Peer Review File

Manuscript Title: Odor motion sensing enhances navigation of complex plumes

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1 (Remarks to the Author):

This manuscript tackles a very exciting topic in system neuroscience: how animals extract directional information from turbulent olfactory environments to perform robust navigation. Using Drosophila has a model system, the authors uncover a new type of cue, odor motion, which can be exploited to direct orientation decisions. Odor motion results from the temporal correlations of odor signals measured between multiple sensors — in the case of Drosophila, two antennae separated by less than 300 um. By drawing a fruitful analogy with insect vision, the authors propose that the detection of odor motion is achieved by an algorithm similar to the Hassenstein-Reichardt correlator (HRC) model — a model that has inspired research in the field of visual motion detection for more 60 years. The manuscript convincingly establishes that odor motion is a source of directional information that has been overlooked until now. Using a virtual-reality paradigm, the authors provide strong evidence that odor motion can direct the orientation of walking flies in odor plumes. Based on this result, the reader is left with the exciting idea that odor motion might direct insect flight behavior as well as the navigation of terrestrial animals beyond invertebrates.

The findings of this manuscript rely on one of the smartest use that I have seen of virtual-reality (VR) paradigms in olfaction. By engineering patterns of optogenetics-driven activity in the odorant receptor neurons, the authors are able to probe the ability of Drosophila to process spatio-temporal olfactory information collected by their left and right antennae. This approach is remarkable because it enables the authors to test predictions derived from the hypothesis that the olfactory system of the adult fly functions in a way similar to the HRC model. Besides being elegant, the VR approach is powerful because it creates experimental conditions where specific quantitative predictions can be made. This idea is well illustrated in Figure 5 by the replay of temporally reversed fictive odor ribbons to Orco>Chrimson flies. To disentangle the inherent correlation between wind sensing and the olfactory information comprised in odor plumes, the authors take advantage of a series of non-intuitive stimulation protocols derived from the study of visual motion detection. The wit behind the experimental design is truly commendable!

The manuscript includes two components: (1) a technical analysis leading to the identification of odor-motion information in odor plumes and the creation of optogenetic stimulation protocols to present flies with controlled odor motion; (2) a series of experimental manipulations aiming to demonstrate that Drosophila are capable of making use of odor motion to orient and that this source of sensory information is significant compared to other navigation strategies (e.g., gradient sensing through the detection of small gradients between the left and right antennae). The first component of the work is expertly conducted: its presentation is relegated to the methodology part of the manuscript. While this part of the manuscript is essential, its level of technicality will only be fully appreciated by a small group of specialists. The presentation and discussion of the 4 correlated noise stimuli used in Figure 4 will be difficult to follow by a board readership. A more intuitive discussion of the values of the correlation matrix would be helpful. Except for Figure 4, the authors do an admirable job at guiding the reader through the logic of their sophisticated analysis.

The main emphasis of the manuscript is placed on the experimental examination of the role of odor motion sensing in free moving Drosophila. Below, I will focus my discussion on the approach and results associated with the experimental manipulations. While the authors report a convincing set of observations supporting the idea that odor motion alone is sufficient to direct navigation decisions, the relative importance of this mechanism compared to other strategies used by the fly is not entirely clear. In particular, the results of Figure 5 suggests that the contribution of odor motion might be modest. In principle, the relative importance of different sensorimotor mechanisms that have been proposed in published work by the Wilson lab (DOI: 10.1038/nature11747), the Nagel lab (DOI: 10.7554/eLife.37815) and the authors themselves (DOI: 10.7554/eLife.57524) could be tested in agent-based simulations. As part of Figure 5, the authors turn to such simulations to test the role of odor motion, but the set of basic navigation rules included in their model is too crude to reproduce realistic fly behavior.

Together the results of this manuscript reveal that odor timing can be combined with bilateral sensing to extract directional information from odor plumes. These results invite the field to search for the neural correlates of a circuit equivalent to Hassenstein-Reichardt detector in the fly olfactory system. Drosophila is perfectly suited to pursue this exciting research goal given the existence of a circuit diagram of its antennal lobe and the ongoing reconstruction of higher-order olfactory centers.

Comments and suggested improvements:

[General comment about the assay] The assay consists of top and bottom glass surfaces with acrylic sidewalls. It is stated in the caption of Supp. Figure 3 that "it is difficult to distinguish flies walking on the top and bottom surface of the assay." Unless the reviewer is mistaken, the analysis pools the response of flies that are standing upward on the surface of assay or that are lying upside-down from the ceiling of the assay. Is there any clear evidence that flies respond to the odor plumes in the same way in an upright and inverted (upside-down) orientation? If so, such evidence should be discussed in the manuscript. If not, Teflon coating could be applied to the wall to prevent flies from climbing on the ceiling of the assay so that the behavioral characterization could be limited to flies in the upright position.

[Figure 2] Responses to ON and OFF edges is predicted to differentiate between direction sensing and the more classic mechanism of gradient sensing. This experimental paradigm is excellent.
- In panel 2F, the turning bias is quantified as the sign of the change in orientation over a time window from 150 ms and 300 ms after the bar onset. How was this time window established? How does a reaction time from 150 ms and 300 after the bar onset fit with previously published data for Drosophila? For flight behavior, van Breugel and Dickinson (DOI: 10.1016/j.cub.2013.12.023) have shown that the surge response is on the order of 200 ms, which is consistent with a 150-300 ms window used in the present manuscript. However, the response to plume loss involves a

sensorimotor delay on the order of 500 ms in flying flies. This indicates that 300 ms might be too short for the OFF edge response. The response time observed in the present study should be discussed in light of these results together with those reported by the Nagel lab (DOI: 10.7554/eLife.37815). While I do not anticipate that the results of Figure 2 would change significantly, a more careful inspection of the temporal characteristics of the window averaging could impact the results of the controls shown in Supp. Figures 3a and 4.
- In Figure 2, the authors use a bar speed of 15 mm/s. Could a rationale be given for this speed of the moving bar? Was this value found to be optimal through a process of trial and error? More generally, could the estimation of the delay timescale of the HRC model derived in Figure 4 be used to make predictions about the performances of flies responding to different speed of the moving bars presented in Figure 2 and Supp. Figure 4?

[Supp. Figure 3]
- The authors rule out that flies with a single antenna can response to pure odor motion. This result represents an important sanity check. It would be useful to extend this control with single antenna flies to the correlated noise stimuli presented in Figure 4..
- The author state in the caption of Supp. Figure 3 that "right- and left-antenna ablated flies are pooled." Given that adult flies display handedness (e.g., DOI: 10.1073/pnas.1500804112) pooling of the behavior mediated by the left and the right antenna is suboptimal. The two groups of flies should have been tested in separate trials.
- The Or42b>Chrimson behavior shown in Supp. Figure 3 is nice addition, but its purpose is unclear. In addition, there must a typo in panel a of Supp. Figure 3 where Or42b>Chrimson flies are labeled as “single antenna”. The fact that the Or42b>Chrimson flies with unilateral olfactory input could respond to odor motion with a turning bias would go against the model proposed by the authors. In addition, the authors should quantify their data for Or42b>Chrimson flies with the same graph shown in Figure 2f for the turning bias versus orientation response.

[Supp. Figure 4]
- It would be important for the authors to further the correlation between the bar speed and the ON and OFF edge responses observed behaviorally. Can the HRC model account for the difference in the performances to the ON and OFF edge responses? Is it possible that stronger OFF edge responses would be found if the 150-300 ms time window used in the data analysis is extended beyond 300 ms? (see discussion of Figure 2) In addition, it would be very informative to characterize the behavior to faster bar speeds that the fly cannot keep up with and bar speeds that are too slow (<1 mm/s) to elicit an ON-edge response. These behavioral observations would provide additional conditions to test the consistency of the HRC model derived in Figure 4.

[Figure 3] In this figure, the authors start by hypothesizing that the response to the combination of odor-motion and wind signals obeys a summation rule. Their experimental observation corroborate this hypothesis. Again, this hypothesis-driven approach is very effective.
- The protocol of Figure 3 relies on the combination of one value of odor motion (bar speed) and wind speed. From the results of Figure 3d, it appears that odor motion dominates over wind sensing for the experimental conditions chosen by the authors. The authors should push the analysis further to determine whether the combination of wind and odor-motion sensing is dependent on the strength of each sensory cue. For slower or faster bar speeds that produce weaker behavioral

responses (see results of Supp. Figure 4), does wind sensing become dominant over odor motion?

[Figure 4]
- On line 158, it is stated that data from ON and OFF edges are pooled in the analysis. Since the results of Supp. Figure 4 show that the behavioral response to the ON and OFF edge is asymmetrical, pooling data from ON and OFF edges does not seem to be legitimate. Both conditions should be kept separate or the equivalence of the ON and OFF edge response should be established for the stimulation protocols of Figure 4.

