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Odor localization requires visual feedback during free flight in *Drosophila melanogaster*

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Summary

Adult fruit flies follow attractive odors associated with food and oviposition sites through widely varied visual landscapes. To examine the interaction between olfactory and visual cues during search behavior, we recorded three-dimensional flight trajectories as individuals explored controlled sensory landscapes. When presented with the source of an attractive odor invisibly embedded in the floor of a 1 m arena, flies spend most of their time hovering back and forth over the source when flying within a randomly textured visual background but fail to localize the source when searching within a uniform white surround. To test whether flies are associating unique features of the visual background with the strength of odor cues, we flew them within arenas containing evenly spaced vertical stripes. Flies readily localized the odor when flying within visual landscapes lacking azimuthal landmarks provided that vertical edges were present. Flies failed to localize odor when flying within a background pattern consisting of horizontal stripes. These results suggest that, whereas flies do not require spatially unique visual patterns to localize an odor source, they do require visual feedback generated by vertical edges. Quantitative shifts in several components of flight behavior accompanied successful odor localization. Flies decrease flight altitude, turn more often and approach visually textured walls of the arena near an odor source. A simple model based on the statistics of flight behavior supports the hypothesis that a subtle influence on these behaviors is sufficient to lead a fly to its food.

Key words: *Drosophila*, insect, free flight, olfaction, vision, chemotaxis, optomotor, collision avoidance, flight control.

Introduction

To localize food sources and oviposition sites successfully during flight, animals must integrate visual, olfactory and mechanosensory information. Tracking an odor during flight becomes more difficult in the absence of wind, which provides the mechanosensory anemotactic cues that many animals use to navigate upwind through odor plumes (Belanger and Willis, 1996). However, in the absence of a background of uniform air flow, local concentration gradients and the spatial structure of cohesive plume fragments can provide information for identifying the position or direction of a desired olfactory target (Fraenkel and Gunn, 1961). Fruit flies – like most flying animals – rely extensively on visual feedback to control several key components of flight behavior, including body posture (Götz et al., 1979), flight speed (David, 1979), course heading (Wolf and Heisenberg, 1990), obstacle avoidance (Tammero and Dickinson, 2002a) and landing (Borst and Bahde, 1988). For a fly to approach and land upon the origin of an attractive odor, olfactory input must somehow modulate, compete with or override visual controls that would otherwise maintain stable flight.

Whereas physiological mechanisms of visual motion detection (Borst and Haag, 2002) and odor discrimination (Carlson, 2001), have been studied independently, we know little about how the fly's nervous system integrates these two sensory processes to guide or bias an animal's movement towards an odor source. In this study, we investigate the effects of varying the spatial distribution of both visual and olfactory cues on the control of free flight. We begin by comparing odor localization within a richly textured visual background with uniform white surroundings and proceed with experiments that examine the influence of specific visual features, such as vertically or horizontally oriented edges. Our results show that olfactory acuity depends upon the complexity of the visual landscape and that vertical edges provide an essential visual cue for chemotactic behavior in *Drosophila*. We discuss possible behavioral and physiological mechanisms for these features of visual–olfactory fusion.

Materials and methods

Animals

All experiments were performed on 2–3 day-old *Drosophila melanogaster* (Meigen), from a laboratory stock maintained at The University of California – Berkeley. Animals were maintained on a 12 h:12 h L:D photoperiod and tested 5 h after the onset of subjective day. To motivate long flight sequences, flies were starved for 4 h and adapted to the light level of the arena for 2 h prior to each experiment. Upon being released in the arena, each fly generated a continuous flight sequence lasting from several seconds to several minutes before landing on the floor, at which point data collection was terminated. We individually tested 110 male and 108 female flies. We found no behavioral differences between males and females. Some flies provided more than one flight sequence.

Free-flight tracking and experimental treatments

The geometry of the flight arena and tracking cameras is described in more detail elsewhere (Tammero and Dickinson, 2002a). A circular arena (1 m diameter × 0.6 m height) was illuminated from above with an array of infrared light-emitting diodes (Fig. 1A). Flight trajectories were monitored by infrared-sensitive cameras and sampled at 60 Hz using a custom-modified version of commercially available software, Trackit 3-D (Fry et al., 2000). Flight trajectories were analyzed off-line using custom software routines written in MATLAB v.6. We included all flight sequences greater than 5 s in the analyses. The walls of the arena were backlit with a circular array of halogen lamps. Mean illuminance within the arena was 15 lux for all experiments. For experimental treatments with an attractive odorant, a 0.5 ml microphage tube filled with apple cider vinegar was embedded in the floor midway between the center and the wall of the arena. The microfuge tube was painted black and mounted flush with the black arena floor to minimize its visibility.

The walls of the arena were lined with either a uniform white panorama or printed black-and-white patterns. Five different visual landscapes were used for these experiments (Fig. 1B–F): (1) a random array of individual squares each subtending 5° at the center of the arena (50% probability of black), (2) a uniform white arena, (3) three thick vertical stripes (each subtending 15°) spaced 120° apart, (4) an alternating array of black-and-white 5° vertical stripes and (5) a similar array of horizontal stripes (Fig. 1B–F, respectively). The floor of the arena consisted of black flock paper, which enhanced the contrast of the bright fly on the dark background. The ceiling consisted of a cylinder of dense black cloth, which inhibited an upward phototactic escape response when flies were placed in the arena. The dark floor and ceiling also served to form contrasting horizontal edges with the walls, providing flies with stabilizing cues in all visual landscapes, including the uniform white surround. To avoid interference from residual odor stimuli, the visual background patterns and the floor of the arena were replaced between odor and non-odor treatments.

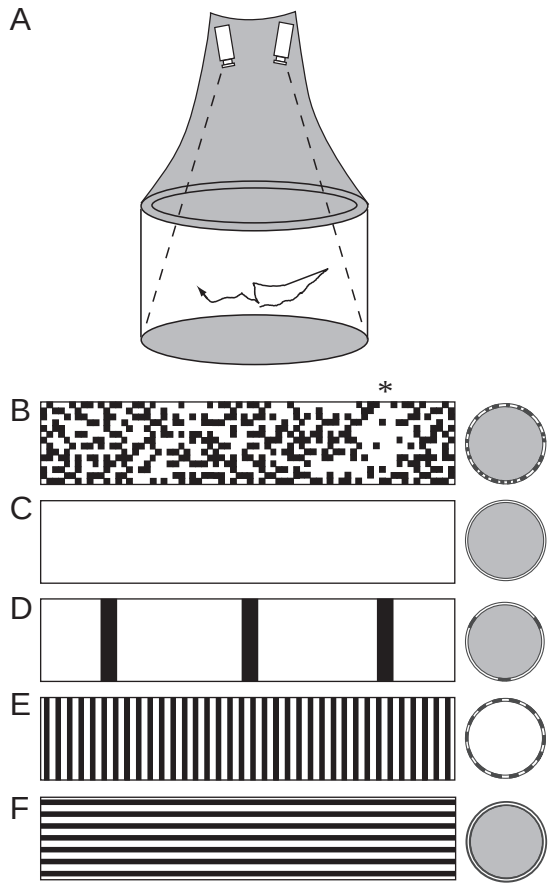

Fig. 1. Geometry of free-flight arena and experimental visual patterns. (A) Flies explored an arena 1 m in diameter, 0.6 m high. A pair of video cameras tracked *Drosophila* flight trajectories within a conical region of the arena (broken lines). (B–F) Different black-and-white patterns, printed on white paper, formed experimental visual landscapes. In all cases, the ceiling and floor of the arena were left black, forming two contrasting vertical edges. Circular icons, representing the top view of each visual treatment, are used in subsequent figures. The asterisk indicates a region of pattern that may have biased trajectory distributions (see Materials and methods).

Results

Uniform white and random checkerboard backgrounds

Flies flying within the randomized black-and-white checkerboard panorama showed a weak directional preference for a region in the background pattern that, by chance, had a large proportion of white squares; see asterisk in Fig. 1B (Fig. 2A,E). Such preferences vary depending on the precise pattern of black-and-white squares used. However, when flying within the uniform white visual panorama, flies showed no directional preference and spent most of their time flying back and forth across the center of the arena (Fig. 2B,F).

