Motor output reflects the linear superposition of visual and olfactory inputs in *Drosophila*

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Summary

Animals actively seeking food and oviposition sites must integrate feedback from multiple sensory modalities. Here, we examine visual and olfactory sensorimotor interactions in *Drosophila*. In a tethered-flight simulator, flies modulate wingbeat frequency and amplitude in response to visual and olfactory stimuli. Responses to both cues presented simultaneously are nearly identical to the sum of responses to stimuli presented in isolation for the onset and duration of odor delivery, suggesting independent sensorimotor pathways. Visual feedback does, however, alter the time course of the odor-off response. Based on the physiology of the flight motor system and recent free-flight analyses, we present a hypothetical model to account for the summation or superposition of sensorimotor responses during flight.

Key words: vision, olfaction, fruit fly, *Drosophila*, sensorimotor integration, sensory fusion, optomotor, multimodal integration.

Introduction

As expected of a cosmopolitan generalist, *Drosophila melanogaster* is remarkably adept at finding sources of odor in widely varying visual environments. The detection, discrimination and directed search for attractive signals within complex sensory landscapes represents multi-step processes spanning many physiological systems. Spatially structured information from the visual system is integrated with input from other modalities such as olfaction, translated into motor commands and finally transformed by the musculoskeletal system into locomotor maneuvers that direct the fly towards its goal. For *Drosophila*, visuo-olfactory integration is particularly important for search behavior. Recent free-flight analyses show that animals readily localize an odor source against a textured visual background but fail to do so when the target is presented within a uniform visual surround (Frye et al., 2003). Specifically, visual feedback from the apparent motion of vertical edges appears necessary for successful odor localization. These results suggest that attractive odor must somehow suppress or modify visually elicited collision avoidance maneuvers that would otherwise bias an animal's trajectory away from the odor source.

The necessary interaction between visual and olfactory feedback might occur at various levels within the nervous system. For example, integration of multiple sensory modalities within the mushroom bodies is critical in context-dependent learning during flight in *Drosophila* (deBelle and Heisenberg, 1994). Other higher order regions within the protocerebrum, such as the lateral horn, also receive visual and olfactory input from primary sensory regions. Alternatively, feedback signals may be integrated within specialized descending networks that supply the flight motor within the thoracic ganglia. Sensory fusion may arise within individual multimodal descending neurons or may be distributed among parallel unimodal pathways. Notwithstanding the anatomical locus of sensory fusion, understanding how vision and olfaction interact to coordinate motor reflexes in *Drosophila* will accelerate efforts to link molecular mechanisms to the systems-level neural processes responsible for complex behaviors in flies and other animals.

In the present study, we investigated the interaction between olfactory and visual stimuli on the motor control of wing kinematics during flight. Our results show that flies exhibit robust and stereotyped bilateral increases in wingbeat amplitude and frequency when presented with a frontal stream of vinegar vapor. Patterns of lateral visual expansion elicit rapid, reflexive modulations of wing kinematics that direct flies away from an apparent collision. When presented with both sensory cues simultaneously, motor responses show linear superposition. We propose a model for how visual and olfactory feedback is structurally integrated within the musculoskeletal system to functionally bias *Drosophila* flight trajectories towards odor in free flight.

Materials and methods

Experiments were performed on 2–3-day-old female wild-type *Drosophila melanogaster* (Meigen) from a laboratory stock maintained at University of California, Berkeley. Flies were reared on standard artificial diet, maintained on a 12 h:12 h L:D photoperiod and tested within 6 h of the onset

of subjective day. Under cold anesthesia, each fly was tethered to a short segment of tungsten wire glued at the dorsal junction of the head and thorax; the head was immobilized to allow precise measurement of image motion on the retina.

We tested flies' motor responses to visual and olfactory stimuli during intact tethered flight within a tethered flight simulator. A detailed description of this apparatus is available elsewhere (Lehmann and Dickinson, 1997), and only a brief account is given here. Flies were suspended between an optical sensor and an infrared light-emitting diode (Fig. 1A). The beating wings cast a shadow onto the sensor. Associated electronic components track the motion of both wings and measure the amplitude and total frequency of each wing stroke. While tethered in place, flies attempt to steer by modulating the difference between the left and right wingbeat amplitude (ΔWBA). To create closed-loop conditions, the time varying ΔWBA signal is coupled to the angular velocity of a visual pattern created by a cylindrical array of green LEDs (Fig. 1A).

We allowed flies to control the velocity of an expanding flow field centered laterally. Any change in ΔWBA resulted in a vertically striped pattern moving horizontally across the front and rear visual fields, generating opposing poles of expansion and contraction (Fig. 1C). The direction of motion was inverted with respect to the ΔWBA signal (hence, a turn

directed away from the pole of expansion resulted in reduced expansion velocity). Under these conditions, flies show a strong tendency to minimize the velocity of the expansion/contraction. Periodically, we simulated a collision stimulus by adding an impulsive bias to the closed-loop feedback signal such that if the fly did not respond at all, the visual pattern would expand from the right at constant velocity for the duration of the bias. This stimulus regime generates robust and repeatable collision avoidance reflexes composed of rapid changes in ΔWBA stroke amplitude.

