The Journal of Experimental Biology 213, 3625-3635

doi:10.1242/jeb.040402

Olfactory modulation of flight in *Drosophila* is sensitive, selective and rapid

Vikas Bhandawat[1,*], Gaby Maimon[2], Michael H. Dickinson[2] and Rachel I. Wilson[1,†]

[1]Department of Neurobiology, Harvard Medical School, 220 Longwood Avenue, Boston, MA 02115, USA and [2]Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA

*Present address: Duke Department of Biology, 125 Science Drive, Durham, NC 27708, USA

†Author for correspondence (rachel_wilson@hms.harvard.edu)

Accepted 14 July 2010

SUMMARY

Freely flying *Drosophila melanogaster* respond to odors by increasing their flight speed and turning upwind. Both these flight behaviors can be recapitulated in a tethered fly, which permits the odor stimulus to be precisely controlled. In this study, we investigated the relationship between these behaviors and odor-evoked activity in primary sensory neurons. First, we verified that these behaviors are abolished by mutations that silence olfactory receptor neurons (ORNs). We also found that antennal mechanosensors in Johnston's organ are required to guide upwind turns. Flight responses to an odor depend on the identity of the ORNs that are active, meaning that these behaviors involve odor discrimination and not just odor detection. Flight modulation can begin rapidly (within about 85 ms) after the onset of olfactory transduction. Moreover, just a handful of spikes in a single ORN type is sufficient to trigger these behaviors. Finally, we found that the upwind turn is triggered independently from the increase in wingbeat frequency, implying that ORN signals diverge to activate two independent and parallel motor commands. Together, our results show that odor-evoked flight modulations are rapid and sensitive responses to specific patterns of sensory neuron activity. This makes these behaviors a useful paradigm for studying the relationship between sensory neuron activity and behavioral decision-making in a simple and genetically tractable organism.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/213/21/3625/DC1

Key words: *Drosophila*, flight, olfaction, odors, olfactory receptor neurons, antenna, insect, anemotaxis, Johnston's organ.

INTRODUCTION

Drosophila melanogaster is a useful model organism for studying olfaction, in part because it offers powerful genetic tools for manipulating neural activity in the olfactory system (Holmes et al., 2007; Luo et al., 2008; Olsen and Wilson, 2008). In addition, it is feasible to perform electrophysiological recordings from identified *Drosophila* olfactory neurons *in vivo*. Considerable progress has already been made in describing how odors are represented by neural activity in this organism (Berry et al., 2008; Fiala, 2007; Hallem and Carlson, 2006; Wilson, 2007). An important current challenge is to understand the relationship between sensory neuron activity and behavior.

In this study, we address this issue in the context of a behavior where flies are true virtuosos: namely, the rapid and precise control of flight (Borst and Haag, 2002; Frye and Dickinson, 2001). When free-flying *Drosophila* encounters an attractive odor, it surges forward and turns upwind (Budick and Dickinson, 2006). One virtue of studying olfaction in the context of this behavior is that flight maneuvers can be very rapid. For example, visually guided flight maneuvers can occur within tens of milliseconds (Collett and Land, 1975; Land and Collett, 1974; Tammero and Dickinson, 2002). Because the motor component of flight is fast, studying these behaviors should help place a useful bound on the time required for sensory neurons to encode and process olfactory information.

Another virtue of using flight for this purpose is that it can be studied under experimental conditions where the stimulus is highly controlled. *Drosophila* can fly for hours when tethered to a pin (Götz, 1987). Tethering is useful because it allows odor stimuli to be presented at a fixed concentration and air speed. This permits a precise comparison between neural and behavioral responses to the same stimuli.

Several studies have shown that odor stimuli cause tethered *Drosophila* to increase their wingbeat frequency and amplitude, and/or to modulate their flight direction (Chow and Frye, 2008; Duistermars et al., 2009a; Duistermars et al., 2009b; Duistermars and Frye, 2008; Frye and Dickinson, 2004; Guo and Gotz, 1997; Wolf and Heisenberg, 1991; Xi et al., 2008). In this study, our broad aim was to investigate the relationship between these flight behaviors and primary sensory neuron activity. Specifically, we focused on three questions. What primary sensory neurons can elicit these behaviors? How rapidly do flight maneuvers occur after the onset of neural activity? Finally, are different components of these maneuvers evoked independently, or are they triggered by the same command circuit? These questions are fundamental to understanding what these behaviors tell us about the ability of flies to detect and discriminate odors.

MATERIALS AND METHODS

Fly strains

Unless otherwise mentioned, experiments were performed using laboratory cultures of *Drosophila melanogaster* Meigen established several years ago from 200 wild-caught individuals. This strain is similar to that used by several previous studies of olfactory modulation in tethered flying *Drosophila* (Chow and Frye, 2008; Duistermars et al., 2009a; Duistermars et al., 2009b; Duistermars and Frye, 2008; Frye and Dickinson, 2004). For convenience, we refer to this strain as 'wild'. *Or83b*$^{-/-}$ flies (allele *Or83b*^{2}) and the

control strain for this mutant ($Or83b^{+/+}$) were kindly provided by Leslie Vosshall and were generated in a w^{1118} background (Larsson et al., 2004). $Or42b^{-/-}$ flies (allele $Or42b^{EY14886}$) were obtained from the Bloomington stock center and were back-crossed by us for 10 generations to w^{1118} (Bhandawat et al., 2007). $Or42b^{-/+}$ heterozygotes were the progeny of a cross between the back-crossed $Or42b^{EY14886}$ flies and w^{1118} flies. In pilot experiments, we systematically compared the flight kinematics of the wild flies and the inbred flies we used in this study, focusing here on the inbred strain (w^{1118}) which constitutes the background for all the mutants we used. These experiments confirmed that odors elicit grossly similar flight modulations in wild flies and w^{1118} flies. However, the kinematics of these responses differed systematically in wild flies and w^{1118} flies (supplementary material Fig. S1). In particular, these strains differed in their maximum wingbeat frequency (supplementary material Fig. S2).

Culture conditions

We found that if a fly flew robustly, it generally also responded to odors in flight, but the fly's ability to maintain flight depended on culture density. We began our pilot experiments with conventional 'dense' cultures, where we allowed three wild females to lay eggs in a 175 ml bottle for 10 days before removing them from the bottle. We estimate that these dense cultures contained ~1500 eggs, assuming that each female lays ~50 eggs per day (Ashburner et al., 2004). We found that only 15 of 22 flies from dense cultures flew when tethered in our apparatus, and these stopped flying after only 10±7 trials. The wingbeat frequency of these flies was low (196±21 Hz; mean ± s.d.). Data from these flies are not included in this study. Next, we conducted a similar pilot experiment with 'sparse' cultures, with only 50–200 eggs in a 175 ml bottle. To obtain this egg density, 15–20 adult wild females were allowed to lay eggs for ~5 h at 25°C. After 5 h, we counted the number of eggs in each bottle and adjusted it by either scooping out some eggs or allowing the flies to continue to lay eggs. We found that a significantly higher fraction of flies from sparse cultures flew when tethered in our apparatus (20/20, $P<0.01$; contingency test). On average, they flew for more trials before stopping for the first time (19±10 trials, $P<0.005$) and their wingbeat frequency was higher (212±7 Hz, $P<0.01$). Data from these pilot experiments with sparse cultures are included in this study.

Cultures were maintained at 25°C and ~50% humidity on conventional cornmeal–agar medium. For our experiments, we used females from these cultures aged 3–5 days. We did not attempt to control the temperature or humidity of the room where we performed our experiments (typically 20–21°C and 30–50% humidity). Cultures were maintained on a 12 h:12 h light:dark cycle, and all experiments were performed within the 3 h before the start of the flies' subjective night. We tried starving the flies for 4–6 h before the experiment, but we found this did not improve flight robustness or odor responses, and longer starvation only resulted in a loss of robust flight.

Fixed-tether apparatus

In the fixed-tether apparatus, the fly is not allowed to rotate, and wing movements are monitored optically (Fig. 1A). Other details of this paradigm are described elsewhere (Lehmann and Dickinson, 1997). We anesthetized the fly by cooling it, and then attached the fly to a tungsten wire (0.5 mm diameter) inserted into a holder. The attachment was made at the anterior-dorsal end of the thorax with UV-fixable epoxy resin (Kemxert). Glue was not allowed to touch the head. We typically tethered several flies at once. Flies were allowed to rest for at least 15 min after tethering to ensure sufficient recovery from anesthesia. During the rest period, the tethered flies were stored in a scintillation vial containing damp tissue paper to prevent them from dehydrating. We prevented flies from flying during the rest period by inducing them to grasp a small piece of tissue paper with their legs; this reflexively inhibits flight.