[Figure 5] Here the authors aim to show that odor motion provides directional cue complementary to odor gradients and wind motion, and enhances navigation in complex odor plumes. While this statement represents an important conclusion of the study, it is not strongly supported by the data presented in Figure 5.
- While I appreciate the elegance of the stimulation design tested in Figure 5d, are the behavioral results not an extension of the ON/OFF edge responses presented in Figure 2 in presence of wind? How does this protocol go beyond Figures 2 and 3?
- In panels 5e and 5h, the authors use two paradigms to characterize the contribution of odor-motion to the navigation of free moving flies. Consistent with the rest of the work, the design of the VR stimulation protocols is clever. However, the interpretation of the results is not straightforward due to the very low success rates of the flies. For the comparison between the forward and temporally reversed plumes, only 3% out of 295 flies managed to reach the source with the forward plume. Less than 9 flies were successful. The temporally reversed plume structure leads to a reduction of the success rate to 2 (?) flies, prompting the authors to conclude that odor motion sensing enables flies to navigate the forward plume structure. Although the difference between forward and reversed plumes is significant, flies do not navigate the plume replay accurately. A more appropriate conclusion seems to be that odor-motion sensing contributes to very weak navigation behavior. The same applies to the outward and inward bar protocol of Figure 5d, though the success rate is modestly higher (less than 10% of the flies successfully reach the source). The authors should strengthen these results to support one of the major conclusions of their study.
- On line 259 it is stated that "with an eye toward practical applications" the authors used "in silico experiments to explore the impact of odor motion sensing for robots obeying simplified navigation algorithm." While using a simplified navigation algorithm might be a useful start, the authors are clearly equipped to test the contribution of odor-motion sensing to a much more realistic model combining mechanisms of sensorimotor control published by their lab and others (e.g., DOI: 10.7554/eLife.37815). The behavior of the fictive robots (Figure 5k) appears to be dramatically different from the trajectories of real flies shown in Figure 5h. It would be important to demonstrate the relative contribution of odor-motion sensing in the context of a model that is reasonably good at accounting for the navigation of real flies.
- Minor: the statistical significance of the t-test should be indicated in panel 5f.

Referee #2 (Remarks to the Author):

Kadakia and colleagues investigate the ability of walking Drosophila to use temporal correlations between activity in their two antennae to navigate odor plumes. Using patterned optogenetic activation (artificial and based on simulated plumes) of the olfactory receptor neurons (ORN) in the antenna, they find that flies will turn towards the antenna that is activated first (counterturning against the direction to the fictive “odor motion”), which would be expected to promote navigation towards a fictive odor point source. The authors describe the underlying computations as being algorithmically analogous with those in motion vision detection circuits.

This paper provides a series of convincing experiments demonstrating that temporal correlations in bilateral antennal activity can bias fly walking behavior under constrained, well-controlled experimental settings. The larger question is whether, given the size of these effects and the conditions in which flies would be expected to encounter odor motion in the natural world, whether information from antennal correlations contributes significantly to navigation of natural plumes by Drosophila. If the latter is the case, this study would be an important and novel contribution to a relatively well-developed and mature field of study on neural mechanisms of insect plume navigation.

Several additional pieces of data or analysis would substantially strengthen the case that antennal correlations are used in natural plume navigation:

1. Evidence that temporal correlations in bilateral antennal activation (in at least some positions) in a naturalistic odor plume fall within the range of magnitudes and timing differences (Figure 4b, Supplementary Figure 4) that the authors show can elicit turning. Simultaneous bilateral recordings from a specific identified ORN type in each antenna in response to a naturalistic plume (which the authors have experience generating from their behavioral assays) would help to clarify if correlations of the necessary temporal scale occur in response to natural odor stimuli.

2. Additional consideration and discussion of the mechanisms by which the olfactory circuit architecture and bilateral PN signaling could feasibly transmit relative timing information between antennae to a downstream decoder is warranted. This is because, whereas flies can obviously perform the antennal comparison across many trials of precise, repeated optogenetic “motion”, it would be a computational feat by the fly to extract reliable timing differences between antennae on the order of ~16-25 ms (based on estimated odor motion of 10-15 mm/s). First, ORNs are noisy and the latency to the first spike of an odor-evoked response varies considerably across the ORN population (~40-50 ms, Bhandawat et al. 2007). Because of this, PNs averaging over many noisy ORNs have relatively long integration windows (estimated ~30 ms, Jeanne & Wilson, 2015), on a similar timescale to odor motion antennal timing differences. Second, ORNs project bilaterally, with PNs receiving an estimated ~60% of presynaptic input from ipsilateral ORN population and ~40% of its presynaptic input from the contralateral population (Gaudry et al. 2013, Tobin et al.). This asymmetry leads to a ~30% difference in response strength between ipsilateral and contralateral PNs to a lateralized odor input. A downstream detector would have to extract these bilateral differences on a finer timescale than the timing difference between antennae. Thus, it’s likely that strong antennal activation with repeated, reliable timing differences would be required to have any significant impact on behavior. This also strongly motivates point 1 above to establish the magnitude and temporal properties of antennal correlations in naturalistic situations.

3. A critical set of experiments is in Figure 5d-i. Only a single metric is extracted from this rich

dataset (fraction of flies at the source), and the vast majority of flies do not reach the source in either experimental or control condition (<5%). This observation suggests that flies are not navigating the fictive plumes particularly well by this metric and additional behavioral outputs should be examined. Based on the authors' model, the improved navigation to the fictive source in the experimental plumes (as compared to control plumes) presumptively depends on enhanced lateral movement towards the midline, but not increased upwind navigation. To test this prediction, the authors could analyze the walking trajectories further to determine the relative rates of upwind and lateral displacement (towards the midline) during encounters with fictive odor (light) between the experimental and control plumes.

4. Can the fictive plume experiments (Figures 5d, 5g) be repeated in the absence of laminar wind as an additional navigational cue? Rather than examine the ability of flies to navigate to the upwind source, the percentage of flies that navigate to the center zone (along the lateral axis) of the fictive plume would be compared for normal/outward versus reverse/inward fictive conditions.

Minor Points:

1) In Figure 4, the correlator τ is fit with both ON and OFF edges. However, Supplementary figure 5 shows that only ON edges evoked major turning bias in the presence of wind. Does this effect the effectiveness of this model?
2) In earlier figures, the turning bias is fit to the sine of the angle of the fly with respect to the stimulus motion (equivalent to a linear fit to the distance of the antennae along the direction of motion, or the time delay between stimulation of the antennae). However, the correlator model in Fig 4b suggests a sublinear fit of turning bias to ΔT. Does accounting for this remain consistent with the previous sine fits?
3) Supplementary Fig 1a: It would be helpful to make the orientation references of the fly consistent with the ones shown in the main text (and Supplementary Fig 5).
4) Supplementary Fig 3c, d: The text in the figure might be mislabeled ("single sntenna"). Are the antennae intact in this experiment?
5) As in Fig 3a, it would be useful to have a small pair of arrows on the fly schematic in Fig 1c indicating the sign of the turning directions. The figure legend clarifies the schematic, but it would be visually helpful to have this additional marker.

Referee #3 (Remarks to the Author):

Kadakia et al. describe an elegant set of experimental and modeling results to argue that Drosophila can and do use the direction of motion of odors to localize plumes. How insects localize complex plumes has been the subject of an extensive literature for decades, and many studies have assumed that the direction of the wind provides the only directional signal. The authors propose that, in addition, Drosophila use the temporal correlation of the odor signal detected between their two antennae to infer the direction of odor motion. The paper describes a series of experiments using virtual reality to deliver fictive odors in freely walking Drosophila, followed by detailed analyses of their behavior and simulations of virtual agents that use this directional cue to solve odor localization tasks.

Overall, I find the paper to be well written, the figures well presented, and the implications of the findings very intriguing. The question they tackle is an important one with wide implications, as it challenges the long-standing assumption that insect navigation in complex plumes relies on wind direction as the only directional cue. I have some specific questions and concerns, as itemized below:

- How would be simulated agents perform if the natural plume were turbulent and intermittent? As reviewed recently by Reddy, Murthy & Vergassola (Ann Rev, 2022), these types of natural plumes are particularly challenging to track because they are intermittent---which is to way, there are large gaps in between detectable odor cues. The simulated plume in Fig. 5 is unpredictable and complex, but it is not intermittent. I'm curious how the agent performs using odor motion cues in intermittent plumes (such as was simulated in Singh et al. arXiv:2109.12434)? And more generally, I would like to understand how the in silico experiment may perform in more, different plume structures other than the particular one the paper currently explores in Fig 5.

- There's an interesting questions of scale here. As the authors have discussed, that olfaction can use bilateral cues (either from both nostrils, or between multiple sensory appendages) has been proposed by many papers before. However, the majority of the prior work has been in (1) larger animals, so that the difference in what's detected by the two sensors is larger; and (2) in terrestrial odor trails, where the odor cues themselves are not changing. One of the surprising parts of the findings in the current paper is that the relatively small Drosophila is able to use the differential signals in their antennae. I'm curious how this scales with the size of the animal and the statistics of the natural plumes? How small does a fly have to be before this type of computation is unreliable for navigation?

- There is a possibility that the direction of motion is computed not between the two antennae per se, but rather from sampling in space, which can be done on a sufficiently long single antenna. This idea has been explored in cockroaches (Lockey & Willis, JEB 2015). Can Drosophila be using this strategy as well? Can this be tested using the fictive VR paradigm? And are there alternative circuit mechanisms (relatives of the HRC model) that might implement such a computation?

- Many animals move their sensory appendages actively to seek information. For instance, Draft et al. JEB 2018 showed that ants use antennae movements to sample the odor trail. The odor cues in the current paper are airborne as opposed to surface bound. Even so, I'm wondering if there is any evidence of Drosophila actively moving their antennae to sample the olfactory environment?

- I feel the last sentence of the abstract is a bit of a reach and not well supported by the (ample and quite convincing) results in the paper. The paper describes odor motion sensing by walking flies in 2D, which would not directly suggest that 'odor direction sensing is /likely used/ throughout the animal kingdom' (emphasis mine).

Minor comments:

- In the second paragraph of the main text, the narrative on the difference between the odor gradient vs the direction of odor motion is clear. This distinction is crucial to the paper, so I think it can be improved with either an addition sentence and/or an additional diagram, which would improve the clarity of the paper for a broad readership.

- In Fig 4b, label the vertical axis.

- Also in Fig 4, is there any hysteresis in the turning bias?