Introducing an odor source hidden in the floor of the random checkerboard arena resulted in a robust and repeatable shift in the spatial distribution of flight paths (Fig. 2C,G). Animals spent most of their time flying back and forth over the odor source, reminiscent of the way they hover over bowls of fruit.

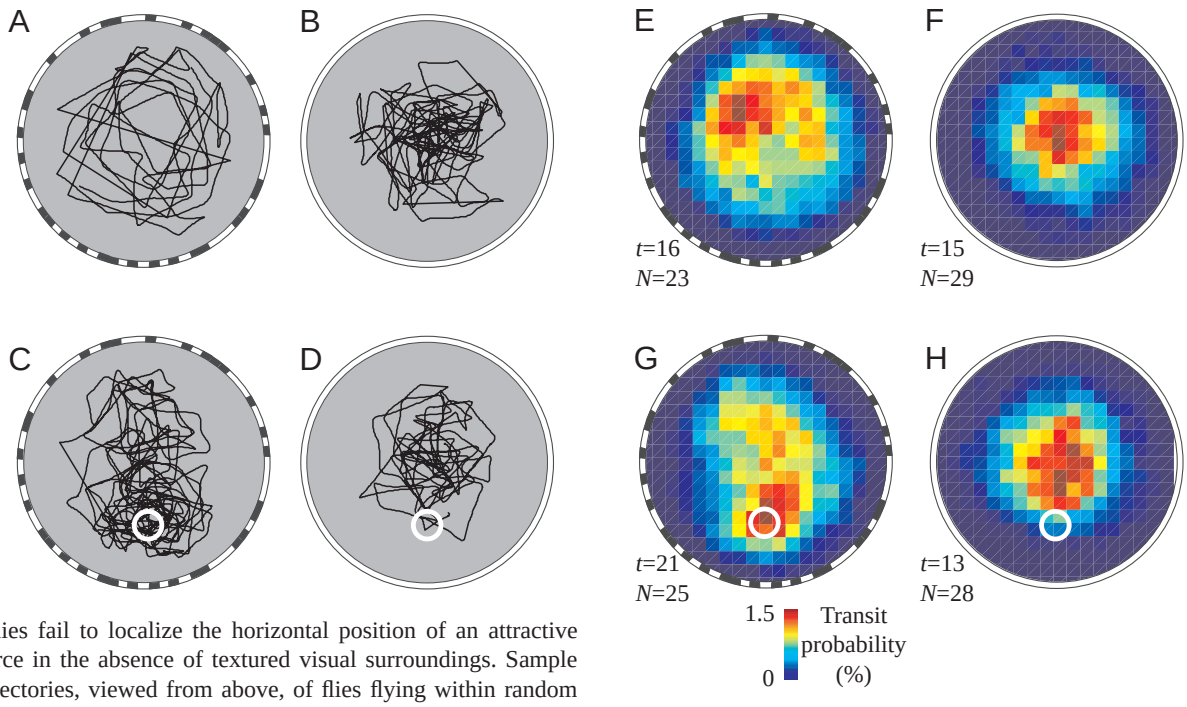

Fig. 2. Flies fail to localize the horizontal position of an attractive odor source in the absence of textured visual surroundings. Sample flight trajectories, viewed from above, of flies flying within random checkerboard (A) and uniform white (B) visual surroundings. Embedding a vial of apple cider vinegar in the floor of the arena (location indicated by white circles) resulted in biased flight trajectories for animals flying within the random (C), but not the uniform (D), treatment. Average position indicated with 2-D histograms plotted in pseudocolor. Position bins are 50 mm×50 mm. Number of flies (*N*) and total flight time in min (*t*) are indicated for each plot.

Neither the presentation of odor nor the uniform and random visual backgrounds qualitatively altered the shape of flight paths (Fig. 2A–D). In the absence of textured visual surroundings, however, flies were unable to localize the horizontal position of the odor (Fig. 2D,H).

Whether flying in textured or uniform visual surroundings, the flight trajectories that fulfilled our criteria of being greater than 5 s in duration were distributed primarily within the lower region of the arena (Fig. 3A,B). Within individual flight bouts, flies gradually maneuvered closer to the floor, bringing them nearer to the odor source over time (Fig. 3C,D). Accordingly, with the presentation of odor, the distributions of trajectory altitude (Fig. 3E,F) shift to lower values for both random checkerboard and uniform visual conditions (Fig. 3G,H). Taken together, the results show that whereas odor localization within the horizontal plane appears to depend upon the complexity of the visual environment, localization in the vertical plane may not. This suggests that the behavioral response to odor comprises separate horizontal and vertical components.

To determine how the flies' ability to localize the horizontal position of an odor source depends on visual landscape, we further analyzed the flight trajectories exhibited in each experimental treatment. *Drosophila* flight behavior consists of stereotypical sequences of straight flight interspersed by rapid turns called 'saccades', which are characterized by a rapid change in the direction of the flight path (Fig. 4A,B). Flies are thought to execute saccadic turns during flight for the same reason that humans use saccadic eye movements – to minimize the duration of motion blur on the retina while reorienting gaze (Schilstra and van Hateren, 1998). Flies also employ saccades to steer away from approaching obstacles (Tammero and Dickinson, 2002a,b). For the analysis of *Drosophila* flight trajectories, saccades were defined as peaks in the angular velocity of the flight path that exceeded 300 deg. s^{-1}. The influence of odor on the spatial distribution of flight trajectories must be due to either a change in flight speed between saccades or changes in the spatial distribution, frequency or magnitude of the saccades themselves.

For each saccade, we measured several characteristic behavioral parameters. Parameters intrinsic to the flight trajectory included 'saccade angle', defined as the angular deviation from the current path at the time of the turn, and 'segment length', defined as the total distance between saccades (Fig. 4C). Extrinsic parameters included 'odor distance', defined as the distance to the odor at each saccade, 'collision distance', defined as the distance to the arena wall along the current heading, and 'arena heading', defined as the point on the arena towards which the fly was headed (Fig. 4C). In experiments in which there was no odor source, odor distance is defined as the distance to the location within the arena where the odor source is normally placed.

Values of saccade angle, velocity, collision distance, segment length and odor distance throughout a sample trajectory within the random checkerboard arena are plotted in Fig. 5. As the fly approached the odor source, indicated by a drop in odor distance (Fig. 5E), it reduced flight velocity and executed short inter-saccade segments (Fig. 5B,C). By

contrast, saccade angles remained relatively constant as the animal neared the odor source (Fig. 5A). The apparent detection of the odor source is also indicated by a marked oscillation of collision distances between low and high values (Fig. 5D). This oscillation indicates a back-and-forth pattern in which the animal approached the wall at a close distance when heading towards the odor but then flew only a short distance towards the far wall before turning back.

A fly's average flight velocity is coupled to the distance it flies between saccades, because translational velocity drops during the execution of each saccade (see fig. 2C in Tammero and Dickinson, 2002a). Accordingly, short inter-saccade flight segments (i.e. low segment length) are characterized by reduced mean flight velocity (Fig. 6). As segment length increases, mean flight velocity spans an envelope within which the fly controls velocity over a range of approximately 200–700 mm s^{-1} (Fig. 6). We found no systematic effects of odor on flight velocity within any of the conditions we tested; flies in all treatments exhibited qualitatively similar envelopes in flight speed with respect to segment length.