After the rest period, the fly was centered below an infrared emitter (PDI-E805-ND; Digi-key, Thief River Falls, MN, USA), and above a pair of photodetector wafers, with one detector beneath each wing (Fig. 1A). The detectors were covered by a mask with a pair of mirror-symmetric wedge-shaped cutouts centered below the fly. For this reason, the shadow cast by the beating wings of the fly produces a time-modulated visual signal incident on the detectors that is dependent on wing stroke position (Götz, 1987). We recorded one raw electrical signal from each detector (see supplementary material Fig. S3). The outputs of the detectors were analyzed in real time by custom electronic circuits ("Wingbeat Analyzer", Electronics Shop, The James Franck Institute, University of Chicago, IL, USA) to yield a measurement of wingbeat frequency (WBF) and separate wingbeat amplitude (WBA) measurement for each wing. Except where otherwise noted, we here report the summed WBA measurements for the two wings (arbitrary units, 1 a.u.=1 V summed output from the right and left detectors).

To induce flight at the beginning of each experiment, we removed the tissue paper held in the fly's legs; it was sometimes also necessary to blow gently on the fly to make it begin flying. During an experiment, a fly would occasionally stop flying, and in these cases we re-initiated flight by gently blowing on the fly. If the fly stopped a second time, we terminated the experiment. At the beginning of each experiment, the fly's position was adjusted so that the output of the detectors had the characteristic shape shown in supplementary material Fig. S3, but WBA measurements were not otherwise calibrated, and so absolute values of WBA should be interpreted with caution.

In order to minimize the salience of visual stimuli in the room, all our flight experiments were performed with dim room lights covered by red filters (Roscolux #26, 12–13% transmission <400 nm, 0% 420–580 nm, 50–85% >620 nm; Rosco Laboratories, Stamford, CT, USA). Raw optical wingbeat signals (supplementary material Fig. S3) were comparable to those observed previously in experiments using identical equipment to study vision-based behaviors (G.M. and M.H.D., unpublished observations).

Rotatable-tether apparatus

In the rotatable-tether apparatus, the fly is allowed to rotate freely in the x–y plane. Wing movements are monitored acoustically and body position is monitored optically. This type of apparatus has been used previously (Bender and Dickinson, 2006a; Bender and Dickinson, 2006b; Duistermars et al., 2009a; Duistermars et al., 2009b; Duistermars and Frye, 2008), but because our modifications were extensive we provide a full description of our setup here.

Flies were anesthetized, glued, and handled as in the fixed-tether experiments, except that flies were tethered to a steel pin (diameter 0.1 mm, length 0.3–0.5 cm). The fly was fixed to the blunt end of the pin, and the sharp end was placed on a jewel bearing (VJ-0469-01; smallparts.com) in the center of a cylindrical rare-earth magnet (1.27 cm dia.×1.27 cm thick). A second magnet was placed 1 cm below the first and concentric to it, and the resulting magnetic field tended to keep the pin parallel to the axis between the centers of the magnets. We found that a dead fly is useful for assessing the alignment of the magnets: gentle blowing causes a correctly oriented dead fly to spin freely.

A single infrared LED was used to illuminate the flies in our video images (ILED-8 from AllElectronics.com; 4 cm from the fly at an angle of 30 deg from the air tube), but otherwise the room was darkened as in our fixed-tether experiments. We used a dental mirror to project the image of the fly's ventral side into a camera (Fire-i camera, Unibrain, 30 frames s^{-1}; San Ramon, CA, USA). Image analysis was performed in Matlab using a custom routine. At the beginning of each experiment, we determined the center of rotation of the fly in the camera coordinates. The center of mass of the fly was calculated on every frame. The center of mass traces a circle around the center of rotation as the fly spins on its tether, and so we could compute the orientation of the fly on each frame by measuring the angle between the line joining the center of rotation to the center of mass and a reference line. Our reference line was the direction of the odor tube. In some trials, the orientation of the fly was relatively constant in the absence of an odor stimulus, but in other trials the fly made occasional spontaneous saccadic turns even in the absence of an odor stimulus.

We also placed a microphone near the fly to record the sound of the wingbeats and thereby to extract WBF. The microphone (MM series matchstick microphone from www.microphones.com) was placed as near to the fly as possible without touching it. The output of the microphone was amplified using an external pre-amplifier and recorded digitally using the 'line in' input of the computer. WBF was extracted from the audio recording in Matlab using a custom routine. To assess the accuracy of our WBF measurement, we simultaneously measured WBF with both the acoustic and optical method in the fixed-tether setup. We found a very close match between the two methods, as shown in supplementary material Fig. S3. We could not accurately measure WBA using the acoustic signal because the amplitude of the signal varies with the orientation of the fly relative to the microphone.

We also confirmed that the WBF response was similar in the fixed- and rotatable-tether setups (supplementary material Fig. S4). This shows that rigidly tethering the fly does not impose a delay on the response of the fly to odors, as compared to a freely rotating tether. As in the fixed-tether experiments, we found that flight in the rotatable-tether setup did not require clear visual cues. Even in low levels of red light without closed-loop visual feedback, flies made spontaneous saccadic turns and spontaneously modulated their WBF, similar to previous studies in which visual cues were present (Bender and Dickinson, 2006b).

Arista clipping

Arista clipping was carried out immediately before a flight experiment, during the time period when the flies were cold-anesthetized for tethering. In half of the flies, both antennal aristae were carefully broken near the base using fine forceps. The remaining half of the flies were cold-anesthetized for a similar amount of time, but the aristae were not removed (these were the 'mock-clipped' flies).

Field potential recordings

Field potential recordings from the antennal funiculus and maxillary palp were performed as described previously (Olsen et al., 2007). Briefly, flies were immobilized at the trimmed end of a plastic pipette tip. The recording electrode was a sharp saline-filled glass electrode inserted into the center of the antennal funiculus or the maxillary palp. A saline-filled glass electrode placed in the eye served as the ground electrode. Signals were filtered at 2 kHz and acquired at 10 kHz using an A-M Systems amplifier (Model 2400; Carlsborg, WA, USA). All analysis was performed in IGOR Pro (WaveMetrics, Portland, OR, USA).

Odor stimulation

Odor stimulation was performed using a custom-made olfactometer described elsewhere (Olsen et al., 2007). The same device was used for behavioral and neurophysiological experiments. In preliminary behavioral experiments, a continuous stream of air was directed at the fly. We found that flies typically stopped flying when subjected to a continuous stream for >10 min. Therefore, we kept the air off except for a 12-s period around each odor stimulus. We used a computer-controlled solenoid valve (#01540-11; Cole-Parmer, Vernon Hill, IL, USA) to switch the air on 4 s before the start of the odor stimulus. The odor pulse was 3 s long, and the air stream remained on for 5 s following odor off. We note that this protocol creates a periodic fluctuation in air flow which may promote flight. The air flow alone also has a small effect on wingbeat dynamics. Specifically, turning on the air tended to produce a small transient increase and then a steady decrease in the WBF (supplementary material Fig. S5), and this accounts for the slowly diminishing WBF during the pre-odor baseline period in some experiments (e.g. Fig. 2). Odor was added to the air stream by switching another solenoid valve that redirected a minor portion of the air stream (9%) through an odor vial before rejoining the main flow 15 cm from the end of the odor tube. The inner diameter of the odor tube was 6.45 mm. The tube was positioned directly in front of the fly (6 mm away) so that the entire fly was enveloped in the air stream.

The flow rates of the major and minor air stream were measured in-line using ball-float flow meters (Cole-Parmer) at a point before the solenoid valve. In all the fixed-tether experiments we used a total air flow rate of 1100 ml min^{-1}, except where otherwise noted (supplementary material Figs S4 and S6). In principle, this should correspond to an air speed of 0.56 m s^{-1} at the outlet of the tube, under the simplifying assumption that air speeds are constant throughout the cross-sectional area of the tube. This air speed is well within the range of air speeds encountered by *Drosophila* in its natural environment (Budick and Dickinson, 2006).

In the rotatable-tether setup, this flow rate caused a strong anemotactic response (Budick et al., 2007), so we performed all the rotatable-tether experiments at a lower flow rate (550 ml min^{-1}) which did not produce anemotaxis. This flow rate should, in principle, produce an air speed of 0.28 m s^{-1} at the outlet of the tube. In pilot experiments, we found that the air speed of the carrier stream has a major impact on the kinetics and magnitude of the odor-evoked flight response (supplementary material Fig. S6).

We used four different odors in this study: methyl salicylate, fenchone, ethyl acetate and a blend that mimics the smell of ripe mangos (a 1:22:5 blend of 2-phenyl-ethanol:acetic acid:ethanol). This blend (referred to as 'mango' henceforth) is reportedly attractive to freely flying *Drosophila* (Zhu et al., 2003). Odor dilutions, when noted, were v/v dilutions in paraffin oil. We confirmed that paraffin oil, by itself, does not evoke a behavioral response (supplementary material Fig. S7). Each odor stimulus tested on a given fly was presented repeatedly in 6–10 consecutive trials for the behavioral experiments, and three trials for the field potential recordings.

Throughout this study, the '0' time point corresponds to the time of valve switching. There is a delay of about 250 ms between this time point and the onset of ORN activity. We used a fast photoionization detector to confirm that this delay mainly represents the time required for the odor pulse to propagate through the tubing of our odor delivery device (data not shown).