- The reverse time experiment is very clever, kudos!

Author Rebuttals to Initial Comments:

Dear reviewers,

Thank you for your comments and critiques. In our revised manuscript, we have address them all. In doing so, we have added new Figure 5 and new figure panels in Figures 4, 6, and 7. We are especially pleased to present new results in new Figure 5a-h, which we believe have substantially strengthened the claims in the paper, showing how plausible these computations are within the responses of olfactory receptor neurons. We look forward to hearing from you.

Sincerely,

Nirag Kadakia, Damon Clark, & Thierry Emonet

Referee #1 (Remarks to the Author):

This manuscript tackles a very exciting topic in system neuroscience: how animals extract directional information from turbulent olfactory environments to perform robust navigation. Using Drosophila has a model system, the authors uncover a new type of cue, odor motion, which can be exploited to direct orientation decisions. Odor motion results from the temporal correlations of odor signals measured between multiple sensors — in the case of Drosophila, two antennae separated by less than 300 um. By drawing a fruitful analogy with insect vision, the authors propose that the detection of odor motion is achieved by an algorithm similar to the Hassenstein-Reichardt correlator (HRC) model — a model that has inspired research in the field of visual motion detection for more 60 years. The manuscript convincingly establishes that odor motion is a source of directional information that has been overlooked until now. Using a virtual-reality paradigm, the authors provide strong evidence that odor motion can direct the orientation of walking flies in odor plumes. Based on this result, the reader is left with the exciting idea that odor motion might direct insect flight behavior as well as the navigation of terrestrial animals beyond invertebrates.

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We sincerely thank the reviewer for the encouraging comments.

The manuscript includes two components: (1) a technical analysis leading to the identification of odor-motion information in odor plumes and the creation of optogenetic stimulation protocols to present flies with controlled odor motion; (2) a series of experimental manipulations aiming to demonstrate that

Drosophila are capable of making use of odor motion to orient and that this source of sensory information is significant compared to other navigation strategies (e.g., gradient sensing through the detection of small gradients between the left and right antennae). The first component of the work is expertly conducted: its presentation is relegated to the methodology part of the manuscript. While this part of the manuscript is essential, its level of technicality will only be fully appreciated by a small group of specialists. The presentation and discussion of the 4 correlated noise stimuli used in Figure 4 will be difficult to follow by a board readership. A more intuitive discussion of the values of the correlation matrix would be helpful. Except for Figure 4, the authors do an admirable job at guiding the reader through the logic of their sophisticated analysis.

We have reworked Fig. 4c-d to portray more visually what is meant by a "positive" or "negative" correlation in a given direction, rather than simply showing the spatiotemporal correlation functions (these are now moved to the supplement). We hope this adds a better intuition that complements the accompanying explanation in the text.

The main emphasis of the manuscript is placed on the experimental examination of the role of odor motion sensing in free moving *Drosophila*. Below, I will focus my discussion on the approach and results associated with the experimental manipulations. While the authors report a convincing set of observations supporting the idea that odor motion alone is sufficient to direct navigation decisions, the relative importance of this mechanism compared to other strategies used by the fly is not entirely clear. In particular, the results of Figure 5 suggests that the contribution of odor motion might be modest. In principle, the relative importance of different sensorimotor mechanisms that have been proposed in published work by the Wilson lab (DOI: 10.1038/nature11747), the Nagel lab (DOI: 10.7554/eLife.37815) and the authors themselves (DOI: 10.7554/eLife.57524) could be tested in agent-based simulations. As part of Figure 5, the authors turn to such simulations to test the role of odor motion, but the set of basic navigation rules included in their model is too crude to reproduce realistic fly behavior.

As we discuss in more detail in the responses that follow, we have addressed this comment by i) providing additional metrics quantifying the importance of odor motion sensing in natural plume navigation, ii) using new virtual complex plume environments that better illustrate the contribution of odor motion sensing in odor navigation, and iii) using more realistic *Drosophila* odor navigation models in agent-based simulations, quantifying how odor motion sensing enables more effective navigation to the source.

Together the results of this manuscript reveal that odor timing can be combined with bilateral sensing to extract directional information from odor plumes. These results invite the field to search for the neural correlates of a circuit equivalent to Hassenstein-Reichardt detector in the fly olfactory system. Drosophila is perfectly suited to pursue this exciting research goal given the existence of a circuit diagram of its antennal lobe and the ongoing reconstruction of higher-order olfactory centers.

Comments and suggested improvements:

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The reviewer is correct that we are pooling flies on the bottom and top surfaces. To maintain the airflow, we use a z-depth of 1 cm – which is ~3X larger than other walking fly assays such as FlyBowl (https://www.janelia.org/open-science/fly-bowl). For this reason, flies can flip to the top surface without climbing the acrylic sidewalls. This has precluded our prior attempts to use Teflon coatings and surfactants to prevent top-surface walking. Nonetheless, to demonstrate that optogenetically-active flies respond to the fictive odor similarly on either surface, we have added data (**New Supp. Fig. 2d**) showing that flies on both surfaces (manually annotated) follow fictive odor ribbons upwind, similarly to previous studies with real odors in the same assay, and have mentioned this in the Methods (lines 84-85).

[Figure 2] Responses to ON and OFF edges is predicted to differentiate between direction sensing and the more classic mechanism of gradient sensing. This experimental paradigm is excellent.

We thank the reviewer for their encouraging comments.

- In panel 2F, the turning bias is quantified as the sign of the change in orientation over a time window from 150 ms and 300 ms after the bar onset. How was this time window established? How does a reaction time from 150 ms and 300 after the bar onset fit with previously published data for Drosophila? For flight behavior, van Breugel and Dickinson (DOI: 10.1016/j.cub.2013.12.023) have shown that the surge response is on the order of 200 ms, which is consistent with a 150-300 ms window used in the present manuscript. However, the response to plume loss involves a sensorimotor delay on the order of 500 ms in flying flies. This indicates that 300 ms might be too short for the OFF edge response. The response time observed in the present study should be discussed in light of these results together with those reported by the Nagel lab (DOI: 10.7554/eLife.37815). While I do not anticipate that the results of Figure 2 would change significantly, a more careful inspection of the temporal characteristics of the window averaging could impact the results of the controls shown in Supp. Figures 3a and 4.

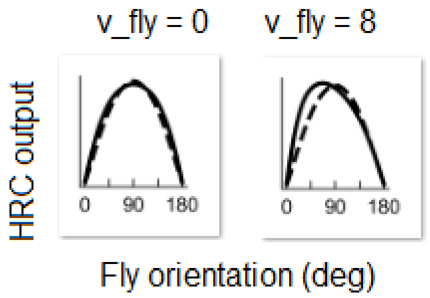
The 150-300 ms was chosen since i) it produced a robust behavioral response, ii) it is consistent with previous behavioral delays as noted by the reviewer in the van Breugel & Dickinson paper, and iii) because the limited spatial and temporal resolution of the fly tracking produces an uncertainty of ~150 ms (noted in Methods). However, to motivate our choice, we now include data in **new Supp. Fig. 4c** plotting the behavioral responses as a function of window timing for both ON and OFF edge – indicating a degree of robustness to the precise window definition. We have also added a discussion of this timing window to the caption of Supp. Fig. 4, and its relationship to prior findings, as suggested by the reviewer.

- In Figure 2, the authors use a bar speed of 15 mm/s. Could a rationale be given for this speed of the moving bar? Was this value found to be optimal through a process of trial and error? More generally, could the estimation of the delay timescale of the HRC model derived in Figure 4 be used to make predictions about the performances of flies responding to different speed of the moving bars presented in Figure 2 and Supp. Figure 4?

Due to the projector's update rate of 180 Hz, bar speeds cannot exceed ~30 mm/s without pixels being 'skipped' in successive frames, making it difficult to reliably hit the two antennae in sequence. Indeed, the rationale was a naïve one – antennae are separated by ~0.3 mm, and the timescales of visual motion detectors are ~15-30ms, giving ~15 mm/s. We added a line in the caption of Fig. 2 stating this rationale. Of course, this speed ended up being fortuitous, but since this experiment was an initial "proof-of-concept", we chose to leave the fuller investigation for the following figure with the wider bars in which we probe a range of speeds.

Regarding the use of timing to predict performance to different bar speeds, this is an important point that we need to clarify. As we understand it, the reviewer is observing that, since we have an estimate of the HRC delay timescale τ, and we know which bar speeds flies are sensitive to, we may be able to make predictions about the flies' responses to moving bars shown in Fig. 2 and Supp. Fig. 4. For example, the bar speed corresponding to a latency ΔT that matches the estimated HRC timescale, $\Delta T \sim \tau \sim 20$ should give a strong response. Indeed, this observation is qualitatively supported by our measurements: for a delay $\Delta T \sim 20$ ms, the corresponding bar speed across the antennae for perpendicularly-oriented flies would be 300 um/(20 ms) = 15 mm/s, which we find is the speed giving the largest turning bias for ON and OFF edges (Fig. 2).

Further, we can make a rough prediction of the response as a function of the fly's orientation, θ. Since we have an expression for $\Delta T(\tau, v_{fly}, v_{bar})$ (Methods and Supp. Fig. 6), and we have an expression for HRC output as a function of ΔT, we can predict HRC versus θ for a given bar speed, *assuming a given fly speed*. This is a key point – the delay ΔT and thus the HRC output depend on fly walking speed – and is the reason that we need to compute the delay in the fly's (moving) frame: ΔT in Fig. 4 (see diagram in Suppl. Fig. 6). Given this, we can make a rough estimate of the response for different walking speeds: we get these two plots for $v_{fly} = 0$ mm/s, and the median walking speed $v_{fly} = 8$ mm/s:



where the dashed line is the sinusoid $\sin\theta$. We see that the sinusoid is a reasonable approximation, even for higher walking speeds, albeit slightly skewed.