To test the effects of steady-state odor cues on visually mediated collision avoidance reflexes, we modified the flight arena to deliver a stream of saturated vapor onto the fly's antennae (Fig. 1B). A mass flow controller (model 840; Sierra Instruments, Monterey, CA, USA) delivered a constant velocity stream of air controlled by a solenoid valve to two 30-ml vials containing either distilled water or a 15% solution of apple cider vinegar. Dilute vinegar promptly attracts freely mobile *Drosophila* but it does not necessarily evoke a generalized olfactory response. Saturated water and vinegar vapor were delivered through separate tubes into a pair of 16-gauge hypodermic needles that were in turn sealed into the distal tip section of a clear pipette tip. Thus, the tip of the pipette (4 mm long and <1 mm^3 in volume) served as a

Fig. 1. Experimental apparatus to examine sensorimotor interactions in *Drosophila*. (A) A fly is tethered beneath an infrared diode that casts a shadow of the beating wings onto an optoelectronic wingbeat analyzer (red). The output of the analyzer is coupled with the rotational velocity of the pattern displayed on the wrap-around LED screen (green) such that the fly has closed-loop control of the visual panorama. (B) System to deliver a continuous stream of saturated vapor onto the antennae. A computer-controlled solenoid valve shunts a mass-flow-regulated air supply to either a vial of distilled water (experimental control) or a vial of dilute vinegar solution. (C) A pattern of vertical stripes appears to expand from the right and contract to the left of the fly. The velocity of expansion/contraction is in closed-loop such that if the expansion appeared from the right, a turn to the left would reduce the velocity of expansion and *vice versa*. To examine the strength of visual reflexes, with and without odor, we perturbed the fly's closed-loop control of the expansion/contraction stimulus by adding a 1.25-s bias to the feedback loop (see Materials and methods).
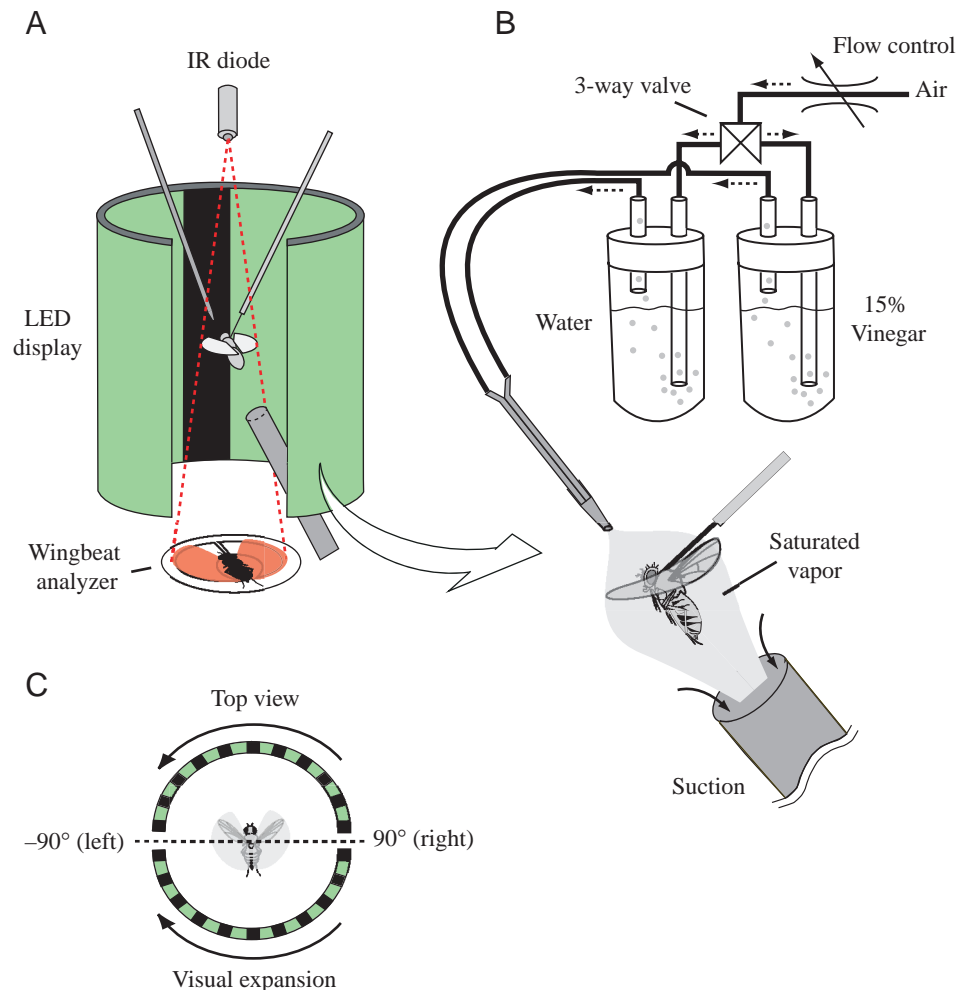
common pore through which the parallel distribution system expelled saturated vapor. The pipette tip was oriented along its long axis with respect to the fly's retina to minimize its apparent size (less than 5°). The apparatus delivered a constant stream of saturated vapor through the pipette tip, and the solenoid valve periodically switched between the odor stimulus (vinegar) and an odor-free stimulus to control for anemotactic cues. The delivery tubes were pre-loaded with saturated vapor before the experiment to minimize the delay from the solenoid switch to the delivery of vapor on the antenna. Restricting odor to separate pathways until the final few cubic millimeters of the delivery system also minimized stimulus delay and ensured a rapid onset of odor cues. Vapor was delivered to the antennae at 280 mm s^{-1}, matching the average airspeed of animals in free flight (Tammero and Dickinson, 2002a). A brass tube placed behind the fly provided gentle suction to remove residual odor stimuli. The tube was placed in the fly's visual blind spot and the suction was not strong enough to affect wing kinematics. We performed several control experiments to ensure that the delivery system did not introduce large mechanical artifacts or side bias. There was no detectable WBA or wingbeat frequency (WBF) response to water/water controls, and switching the position of the vinegar and water vials produced identical results (data not shown).