Data analysis

Except where otherwise noted, all reported data ranges are ± s.e.m. Behavioral signals were filtered at 2 kHz and physiology signals

were filtered at 5 kHz; both were digitized at 10 kHz. Data analysis was performed using custom routines in IgorPro and Matlab. Baseline (pre-odor) WBF and WBA were measured by averaging over the 2-s window prior to the odor pulse. Changes from baseline values ('ΔWBF' and 'ΔWBA') were computed on a trial-by-trial basis by subtracting this 2-s baseline value for each trial from the maximum value during the odor period. For the analysis of flight-surge latency, the neural and behavioral responses were first smoothed (using a 501-point Savitzky–Golay filter) and then differentiated with respect to time. The earliest responses appeared no sooner than 190 ms after nominal stimulus onset, and so we analyzed the 750-ms period after this time point (the 'response period') and also a period of equal duration prior to this time point ('the control period'). We systematically varied the threshold until we found the level for which the probability of threshold crossing during the response period was 10-fold higher than the probability of crossing during the control period. Circular distributions were compared using the circ_kuipertest in Matlab. In supplementary material Fig. S6D, we measured the latency of the WBF response as the time after the nominal odor stimulus onset when the WBF reached 20% of the difference between peak and baseline values, where peak is defined as the maximum response in the odor stimulus period.

RESULTS

Odors evoke a surge in wingbeat frequency and amplitude

In this study, we used two experimental methods for studying tethered flight. In the first method, the fly was rigidly oriented into a stream of air (Fig. 1A). Odors were injected into the air stream using a computer-controlled valve while the wing movements of the fly were monitored with an optical sensor (Fig. 1A). Several studies using this type of apparatus have demonstrated that odors can alter wing kinematics in a manner that is expected to increase flight force (Chow and Frye, 2008; Frye and Dickinson, 2004). These studies showed that odors generally increase both WBF and WBA. All these studies presented odors in conjunction with a closed-loop visual stimulus, and analyzed how olfactory and visual cues interact to modulate flight.

Our initial goal was to see if we could replicate these observations without a closed-loop visual stimulus and under low levels of illumination. We found that under these conditions, flies responded to odors with robust, transient increases in both WBF and WBA (Fig. 1B,C). Responses were generally relatively consistent across multiple stimulus presentations in the same fly (Fig. 1B,C). Thus, odors can evoke a flight surge even without visual feedback. This allows us to study the relationship between ORN activity and flight behavior without a visual stimulus.

Olfactory receptor neurons are required for the surge

Because the flight surge is time-locked to the olfactory stimulus, it seems plausible to interpret it as a consequence of activating ORNs in the antennae and maxillary palps. However, we also considered the possibility that the surge could be purely a response to the small mechanical artifact that invariably occurs when a portion of the air stream is diverted through an odor vial before rejoining the main stream (see Materials and methods). Alternatively, the surge could be mediated by non-olfactory chemoreceptors, such as gustatory receptors on the proboscis and legs.

In order to rule out these scenarios, we tested flies homozygous for a null mutation in the *Or83b* gene (*Or83b*$^{-/-}$). The *Or83b* gene is expressed in the majority of ORNs and is essential for odor-induced electrical activity in the ORNs that normally express it (Larsson et al., 2004). Gene expression is not detected in other tissues (Larsson et al., 2004), and so this manipulation should be selective for the olfactory system.

As a positive control, we used a strain that is genetically identical to the mutant except that the targeting insert is integrated at a different site on the third chromosome, leaving the *Or83b* gene intact (*Or83b*$^{+/+}$) (Larsson et al., 2004). We found that the *Or83b*$^{+/+}$ flies increased their WBF and WBA in response to odor (Fig. 2A), much like *w*^{1118} flies. By contrast, *Or83b*$^{-/-}$ flies did not show a consistent increase in WBF or WBA upon odor stimulation (Fig. 2B). This demonstrates that the odor-evoked flight surge requires ORNs, and is not mediated exclusively by mechanoreceptors or gustatory receptors.

Flight modulation depends on the identity of ORNs activated by an odor

Chemically distinct odors activate different combinations of ORNs in the *Drosophila* antennae and maxillary palps (de Bruyne et al., 1999; de Bruyne et al., 2001). We investigated whether the effect of an odor on *Drosophila* flight depends on the identity of the ORNs that are activated by that stimulus. Alternatively, flight responses might be a reflexive response to any level of ORN activity above a certain threshold. To address this, we selected odor stimuli that activate the olfactory system to a similar overall intensity level, and we compared their ability to evoke flight modulation.

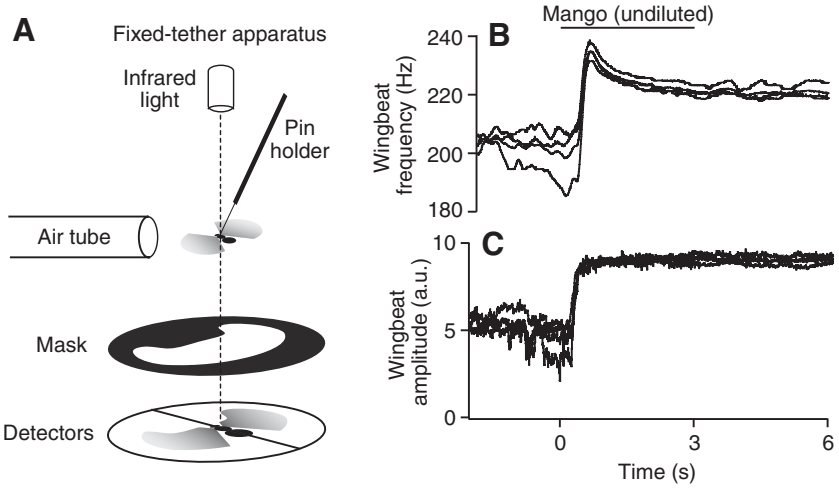

Fig. 1. Odors can evoke a surge in flight. (A) Schematic of the fixed-tether apparatus. The wings of a fly tethered below an infrared light cast an oscillating shadow onto a pair of photosensitive detectors below. The detectors are covered by a mask with a pair of cutouts centered below the fly. As the wing shadow sweeps across the cutouts, the time-varying light signal is converted into an electrical signal and used to compute wingbeat frequency (WBF) and wingbeat amplitude (WBA). Objects are not drawn to scale. (B) Odor increases WBF. Each trace is a different trial from the same wild strain fly. (C) Odor increases WBA. Each trace is a different trial, same fly as in B.

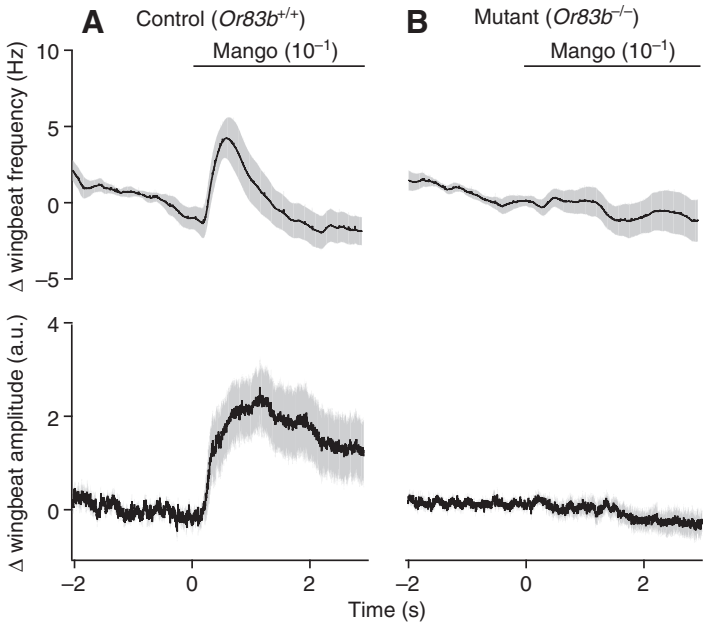

Fig. 2. Olfactory receptor neurons are required for the flight surge. (A) Control flies (*Or83b*$^{+/+}$) responded to odor with increases in WBF and WBA. (B) Mutant flies with nonfunctional ORNs (*Or83b*$^{-/-}$) showed no consistent change in WBF or WBA (ΔWBF and ΔWBA both significantly different from controls at $P<0.05$, $N=13$ for controls and 17 for mutants; *t*-tests). Spontaneous WBF and WBA were not significantly different across genotypes. Mean ± s.e.m. across flies. Note that average WBF values decrease slightly during the baseline (pre-odor) period; this is due to the onset of the air stream 4 s before the odor stimulus, which tends to produce a transient increase followed by a steady decay in the WBF (see supplementary material Fig. S5).