To examine whether visual surroundings and odor affect the distance animals fly between saccades, we plotted segment length against odor distance for each saccade. In the absence of

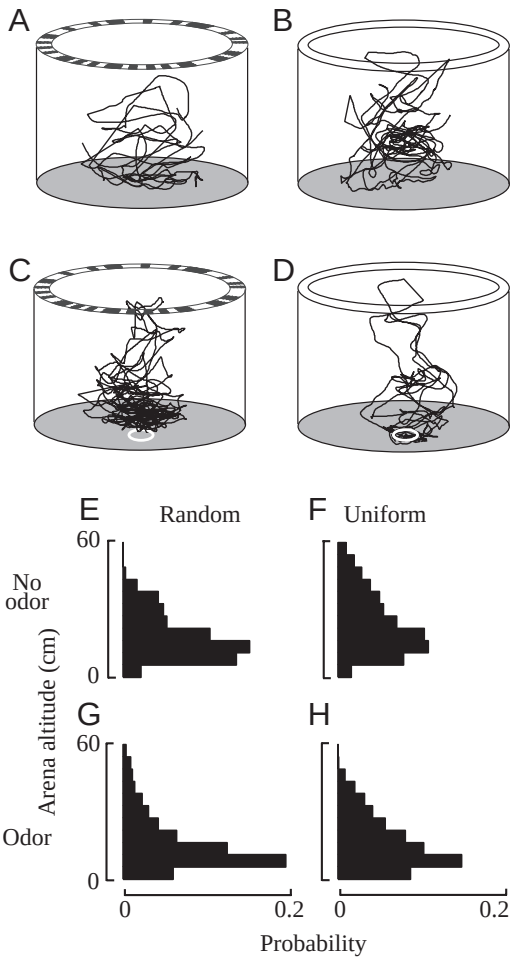

Fig. 3. Flies respond to odor by flying at lower arena altitudes. Sample flight trajectories for visual and olfactory treatments viewed from the side. (A) random checkerboard, (B) uniform, (C) checkerboard plus odor and (D) uniform plus odor. Probability histograms show distributions for altitude in (E) random checkerboard, (F) uniform, (G) checkerboard plus odor and (H) uniform plus odor. Location of odor source indicated by white circles. Numbers of flies and total flight times are the same as in Fig. 2.

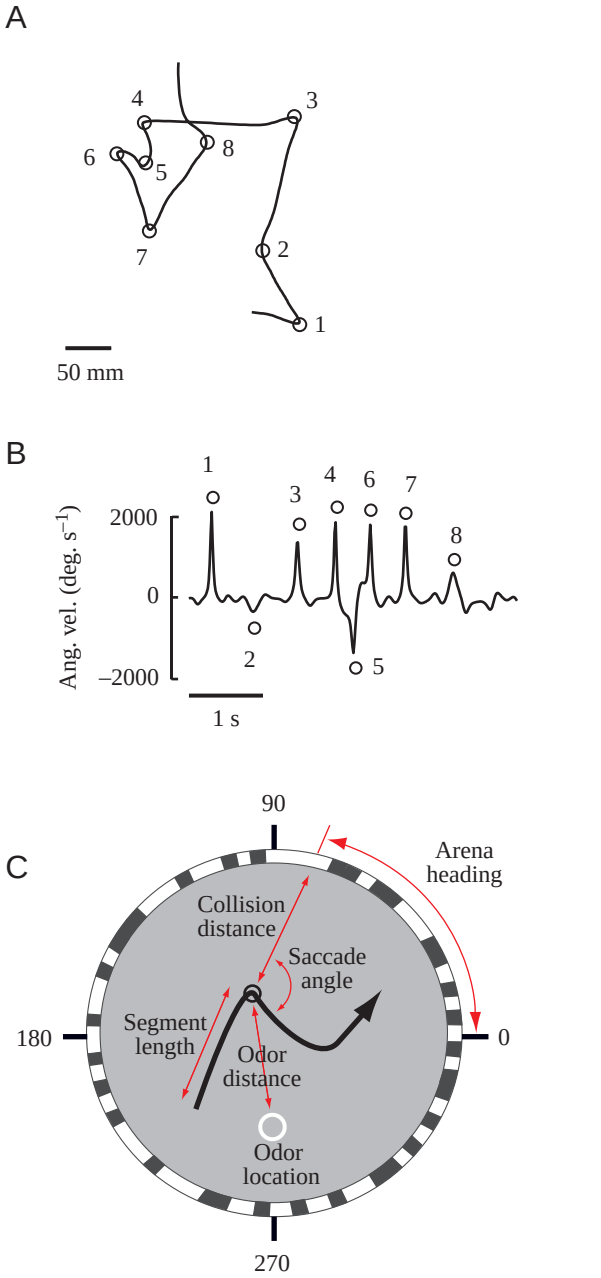

Fig. 4. Measured parameters from fly flight trajectories. (A) Flies exhibit segments of straight flight punctuated by turns of approximately 90°. (B) Saccades are characterized by rapid increases in angular velocity, therefore easily distinguished in time and space. (C) For each saccade, we measured several parameters of flight control, including saccade angle, collision distance, arena heading, segment length and odor distance.

odor, segment length shows no systematic variation for animals that flew within either the random or uniform backgrounds (Fig. 7A,B). These results were expected and serve as a control indicating that the visual features of the arena did not bias segment length between saccades in the absence of odor. Flies exposed to odor executed more saccades near the odor source, and these saccades accompanied short flight segments (Fig. 7C,D). To visualize this result better, we calculated the probability distributions and median segment lengths as functions of odor distance. When flying within both textured and uniform visual conditions, flies saccaded near the odor source with greater probability (Fig. 7E,F). Accompanying this shift in saccade probability, median segment length decreases near the odor source in both visual treatments (Fig. 7G,H). Although the magnitude of this response is greater for flies in the random checkerboard arena, animals in the uniform arena show qualitatively similar responses.

To examine how the spatial distributions of olfactory and visual cues within the arena influence where flies saccade, we plotted collision distance against arena heading for each saccade. In the absence of odor, collision distance shows no systematic variation with arena heading for either random checkerboard or uniform visual conditions (Fig. 8A,B). This result indicates that the arena contained no feature that biased the spatial distribution of saccades. By contrast, for flies flying in the presence of an odor within the random checkerboard arena, collision distance decreased for flight segments aimed towards the odor source and increased for segments headed away (Fig. 8C). When the checkerboard pattern was replaced with the uniform white surround, flies showed a much smaller modulation in collision distance. Thus, the influence of odor on the spatial distribution of saccades depends upon the characteristics of the visual background.

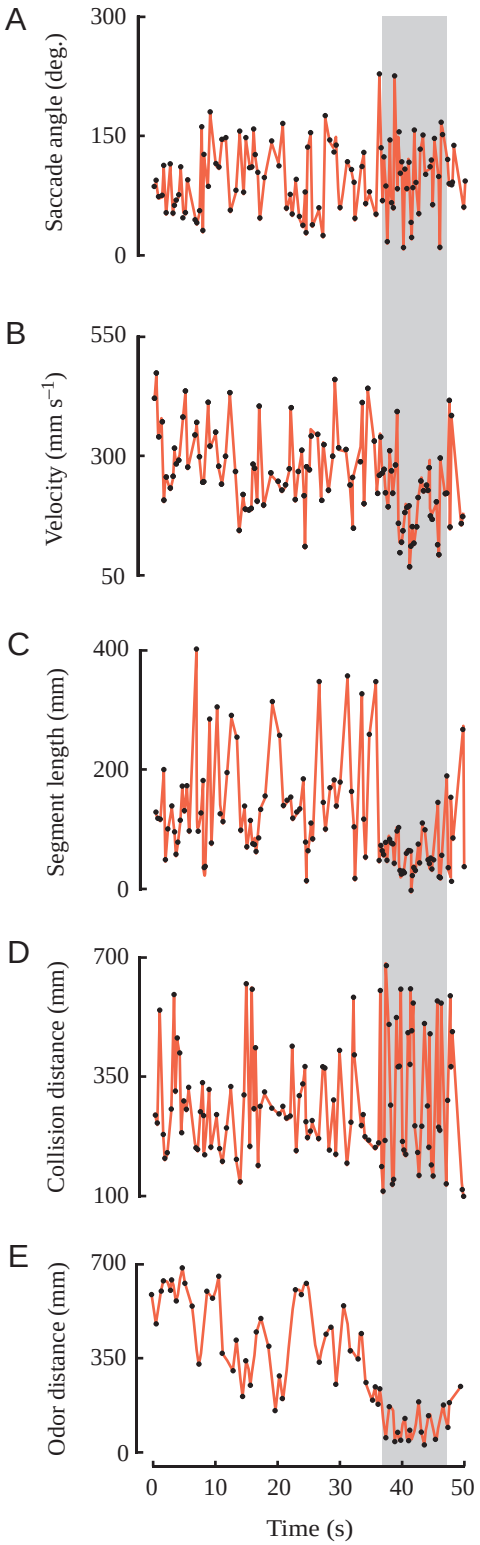

Fig. 5. For a sample flight trajectory within the random checkerboard arena, saccade angle (A) does not fluctuate, whereas velocity (B), segment length (C), and collision distance (D) vary as a fly approaches the odor source (E). The gray bar highlights low odor distance.