For decades, researchers have used tractable open-loop steady-state approximations of naturally dynamic sensory stimuli to study multisensory and sensorimotor integration. Such linear analyses are an integral first step towards a comprehensive understanding of sensorimotor processing by nervous systems. Future work should incorporate more naturalistic olfactory dynamics to examine sensory superposition in non steady-state conditions.

Results

Visual and olfactory mediated motor responses vary independently for the onset and duration of odor stimuli

We used an electronic flight arena (Lehmann and Dickinson, 1997) to test flies' responses to visual and olfactory cues, presented both separately and in combination (Fig. 1). We chose a visual stimulus that produced robust closed-loop motor behavior during tethered flight (Tammero et al., 2004) – a pattern of vertical stripes that expanded from the fly's right and contracted to the left (Fig. 1C). To examine the strength of visual reflexes, we periodically challenged flies by imposing a bias to their closed-loop control of expansion/contraction velocity (see Materials and methods). In response to these experimental perturbations, flies show rapid modulation of the difference in amplitude between the left and right wings (here referred to as ΔWBA) as they attempt to maintain visual equilibrium (Fig. 2A, ΔWBA waveform). Since the time-varying ΔWBA signal is coupled *via* feedback to the motion of the visual display, changes in ΔWBA result in changes in the velocity of visual expansion (Fig. 2A, velocity waveform). The low degree of variance for both the ΔWBA signal and

image velocity indicates that closed-loop motor responses to rapid changes in visual expansion are highly robust (Fig. 2B). Furthermore, the trajectories of these visually mediated motor reflexes do not vary with the simultaneous presentation of odor (Fig. 2C, compare i and iii). In addition to an expanding/contracting visual stimulus, we examined the influence of odor on a suite of visual reflexes including stripe fixation, optomotor stabilization and object avoidance. Odor did not alter either the spatial or the temporal structure of responses to these classes of visual stimuli. For example, as with an expanding flow field, flies that had closed-loop control of either a single vertical stripe or a rotating random checkerboard responded to periodic bias with rapid compensatory changes in ΔWBA (Fig. 2D). Neither of these well-studied visual reflexes were affected by the presentation of odor (Fig. 2D). In addition to experimentally imposed visual bias, odor did not significantly alter the structure of spontaneous saccade-like turns during closed-loop flight (data not shown).

Flies show robust and repeatable motor responses to the presentation of an attractive odor during tethered flight. The total amplitude of the right and left wingbeat (the sum of WBA, hereafter referred to as ΣWBA) as well as wingbeat frequency (WBF) rise monotonically and, on average, reach steady-state levels within 2.5 s following the onset of odor delivery (Fig. 2A,B). At the termination of the 10-s odor stimulus, WBF and ΣWBA responses decay along a slower time course, returning to baseline after approximately 10 s. Rapid changes in visually elicited ΔWBA subtly alter the time courses of ΣWBA and WBF waveforms. There was no detectable WBA or WBF response to water/water controls (data not shown).

The fine structure of motor responses is highlighted in Fig. 2C. At the onset of a stepwise bias in closed-loop feedback, the visual display expands from the right. In response, flies decrease ΔWBA within 300 ms in an effort to turn away from the focus of visual expansion. The magnitude of expansion velocity is rapidly reduced as the fly compensates for the bias and stabilizes pattern motion. However, the flies are not able to completely overcome the imposed bias, resulting in non-zero steady-state velocity (Fig. 2Ci, arrow). At the termination of the visual bias, ΔWBA returns to zero within approximately 300 ms. Thus, the onset and offset of this visual reflex operate on similar rapid time courses.

By contrast, motor responses to the presentation and termination of odor pulses follow slow, asymmetric time courses. For visual closed-loop conditions without visual bias, the onset of odor results in an increase in WBF that approaches a steady-state level within ~2 s (Fig. 2B). In response to odor, ΣWBA first decreases then monotonically increases to a steady-state level (Fig. 2Cii, arrow). We have repeated these experiments several times. The initial downward transient is a consistent feature of vinegar-elicited increases in ΣWBA. This transient may result from mechanical constraints on power production within the flight system (Lehmann and Dickinson, 2001). The mechanical power imparted to the beating wings is roughly proportional

to the cubed product of ΣWBA and WBF and is limited by several factors, including maximum wing amplitude and the performance limits of flight muscle. We suspect that the transient decrease in ΣWBA results from a time-lag between

increased WBF, which is driven by changes in small control muscles, and the regulatory pathway that can enhance mechanical power output of the large power muscles.