We evaluated the overall intensity of each stimulus by measuring local field potentials in the antennae. These measurements provide a rough estimate of the summed ORN response (Carlson, 1996; Olsen et al., 2010). We initially selected three stimuli that produce a similar local field potential response (Fig. 3A): methyl salicylate (10^{-2} dilution), fenchone (10^{-2}) and ethyl acetate (10^{-8}). These three stimuli activate distinct (although partly overlapping) groups of ORNs (Goldman et al., 2005; Hallem and Carlson, 2006). Of these, methyl salicylate produced the strongest increase in wingbeat frequency and wingbeat amplitude, with fenchone eliciting a weaker response (Fig. 3B). Unlike these stimuli, ethyl acetate caused no change in either WBF or WBA (Fig. 3B). Higher concentrations of ethyl acetate evoked much larger ORN responses (Fig. 3A), but still no behavioral responses (Fig. 3B).

Taken together, these results suggest that flight modulations depend on the identity of the ORNs that are activated by an odor

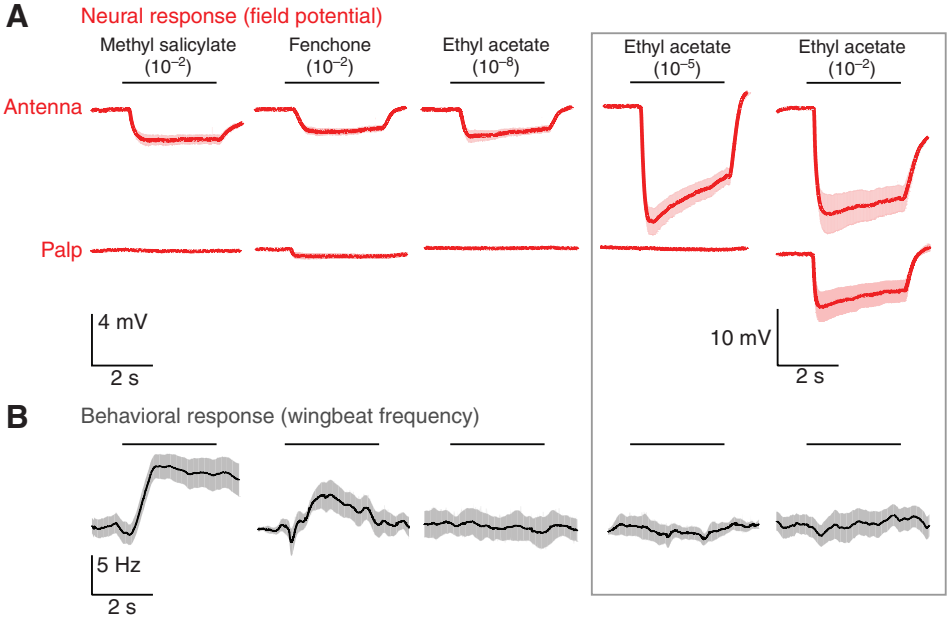

Fig. 3. Flight modulation depends on the identity of ORNs activated by an odor. (A) Field potential recordings from the antenna (top) and the maxillary palp (bottom) measure the summed response of ORNs to each stimulus. Summed responses to methyl salicylate (10^{-2} dilution), fenchone (10^{-2}), and ethyl acetate (10^{-8}) are similar. These odor stimuli activate different populations of ORNs. Box: higher concentrations of ethyl acetate (10^{-5} and 10^{-2}) evoke a larger neural response. Note compressed vertical scale in last column. Mean ± s.e.m. across flies, $N=5$ flies for antennal recordings and six for palp recordings. All individual flies showed similar responses. (B) WBF responses to these stimuli. The first two stimuli elicited a surge but the third does not. Box: even the higher concentrations of ethyl acetate fail to elicit a change in WBF, although the neural responses to these stimuli are up to 10-fold larger. Black lines indicate the means and gray shading, ±s.e.m. across flies, $N=8$ flies. No individual fly showed a WBF response to ethyl acetate in these experiments. Flies are wild strain. Flies of this strain also failed to show robust WBF responses to this odor in the rotatable-tether apparatus (data not shown).

stimulus. Thus, the flight surge is not merely a response to the total intensity of olfactory input. Interestingly, we observed that although ethyl acetate does not evoke a surge in WBF or WBA at any concentration we tested, this odor is clearly detected by the fly, since it modulates the relative amplitude of the right and left stroke amplitudes in the fixed-tether apparatus (supplementary material Fig. S8).

Flight modulation occurs rapidly

We next asked how the speed of behavioral responses compares with the speed of receptor neuron responses. For this comparison, we used methyl salicylate (10^{-2} dilution), a stimulus that elicits a robust neural and behavioral response. The onset of the summed ORN response occurred about 250 ms after the nominal start of the odor stimulus (Fig. 4A). This delay is mainly due to a delay in the odor pulse traveling from the solenoid valve to the fly. Behavioral responses generally began <100 ms after the onset of ORN activity (Fig. 4B).

We quantified the neural and behavioral response onset for each trial by defining a threshold (blue lines in Fig. 4A,B) and measuring the time of first threshold crossing after the odor valve opened. Thresholds were chosen so that the probability of crossing during the response period was 10-fold higher than the probability of crossing during the control period (see Materials and methods). The distribution of response onset times reflects variability across trials and across flies (circles in Fig. 4A,B). The median latency of the neural response was 245 ms, and the median latency of the behavioral response was 330 ms (Fig. 4C). Most of this latency is due to the time required for the odor to travel from the valve to the fly; what is interesting is that the delay between the median neural and behavioral responses is only 85 ms. This figure probably represents an upper bound on the true latency, given that our thresholds are conservative ones. It is also worth noting that the behavioral response time was substantially more variable than the neural response time (Fig. 4A,B), and some behavioral response times are considerably faster than the median. These results show that behavioral responses to odors in *Drosophila* can occur rapidly after the onset of ORN activity.

Odors evoke a surge and an upwind turn in a freely rotating fly

The fixed-tether apparatus has the virtue of keeping the fly in a precisely defined position relative to the air stream. However, allowing the fly to rotate allows the experimenter to observe how it orients its body relative to the wind direction. Free rotation can be achieved by attaching the fly to a pin aligned within a magnetic field, thereby allowing the fly to rotate about its yaw axis whenever it generates asymmetric forces with its two wings (Fig. 5A). This type of rotatable-tether apparatus has been used previously to study how a fly turns in response to visual, mechanosensory and olfactory stimuli (Bender and Dickinson, 2006a; Bender and Dickinson, 2006b; Budick et al., 2007; Duistermars et al., 2009a; Duistermars et al., 2009b; Duistermars and Frye, 2008).

Consistent with these previous studies, we observed that flies tended to turn upwind when odor was added to the air stream (Fig. 5B,C). The upwind turn was generally also accompanied by a flight surge, as indicated by an increase in WBF (Fig. 5D). Upwind turns often consisted of rapid saccades that moved the fly into a precisely upwind orientation (Fig. 5B). This observation motivated us to ask what sensory neurons signal the direction of the odorized wind.

Odor-evoked turning requires mechanosensory input from the antennae

The direction of the odorized air stream could in principle be inferred from ORN activity alone. If one antenna is partially shielded from the air stream by the fly's head, the air speed at the two antennae would probably be different, meaning the flux of odor molecules would be bilaterally asymmetric. Because ORN responses vary with the flux of odor molecules (Kaissling, 1998; Rospars et al., 2000), the ORNs on the antennae experiencing the higher air speed would be expected to respond more strongly.

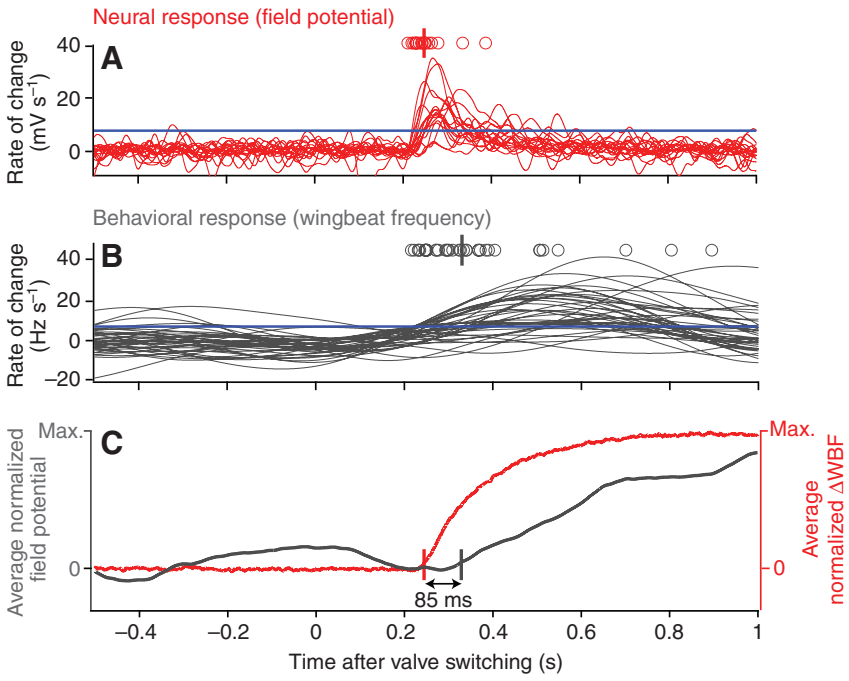

Fig. 4. The flight surge follows the receptor neuron response with a short latency. (A) Field potential recordings from the antenna measure the summed response of antennal neurons; the rate of change of the field potential is shown here. Each trace is a different trial (pooled trials from five flies). Circles show the timing of response onset (threshold crossing) for each trial, with the median indicated by the vertical tick. The threshold is shown in blue. (B) Behavioral responses to the same stimulus; the rate of change of the wingbeat frequency is shown here. Each trace is a different trial (pooled trials from eight flies). (C) A comparison between the mean time course of the neural and behavioral responses, averaged across all trials. Responses were normalized to the same maximum before averaging. Vertical ticks are median time of threshold crossing from A and B. The odor is methyl salicylate (10^{-2}): the strain is wild.