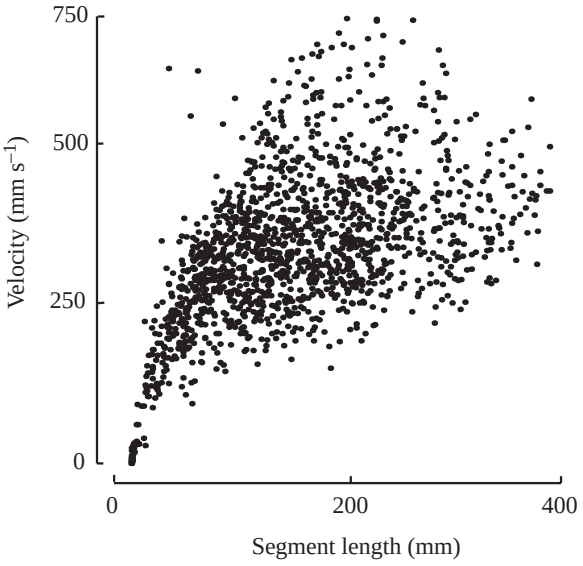

Fig. 6. Flight speed between saccades varies within an envelope across segment length. Each point represents the mean velocity between two saccades. Data plotted from the random checkerboard treatment. Numbers of flies and total flight time are the same as in Fig. 2.

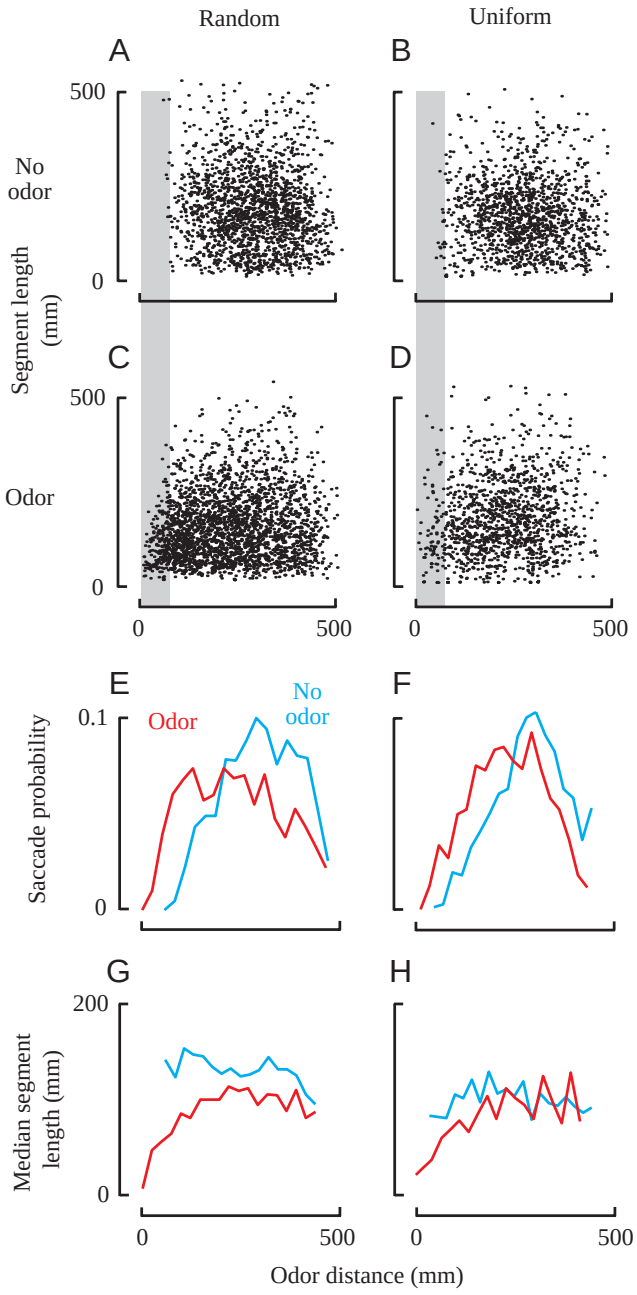

Fig. 7. Segment length between saccades is reduced near the odor source. Segment length plotted against odor distance for each saccade exhibited by flies flying in (A) random, (B) uniform, (C) random checkerboard plus odor and (D) uniform background plus odor. A greater proportion of saccades exhibited in odor treatments occurs near the source, and saccades exhibited near the source follow short segments (gray bars highlight data points within a 75 mm radius of the odor source). Probability distributions for each saccade were generated by binning segment length according to odor distance: (E) random checkerboard background and (F) uniform white background plus odor (red) or minus odor (blue). Median segment length was calculated for each 30 mm bin of odor distance: (G) random checkerboard background and (H) uniform white background plus odor (red) or minus odor (blue). Numbers of flies and total flight time are the same as in Fig. 2.

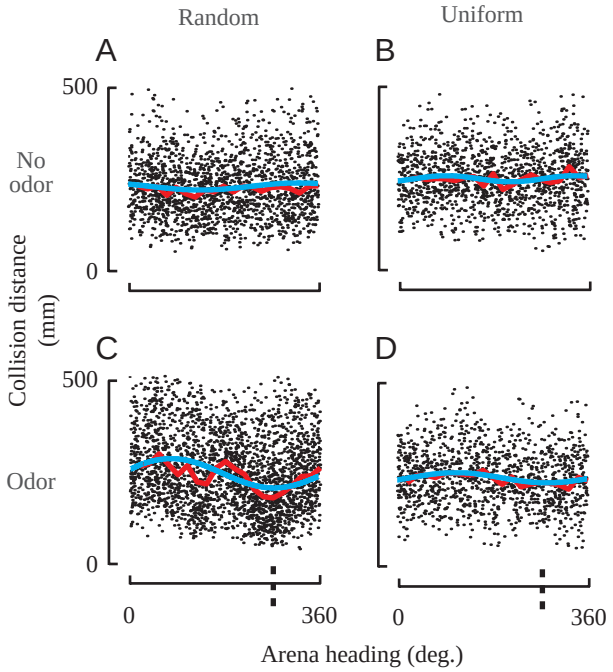

Fig. 8. Flies orient towards an odor source located near the wall at 270° (indicated by broken lines) when flying within visually textured surroundings. For each saccade, collision distance is plotted against arena heading, with median collision distance for bins in arena heading indicated in red and optimized sine fit indicated in blue. The amplitude of the sinusoidal fits indicates the distance between the peak in the average transit distribution and the center of the arena (for details, see Appendix). Random checkerboard (A), uniform (B), random plus odor (C) and uniform plus odor (D) treatments. Numbers of flies and total flight time are the same as in Fig. 2.

Homogeneous backgrounds of vertical stripes

During tethered flight, *Drosophila* can learn to recognize specific elements of a random checkerboard pattern (Dill et al., 1993). Pattern recognition enables them to ‘recall’ the spatial orientation of a visual pattern associated with an olfactory stimulus (Guo and Götz, 1997). These findings support the hypothesis that flies match spatially unique elements of the visual world with the strength of olfactory stimuli and might explain why flies in our experiments required a textured visual surround to localize an odor. To examine this possibility, we flew animals against two spatially uniform background patterns. The first pattern was composed of three thick vertical stripes separated by 120°. This background provides three obvious visual landmarks, which are visually indistinguishable from one another (Fig. 1D). The second pattern was composed of evenly spaced 5° vertical stripes (Fig. 1E) and contained neither salient landmarks nor spatially unique regions.