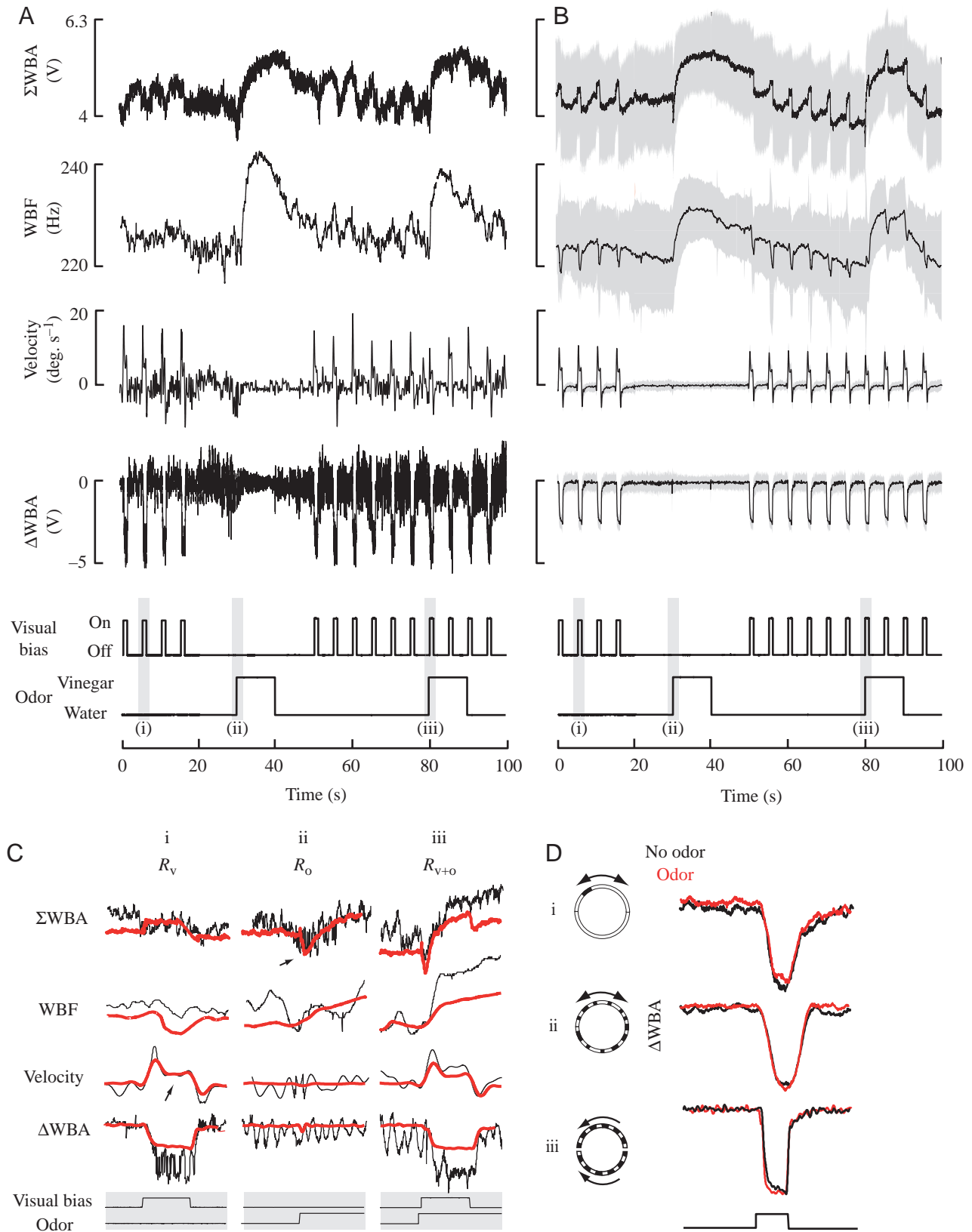In response to repeated stepwise presentation of a bias in

image expansion centered to the right, flies turn away from the focus of expansion by tonically increasing the amplitude of the right wing and decreasing that of the left (Fig. 3, top panel, red lines). During concurrent presentation of odor, these changes in visually mediated ΔWBA are superimposed upon a bilateral elevation of ΣWBA (Fig. 3, top panel, black lines). Aside from the shift in baseline, neither the time course nor magnitude of visually elicited steering reflexes (ΔWBA) are affected by the presentation of odor (Fig. 3, bottom panel). These results suggest that the visual and olfactory stimuli elicit independent motor responses. By visual inspection, motor responses to the two stimuli presented together appear to represent the sum of responses to stimuli presented in isolation (Fig. 2B). To examine this explicitly, we tested for linear superposition, in which the following relationship must be satisfied:

$$R_v + R_o = R_{v+o}$$

where R_v and R_o represent the responses to visual and olfactory stimuli, respectively, and R_{v+o} is the response to the same stimuli presented simultaneously. The average motor responses to visual bias presented in the absence of an odor stimulus include stereotyped, rapid modulations in ΔWBA and resultant changes in image velocity (Fig. 4i). In response to an odor without visual bias, there is no change in ΔWBA, whereas total ΣWBA and WBF rise tonically towards the steady-state value, followed by a slow decay upon termination of the stimulus (Fig. 4ii). The mathematical addition of these separate visual and odor responses produced waveforms nearly indistinguishable from those resulting from the concurrent presentation of the two stimuli (Fig. 4iii). This indicates a linear interaction between the motor responses elicited by visual and olfactory pathways during presentation of the odor pulse.

Visual feedback alters the time course of odor-off responses

The presentation of odor has no effect on either experimentally imposed reflexive visual responses (Figs 3, 4iii) or closed-loop visual control (Fig. 4ii). However,

at the termination of the odor stimulus, the time course of WBF and ΣWBA responses depends upon the visual conditions. This is indicated by a violation of linear superposition immediately after the odor is switched off (Fig. 4iii, end of trace). Just before the odor pulse terminates, the ΣWBA and WBF responses are nearly identical in the presence or absence of visual bias (Fig. 5). However, after the odor is switched off, these motor responses return to baseline much more slowly in experimental treatments that include periodic visual bias (Fig. 5, red lines). This indicates that visual feedback somehow alters the time course of the odor-off response.