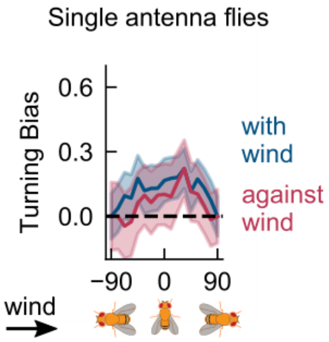
To sum up, the HRC timescale does give predictions about the responses to different bar speeds, and these predictions are consistent with our data, but the predictions depend also on the fly walking speed. We do not include these plots in the main text since our main motivation is to first use the bars to illustrate direction selectively (where the response curves are treated qualitatively to show that flies respond to motion, not gradients), and then to calculate ΔT in the fly frame to determine τ. In this latter case, by working with the bars in the fly frame rather than lab frame, we can precisely take into account fly walking speed.

[Supp. Figure 3]

- The authors rule out that flies with a single antenna can response to pure odor motion. This result represents an important sanity check. It would be useful to extend this control with single antenna flies to the correlated noise stimuli presented in Figure 4.

We opted not to present in the paper the data of correlated noise stimuli with single antenna flies, since these stimuli all have wind flowing. Removing one antenna does not entirely suppress wind sensing, but it has measurable effects (Suver et. al. *Neuron* 2019), making the results less directly interpretable.

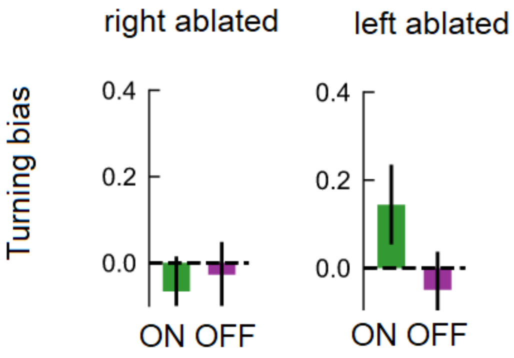
Nonetheless, we include results here for the reviewer's benefit. Shown is turning bias for single-antenna flies, for correlated noise stimuli (compare to the left plot of Fig. 4e) The number of encounters is 465 and 320 for with- (blue) and against-wind (red) responses. Consistent with our other findings, there is no difference for with- and against-wind responses.



- The author state in the caption of Supp. Figure 3 that "right- and left-antenna ablated flies are pooled." Given that adult flies display handedness (e.g., DOI: 10.1073/pnas.1500804112) pooling of the behavior mediated by the left and the right antenna is suboptimal. The two groups of flies should have been tested in separate trials.

Yes, as suggested by the reviewer, the experiments were done separately. However, during experiments, flies can be on the top or bottom surface, which scrambles the sign of the behavior. More specifically, if flies have an intrinsic CCW bias, for example, this would appear as a CCW bias on the bottom surface but a *CW bias* if the flies flip to the top surface. This prevents us from making any judgments about how a behavioral bias is correlated to which antenna is ablated. For this reason, we pooled the data in the manuscript presentation.

Still, for the reviewer's benefit, we plot here the corresponding plots for the L-ablated and R-ablated flies. The results indicate that our behavioral result is maintained (overall, no direction sensing for single antenna optogenetically-active flies) in both cases (p > 0.05 all cases; N = 354, 295 for right-ablated and left-ablated, respectively).



- The Or42b>Chrimson behavior shown in Supp. Figure 3 is nice addition, but its purpose is unclear. In addition, there must a typo in panel a of Supp. Figure 3 where Or42b>Chrimson flies are labeled as "single antenna". The fact that the Or42b>Chrimson flies with unilateral olfactory input could respond to odor motion with a turning bias would go against the model proposed by the authors. In addition, the authors should quantify their data for Or42b>Chrimson flies with the same graph shown in Figure 2f for the turning bias versus orientation response.

Yes, that is a typo – now fixed. As for its purpose, the Or42>Chrimson is an important piece of evidence since Or42b projects to a strongly appetitive glomerulus, so its ethological relevance is more obvious than a blanket stimulation of nearly all Ors. This data also shows that a small subset of ORNs may be activated to obtain the odor motion turning bias. In the revised manuscript, we make the importance of this data clearer. As suggested, we have now added the Or42b>Chrimson turning bias vs. orientation data to **new Supp. Fig. 4b**.

[Supp. Figure 4]

- It would be important for the authors to further the correlation between the bar speed and the ON and OFF edge responses observed behaviorally. Can the HRC model account for the difference in the performances to the ON and OFF edge responses? Is it possible that stronger OFF edge responses would be found if the 150-300 ms time window used in the data analysis is extended beyond 300 ms? (see discussion of Figure 2) In addition, it would be very informative to characterize the behavior to faster bar speeds that the fly cannot keep up with and bar speeds that are too slow (<1 mm/s) to elicit an ON-edge response. These behavioral observations would provide additional conditions to test the consistency of the HRC model derived in Figure 4.

The HRC model relies on only 2nd order correlations, and cannot account for the differences between ON and OFF responses – it is symmetric to these responses (as we derived in *Methods: HRC output versus ΔT for traveling edges*, lines 722-766). ON/OFF differences therefore suggest a sensitivity to higher-order correlations. We have added further text in the Discussion in lines 388-396 noting the incompleteness of the HRC model for odor motion sensing, as suggested by these ON/OFF asymmetries.

Secondly, we have explored the calculation window in **new Supp. Fig. 4c**, and did not find evidence that responses increased when the window was extended. See also the response to the above, related comment.

Thirdly, as suggested by the reviewer we have added data for fast bars (20, 30 mm/s) to **Supp. Fig. 4a**. Due to the resolution of our assay, 30 mm/s is the fastest speed we can deliver without losing antennal resolution (pixels get skipped on frame updates). We find that the counterturning responses reduce with increasing speed (20-30 mm/s), consistent with a correlation-based model. We mention this in the text in lines 105-113. In addition, this data is all consistent with our correlation stimuli (Supp Fig. 7d), which shows that for speeds of <15 mm/s and >28 mm/s, the direction selectivity reduces. Note, however, that the ON responses reduce in magnitude but are still significant for 30 mm/s (while OFF responses are abolished), suggesting that the i) velocity tuning curves are fairly broad for ON edges, and that ii) higher order correlations not explained by the HRC model are being computed (since ON and OFF responses differ). This is mentioned in the Discussion (lines 388-396).

We did not add bars slower than 1 mm/s since our quantification throughout the manuscript relies on the fly's response when the bar *passes over the fly* rather than when it walks into a static odor region. We ensure this by enforcing that the projection of the fly's velocity along the bar direction is less than the bar's velocity (not possible for 0 mm/s). We do this since we are interested in the responses to moving dynamic plumes, rather than responses when a fly walks into an odor patch – i.e. responses to independent odor motion, not motion induced by fly locomotion.

[Figure 3] In this figure, the authors start by hypothesizing that the response to the combination of odor-motion and wind signals obeys a summation rule. Their experimental observation corroborate this hypothesis. Again, this hypothesis-driven approach is very effective.

- The protocol of Figure 3 relies on the combination of one value of odor motion (bar speed) and wind speed. From the results of Figure 3d, it appears that odor motion dominates over wind sensing for the experimental conditions chosen by the authors. The authors should push the analysis further to determine whether the combination of wind and odor-motion sensing is dependent on the strength of each sensory cue. For slower or faster bar speeds that produce weaker behavioral responses (see results of Supp. Figure 4), does wind sensing become dominant over odor motion?

We note that the dominance of wind over odor or vice versa should be accounted for by the summation model inherently. That is, if the response to bars in the absence of wind is weak, then summing it with the flash + wind (no odor motion) should give a response mostly dominated by the wind response, and vice versa. This then predicts that, as the reviewers suggests, for bars that are faster and elicit a weaker direction selective response (e.g. 30 mm/s in Supplementary Fig. 4), the summation model should give a response that resembles more the wind + flash response (Fig. 3b; first plot).

To this end, we repeated the wind + odor experiments with the faster bar speed 30 mm/s, for which the ON edge response in the absence of wind is smaller. Applying the additive model, we find satisfactory predictions for the parallel, antiparallel and perpendicular bars (**Supp. Fig. 6**). A key check is that the amplitude of the perpendicular response is weaker than the original plot (Fig. 3d; green), and its peak is very near the predicted peak of 158^{o} (compare to 145^{o} in Fig. 3d with 15 mm/s bars). This indicates that the summation model is, though not perfect, consistent with the reduced responses for 30 mm/s.

[Figure 4]

- On line 158, it is stated that data from ON and OFF edges are pooled in the analysis. Since the results of Supp. Figure 4 show that the behavioral response to the ON and OFF edge is asymmetrical, pooling data from ON and OFF edges does not seem to be legitimate. Both conditions should be kept separate or the equivalence of the ON and OFF edge response should be established for the stimulation protocols of Figure 4.

We neglected to state that the pooled data in line 158 was from only 10-15 mm/s, for which The ON and OFF responses are not significantly different (Fig. 2f). We have clarified this in the text in line 169.

[Figure 5] Here the authors aim to show that odor motion provides directional cue complementary to odor gradients and wind motion, and enhances navigation in complex odor plumes. While this statement represents an important conclusion of the study, it is not strongly supported by the data presented in Figure 5.

- While I appreciate the elegance of the stimulation design tested in Figure 5d, are the behavioral results not an extension of the ON/OFF edge responses presented in Figure 2 in presence of wind? How does this protocol go beyond Figures 2 and 3?