When flying within the three-stripe background in the absence of odor, flies approached one stripe, then saccaded towards another in turn, thus exhibiting roughly triangular flight trajectories and average transit distributions (Fig. 9A,C). Within a panorama of evenly spaced vertical stripes, flies did not show such directional preferences and flew more uniformly

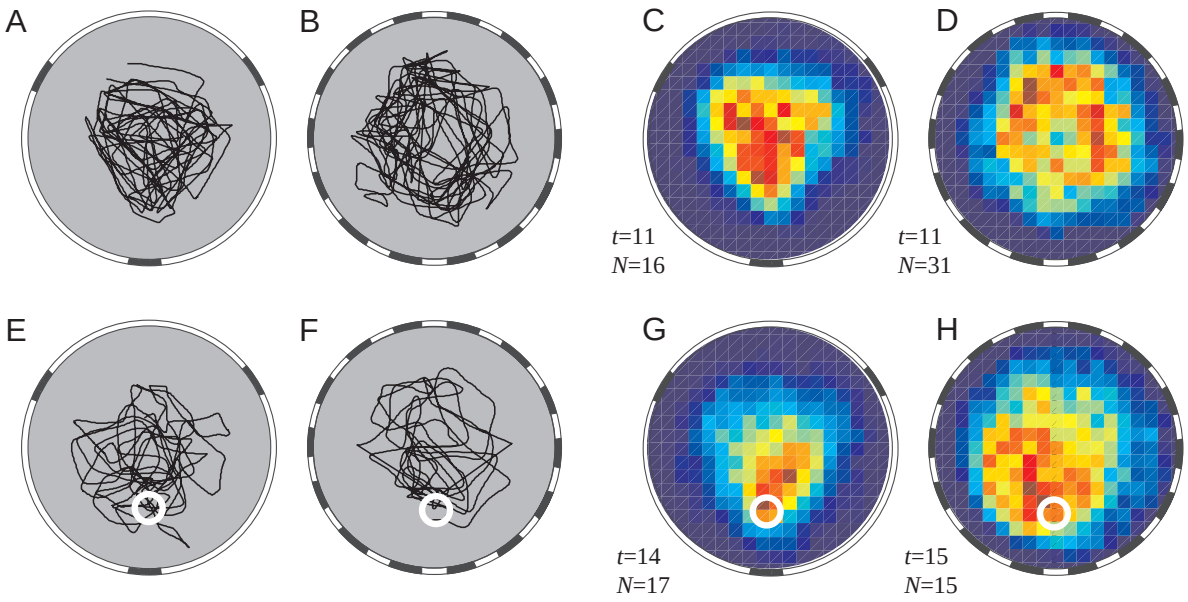

Fig. 9. Flies localize odor within backgrounds of uniformly spaced vertical stripes. Sample flight trajectories from flies flying within arenas of (A) three thick vertical stripes and (B) evenly spaced vertical stripes. Embedding a vial of apple cider vinegar in the floor of the arena (location indicated by white circles) resulted in biased flight trajectories for animals flying within both three-stripe (G) and multiple vertical stripe (H) treatments. Average position indicated with 2-D histograms plotted in pseudocolor. Position bins are 50 mm×50 mm. Number of flies (*N*) and total flight time in min (*t*) are indicated for each plot.

throughout the arena (Fig. 9B,D), similar to the pattern in the random checkerboard. When an odor was placed near one of the three vertical stripes, flies biased their orientation towards that stripe and away from the others (Fig. 9E). This resulted in a shift in the average transit distribution to the odor source (Fig. 9G). Similarly, within an arena of evenly spaced vertical stripes, animals shifted their flight path towards the odor (Fig. 9F,H). As before (Fig. 3), flies respond to the presence of an odor source on the floor by reducing altitude (Fig. 10). Collectively, these results show that flies do not require spatially unique visual patterns to localize the horizontal position of an odor source. These experiments do not exclude the possibility that flies use landmarks for odor localization under some conditions.

Consistent with results from the randomly textured and uniform visual treatments, animals exposed to odor within any arena containing vertical stripes saccaded with greater probability near the odor source than they would otherwise (Fig. 11A,B). Flies also reduced segment length and flight speed so that they tended to stay near the odor source (Fig. 11C,D). The strength of these odor responses in each visual treatment is consistent with the degree to which the mean transit distributions are biased towards the horizontal location of the odor. For example, the shift in odor distance probability is strongest for animals flying in the three-striped arena (Fig. 11A), the visual landscape in which the animals most effectively localized the odor (Fig. 9). The strength of odor localization in this visual treatment is likely to be enhanced because animals tend to spend more time near individual stripes than between them. Animals flying within visual backgrounds containing vertical stripes also saccaded closer to the wall (i.e. lower collision distance) when approaching the odor source than when flying away (Fig. 12). The results presented thus far suggest that, whereas flies require visual feedback to approach and localize an odor source, they do not rely solely on visual landmarks within the visual world. Instead, feedback generated by evenly distributed vertical edges alone is somehow sufficient to allow flies to find the source of an attractive odor.

Horizontally striped background

To examine whether odor localization requires image motion generated by vertical edges, we flew animals in an arena of evenly spaced horizontal stripes (Fig. 1F). The structure of flight trajectories exhibited by animals flying within this treatment is qualitatively different to those produced in all other visual conditions. In addition to flying much closer to the walls, flies responded to the horizontal stripes by flying along curved paths, which seldom crossed the center of the arena (Fig. 13A,C). Remarkably, when flying in an arena of horizontal stripes in the presence of odor, flies displayed the pattern of straight flight sequences interspersed with saccades that is characteristic of other visual treatments (Fig. 13B). However, the mean transit distribution was not shifted towards the odor (Fig. 13D).

In the absence of odor, flying within a horizontally striped arena, flies maintained lower altitude than when flying within a background of vertical stripes (Fig. 13E,G). Aside from

changes in the shape and spatial distribution of flight trajectories in the absence of odor, the effects of odor on altitude, segment length, and collision distance for flies flying within a horizontally striped arena were qualitatively similar to those measured within the uniform visual treatment (Fig. 8). Within an arena of horizontal stripes, flies responded to odor by decreasing altitude (Fig. 13F,H) and flying shorter segment lengths near the odor source (Fig. 13I,J). Coupled with their failure to localize the horizontal position of the odor source, flies within the horizontally striped arena showed only a weak modulation of collision distance with heading (Fig. 13K,L). These results suggest that visual feedback from vertically oriented edges is a critical cue both for flight control in general and for successful odor localization in the horizontal plane.

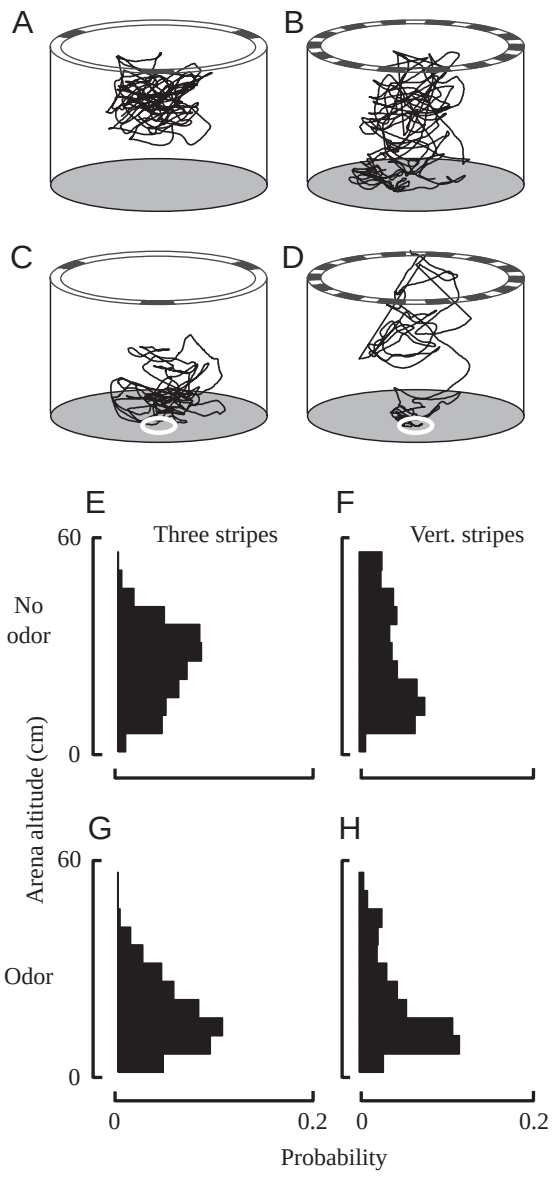

Fig. 10. Flies respond to odor by flying lower in the arena within vertical striped visual conditions. (A–D) Sample flight trajectories for visual treatments and odor viewed from the side (odor location indicated by white circles). (E–H) Average probability distributions for altitude (number of flies and total flight time are the same as in Fig. 9).