To test whether constant visual feedback was sufficient to alter the time course of the odor-off response, we compared responses to odor pulses during unbiased visual closed-loop conditions with those presented in a motionless arena of equal luminance. In the absence of visual feedback, odor pulses elicit increases in mean ΣWBA and WBF (Fig. 6). Thus, visual motion is not required for flies to generate typical motor responses to odor. However, under visual closed-loop conditions, the odor responses are substantially altered. Although the rise-time of the odor responses does not differ significantly between the two treatments, the odor-elicited changes in wing kinematics take considerably longer to return to baseline in the absence of visual feedback (ΣWBA and WBF, Fig. 6). These results suggest that visual feedback somehow resets the system's motor responses to odor.
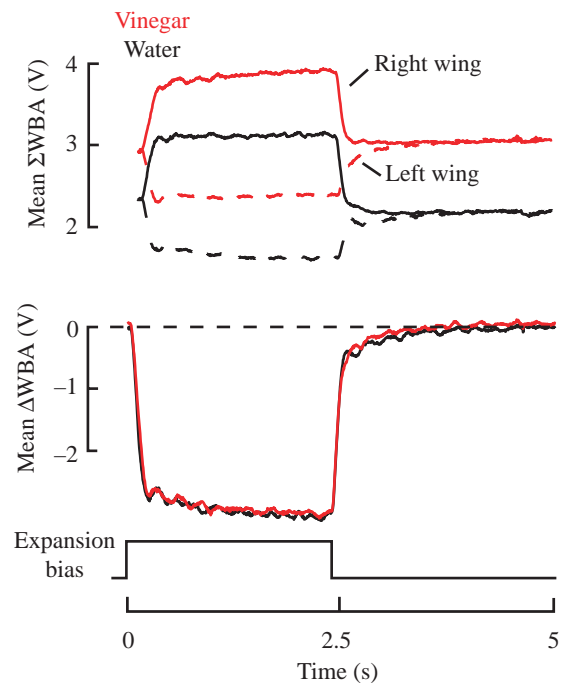

Fig. 3. Odor does not alter the magnitude or time course of steady-state collision avoidance reflexes in closed-loop conditions. Flies were presented with a 2.5-s bias in expansion, with and without concomitant presentation of 30-s odor pulses. Each trace represents a time series average. Each fly received three visual trials within each of nine consecutive odor trials (N=16 flies).

Fig. 2. Visual and olfactory stimuli evoke robust, repeatable, motor responses. (A) Sample responses to a regime of 1.25-s visual bias, 10-s odor and both stimuli presented simultaneously. Onset of stimuli is indicated in last two rows. (B) Time series averages to multisensory stimulus patterns. Gray fill shows the envelope of S.D. (C) Time-expanded responses. The shaded regions in the last two rows of A and B correspond to the data presented in C and D. Sample responses from A (indicated by thin black lines) are overlaid with mean responses from B (indicated by thick red lines). The arrow in column (i) indicates phasic modulation of image velocity (see text). The arrow in column (ii) highlights a transient decrease in WBA at the onset of the odor pulse (see text). (D) Odor did not alter the mean responses to imposed bias during closed-loop control of a single vertical stripe (i; N=10), a rotating random checkerboard pattern (ii; N=10) or an expanding flow field centered laterally (iii; N=10). R_v, response to visual stimulus; R_o, response to olfactory stimulus; R_{v+o}, response to visual and olfactory stimuli simultaneously.
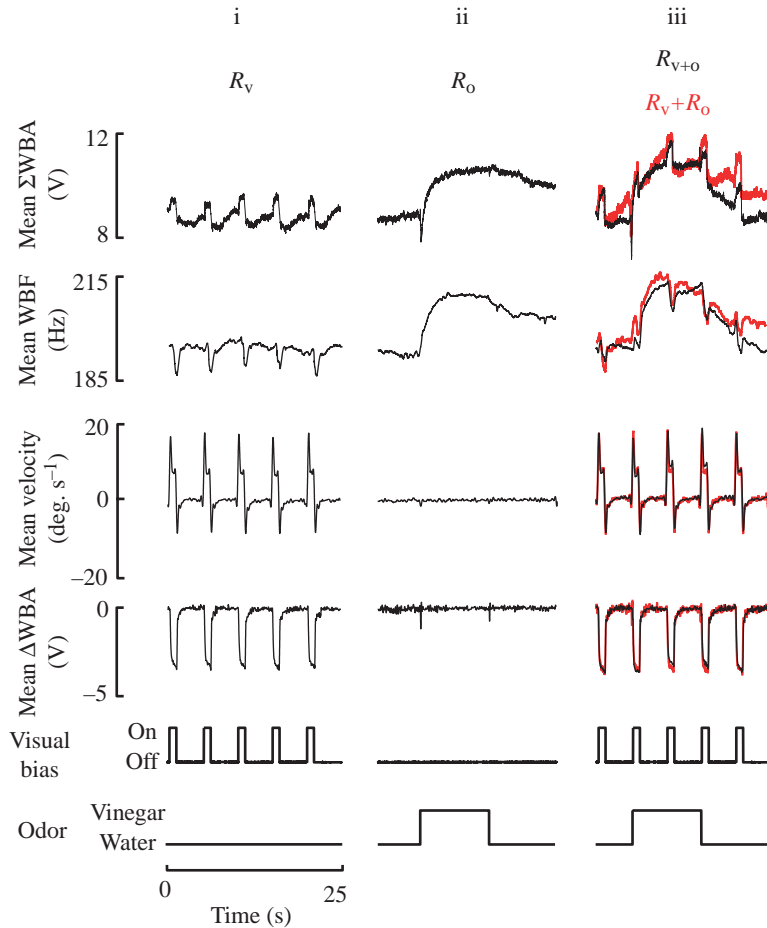
Fig. 4. During the duration of an odor pulse, motor responses to visual and olfactory cues represent the linear superposition of responses to each stimulus presented alone. Data sets are segregated into responses to visual bias during control water vapor delivery (i; R_v), odor in the absence of visual bias (ii; R_o), both stimuli simultaneously (R_{v+o}; indicated by black lines, iii), and the sum of responses to each stimulus presented alone ($R_v + R_o$; indicated by red lines, iii). $N=22$.
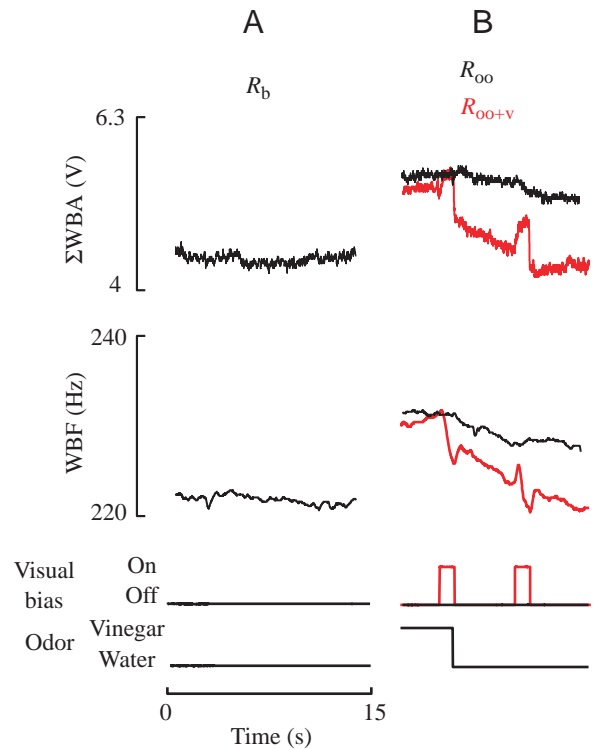
Fig. 5. Visual feedback alters the time course of the odor-off response. (A) Time series average of closed-loop control without either visual bias or odor. (B) Average odor responses without visual bias (black lines) decay along a slower time course compared with odor responses coupled with impulsive visual bias (red lines). $N=22$. R_b, response to baseline; R_{oo}, response to odor-off; R_{oo+v}, response to odor-off and visual bias.