Though Fig. 5d (now Fig. 6d) has moving bars in addition to laminar wind, similar Fig. 3, the environment in Fig. 5d (now Fig. 6d) exhibits a non-trivial spatial structure that is absent in Fig. 3. (Fig. 2 has no wind.) As such, the question being asked is different: it is not simply what are the immediate responses to an edge, but how do these responses integrate over time to contribute to the goal-directed task of finding the (virtual) odor source. The conclusion is not trivial, since flies could alternatively learn the plume structure (for example, by probing the diagonal edges), without using odor motion. For this reason, the plume is illustrative of the value of odor motion sensing in naturalistic environments (but is still followed up by a more realistic plume in new Fig. 6g). In the revised manuscript, we have emphasized this point in lines 311-314.

- In panels 5e and 5h, the authors use two paradigms to characterize the contribution of odor-motion to the navigation of free moving flies. Consistent with the rest of the work, the design of the VR stimulation protocols is clever. However, the interpretation of the results is not straightforward due to the very low success rates of the flies. For the comparison between the forward and temporally reversed plumes, only 3% out of 295 flies managed to reach the source with the forward plume. Less than 9 flies were successful. The temporally reversed plume structure leads to a reduction of the success rate to 2 (?) flies, prompting the authors to conclude that odor motion sensing enables flies to navigate the forward plume structure. Although the difference between forward and reversed plumes is significant, flies do not navigate the plume replay accurately. A more appropriate conclusion seems to be that odor-motion sensing contributes to very weak navigation behavior. The same applies to the outward and inward bar protocol of Figure 5d, though the success rate is modestly higher (less than 10% of the flies successfully reach the source). The authors should strengthen these results to support one of the major conclusions of their study.

These are good points. In the revised manuscript, we have addressed these together with the following additions:

a) quantifying the ratio of successful flies with more care, b) providing additional metrics indicating that odor motion enhances finding the odor source, and c) taking additional data using a plume that covers a larger portion of the arena than our original plume.

The results for a-c are summarized below:

a) Upon further inspection, we found that our initial plots had misrepresented the low percentage of tracks reaching the source. Oftentimes, a single fly's trajectory is split into multiple "tracks" in our dataset, due to tracking ambiguities when flies bump into one other, jump, or reach any of the assay borders. We found that our success ratio was artificially deflated since we had included artificially truncated tracks as failures.

To address this, we re-calculated the percentage of tracks reaching the source, but now removed those that lasted 30s or less, unless they reached the upwind end of the assay before then. We also stress that, as before, only tracks beginning in the back 50 mm of the arena were considered. This ensures that a fly has sufficient time to explore the plume and to attempt to find the source from the back of the arena. With this definition, for the inward/outward bar stimulus, the percentage of tracks getting to the source was 56% and 28% (28 out of 50 and 12 out of 43 tracks, respectively) -- now plotted in **updated Fig. 6f (previously Fig. 5).** The percentage getting to the source in the natural plume (see (c) below) was also substantially higher.

b) The percentage of tracks reaching the source is just one metric, and is only partially representative of the valuable role of odor motion in enabling complex plume navigation. To give a fuller picture, we now also plot the histogram of lateral positions in the plume, for both the upwind (x < 100 mm) and downwind (x > 200 mm) halves of the arena, in **updated Fig 6f, 6i**. The clear difference between the natural (purple) and reversed plumes (green) in the upwind half indicates that increased lateral motion underlies flies' ability to localize the source.

Moreover, we also plot the average drift of flies toward the plume centerline and in the upwind direction (**new Supplementary Fig. 11a**). We find that the drift is enhanced for the outward bars vs. in the inward bars, indicating that the benefits of odor motion sensing derive from enhanced lateral motion toward the plume centerline.

c) The natural fictive plume used in old Fig. 5g-I was derived from a visualized plume presented in our previous work (Demir*, Kadakia*, et al *eLife* 2020). For the purposes here, this plume is suboptimal in that it does not subtend a large region of the arena – the lateral extent at the downwind end is only 60 mm of the cross-sectional 180 mm width. This lessens the presumed role of centerline drift provided by odor motion sensing. We thus generated a different visualized plume in the same arena using a protocol very similar to the one described in (Demir*, Kadakia*, et al *eLife* 2020) – i.e. by injecting smoke into the wind tunnel and perturbing the airflow with lateral jets at the upwind end. To get the wider lateral extent, we slightly adjusted the parameters of the airflow, (now described in Methods). This plume was imaged and then presented as a fictive plume optogenetically (**updated Fig. 6g**).

In this case, the percentage getting to the source from the back 50 mm was 32% (22 of 69 tracks) vs 14% (13 of 91 tracks) for forward and backward playback, respectively (**updated Fig. 6i**). In general, these complex plumes are difficult to navigate on foot, and it is often the case that flies hit the arena borders before getting to the source. Finally, as in the lateral bars plume, we also show that navigation gains are aided by increased lateral motion toward the plume centerline (**new Fig. 6l** and **new Supplementary Fig. 11b).**

- On line 259 it is stated that "with an eye toward practical applications" the authors used "in silico experiments to explore the impact of odor motion sensing for robots obeying simplified navigation algorithm." While using a simplified navigation algorithm might be a useful start, the authors are clearly equipped to test the contribution of odor-motion sensing to a much more realistic model combining mechanisms of sensorimotor control published by their lab and others (e.g., DOI: 10.7554/eLife.37815). The behavior of the fictive robots (Figure 5k) appears to be dramatically different from the trajectories of real flies shown in Figure 5h. It would be important to demonstrate the relative contribution of odor-motion sensing in the context of a model that is reasonably good at accounting for the navigation of real flies.
- Minor: the statistical significance of the t-test should be indicated in panel 5f.

In the revised manuscript, we have added into **new Fig. 6j-l** a second simulation quantifying the performance of agents obeying the fly navigation algorithm we found in a previous work (Demir*, Kadakia*, et al *eLife* 2020). We found that there is a statistically significant improvement in performance with a large effect size when direction sensing is added (25% successful agents without odor motion sensing increases to 34% of agents with odor motion sensing). This is enabled by enhanced motion toward the plume centerline, consistent with previous results. We believe this adds generality to our findings for practical applications.

Referee #2 (Remarks to the Author):

Kadakia and colleagues investigate the ability of walking Drosophila to use temporal correlations between activity in their two antennae to navigate odor plumes. Using patterned optogenetic activation (artificial and based on simulated plumes) of the olfactory receptor neurons (ORN) in the antenna, they find that flies will turn towards the antenna that is activated first (counterturning against the direction to the fictive "odor motion"), which would be expected to promote navigation towards a fictive odor point source. The authors describe the underlying computations as being algorithmically analogous with those in motion vision detection circuits.

This paper provides a series of convincing experiments demonstrating that temporal correlations in bilateral antennal activity can bias fly walking behavior under constrained, well-controlled experimental settings. The larger question is whether, given the size of these effects and the conditions in which flies would be expected to encounter odor motion in the natural world, whether information from antennal correlations contributes significantly to navigation of natural plumes by Drosophila. If the latter is the case, this study would be an important and novel contribution to a relatively well-developed and mature field of study on neural mechanisms of insect plume navigation.

Several additional pieces of data or analysis would substantially strengthen the case that antennal correlations are used in natural plume navigation:

We thank the reviewer for this insightful comment and suggestion, which prompted us to add a new figure that substantially strengthens the paper.

1. Evidence that temporal correlations in bilateral antennal activation (in at least some positions) in a naturalistic odor plume fall within the range of magnitudes and timing differences (Figure 4b, Supplementary Figure 4) that the authors show can elicit turning. Simultaneous bilateral recordings from a specific identified ORN type in each antenna in response to a naturalistic plume (which the authors have experience generating from their behavioral assays) would help to clarify if correlations of the necessary temporal scale occur in response to natural odor stimuli.

The reviewer raises two important points (here and in the next comment) about i) whether ORNs can detect correlations in natural odor signals, given the HRC timescales we have inferred, and ii) whether these correlations could be extracted and preserved downstream, given the known sources of temporal variability in the olfactory circuit. We agree that a fuller consideration of these points would strengthen our arguments.

On the first point, our electrophysiological setup prevents us from recording two ORNs simultaneously. Moreover, dual recordings would prevent ground truth knowledge of the stimuli, since we are not able to measure stimuli at both antennae simultaneously. To sidestep this limitation, but still address the reviewer's concerns, we adopted a related approach. We recorded electrophysiological responses of single ORNs (in either antenna) to short (50 ms) odor pulses. These pulses were repeated for many presentations in many different ORNs. Then we manually shifted the two spike trains measured from different ORNs in different trials by $-\Delta T/2$ and $\Delta T/2$, respectively. This procedure simulates an odor that hits both antennae sequentially – the latter ORN is hit ΔT later – i.e. it simulates a traveling odor signal.

Finally, we calculated the inter-antennal correlations between these two shifted signals. In this way we are able to explore the effect of changing the velocity of the odor packet by changing ΔT.

Our main point is that the correlation between a left and right ORN to a left- or right-moving odor signal is equivalent to the correlation between two different ORNs (on either antenna) for the *same* odor signal, but where one is manually delayed in time. By doing this for many presentations and many ORNs, we can get a full picture of the variability in correlations – essentially we are taking many possible combinations of "left' and "right" ORNs, for various odor speeds. As we are using actual ORN spike recordings, and passing these through the HRC model, we are retaining ORN signaling noise in these computations across ORNs and trials.