Simulated flight trajectories

We found no systematic effects of odor on saccade angle, saccade interval or flight velocity. We did, however, find that odor influenced the spatial distribution and distance flown between saccades. To test whether subtle modulation of these two parameters alone is sufficient to explain a fly's ability to localize odor, we constructed a simple model based on the statistics of free-flight behavior. We selected three components of search behavior to simulate – saccade direction, saccade angle and the distance flown between saccades (i.e. segment length). Gaussian functions for saccade direction and saccade angle were fit to probability distributions exhibited by real flies. Each simulated sequence began with a position and heading selected at random from these distributions. The collision distance along the current heading determined segment length whereas the angle of approach to the oncoming arena wall determined the probability with which the subsequent saccade would be directed towards the right or left (for details, see Appendix).

Given these conditions, the simulation produces individual trajectories that are qualitatively similar to those of real flies (Fig. 14A). Each simulated trajectory is composed of 200 saccades. One hundred iterations of replicating an animal flying in the absence of odor within the random checkerboard

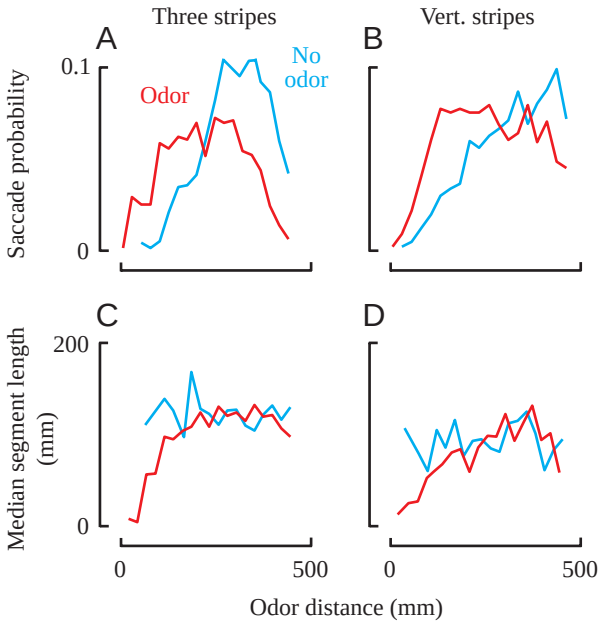

Fig. 11. Segment length between saccades is reduced near the odor source in vertically striped treatments. (A,B) Probability distributions for each saccade were generated by binning segment length according to odor distance (raw data not shown). (C,D) Median segment length was calculated for each 30 mm bin of odor distance. Odor treatments are indicated in red, whereas non-odor treatments are indicated in blue. Numbers of flies and total flight time are the same as in Fig. 9.

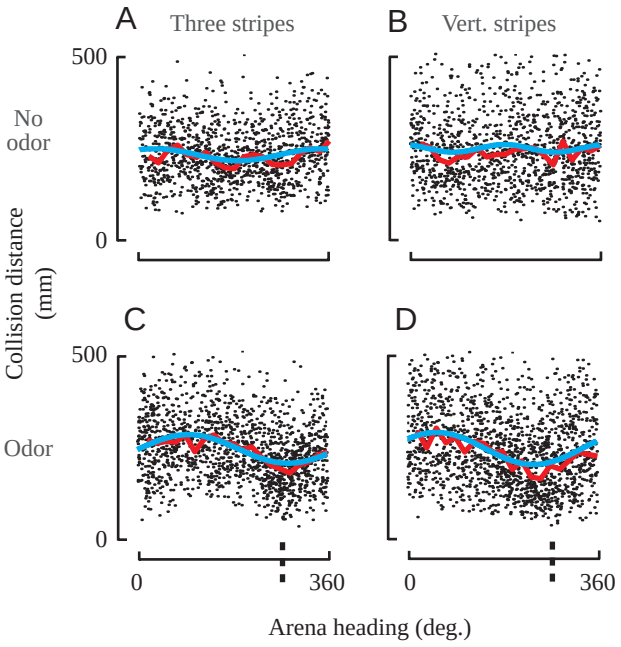

Fig. 12. Flies orient towards an odor source when flying within visual surroundings containing vertically oriented edges (odor location, 270°, indicated by broken lines). For each saccade, collision distance is plotted against arena heading, with median values of collision distance for bins in arena heading indicated in red and optimized sine fit indicated in blue (see Appendix). (A,B) Non-odor treatments, (C,D) odor treatment. Numbers of flies and total flight time are the same as in Fig. 9.

arena produce an average transit distribution centered within the arena (Fig. 14A, right column). Because we have not quantified the diffusion gradient or filamentous distribution of odor in the arena, we did not attempt to model its physical structure. Instead, we simulated the effects that increased odor strength has on locomotor behavior. The influence of odor on flight trajectory was modeled in two ways. In one case (odor influence 1), segment length was reduced by 30% for saccades occurring within a 75 mm radius of the odor source. In the other case (odor influence 2), collision distance was scaled by the sine of arena heading in a manner exhibited by real flies (for details, see Appendix). For simulations in which segment length was reduced for saccades near the odor source (odor influence 1), the mean transit distribution shifts just slightly towards the location of the odor (Fig. 14B). For simulations in which collision distance was modulated as a continuous

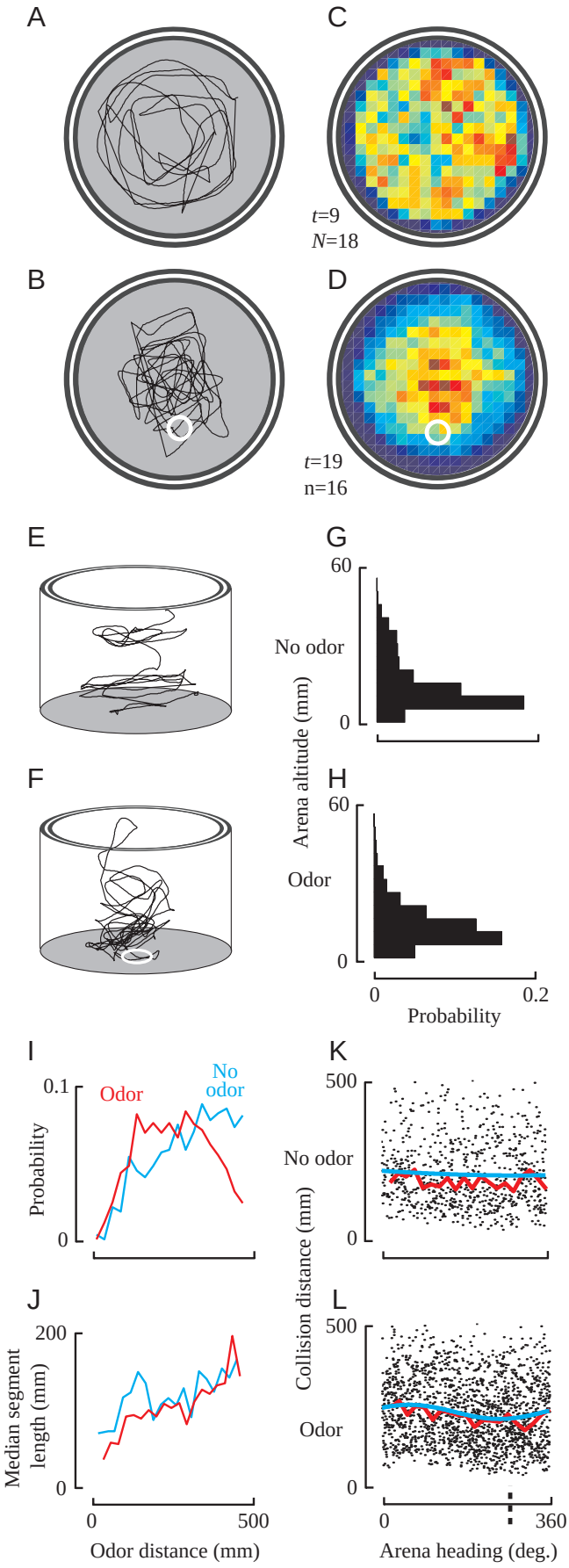

Fig. 13. Within a background of horizontal stripes, flies show qualitatively different flight trajectories compared with other visual conditions and fail to localize an odor source. (A,B) Sample flight trajectories and (C,D) mean transit distributions indicate that flies fly curved paths close to the wall. (E–H) Flies respond to odor by reducing altitude, as in Fig. 3. Probability distributions (I) and median segment length (J) shift for animals in odor as in other visual conditions. (K,L) Modulations in collision distance are weak for animals tested in odor. Median segment length and sine fits are determined as in Figs 8, 12.