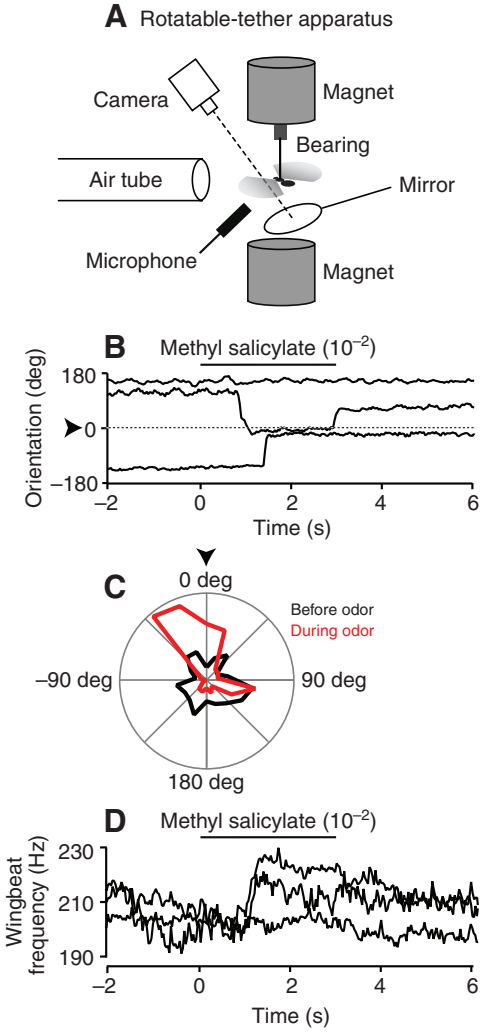

Fig. 5. Odors can evoke a turning response in flight. (A) Schematic of the rotatable-tether apparatus. The fly is tethered to a pin which is suspended between two magnets. The pin rests on a jewel bearing, allowing the fly to rotate freely in the *x*–*y* plane. A camera records the image of the fly, and the *x*–*y* orientation is extracted from this image. A microphone records the sound of the fly's wingbeats, and this audio signal is used to compute WBF. (B) Three representative trials showing how the fly's heading direction can change over time in response to odor. Upwind direction corresponds to 0 deg. (C) Polar histogram of the heading direction of the fly in the 3 s prior to odor (black) and the 3 s during odor stimulation (red). Data for the histogram was accumulated over 30 trials from five flies. Upwind direction is 0 deg (arrowhead). (D) Odor also evoked an increase in WBF in the trials shown in B. The strain is wild.

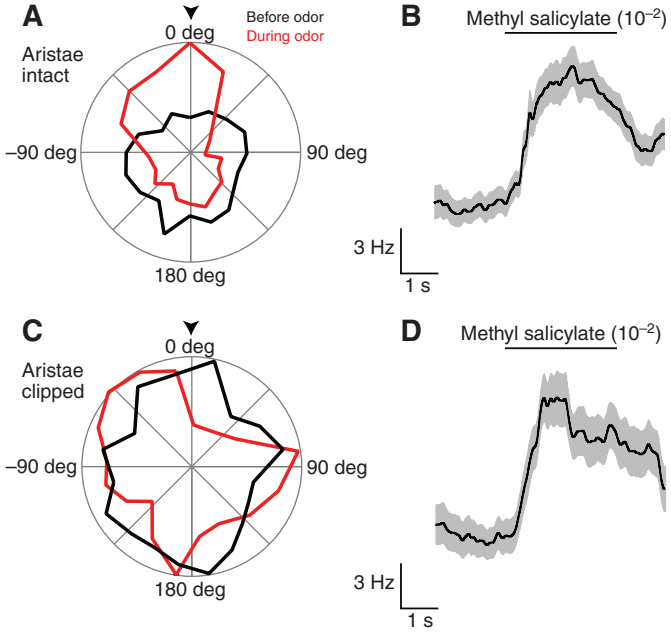

Fig. 6. Odor-evoked turning requires input from antennal mechanosensors. (A) In 'mock-clipped' flies the odor elicited upwind turning. Polar histogram shows that the two orientation distributions are significantly different during the odor period and the pre-odor period (N=22 trials from 17 flies, P<0.001, circular Kuiper's test). (B) In the same trials, these flies also increased WBF in response to the odor stimulus. Mean ± s.e.m. across flies. (C) When the aristae were clipped off, the same stimulus did not elicit upwind turning (N=110 trials from 15 flies, distributions not significantly different). (D) In the same trials, this stimulus did elicit an increase in the WBF. Mean ± s.e.m. across flies. The strain is wild.

Alternatively, sensing the direction of the odorized air stream might require additional types of neurons. Even in the absence of odors, tethered flies turn upwind if the air speed is sufficiently high (Budick et al., 2007). This phenomenon is called anemotaxis, and it demonstrates that olfactory cues are not required for upwind orientation. Anemotaxis in the absence of odors must require Johnston's organ neurons (JONs), because stabilizing the rotation of the antenna with glue essentially eliminates this behavior (Budick et al., 2007). This suggests that JONs might also be involved in odor-evoked upwind turns.

JON responses to air movement are reduced by removing the antennal arista, because the arista acts as a lever that rotates the antenna in the presence of coherent air movement (Gopfert and Robert, 2001; Manning, 1967; Yorozu et al., 2009). To investigate the role of the JONs in the olfactory turning behavior, we therefore clipped both aristae. Control flies were 'mock clipped', meaning that they were anesthetized and handled in the same way but their aristae were untouched.

The mock-clipped flies responded normally to an odor stimulus, turning upwind and increasing their WBF (Fig. 6A,B). The flies with clipped aristae did not orient upwind in response to the odor stimulus (Fig. 6C). However, these flies did increase their WBF (Fig. 6D). Together, these results suggest that mechanosensory cues mediated by JONs are required to guide the odor-evoked upwind turn, but are not involved in the odor-evoked surge.

Olfactory input to a single glomerulus can trigger both turn and surge behavior

Our results in this study, together with previous studies, demonstrate that a wide variety of odor stimuli can elicit flight maneuvers (Budick and Dickinson, 2006; Chow and Frye, 2008; Duistermars et al., 2009a; Duistermars et al., 2009b; Duistermars and Frye, 2008; Frye and Dickinson, 2004; Guo and Gotz, 1997; Wolf and Heisenberg, 1991; Xi et al., 2008). However, in all these cases the odor stimuli were presented at relatively high concentrations, ranging from pure odor to 100-fold diluted. Concentrated odors produce input to many spatially distributed glomeruli. In order to define the relationship between ORN activity and odor-evoked flight behaviors, it would be useful to understand whether these behaviors can be elicited by stimulation of defined ORN types.

