which an animal might track a fragmented odor plume within varied visual landscapes without the need for high-order object-recognition computations.

LIST OF ABBREVIATIONS

WBF	wing-beat frequency
Δ WBA	difference between left and right wing-beat amplitude, equal to left minus right, proportional to yaw torque, positive values denote right turns
Σ WBA	total wing-beat amplitude, equal to left plus right wing-beat amplitude

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