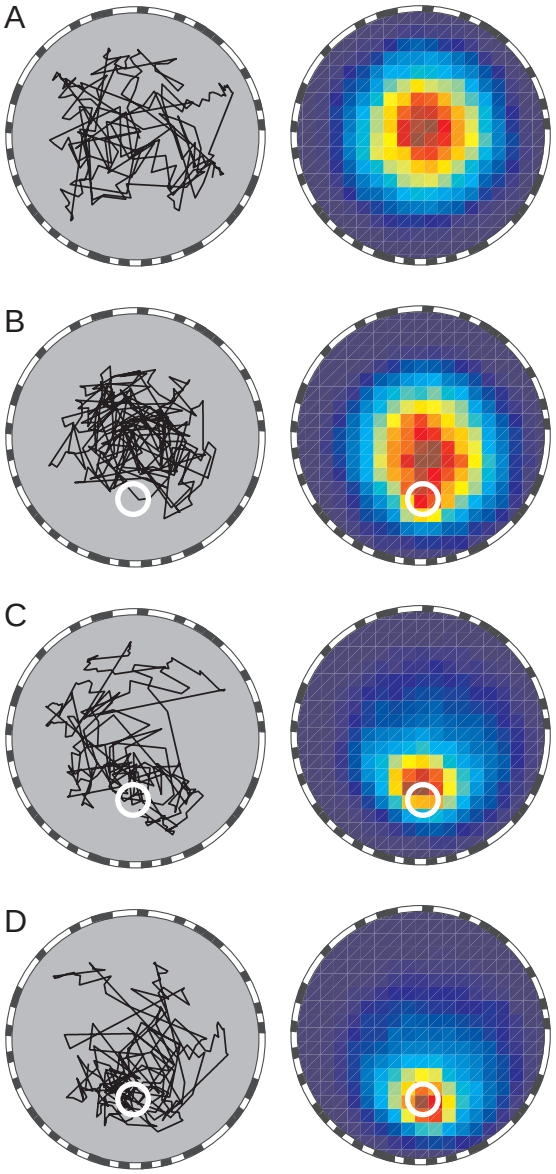

Fig. 14. Flight simulations based on trajectory statistics exhibited by real flies show that modulation of segment length and collision distance is sufficient to account for odor localization. Individual trajectories are composed of 200 saccades, and average spatial distributions resulting from 100 simulations are plotted in pseudocolor as in Fig. 2. (A) No odor modulation of saccade statistics. (B) For saccades that occur by chance near the odor source, segment length is reduced. (C) Collision distance scaled as a sine function of approach angle, as exhibited by real flies (see Appendix). (D) Both segment length and collision distance modulated by odor.

function of arena heading (odor influence 2), the mean transit distributions shift more notably towards the odor (Fig. 14C). For simulations in which odor influenced both segment length and collision distance (odor influence 1 and 2), the shift in transit distribution is also strong (Fig. 14D). The model suggests that, whereas flying short segments when odor concentration is high can help an animal remain near a source, a more robust strategy for odor localization is to maintain heading when flying towards the odor source and turn sooner if flying away.

For experimental treatments in which flies localized the odor source successfully, the transit distribution peak lies slightly centripetal (towards the center) of the location of the odor source (Figs 2G, 9G,H). This could result from competing influences of the attractive odor and the visual expansion cues. As the fly approaches the odor (and the arena walls), visual collision cues increase, causing the fly to saccade before reaching the odor source. Results from the simulations support this idea. For the simulation in which the spatial distribution of saccades was determined by an odor-modulated visual parameter (collision distance, Fig. 14C), the peak distribution was slightly offset from the location of the odor source. However, for simulated saccade distributions mediated by both visual cues and the proximity of the odor source (a visually independent parameter), the mean transit is coincident with the odor source.

Discussion

The results of these experiments suggest that *Drosophila melanogaster* require visual feedback to localize the horizontal location (Fig. 2), but not the vertical displacement (Figs 3, 13), of an attractive odor source. Consistent with this finding, the visual cues that flies use to localize odor emerge from vertical, not horizontal, edges (Figs 2, 9, 13). Odor tracking in the horizontal plane has two components, an increase in saccade rate (decrease in segment length) near the odor source and changes in the spatial distribution of saccades that depend upon heading. The first is reminiscent of a non-directional kinesis. Animals fly short distances between saccades if they happen, by chance, to fly close to an odor source, regardless of the visual landscape (Figs 7, 11, 13). The second component is a visually dependent taxis in which flies more closely approach a textured visual background before saccading away if they are flying towards the odor source. Conversely, when headed away from the odor they saccade earlier and at a greater distance from the textured background (Figs 8, 12). By simulating the effects of odor on the distance between saccades and their spatial distribution, we showed that a combination of these behavioral algorithms could explain how flies localize odor during free flight (Fig. 14).

Limitations of the tracking system may underestimate odor responses

Although the flight arena used in this study was nearly identical to that used by Tammero and Dickinson (2002a), differences in the video tracking systems contributed to small discrepancies in the data presented in the two studies. For both studies, stereo cameras viewed the arena from above, producing a cone-shaped viewing area (Fig. 1A). Therefore, trajectories near the walls might not be visible if the fly is flying high in the arena. This optical constraint generates fragmented trajectories at elevated regions in the arena as the fly moves in

and out of the field of view. The off-line tracking system used by Tammero and Dickinson analyzed individual images from each camera separately, allowing limited 3-D interpolation if the fly briefly disappeared from one camera's view. In effect, the viewable 'cone' was wider in their experiments than in ours, minimizing the centering bias for trajectories high in the arena. The tracking system used here was advantageous in that it operated in real time at 60 Hz, enabling us to perform more experimental trials at higher temporal resolution. However, this system records the fly's position only if it appears in both camera views, thus it is impossible to improve the spatial bias imparted by the camera geometry.

Because an analysis of saccades requires a minimum trajectory length, we only analyzed sequences greater than 5 s. This strategy underestimates the magnitude of altitude responses to odor since our database contains fewer trajectories near the top of the arena. Nevertheless, the results presented here show that animals consistently respond to odor by shifting their altitude towards lower regions of the arena (Figs 3, 10, 13). Additionally, we performed control experiments on a total of 36 flies with the uniform, random checkerboard and three-stripe visual backgrounds using the off-line reconstruction system. The results support the findings of Tammero and Dickinson (2002a) for spatial comparisons between uniform and textured background and also show shifts in altitude, mean transit distribution and collision distance in response to odor that are identical to those presented here.

Kinesis and taxis strategies for odor tracking

An animal tracking an attractive chemical odorant homes in on its source by either modulating its speed or turning rate with respect to the strength of an odor cue (kinesis) or by continuously orienting up the concentration gradient in still air (taxis) (Fraenkel and Gunn, 1961) or up the plume in turbulent air (Vickers, 2000). In kinesis, the direction that the animal turns is selected randomly, but either turn rate or translational velocity is proportional to the strength of the stimulus. Thus, the animal's average position is maintained near the odor source. In a taxis, the animal turns in the direction of the stimulus and maintains heading, thus directing its locomotion and average position towards the source (Dunn, 1990). A fruit fly's average position during flight is determined primarily by where the animal executes saccades (Tammero and Dickinson, 2002a). Therefore, to understand the behavioral strategies and neural mechanisms underlying odor localization in flies, we must consider how olfactory cues affect where and when a fly saccades.