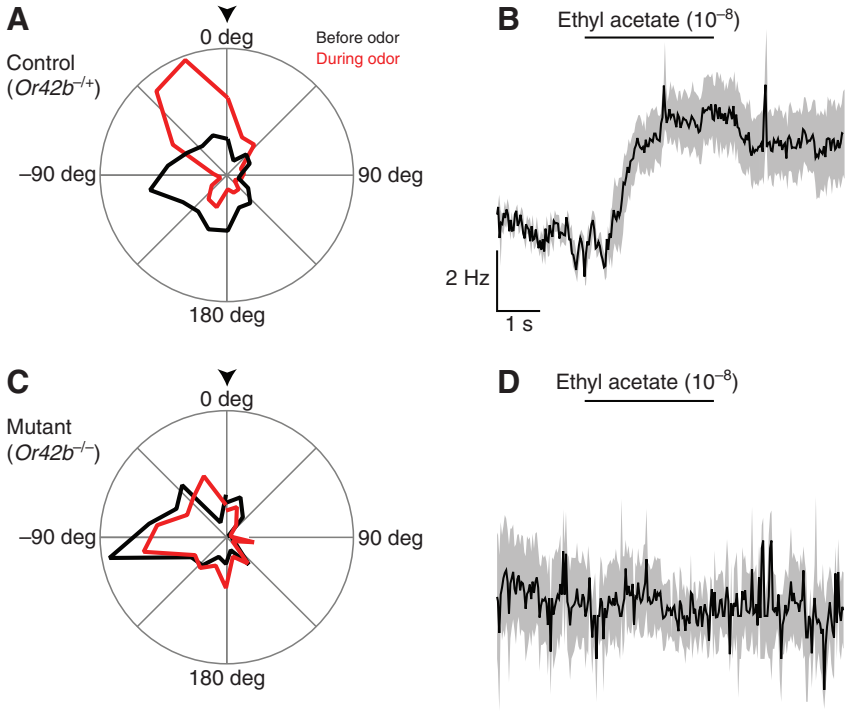

Fig. 7. Input to a single glomerulus can trigger flight modulation. (A) Polar histogram of heading direction for control flies (*Or42b*$^{+/-}$). The odor stimulus elicited upwind turning (N=64 trials from nine flies, P<0.005, circular Kuiper's test). (B) In the same trials, these flies also increased WBF in response to the odor stimulus. Mean ± s.e.m. across flies. [Note that this stimulus did not elicit a WBF change in wild flies in the fixed-tether apparatus (Fig. 3B) or in the rotatable-tether apparatus (data not shown). Thus, the WBF surge in this genotype likely reflects differences in genetic background.] (C) This stimulus did not elicit turning of flies harboring a mutation in a high-affinity ethyl acetate receptor (*Or42b*$^{-/-}$, N=61 trials from 10 flies, distributions not significantly different). (D) In the same trials, the odor also failed to elicit a WBF change (the scale for B and D is the same). Mean ± s.e.m. across flies.

We therefore asked whether these behaviors could be elicited by a minimal ORN activity pattern – namely, stimulation of a single ORN type. For these experiments we chose the odor ethyl acetate, which is a high-affinity ligand for one *Drosophila* odorant receptor (Or42b). At low concentrations, this odor activates Or42b fairly selectively (Hallem and Carlson, 2006; Olsen et al., 2010). We used a dilution well within the range where this odor is selective (10^{-8}). Even at this low concentration, we found that ethyl acetate caused flies in the rotatable-tether apparatus to orient upwind (Fig. 7A) and to increase their WBF (Fig. 7B). This suggests that ORN input to a single glomerulus is sufficient to elicit turns and surges.

In order to confirm that this stimulus is acting through a single receptor, we also tested flies bearing a mutation in the *Or42b* gene (*Or42b*$^{-/-}$). This mutation abolishes the odor responses of the ORNs that normally express this gene (Bhandawat et al., 2007). In the *Or42b*$^{-/-}$ flies, ethyl acetate (10^{-8}) failed to elicit any turning (Fig. 7C). The odor-evoked increase in WBF was also absent, as expected (Fig. 7D).

These results confirm the essential role of ORNs in the turning and surging behaviors. Furthermore, they suggest that both turning and surging can be elicited by selective stimulation of a single ORN type.

Surge and turning can occur independently

Finally, we examined the relationship between the odor-evoked turn and surge behaviors, with the goal of understanding how ORN signals might drive central circuits. On average, we observed that the increase in the WBF began at about the same time as the upwind turn (Fig. 8A–C). However, although the average latency of these two behaviors was similar, the two behaviors could occur independently and at different times within an individual trial. In some trials, odor evoked a turn without any surge in WBF (Fig. 8D,E). In other trials, the fly responded with surge but no turn (Fig. 8F). In most trials, there was both a surge in WBF and an upwind turn (Fig. 8G, see also Fig. 5), but changes in heading direction and wingbeat frequency did not necessarily occur at the same time.

On individual trials, the WBF response generally occurred consistently, and tended to follow the kinetics of the average WBF response. By contrast, the turning response showed greater trial-to-trial variation in its latency, speed and precision. In some trials, the fly made a fast, precise, saccade-like turn into the headwind (Fig. 5B), whereas in other trials the turn involved several saccade-like steps (Fig. 8E) or a gradual progression toward the headwind (Fig. 8D,G). In some trials the fly rapidly turned away from the headwind after the odor was removed (Fig. 8E), whereas in other trials it continued to orient into the headwind for several seconds after the odor had disappeared (Fig. 8D,G).

Together, these results imply that odors modulate stroke frequency and heading direction independently. Another piece of evidence supporting this conclusion is our observation that a particular odor can modulate orientation without evoking changes in wingbeat frequency (compare Fig. 3 and supplementary material Fig. S8). Taken together, these findings suggest that ORN signals trigger surges and turns by activating parallel central command circuits, rather than by activating a single command that modulates both power muscles and steering muscles. The comparative variability of the turning response may reflect the influence of cues that we have not adequately controlled, or variations in the fly's internal state.

DISCUSSION

Odor-evoked flight maneuvers are sensitive, specific and multimodal

One of our central aims in this study was to establish what minimal patterns of primary sensory neuron activity are necessary and sufficient to elicit odor-evoked flight behaviors. First, we confirmed that a genetic mutation that silences the majority of ORNs is sufficient to abolish the odor-evoked surge in wingbeat frequency and amplitude. This demonstrates that the surge is not purely a response to the small mechanical artifact that accompanies the olfactory stimulus. It should be noted that this mutation silences most ORNs, but a few ORN types are unaffected by this mutation

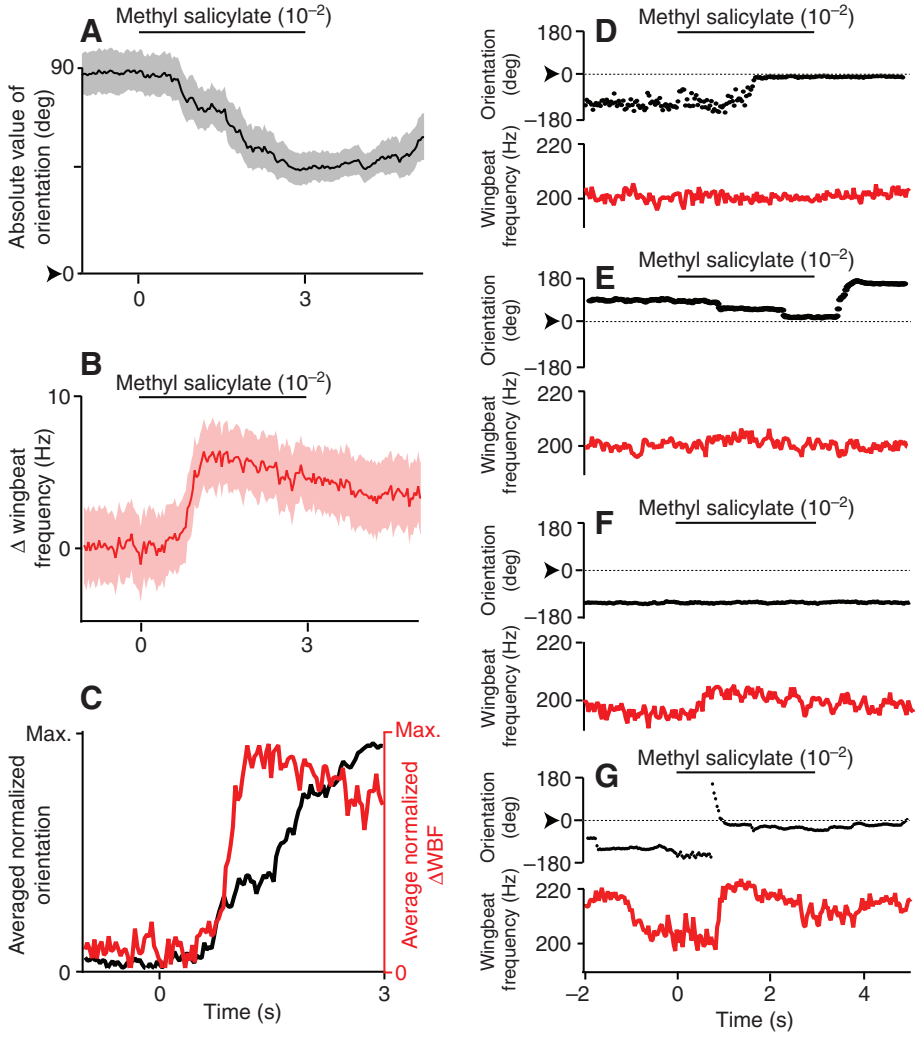

Fig. 8. Surge and turning response are independent behaviors. (A) Average time course of the fly's heading direction (N=5 flies, mean ± s.e.m. across flies). The absolute value of the orientation ranges from 0 to 180 deg, with a baseline mean of 90 deg, as expected from a random distribution of headings. On average, odors do not elicit a full orientation to 0 deg because in some trials the fly did not turn, and in other trials it turned incompletely. (B) Average time course of the WBF response (N=5 flies, mean ± s.e.m. across flies). (C) A comparison between the time courses of the average normalized orientation and ΔWBF. (D,E) Two examples of trials in which the fly turned without changing WBF. This occurred in seven of 30 cases. (F) A trial in which the fly did not turn, but did increase WBF. This occurred in nine cases. (G) A trial in which both turning occurred and WBF changed. This occurred in 11 cases. In a few cases, there was neither a surge nor a turn. The strain is wild.