Our results are as follows:

1. Across all ORNs and presentations (**new Fig. 5a)**, the first spike has a variability of ~5 ms – lower than some prior studies, but in line with our own (Martelli et al , *J. Neurosci*. 2011). This strongly suggests that timing at ORNs is sufficient to preserve directional HRC response (but other downstream transformations and noise sources are discussed in Reviewer's comment #2 below).
2. By choosing a random pair of ORNs and calculating the correlation as described above, we find the computed HRC response is highly directional (+ for $\Delta T > 0$ and - $\Delta T < 0$) as long as $|\Delta T| > \sim 11$ ms (**new Fig. 5b and Fig. 5d**). It is interesting to note that this is less than the HRC filter timescale of $\tau = 15$ ms. This is not an accident, and we show this mathematically (see Methods lines 843-904 and **new Supp. Fig. 9**).
3. Across multiple odor presentations, the HRC response is highly directional to *each* presentation, indicating that directional information is preserved on a whiff-by-whiff basis: averaging over whiffs is not required to infer odor direction (**Fig. 5c**).
4. We also repeat this procedure for a naturalistic odor signal (**Fig. 5f-h**), with timescales and concentrations spanning an order of magnitude, using data from our previous study (Gorur-Shandilya*, Demir*, et. al. *eLife* 2017). We find that, assuming an inter-antennal latency of $\Delta T = 15$ ms (i.e. we correlate between one ORN recording to the naturalistic stimulus and a different recording to the same stimulus shifted by ± 15 ms), our HRC model can infer odor direction for more than 90% of the individual whiffs in this naturalistic signal. This again supports our claim that averaging over repeated presentations is not required to infer odor direction from natural stimuli.

2. Additional consideration and discussion of the mechanisms by which the olfactory circuit architecture and bilateral PN signaling could feasibly transmit relative timing information between antennae to a downstream decoder is warranted. This is because, whereas flies can obviously perform the antennal comparison across many trials of precise, repeated optogenetic "motion", it would be a computational feat by the fly to extract reliable timing differences between antennae on the order of ~16-25 ms (based on estimated odor motion of 10-15 mm/s). First, ORNs are noisy and the latency to the first spike of an odor-evoked response varies considerably across the ORN population (~40-50 ms, Bhandawat et al. 2007). Because of this, PNs averaging over many noisy ORNs have relatively long integration windows (estimated ~30 ms, Jeanne & Wilson, 2015), on a similar timescale to odor motion antennal timing differences. Second, ORNs project bilaterally, with PNs receiving an estimated ~60% of presynaptic input from ipsilateral ORN population and ~40% of its presynaptic input from the contralateral population (Gaudry et al. 2013, Tobin et al.). This asymmetry leads to a ~30% difference in response strength between ipsilateral and contralateral PNs to a lateralized odor input. A downstream detector would have to extract these bilateral differences on a finer timescale than the timing difference between antennae. Thus, it's likely that strong antennal activation with repeated, reliable timing differences would be required

to have any significant impact on behavior. This also strongly motivates point 1 above to establish the magnitude and temporal properties of antennal correlations in naturalistic situations.

We agree with the reviewer that it is not obvious how directional information at timescales ~15 ms is preserved downstream, given the known variability in the fly olfactory circuit. To address this question we repeated our investigations while simulating further sources of variability:

1. We did not find the quantification of first-spike variability across ORNs in Bhandawat et. al., 2007 (their Fig. 1a does show spike variability across many presentations in same ORN, but not multiple ORNs). However, we found this data in Jeanne & Wilson 2015, which presented the cumulative # of first spikes/ORN over time across a population of ORNs feeding into the same glomerulus (their Fig. 4b). The time it takes this graph to reach ~1 indicates the spread of first spike times across ORNs. The plot shows a trend that is approximately linear between 15 and 60 ms, reaching ~1 at 60 ms. This suggests spikes are jittered up to $\pm(60 - 15)/2 \sim \pm$ 23ms approximately uniformly (i.e. a uniform distribution gives linear cdf). Such a uniform distribution would have a SD of ~13 ms, while we have found jitters closer to SD = 5 ms (**new Fig. 5a**). This discrepancy is probably due to technological advances in controlled odor delivery, including some of our own work (e.g. Gorur-Shandilya, et. al. *J. Exp. Biol.* 2019, Martelli et al, *J. Neurosci.* 2011). Nevertheless, as a worst-case-scenario, we repeated our investigations with spike jitters randomly chosen from a uniform distribution [-30, 30] added to the measured ORN recordings. Specifically, each spike was jittered independently anywhere between -30 and +30 ms, chosen uniformly.
2. We mimicked the 60/40 crossover split of ipsi- and contra-lateral ORN-PN projections by replacing the "left" ORN output with L_{mix} = 0.6*left + 0.4*right and the "right" ORN output with R_{mix} = 0.6*right + 0.4*left.
3. We mimicked PN responses by integrating L_{mix} and R_{mix} with exponential lowpass filters with 30ms timescales to produce L_{PN} and R_{PN}.

With all these additional factors, we then re-calculated the HRC responses, now using L_{PN} and R_{PN}, which incorporate the 3 transformations and sources of variability. We find that the HRC output is remarkably robust (**new Fig. 5e**). While the minimal latency that can be detected is increased now from ΔT = 11 to ΔT = 21 ms, this is far lower than the naïve expectation that all responses below 30 ms would be washed out. Note that the uncertainty in **new Fig. 5e** represents the *standard deviation* of the responses, not the standard error of the mean – thus, the directions of *individual pulses* with latencies above 21 ms are expected to be discernible. This finding is in keeping with robust motion detection in visual systems, in which signals can be filtered with relatively slow low-pass filters, but directional correlations can be detected on much faster timescales (Salazar-Gatzimas et al., 2016).

3. A critical set of experiments is in Figure 5d-i. Only a single metric is extracted from this rich dataset (fraction of flies at the source), and the vast majority of flies do not reach the source in either experimental or control condition (<5%). This observation suggests that flies are not navigating the fictive plumes particularly well by this metric and additional behavioral outputs should be examined. Based on the authors' model, the improved navigation to the fictive source in the experimental plumes (as compared to control plumes) presumptively depends on enhanced lateral movement towards the midline, but not increased upwind navigation. To test this prediction, the authors could analyze the walking trajectories further to determine the relative rates of upwind and lateral displacement (towards the midline) during encounters with fictive odor (light) between the experimental and control plumes.

This is a good point, also raised by Reviewer #1. Please also see our detailed response to Reviewer #1 above. Following the suggestion of the reviewer we have now calculated additional metrics of the behavior, which show that odor motion detection results in increased lateral motion toward the plume centerline (**new Fig. 6f, 6i, 6l** and **new Supplementary Fig. 11b).**

4. Can the fictive plume experiments (Figures 5d, 5g) be repeated in the absence of laminar wind as an additional navigational cue? Rather than examine the ability of flies to navigate to the upwind source, the percentage of flies that navigate to the center zone (along the lateral axis) of the fictive plume would be compared for normal/outward versus reverse/inward fictive conditions.

The added metrics in **updated Fig. 6f and 6i** should address the reviewers' concern that source-finding is enabled by biased motion toward the plume centerline. If we were to run these experiments without laminar wind, their centering would really just be an extrapolation of the results in Fig. 2b-d (odor motion with frequency odor hits, no wind). However, our goal in in Fig. 5 is to investigate how odor motion sensing and wind direction sensing together enable plume source navigation in a spatiotemporally complex plume. Thus, we have not included results with a plume in the absence of wind.

Minor Points:

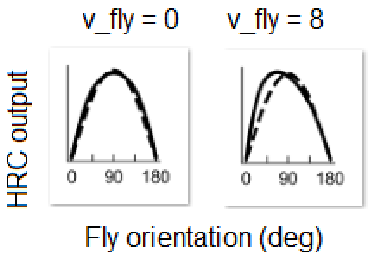
1) In Figure 4, the correlator τ is fit with both ON and OFF edges. However, Supplementary figure 5 shows that only ON edges evoked major turning bias in the presence of wind. Does this effect the effectiveness of this model?

Our assumption is that the HRC model describes the odor-motion-only response. Indeed, with wind, the HRC model is incomplete since the ON and OFF responses are different. In the revised manuscript, we now highlight this asymmetry in the discussion.

2) In earlier figures, the turning bias is fit to the sine of the angle of the fly with respect to the stimulus motion (equivalent to a linear fit to the distance of the antennae along the direction of motion, or the time delay between stimulation of the antennae). However, the correlator model in Fig 4b suggests a sublinear fit of turning bias to ΔT. Does accounting for this remain consistent with the previous sine fits?

This is an interesting point. Indeed, we have an expression for $\Delta T(\tau, v_{fly}, v_{bar})$ (Methods and Supp. Fig. 6), and we have an expression for HRC output as a function of ΔT. Combining these, we can make predictions of HRC versus θ for a given bar speed. However, this must *assume a given fly speed* v_{fly}. This is a key point – the delay ΔT and thus the HRC output depend on fly walking speed – and is the reason that we need to move to the relative frame of the moving to estimate ΔT in Fig. 4 (see diagram in Suppl. Fig. 6).

Still, we can make an estimate for different walking speeds. Combining the expression for $\Delta T(\tau, v_{fly}, v_{bar})$ and HRC(ΔT), we get these two plots for $v_{fly} = 0$ mm/s, and the median walking speed $v_{fly} = 8$ mm/s:



where the dotted line is the sinusoid $\sin\theta$. We see that the sinusoid is a good approximation, even for higher walking speeds, albeit slightly skewed.

3) Supplementary Fig 1a: It would be helpful to make the orientation references of the fly consistent with the ones shown in the main text (and Supplementary Fig 5).

We have now plotted this figure to be consistent with Supplementary Fig. 5, which also has wind.