Discussion

During tethered flight, *Drosophila* respond to the frontal presentation of an attractive odor with bilaterally symmetric increases in both wingbeat frequency and amplitude (WBF and ΣWBA, Figs 2, 6). Under visual closed-loop conditions, flies respond to a lateral expansion bias with collision avoidance maneuvers composed of rapid, asymmetric modulations of wingbeat amplitude that reduces expansion velocity (ΔWBA, Fig. 2). The presentation of odor does not affect the magnitude or time course of this visual reflex (Fig. 3). Furthermore, the motor responses to visual and olfactory stimuli presented together closely approximate the superposition of responses to each stimulus presented in isolation – indicating a linear interaction (Fig. 4). However, the odor-off response decays to baseline more rapidly when coupled with closed-loop visual feedback (Figs 5, 6). The linear superposition of visual and olfactory odor-on responses could bias a fly towards a visual feature associated with an odor cue as the fly enters the plume.

Visual feedback enhances the temporal dynamics of the odor-off response as the fly leaves the plume. To explain the interactions between these sensory-motor responses, we propose a model in which visual and olfactory signals are targeted to separate muscle groups within the flight motor.

Free-flight odor-search behavior in Drosophila

Decreased ΔWBA coupled with increased ΣWBA and WBF in response to an odor cue (Fig. 2) could result in both a decrease in saccade rate and an increase in forward velocity in free-flight conditions. However, when encountering an odor source in still air during free flight, flies do just the opposite, they saccade more frequently and, as a result, their average flight velocity decreases (Frye et al., 2003). There are several possible explanations for this apparent discrepancy. Here, we stimulated flies with long, frontally directed odor pulses in order to examine the influences of steady-state odor cues on dynamic visual reflexes. Of course, flies do not normally

encounter such experimentally tractable conditions. Rather, odor responses in free flight are likely to represent successive responses to the rapid onset, duration and offset of odor cues as the fly repeatedly approaches, flies past and turns back towards the odor plume. Therefore, an odor-dependent increase in free flight saccade rate may be consistent with the elevated frequency of attempted turns observed between odor pulses in tethered animals (Fig. 2). Flight velocity slows during a free flight saccade (Fry et al., 2003; Tammero and Dickinson, 2002b); therefore, average flight velocity is reduced whenever an animal executes frequent saccades, such as when approaching an odor source. Whether the odor-dependent increases in WBA and WBF during tethered flight result in increased forward thrust corresponding to increased free flight velocity remains to be tested directly.