(Larsson et al., 2004; Olsen et al., 2007). Thus, it might be possible to find odor stimuli that elicit flight responses even in these mutants.

We also found that an odor stimulus that evokes activity in a single ORN type can produce both surge and turning behavior. The odor stimulus we used in these experiments elicits a relatively weak response in these ORNs (~20 spikes s^{-1}) (Olsen et al., 2010). Because the surge begins only about 85 ms after the onset of ORN activity, each of these ORNs is likely to fire only a handful of spikes before the behavioral response occurs.

We also found that odors that activate different ORN types also differ in their tendency to elicit a flight surge. This was true even though these odors were matched for intensity, meaning that they elicited the same levels of total ORN activity. Thus, these flight maneuvers are not a simple consequence of ORN activity *per se*. Rather, the decision to surge depends on the identity of the ORNs that are active. This finding is reminiscent of a recent report that the locomotor responses of freely walking *Drosophila* depend on the identity of the ORNs that are activated by an odor (Semmelhack and Wang, 2009).

Finally, we found that antennal aristae are also required for an odor to elicit an upwind turn. Because the aristae do not contain ORNs (Stocker, 1994), this result implies that ORNs alone do not provide enough spatial information to guide the turn. This would suggest that ORNs merely gate a turn which is guided by other sensory neurons. The antennal aristae are crucial to the normal function of JONs, which are the mechanosensory neurons that encode the movement of the antennal funiculus (Gopfert and Robert, 2001; Manning, 1967; Yorozu et al., 2009). The direction of air particle movement could, in principle, be deduced on the basis of bilateral comparisons between JONs in the two antennae. This comparison would then guide the turn, and olfactory-mechanosensory integration would gate the turn.

If air speeds are sufficiently high, a tethered fly will turn upwind even in the absence of odors, and JONs are required for this behavior (Budick et al., 2007). Our findings thus reinforce the conclusions of Budick et al. that a flying *Drosophila* senses headwind direction primarily *via* input from its JONs – at least in the absence of visual inputs.

Another study has reported that orienting into an odor plume is only modestly impaired by clipping the aristae (Duistermars et al., 2009a; Duistermars et al., 2009b). Stabilizing the funiculus with glue had a larger effect, although it did not abolish orienting behavior. This may be due to the fact that this study used a much lower flow rate than we did (7 ml min^{-1} *versus* 550 ml min^{-1}; M. A. Frye, personal communication), and this may have minimized the contribution of mechanosensory cues. Also, this study used a vacuum below the fly to create a discrete narrow odor plume, and so spatial olfactory cues may have been stronger than in our experiments.

Odor-evoked flight behaviors are rapid

A second aim of this study was to investigate how rapidly flight maneuvers occur after the onset of ORN activity. We found that the difference between the median ORN response onset time and the median surge onset time was 85 ms. This probably represents only an upper bound, because the fly's behavioral response time was substantially more variable than its neural response time, and a few flies surged <20 ms after the fastest ORN response we recorded.

This measurement also places an upper bound on the latency of odor discrimination. This is because ethyl acetate (10^{-8}) elicits no surge in this experimental configuration (wild flies, fixed-tether apparatus), even though it produces a level of summed ORN activity that is similar to the ORN activity evoked by the odor in the latency experiments (methyl salicylate, 10^{-2}). Thus, by the time the fly shows a behavioral response to methyl salicylate, it has not only detected that an odor is present, but it has also distinguished odor identity based on the pattern of ORN activity elicited by the odor.

In the brain, ORN axons form synapses that selectively transmit the onset of an ORN spike train (Kazama and Wilson, 2008), and central neurons directly postsynaptic to ORNs are particularly sensitive to the beginning of an odor stimulus (Bhandawat et al., 2007). Thus, the flight surge may be triggered by just the first few spikes in the ORN population. Consistent with this idea, we found that activating a single ORN type at a rate of ~20 spikes s^{-1} is sufficient to elicit a behavioral response. This leaves time for each responding ORN to fire just a few spikes before the behavioral response onset.

Flight in Diptera is controlled by two kinds of muscles, direct muscles that insert at the base of the wing and indirect muscles that move the wing by contracting the thoracic cavity (Dickinson and Tu, 1997). Previously, it was proposed that olfactory modulation of flight occurs through modulation of the indirect muscles (Frye and Dickinson, 2004). However, given our finding that odor-evoked flight responses typically begin within 85 ms of the onset of sensory neuron activity (and even faster in some cases), they seem unlikely to be triggered by the indirect musculature alone, which is recruited more slowly than the direct musculature. Rather, the speed of these olfactory responses may reflect a role for the direct muscles, which can modulate wingbeat amplitude on a faster time scale (Dickinson and Tu, 1997).

Parallel command circuits link primary sensory neurons to motor neurons

A third aim of this study was to determine whether odor-evoked surges and turns are evoked independently, or whether they always occur together. At least in principle, the fly should be able to command these components independently because they are mediated by different muscle groups. Whereas the surge is mediated by the power muscles (Frye and Dickinson, 2004), turning is mediated by the steering muscles, which unlike the power muscles can be modulated asymmetrically (Heide, 1983; Heide et al., 1984; Levine, 1973). If the result of the fly's odor discrimination decision was a single command to both these muscle groups, they would always occur together. Contrary to this, we observed that odor-evoked turns can occur independently from odor-evoked changes in wingbeat frequency on a trial-to-trial basis. Moreover, turns were more variable than surges, and clipping the aristae eliminated the turn response without eliminating the surge. We also observed that a particular odor stimulus can evoke turns (or suppression of turns) without evoking changes in wingbeat frequency. These results suggest that ORN activity leads to surging and turning *via* independent commands to the turning muscles and power muscles.

Olfactory modulation of tethered flight: methodological findings

We also report several methodological findings (see Materials and methods and the supplementary material). These results have important implications for investigators using this experimental approach. First, we found that odors can modulate flight even in the absence of a closed-loop visual stimulus. This is useful because it considerably simplifies the apparatus needed for these experiments.

Second, we found that olfactory modulation of flight is robust in inbred laboratory strains. This is important because previous studies have mainly used wild strains, which are not convenient for transgenesis. However, we found that not all strains fly equally well. For example, our results suggest that wild flies are capable of a higher maximum stroke frequency than w^{1118} flies. In the absence of odors, these two strains have the same stroke frequency, but w^{1118} flies have a smaller dynamic range for an odor-evoked surge. This is important because w^{1118} is probably the most common genetic background for transgenesis, and so in order to take advantage of the *Drosophila* genetic toolbox it is most convenient to work in this background.

Third, we found that air speed is a critical factor. To begin with, higher flow rates (corresponding to higher air speeds) produced lower wingbeat frequencies. This may reflect modulation of flight by antennal mechanosensors (Heide et al., 1984). Moreover, the odor-evoked surge in wingbeat frequency and amplitude was larger at high flow rates. This is probably due to the fact that high air speeds produce a larger flux of odor molecules across the antennae and palps, which results in stronger ORN activation (Kaissling, 1998; Rospars et al., 2000). Finally, high flow rates decreased the latency of the behavioral response. This is probably due to the fact that a high air speeds shortens the time between odor valve switching and the arrival of the odor pulse at the fly. It may also reflect increased ORN activity at higher air speeds. Higher air speeds are also known to promote upwind turns in the absence of odors (Budick et al., 2007).

A previous study using the rotatable tether apparatus showed that flies cannot reliably orient in a narrow plume without a strong visual stimulus marking the location of the plume (Duistermars and Frye, 2008). However, this study used a much lower air flow rate than we did to deliver odor to the fly, and thus mechanosensory spatial cues were probably weaker. Our results support the conclusion of Budick et al. that a fly can make reliable upwind turns even in the absence of strong visual cues, provided that mechanosensory stimuli are sufficiently strong (Budick et al., 2007).

ACKNOWLEDGEMENTS

We thank K. I. Nagel for help in data acquisition, assistance in writing the image analysis software, and helpful conversations. We are grateful to members of the Wilson lab for feedback on the manuscript. This work was funded by a grant from the NIH (R01DC008174), a McKnight Scholar Award, a Sloan Foundation Research Fellowship, and a Beckman Young Investigator Award (to R.I.W.), together with a grant from the NSF (FIBR 0623527, to M.H.D.). V.B. was partially supported by Charles A. King Trust Postdoctoral Fellowship. G.M. was supported by a Caltech Della Martin Fellowship. R.I.W. is an HHMI Early Career Scientist. V.B. and R.I.W. designed the experiments. V.B. collected the data. Pilot experiments were performed by V.B. with assistance from G.M. in the laboratory of M.H.D. Both G.M. and M.H.D. provided technical support and intellectual contributions. V.B. and R.I.W. wrote the manuscript. Deposited in PMC for release after 12 months.