4) Supplementary Fig 3c, d: The text in the figure might be mislabeled ("single sntenna"). Are the antennae intact in this experiment?

That was a typo, fixed. The antenna are intact, and the flies still display directional behavior.

5) As in Fig 3a, it would be useful to have a small pair of arrows on the fly schematic in Fig 1c indicating the sign of the turning directions. The figure legend clarifies the schematic, but it would be visually helpful to have this additional marker.

In the revised manuscript, we have added cartoons to 1c and 1e indicating the turning directions in different regimes of the plot.

Referee #3 (Remarks to the Author):

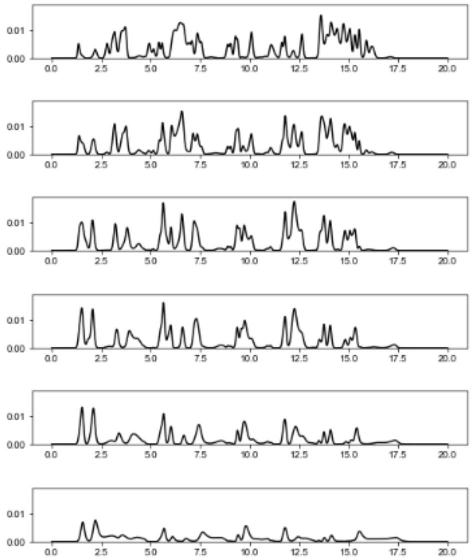
Kadakia et al. describe an elegant set of experimental and modeling results to argue that Drosophila can and do use the direction of motion of odors to localize plumes. How insects localize complex plumes has been the subject of an extensive literature for decades, and many studies have assumed that the direction of the wind provides the only directional signal. The authors propose that, in addition, Drosophila use the temporal correlation of the odor signal detected between their two antennae to infer the direction of odor motion. The paper describes a series of experiments using virtual reality to deliver fictive odors in freely walking Drosophila, followed by detailed analyses of their behavior and simulations of virtual agents that use this directional cue to solve odor localization tasks.

Overall, I find the paper to be well written, the figures well presented, and the implications of the findings very intriguing. The question they tackle is an important one with wide implications, as it challenges the long-standing assumption that insect navigation in complex plumes relies on wind direction as the only directional cue. I have some specific questions and concerns, as itemized below:

We thank the reviewer for the encouraging comments.

- How would be simulated agents perform if the natural plume were turbulent and intermittent? As reviewed recently by Reddy, Murthy & Vergassola (Ann Rev, 2022), these types of natural plumes are particularly challenging to track because they are intermittent---which is to way, there are large gaps in between detectable odor cues. The simulated plume in Fig. 5 is unpredictable and complex, but it is not intermittent. I'm curious how the agent performs using odor motion cues in intermittent plumes (such as was simulated in Singh et al. arXiv:2109.12434)? And more generally, I would like to understand how the in silico experiment may perform in more, different plume structures other than the particular one the paper currently explores in Fig 5.

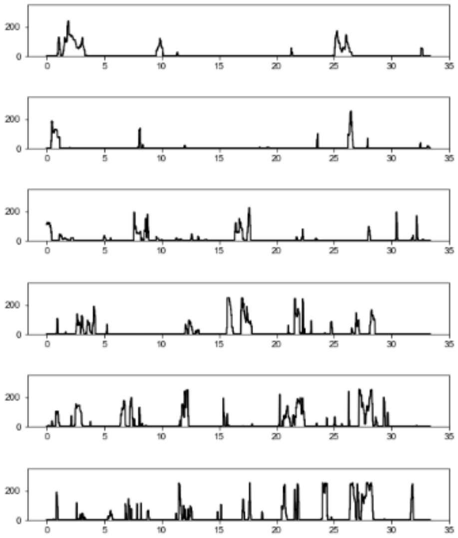
We have examined the statistics of the odor in our simulated plume shown in Fig 6a. First, we plot a few illustrative time traces at the downwind end of the plume, beginning at the centerline (top plot) and moving outward (bottom plot).



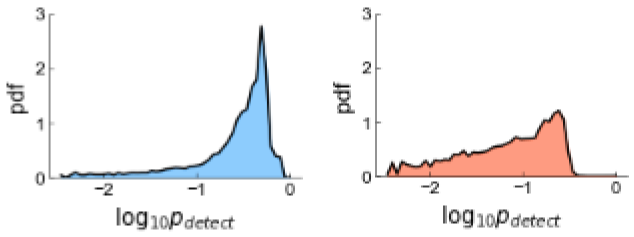
The signal in this simulation does have some temporal gaps, but the reviewer is right in that it is rather continuous, and can lead to long encounters lasting several seconds with blank periods that are shorter. However, this depends also on the detection threshold one would choose.

To quantify this, we calculated the average percentage of time the signal is above threshold (we call this percentage p_{detect}, using a threshold of 1e-3 as in the simulation). We find that $p_{\text{detect}} = 0.20$ averaged over the whole plume. In the center of the plume, $p_{\text{detect}} = 0.65$. So, while the plume does have a degree of intermittency, the animal is in the signal a substantial amount of time, especially near the centerline.

Thus, to explore plumes that are more intermittent – in which the navigator spends less time in the signal – we have now generated a second plume by imaging smoke in our wind tunnel (a snapshot of the plume is shown in **new Fig. 6g**), and then used this as the signal in a new set of experiments (**new Fig. 6hi**) and simulations (**new Fig. 6j-l**). We generated the plume using the same experimental paradigm as in our prior study (Demir*, Kadakia*, et al, *eLife* 2020). Here, the illustrative time traces at the downwind end of the plume, beginning at the centerline (top plot) and moving outward (bottom plot) are:



In this new plume, we now get p_{detect} = 0.07. Moreover, in the center of the plume, $p_{\text{detect}} = 0.25$. Quantitatively, we plot below the histogram of p_{detect} on a log scale in the plume shown in **new Fig. 6a** (below left) and in the new plume shown in **new Fig. 6g** (below right):



A substantial fraction of the new plume (**new Fig 6g**) has p_{detect} lower than 10^{-1}, while in the plume shown in **new Fig 6a** we mainly have $p_{\text{detect}} > 10^{-1}$. The highly intermittent structure of the new plume is also clear from the snapshot in **new Fig. 6g.**

In this new intermittent plume, we simulated agents using a more bio-inspired navigation algorithm, derived from our earlier study (Demir*, Kadakia*, et al, *eLife* 2020). In this navigation model, agents increase the upwind bias of their turns as the frequency of odor hits increases. We added odor motion sensing by allowing agents to bias their motion against, not the wind alone, but the sum of the wind and odor motion directions. We find that, much like for the original plume (**new Fig. 6a**), agents can navigate to the source successfully without odor motion sensing, but that odor motion sensing markedly increases their performance (**new Fig. 6j-l**). This indicates that even with intermittent information, odor motion can play a significant role in odor plume navigation for virtual agents.

To intuit navigation performance over a wider range of plume parameters, we point to our theoretical analysis in the last section of the Methods and Supp. Fig. 13, which uses the same model of odor dispersion as the one used in the paper mentioned by the reviewer (Singh et al. arXiv:2109.12434): correlated random walks of odor packets that are also diffusing. These calculations indicate how the value of the odor correlator – which provides odor motion information – depends on the molecular diffusivity, packet speed, and packet correlation time.

- There's an interesting question of scale here. As the authors have discussed, that olfaction can use bilateral cues (either from both nostrils, or between multiple sensory appendages) has been proposed by many papers before. However, the majority of the prior work has been in (1) larger animals, so that the difference in what's detected by the two sensors is larger; and (2) in terrestrial odor trails, where the odor cues themselves are not changing. One of the surprising parts of the findings in the current paper is that the relatively small Drosophila is able to use the differential signals in their antennae. I'm curious how this scales with the size of the animal and the statistics of the natural plumes? How small does a fly have to be before this type of computation is unreliable for navigation?

The reviewer raises some interesting points here. In general, the ability to sense odor motion will depend on a) that there is correlated motion of odor packets on the scale of the distance between the sensors, and b) the precision in time of the correlator used to detect that motion. The HRC model computes direction by calculating correlations across space and time, and the regimes over which the computation is reliable (the odor speeds to which the HRC responds) is a direct function of the internal timescales of the HRC (we found ~20 ms; Fig. 4b). Thus, in principle, odor direction can be computed even for animals smaller than flies, provided that the internal timescale of their correlation-based computation decreases as well. In other words, the hypothesis is that the HRC-like motion detector in other animals (if it exists) would have a timescale that is related to the size of the nostril/nare/antenna spacing and to the range of speeds of odor motion it has evolved to detect. It would indeed be interesting to see how correlator timescales depend on nostril/antennal spacing – we now note this in the Discussion in lines 418-424.

We also point to our new electrophysiology results (**new Fig. 5**), which estimate HRC responses for measured *Drosophila* ORNs. All the results there show that, despite various sources of noise, extracting the direction of the odor signal is a very robust computation, even below the HRC filter timescale (assumed 15 ms there).

- There is a possibility that the direction of motion is computed not between the two antennae per se, but rather from sampling in space, which can be done on a sufficiently long single antenna. This idea has been explored in cockroaches (Lockey & Willis, JEB 2015). Can Drosophila be using this strategy as well? Can this be tested using the fictive VR paradigm? And are there alternative circuit mechanisms (relatives of the HRC model) that might implement such a computation?