Underlying neural pathways

A striking feature of the flight control architecture in flies is vast sensory-to-motor convergence. Feedback from tens of thousands of peripheral sensory and central brain neurons is collected, integrated and ultimately filtered through the activity of no more than 17 pairs of muscles that control the steering motions of the wings. The flight control system is therefore compacted into relatively few neurons, making flies particularly useful for studying the neurobiology of complex behavior (Frye and Dickinson, 2001). *Drosophila melanogaster*, in particular, has emerged as a key model system to investigate molecular-genetic, developmental and physiological determinants of olfactory discrimination (Stensmyr et al., 2003; Vosshall, 2000), visual motion detection (Barth et al., 1997; Gibbs et al., 2001; Juusola and Hardie, 2001; Wolf and Heisenberg, 1990), as well as associative learning and memory formation (Connolly et al., 1996; deBelle and Heisenberg, 1994; Guo and Götz, 1997; Pascual and Preat, 2001). Recent advances in targeted genetic manipulations such as the pGAL4 enhancer-trap system (Brand and Perrimon, 1993) have catalyzed the identification of anatomical sites of multimodal integration (Ito et al., 1998), functional roles of central brain structures, and sensorimotor synapses and peripheral sensory pathways involved in gross locomotor performance (Kitamoto, 2001; Strauss, 2002).

Unfortunately, we are as yet unable to interpret many of these advances within the framework of the systems-level neural mechanisms of multimodal integration simply because many sensorimotor interactions in flies have not been quantitatively characterized at the behavioral level. Those that have been in *Drosophila* share a common theme – flight behaviors are orchestrated by parallel sensorimotor processes. For example, the descending giant fiber
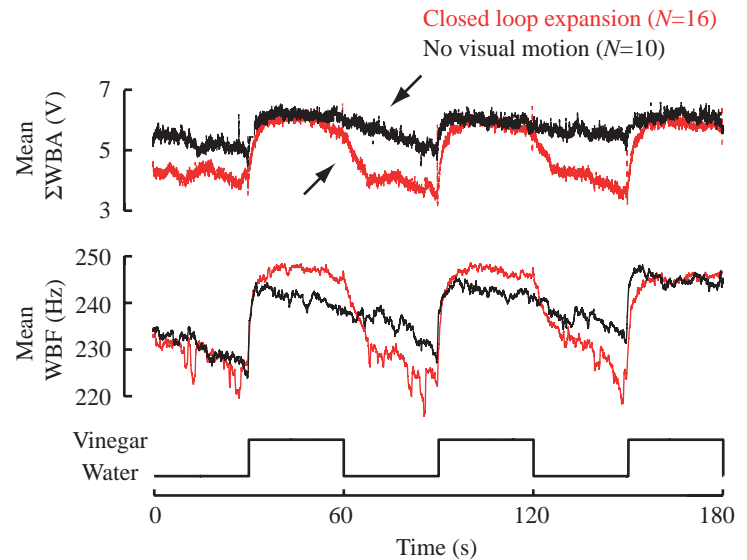

Fig. 6. Unbiased closed-loop visual feedback is sufficient to alter the time course odor-off responses. Flies were presented with 30-s odor pulses. Time series averaged responses to odor presented during visual closed-loop conditions (red lines) decay more rapidly between odor pulses (example highlighted with arrows) than odor responses in the absence of any visual motion (black lines).
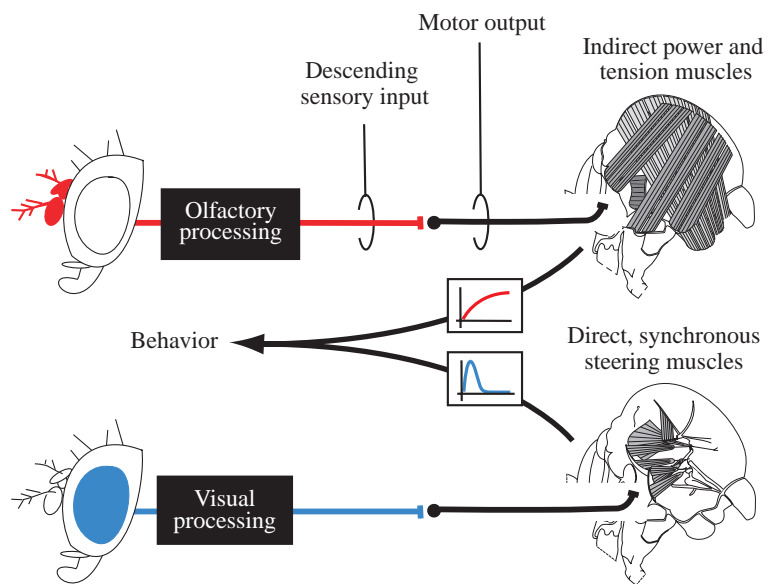

Fig. 7. A schematic model to describe neural mechanisms by which multisensory input is linked to motor output. Visual (blue) and olfactory (red) feedback projects along separate neural pathways to the flight motoneurons of the thorax. Olfactory feedback is selectively targeted to the motoneurons of both the indirect power muscles and constitutively active steering muscles – resulting in tonic elevation in wingbeat frequency (WBF) and sum of wingbeat amplitude (ΣWBA) in response to odor. Visual feedback activates steering muscles that initiate rapid, phasic changes in wingbeat amplitude resulting in collision avoidance maneuvers. The superposition of both motor responses could alter body posture or heading to bias flies' overall flight trajectory towards visual features associated with attractive odorants.

initiates a flight escape response to visual stimuli (Tannouye and Wyman, 1980; Trimarchi and Schneiderman, 1995b). A similar behavioral response to noxious olfactory stimuli is mediated by a parallel, as yet unidentified, descending pathway (Trimarchi and Schneiderman, 1995a). During flight, patterns of visual expansion presented laterally produce robust collision-avoidance steering maneuvers, whereas expansion centered frontally evokes a landing reflex (Tammero and Dickinson, 2002a). The spatial and temporal tuning properties of these two visual reflexes suggest that they are mediated by separate visuomotor pathways. Additionally, temporal separation of mechanosensory and visually mediated equilibrium reflexes enable the fly to detect and counteract rotational disturbances over a wide range of angular velocities during flight (Sherman and Dickinson, 2003).