REFERENCES

Ashburner, M., Golic, K. G. and Hawley, R. S. (2004). *Drosophila – A Laboratory Handbook*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Bender, J. A. and Dickinson, M. H. (2006a). A comparison of visual and haltere-mediated feedback in the control of body saccades in *Drosophila melanogaster*. *J. Exp. Biol.* **209**, 4597-4606.

Bender, J. A. and Dickinson, M. H. (2006b). Visual stimulation of saccades in magnetically tethered *Drosophila*. *J. Exp. Biol.* **209**, 3170-3182.
Berry, J., Krause, W. C. and Davis, R. L. (2008). Olfactory memory traces in *Drosophila*. *Prog. Brain Res.* **169**, 293-304.
Bhandawat, V., Olsen, S. R., Gouwens, N. W., Schlief, M. L. and Wilson, R. I. (2007). Sensory processing in the *Drosophila* antennal lobe increases reliability and separability of ensemble odor representations. *Nat. Neurosci.* **10**, 1474-1482.
Borst, A. and Haag, J. (2002). Neural networks in the cockpit of the fly. *J. Comp. Physiol. A* **188**, 419-437.
Budick, S. A. and Dickinson, M. H. (2006). Free-flight responses of *Drosophila melanogaster* to attractive odors. *J. Exp. Biol.* **209**, 3001-3017.
Budick, S. A., Reiser, M. B. and Dickinson, M. H. (2007). The role of visual and mechanosensory cues in structuring forward flight in *Drosophila melanogaster*. *J. Exp. Biol.* **210**, 4092-4103.
Carlson, J. R. (1996). Olfaction in *Drosophila*: from odor to behavior. *Trends Genet.* **12**, 175-180.
Chow, D. M. and Frye, M. A. (2008). Context-dependent olfactory enhancement of optomotor flight control in *Drosophila*. *J. Exp. Biol.* **211**, 2478-2485.
Collett, T. S. and Land, M. F. (1975). Visual control of flight behaviour in the hoverfly, Syritta pipiens L. *J. Comp. Physiol.* **99**, 1-66.
de Bruyne, M., Clyne, P. J. and Carlson, J. R. (1999). Odor coding in a model olfactory organ: the *Drosophila* maxillary palp. *J. Neurosci.* **19**, 4520-4532.
de Bruyne, M., Foster, K. and Carlson, J. R. (2001). Odor coding in the *Drosophila* antenna. *Neuron* **30**, 537-552.
Dickinson, M. H. and Tu, M. S. (1997). The function of dipteran flight muscle. *Comp. Biochem. Physiol.* **116**, 223-238.
Duistermars, B. J. and Frye, M. A. (2008). Crossmodal visual input for odor tracking during fly flight. *Curr. Biol.* **18**, 270-275.
Duistermars, B. J., Chow, D. M. and Frye, M. A. (2009a). Flies require bilateral sensory input to track odor gradients in flight. *Curr. Biol.* **19**, 1301-1307.
Duistermars, B. J., Chow, D. M. and Frye, M. A. (2009b). Flies require bilateral sensory input to track odor gradients in flight (ERRATUM). *Curr. Biol.* **19**, 1774-1775.
Fiala, A. (2007). Olfaction and olfactory learning in *Drosophila*: recent progress. *Curr. Opin. Neurobiol.* **17**, 720-726.
Frye, M. A. and Dickinson, M. H. (2001). Fly flight: a model for the neural control of complex behavior. *Neuron* **32**, 385-388.
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.
Goldman, A. L., Van der Goes van Naters, W., Lessing, D., Warr, C. G. and Carlson, J. R. (2005). Coexpression of two functional odor receptors in one neuron. *Neuron* **45**, 661-666.
Gopfert, M. C. and Robert, D. (2001). Biomechanics. Turning the key on *Drosophila* audition. *Nature* **411**, 908.
Götz, K. G. (1987). Course-control, metabolism, and wing interference during ultralong tethered flight in *Drosophila melanogaster*. *J. Exp. Biol.* **128**, 35-46.
Guo, A. and Gotz, K. G. (1997). Association of visual objects and olfactory cues in *Drosophila*. *Learn. Mem.* **4**, 192-204.
Hallem, E. A. and Carlson, J. R. (2006). Coding of odors by a receptor repertoire. *Cell* **125**, 143-160.
Heide, G. (1983). Neural mechanisms of flight control in Diptera. In *Report of the Symposium "Physiology and Biophysics of Insect Flight", BIONA-report 2* (ed. W. Nachtigall), pp. 35-52. Stuttgart: G. Fischer.
Heide, G., Spüler, M., Götz, K. G. and Kamper, K. (1984). Neural control of asynchronous flight muscles in flies during induced flight manoeuvres. In *Insect Locomotion* (ed. M. Gewecke and G. Wendler), pp. 215-222. Berlin and Hamburg: Verlag Paul Parey.
Holmes, T. C., Sheeba, V., Mizrak, D., Rubovsky, B. and Dahdal, D. (2007). Circuit-breaking and behavioral analysis by molecular genetic manipulation of neural activity in *Drosophila*. In *Invertebrate Neurobiology* (ed. G. North and R. J. Greenspan), pp. 19-52. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
Kaissling, K. E. (1998). Flux detectors versus concentration detectors: two types of chemoreceptors. *Chem. Senses* **23**, 99-111.
Kazama, H. and Wilson, R. I. (2008). Homeostatic matching and nonlinear amplification at identified central synapses. *Neuron* **58**, 401-413.
Land, M. F. and Collett, T. S. (1974). Chasing behavior of houseflies (Fannia canicularis). *J. Comp. Physiol.* **89**, 331-357.
Larsson, M. C., Domingos, A. I., Jones, W. D., Chiappe, M. E., Amrein, H. and Vosshall, L. B. (2004). Or83b encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. *Neuron* **43**, 703-714.
Lehmann, F. O. and Dickinson, M. H. (1997). The changes in power requirements and muscle efficiency during elevated force production in the fruit fly *Drosophila melanogaster*. *J. Exp. Biol.* **200**, 1133-1143.
Levine, J. (1973). Properties of the nervous system controlling flight in *Drosophila melanogaster*. *J. Comp. Physiol.* **84**, 129-166.
Luo, L., Callaway, E. M. and Svoboda, K. (2008). Genetic dissection of neural circuits. *Neuron* **57**, 634-660.
Manning, A. (1967). Antennae and sexual receptivity in *Drosophila melanogaster* females. *Science* **158**, 136-137.
Olsen, S. R. and Wilson, R. I. (2008). Cracking neural circuits in a tiny brain: new approaches for understanding the neural circuitry of *Drosophila*. *Trends Neurosci.* **31**, 512-520.
Olsen, S. R., Bhandawat, V. and Wilson, R. I. (2007). Excitatory interactions between olfactory processing channels in the *Drosophila* antennal lobe. *Neuron* **54**, 89-103.
Olsen, S. R., Bhandawat, V. and Wilson, R. I. (2010). Divisive normalization in olfactory population codes. *Neuron* **66**, 287-299.
Rospars, J. P., Krivan, V. and Lansky, P. (2000). Perireceptor and receptor events in olfaction. Comparison of concentration and flux detectors: a modeling study. *Chem. Senses* **25**, 293-311.
Semmelhack, J. L. and Wang, J. W. (2009). Select *Drosophila glomeruli* mediate innate olfactory attraction and aversion. *Nature* **459**, 218-223.
Stocker, R. F. (1994). The organization of the chemosensory system in *Drosophila melanogaster*: a review. *Cell Tissue Res.* **275**, 3-26.
Tammero, L. F. and Dickinson, M. H. (2002). Collision-avoidance and landing responses are mediated by separate pathways in the fruit fly, *Drosophila melanogaster*. *J. Exp. Biol.* **205**, 2785-2798.
Wilson, R. I. (2007). Neural circuits underlying chemical perception. *Science* **318**, 584-585.
Wolf, R. and Heisenberg, M. (1991). Basic organization of operant behavior as revealed in *Drosophila* flight orientation. *J. Comp. Physiol. A* **169**, 699-705.
Xi, W., Peng, Y., Guo, J., Ye, Y., Zhang, K., Yu, F. and Guo, A. (2008). Mushroom bodies modulate salience-based selective fixation behavior in *Drosophila*. *Eur. J. Neurosci.* **27**, 1441-1451.
Yorozu, S., Wong, A., Fischer, B. J., Dankert, H., Kernan, M. J., Kamikouchi, A., Ito, K. and Anderson, D. J. (2009). Distinct sensory representations of wind and near-field sound in the *Drosophila* brain. *Nature* **458**, 201-205.
Zhu, J., Park, K. C. and Baker, T. C. (2003). Identification of odors from overripe mango that attract vinegar flies, *Drosophila melanogaster*. *J. Chem. Ecol.* **29**, 899-909.