The distance a fly covers between saccades (i.e. segment length) varies between 50 mm and 400 mm, but, when flies are within approximately 75 mm of the odor source, they reduce segment length (Figs 5, 7, 11, 13). A succession of short segments helps maintain the fly's position near the odor. Individuals show this response, to varying degrees, in each of the visual landscapes we tested (Figs 7, 11, 13). This suggests that, whereas on average flies may be unable to orient towards the odor without appropriate visual cues, those that happen by chance to approach the odor source respond by decreasing segment length. To explore whether this strategy could enable flies to localize an odor source over time, we modeled a kinesis in which simulated trajectories passing within a 75 mm radius of the odor source were assigned a shifted distribution of segment lengths – the lower 30% values exhibited by real flies. Our simulations suggest that a visually independent 'chemokinesis' can subtly bias a fly's average position towards the odor source (Fig. 14B) but the effect is not strong.

Flies flown in an arena containing high-contrast vertical edges maintain course heading to closely approach the arena walls when they are aimed towards the odor whereas they saccade farther from the looming wall when aimed away (Figs 8, 12). We modeled this taxis component of odor localization by scaling collision distance as a sinusoidal function of arena heading, according to the responses exhibited by real flies (see Appendix; Figs 8, 12). This simulation of chemotaxis produces a strong shift in the average transit distribution directly over the odor source (Fig. 14C). In combination, the simulated effects of odor on both segment length (non-directional kinesis) and collision distance (directional taxis) result in a narrowly tuned average transit distribution, centered over the odor source (Fig. 14D). This model is based on a small subset of control parameters and suggests that a subtle modulation of a single output parameter – the spatial distribution of saccades – is sufficient to explain how flies locate the source of an attractive odor in flight.

Our results indicate that the fly's olfactory system is able to distinguish whether it is moving towards or away from an odor source. There are several features of the odor plume that might permit such discrimination, such as the spatial and temporal distribution of cohesive plume fragments and a diffuse spatial gradient in odorant concentration. During walking, *Drosophila* are able to orient within a gradient in odor concentration delivered to their two antennae (Borst and Heisenberg, 1982). Additionally, flies tethered in the dark maintain a net forward flight orientation when stimulated with an oncoming odor plume (Wolf and Heisenberg, 1991). As yet, however, there is no definitive evidence that fruit flies detect spatial gradients in free flight.

The visual estimation of collision distance

Honeybees (*Apis mellifera*) use the perceived speed of image motion both to regulate their distance to visual surroundings (Kirchner and Srinivasan, 1989) and as a flight odometer (Srinivasan et al., 1997). *Drosophila* use similar visual processes to regulate ground speed and altitude during free flight (David, 1979). At least in theory, an animal moving at known speed can use the apparent motion of the visual world generated during straightforward flight to determine the distance to an object (Srinivasan, 1993). As shown for bees (Srinivasan et al., 1997), we might expect that flies use patterns of image motion to regulate their distance to objects (i.e. collision distance). If this is true, we might expect that flies would fail to estimate distance within a visual background of horizontal stripes that generates little apparent image motion.

Indeed, flies fly much closer to the walls within the horizontal striped arena than in any other visual condition we tested (Fig. 13). In addition, the curved flight paths exhibited in this visual treatment suggest that flies are not simply lacking important stabilizing cues, rather the pattern of horizontal stripes somehow perturbs fundamental components of flight control. Flies fly straight trajectories within every other visual condition we tested, including the uniform white surround, which contains very few stabilizing cues.

The importance of vertical edges is especially evident for flies seeking the source of an attractive odor. Although they fly closer to the walls in a horizontally striped arena when presented with odor (Fig. 13L), flies fail to selectively maintain their position near the odor source (Fig. 13B,D). These results suggest that flies somehow require feedback generated by the motion of vertical edges to maintain and modulate collision distance as they search for an attractive odor.

Using image motion to control the distance to objects, however, is not the only visual behavior that might be influenced by olfactory cues in chemotaxis. Another possibility is that flies stabilize their gaze upon a visual object when headed towards the odor source – in essence 'ignoring' global image motion in order to approach the object. Our results show that *Drosophila* does not need to associate a unique visual pattern with the strength of an odor cue, rather they may be using only general forms of visual landmarks to maintain their current heading if odor cues are strong.

Olfactory influence on orientation and collision avoidance

As a fly approaches visual objects during flight, the object's image on the retinae expands. The combination of horizontal and vertical image expansion is a powerful stimulus for collision-avoidance saccades in *Drosophila* (Tammero and Dickinson, 2002a). How then are flies able to override a collision avoidance reflex to approach and land on an enticing visual object? The answer might lie in the geometry of image motion during free flight as well as parallel neural processes operating within the flight control system. An animal flying straight through a visual landscape that is infinitely distant experiences image expansion radiating from a pole located along its heading. In the absence of yaw or side slip, the pole is imaged on the frontal region of the retina. Any simultaneous rotation or side slip will shift the pole of expansion in the direction opposite to the rotation, towards lateral regions of the eye (Srinivasan, 1993). In *Drosophila*, image expansion on lateral regions of the retina triggers collision-avoidance saccades aimed away from the expanding stimulus, whereas expansion in the frontal region triggers motor responses involved in landing (Tammero and Dickinson, 2002b). Olfactory input may modulate the output of these parallel motor pathways to differing degrees, thus 'biasing' an animal's tendency to maintain heading and approach an expanding visual image.

Flight behavior in flies emerges from a vast sensory-to-motor convergence. Feedback from tens of thousands of peripheral sensory and central brain neurons is ultimately filtered through the activity of just 17 pairs of muscles that control the steering motions of the wings (Borst and Dickinson, 1999; Frye and Dickinson, 2001). Wherever visual and olfactory fusion takes place within this sensorimotor cascade, it is a crucial process in the control of free-flight behavior in *Drosophila*. Odor might directly influence complimentary visual processes such as distance estimation, object fixation and collision avoidance. Alternatively, indirect olfactory modulation of the motor circuits that control the distance they fly between visually elicited saccades might explain how flies localize attractive odor sources during flight in varied visual landscapes.

Appendix

Details of the computational simulation of flight trajectories

As a fly cruises straight across the arena at constant velocity, the rate of frontal image expansion increases by roughly the square of the distance to the visual image (defined here as collision distance) (Gabbiani et al., 1999). In our simulation (implemented with custom routines written in MATLAB v.6), the initial position and heading were selected at random, and flight velocity was constant at $300\,\mathrm{mm\,s^{-1}}$. The simulation randomly selected values for (1) segment length between saccades, (2) saccade direction and (3) heading from Gaussian distributions fit to probability densities exhibited by real flies (see below).

For the current (i^{th}) saccade, the length of the j^{th} flight segment (L_j) was determined by:

$$L_j = \beta_i^2 c\,, \tag{A1}$$

where β is the collision distance at the i^{th} saccade, and c is a constant used to simulate the effects of odor. In response to odor, real flies show reduced collision distances when headed towards the odor source (e.g. arena angles near 270°) and *vice versa* when flying away (e.g. Fig. 8C). We approximated the modulation of collision distance (β) as a function of arena heading (γ) with the following periodic equation:

$$\beta(\gamma) = a[\sin(\omega\gamma + \phi)] + O\,, \tag{A2}$$

where *a* represents response amplitude, ω defines cycle period, ϕ is the phase, and *O* is an offset in collision distance. We used a Nelder–Mead nonlinear minimization routine written in MATLAB to fit *a*, ϕ and *O* to the distribution of $\beta(\gamma)$ exhibited by real flies (see blue lines in Figs 8, 12). For the model, parameter values were means of optimized function fits to the random checkerboard, three vertical stripe and alternating vertical stripe data sets.

For real flies, the direction that an animal saccades is tightly correlated with the angle between the current heading and a line perpendicular to the tangent at the interception with the arena wall (Tammero and Dickinson, 2002a). For example, a fly approaching the arena wall on its left will very likely exhibit a saccade to its right. Therefore, saccade direction was chosen at random from a probability distribution exhibited by real flies (see fig. 7 in Tammero and Dickinson, 2002a). The probability

distribution was approximated with a half-Gaussian function centered at –40° with an S.D. of 194. Therefore, an approach angle of –40° corresponds to a saccade to the left 80% of the time. Saccade amplitude was randomly chosen from a normal distribution generated with a Gaussian function (mean=90° and S.D.=52).

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