Neuromuscular mechanisms for visuo-olfactory sensorimotor interactions in Drosophila

The linear superposition of visual and olfactory motor responses (Fig. 4) may reflect a confluence of sensory reflexes projecting along separate but parallel sensorimotor pathways. Alternatively, sensory input may be fused within individual neurons that preserve the linear independence of multimodal input. In either case, specialized descending neurons (DNs) that carry input from the brain to the thoracic flight motor neuropile undoubtedly play a central role in coordinating visual and olfactory mediated flight behaviors. Tonic DN activity is thought to organize and tune wingbeat synchronous mechanosensory reflexes reverberating within the thorax to control flight muscles (Heide, 1983). Aside from the giant fiber system, physiological properties of premotor DNs in fruit flies are completely unknown. However, in larger blowflies, a class of unidentified premotor DNs shows receptive field specificity for patterns of visual expansion centered frontally and might coordinate landing responses (Borst, 1991). In addition, anatomically identified DNs in *Sarcophaga* show specificity for motion within small patches of the visual space (Gronenberg and Strausfeld, 1992) and are thought to control visuomotor reflexes. Although there is no evidence to date of parallel olfactory encoding in these neurons, some do show multimodal processing. For example, spiking responses to visual motion are gated by mechanosensory feedback generated by wind on the antennae (Gronenberg and Strausfeld, 1990). Comparative studies of analogues in other insects serve as models to motivate future work in flies. Descending cells with inputs residing within the deutocerebrum of gypsy moths (*Lymantria dispar*) show amplified responses to visual motion that are gated by olfactory stimuli (Olberg and Willis, 1990). To our knowledge, this is the only reported example of an identified descending neuron that is selective for visual and olfactory cues. However, cells that integrate visual and mechanosensory information in other insects are not uncommon (Baader et al., 1992; Gronenberg et al., 1995; Olberg, 1981; Rowell and Reichert, 1986). Examining the physiological properties of as yet unidentified DNs in *Drosophila* will help define physiological mechanisms of sensorimotor superposition.

However, based on the functional organization of the musculoskeletal system, we propose that visual and olfactory feedback is carried by separate populations of DNs that project to distinct sets of wing control muscles (Fig. 7). Flight muscles can be roughly categorized into two groups (so-called direct and indirect) that either insert directly at the wing base or move the wings indirectly by deforming the thorax (Dickinson and Tu, 1997). The large powerful indirect flight muscles (IFMs) are not controlled on a contraction-by-contraction basis. Rather, they are stretch activated and create a myogenic rhythm through the mechanical resonance of antagonistic pairs. Another set of smaller indirect control muscles are thought to stiffen the thorax and vary its resonant properties, thus altering the mechanical action of the IFMs to effect changes in wingbeat amplitude and frequency. Whereas the indirect muscle groups do not coordinate cycle-by-cycle changes in wing kinematics, tonic variation in neural drive is correlated with gross changes in ΣWBA and WBF (Heide, 1983). We suggest that the relatively slow odor-mediated changes in bilateral ΣWBA and WBF (Fig. 2) are coordinated by tonic descending input targeted to select indirect muscles.

Modulation of power muscle activity *via* descending pathways is characteristically slow, operating on a time scale spanning hundreds of individual wing strokes (Dickinson et al., 1998), however *Drosophila* can turn by 90° within 40 ms, or about eight individual wing strokes (Fry and Dickinson, 2003). Rapid changes in wing motion are coordinated by a specialized group of synchronous (i.e. twitch-type) flight muscles that insert directly onto the wing hinge. The wings beat so fast that steering muscles have only two neural control parameters – whether or not they fire at all within a cycle and at what phase they do. For example, phase shifts in the timing of the first basalare muscle, b1, are correlated with an elevation of the stroke plane, whereas a spike in b2 causes an immediate increase in ipsilateral wingbeat amplitude (Heide and Götz, 1996; Lehmann and Götz, 1996). The rapid changes in ΔWBA in response to lateral expansion (Fig. 2) are most likely coordinated by descending input that drives direct control muscles such as b1 and b2. By contrast, the time course of odor responses suggests that this pathway activates indirect control muscles, as well as the IFMs, to cause slower changes in wing motion. This neuromuscular segregation of visual and olfactory feedback is consistent with the near perfect linearity of these systems at the level of behavioral responses. Functionally, such superposition might bias an animal's body orientation, and as a consequence its flight trajectory, towards visual features associated with attractive odors.

Whereas olfactory and visual responses are linearly superimposed for the onset and duration of odor cues, odor-mediated changes in wingbeat frequency and amplitude outlast the duration of the odor stimulus in the absence of visual feedback (Fig. 6). This is intriguing because it suggests that visual feedback somehow reconfigures or 'resets' motor responses to odor. This phenomenon could also arise from

visual and olfactory feedback targeting different groups of flight muscle motoneurons. For example, rapid, phasic stimulation of muscle b2 in response to visual motion could interfere with the effects of olfactory-elicited tonic stimulation of indirect tension or power muscles. The combined influence of the two motor patterns could return wingbeat amplitude to baseline levels. Thus, as an animal navigates a spatially varying odor plume during free flight, visual feedback may functionally enhance olfactory acuity by shutting down or resetting odor-mediated motor responses. Taken together, the results presented here reveal specific sensorimotor interactions that lay the groundwork for future electrophysiological and molecular-genetic analyses of sensory fusion for complex behavior in flies and other animals.

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