This is an interesting suggestion. Our single antenna experiments indicate that direction sensing is lost when 1 antenna is ablated, so, at least at the resolution of our assay, any differential sensing along 1 antenna does not produce direction selectivity that drives behavior. However, our projector has limited spatial resolution and the length of the antenna is near to the resolution, so we cannot entirely rule out this possibility. Moreover, there is a spatial organization of ORNs along an antenna, so in principle glomeruli could pick up timing differences as an odor signal travels along the antenna length. The Lockey & Willis results are also intriguing, and we suspect that direction sensing along 1 antenna may certainly be a possibility in cockroaches due to i) the scale of the antenna, which is 100X the length of the fly and ii) active sampling. We now mention the possibility of single-antenna direction sensing in lines 427-432.

As far as alternative circuit mechanisms, the fact that different ORN types are arranged with some degree of spatial organization along the antenna (and their downstream glomeruli maintain this separation) suggests that a correlation-based model could work in the hypothetical scenario of within-antenna motion detection. Thus, the main issue is not whether some other mechanism is needed, but whether direction sensing is being computed between glomeruli in a single hemisphere – as opposed to between hemispheres as we investigate in this study.

- Many animals move their sensory appendages actively to seek information. For instance, Draft et al. JEB 2018 showed that ants use antennae movements to sample the odor trail. The odor cues in the current paper are airborne as opposed to surface bound. Even so, I'm wondering if there is any evidence of Drosophila actively moving their antennae to sample the olfactory environment?

We thank the reviewer for this interesting point. Indeed, the fly can move its individual antennae, or sweep its head left or right, which can induce distinct odor motion speeds. Regarding *Drosophila,* we are not aware of published studies of active sampling in adult *Drosophila* or its use in odor navigation. In the revised manuscript, we mention this possibility and limitation in our discussion in lines 425-427.

- I feel the last sentence of the abstract is a bit of a reach and not well supported by the (ample and quite convincing) results in the paper. The paper describes odor motion sensing by walking flies in 2D, which would not directly suggest that 'odor direction sensing is /likely used/ throughout the animal kingdom' (emphasis mine).

We modified the text to indicate just the possibility.

Minor comments:

- In the second paragraph of the main text, the narrative on the difference between the odor gradient vs the direction of odor motion is clear. This distinction is crucial to the paper, so I think it can be improved with either an addition sentence and/or an additional diagram, which would improve the clarity of the paper for a broad readership.

We reworked the explanation in that section (now lines 47-50) to give an example of how one might intuit odor motion, before we present results on this concept.

- In Fig 4b, label the vertical axis.

Done.

- Also in Fig 4, is there any hysteresis in the turning bias?

The correlated stimuli are on for 4 seconds, then off for 4 seconds, repeated for 1 minute total. On the timescale of 4 seconds, fly orientation is sufficiently randomized after the 4 second blank period, so there is little to no dependence on the prior stimulus. This is consistent with our prior study (Demir*, Kadakia* et al, *eLife* 2020), which showed that *Drosophila* upwind orientation driven by the frequency of odor hits is randomized after 5 seconds of no odor hits (Fig. 3C of Demir*, Kadakia* et al, *eLife* 2020).

- The reverse time experiment is very clever, kudos!

Thank you!

Reviewer Reports on the First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

I thank the authors for the thorough discussion included in their rebuttal letter and for sharing additional data that are not included in their manuscript.

In the revised manuscript, a lot of work has been done to address the questions and concerns raised by the reviewers. Many important details were clarified. For example, the addition of panel 4d provides a better intuition about the effects of positive and negative spatio-temporal correlations. Where it was necessary, the data analysis has been improved. For instance, the re-analysis of the trajectories shown in Figure 6e & 6h now reveals behavioral differences that are statistically significant. In Figure 6i-k, the authors capitalized on an agent-based framework previously published by their group to test the role of odor motion sensing in fictive robots.

Generally, the authors went out of their way to follow up and test hypotheses suggested by the reviewers. In particular, they included new evidence based on electrophysiology and numerical simulations to demonstrate that the temporal precision of the ORN responses is sufficient to reliably encode information related to odor directionality. I appreciate the fact that the incompleteness of the HRC model is acknowledged in the discussion. As stated in my first report, the methodology behind this manuscript is superb. The work reports an important mechanism that complements our understanding of olfactory navigation. This manuscript represents a valuable contribution to the field of olfaction and systems neuroscience.

Minor: the authors might want to remove the comment in parenthesis that appears on lines 8-9 of the caption of Figure 2. While I agree that not everyone "might care about fly vision", it is reasonable to expect that you justify the parameters of the methodology used in your behavioral experiments.

Referee #2 (Remarks to the Author):

The additional analyses and textual revisions presented in the revised manuscript by Kadakia and colleagues improve the study and provide better support for their claims. The manuscript rigorously demonstrates that flies have the ability to use temporal correlations in ORN activity between their antennae to bias their movement. We appreciate the clarity and care with which the manuscript is written and presented. The improved tracking and the additional metrics in the new figure 6f, i, and l provide a better picture of the plume/bar tracking. The accompanying supplementary figure 11 also aids in making this point.

We agree that an experiment we requested, bilateral recording of identified ORNs, is a technically difficult experiment. However, it is the most direct method to demonstrate that ORNs in the two antennae experience temporal correlations when situated in a naturalistic plume. We understand the confound that no man-made sensor has the spatial resolution required to independently measure the stimulus at each antenna. This is why we suggest empirical measurement of the distribution of temporal correlations between biological antennae in a naturalistic plume, which would be highly relevant to evaluating the hypothesis. For instance, are the concentrations edges/borders of real odor packets “sharp” enough to induce reliable temporal differences between antennae that reflect odor velocity?

However, we acknowledge the technical challenge involved, and the results of the computational simulation of odor motion by jittering ORN spike trains forward or back are useful. Although not all sources of variance (such as that arising from the motion of the odor between antennae) are accounted for, the analyses show how the algorithm is plausible in the face of ORN timing variability. As the authors note, the other additions to the model (60:40 mixing and temporal filtering) do not largely affect the ability of the HRC model to extract direction, but these details may become more important when considering other ways that a neural implementation will differ from the mathematical model, such as the introduction of noise at each stage of neural transmission. It would be useful if the authors can provide intuition about how the HRC reliably detects timing differences smaller or on the same order as its tau, as this is not obvious to a non-specialist reader.

From a biological perspective, the overarching open question is the significance of odor-evoked antennal correlations for odor-guided navigation by animals in natural conditions, when other salient cues (for instance, wind direction) are available. This is a very challenging hypothesis to prove, one that the data in the manuscript do not directly address. We think it can reasonably be considered beyond the scope of a first report, but the language in the manuscript should show constraint and reflect the current state of the evidence, in particular, in the title and and the last sentence of the abstract. The title as currently written makes it sound as if odor motion sensing is the primary mechanism by which complex plumes are navigated, which of course is certainly not the case. If relevance/applications to robotic navigation are to be invoked, the authors should also include a discussion of the current state-of-the-art for VOC sensors in terms of the detection range of odor velocities supported by their current spatiotemporal resolution, and a comparison of whether gradient sensing or odor motion sensing is more easily implemented. This would provide useful information for helping engineers to prioritize their work in this domain.

Referee #3 (Remarks to the Author):

I thank the authors for their thorough and thoughtful responses to my questions. The revised manuscript is very strong, and I appreciate the authors' addition of several points, including new analyses and discussion points. I think the observations reported here are of broad interest to the scientific community and would be happy to see them published in Nature.

- Bing Brunton

Author Rebuttals to First Revision:

Referee #1 (Remarks to the Author):

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Generally, the authors went out of their way to follow up and test hypotheses suggested by the reviewers. In particular, they included new evidence based on electrophysiology and numerical simulations to demonstrate that the temporal precision of the ORN responses is sufficient to reliably encode information related to odor directionality. I appreciate the fact that the incompleteness of the HRC model is acknowledged in the discussion. As stated in my first report, the methodology behind this manuscript is superb. The work reports an important mechanism that complements our understanding of olfactory navigation. This manuscript represents a valuable contribution to the field of olfaction and systems neuroscience.

Minor: the authors might want to remove the comment in parenthesis that appears on lines 8-9 of the caption of Figure 2. While I agree that not everyone "might care about fly vision", it is reasonable to expect that you justify the parameters of the methodology used in your behavioral experiments.

We are glad that the reviewer is satisfied with our improved manuscript. We thank the reviewer for the feedback and for pointing out the comment to ourselves that we had forgotten to remove from Figure 2. This statement has been removed.

Referee #2 (Remarks to the Author):

The additional analyses and textual revisions presented in the revised manuscript by Kadakia and colleagues improve the study and provide better support for their claims. The manuscript rigorously demonstrates that flies have the ability to use temporal correlations in ORN activity between their antennae to bias their movement. We appreciate the clarity and care with which the manuscript is written and presented. The improved tracking and the additional metrics in the new figure 6f, i, and l provide a better picture of the plume/bar tracking. The accompanying supplementary figure 11 also aids in making this point.

We are glad that the reviewer finds the paper has improved and thank the reviewer for the feedback.

We agree that an experiment we requested, bilateral recording of identified ORNs, is a technically difficult experiment. However, it is the most direct method to demonstrate that ORNs in the two antennae experience temporal correlations when situated in a naturalistic plume. We understand the confound that no man-made sensor has the spatial resolution required to independently measure the stimulus at each antenna. This is why we suggest empirical measurement of the distribution of temporal correlations between biological antennae in a naturalistic plume, which would be highly relevant to evaluating the hypothesis. For instance, are the concentrations edges/borders of real odor packets “sharp” enough to induce reliable temporal differences between antennae that reflect odor velocity?

However, we acknowledge the technical challenge involved, and the results of the computational simulation of odor motion by jittering ORN spike trains forward or back are useful. Although not all sources of variance (such as that arising from the motion of the odor between antennae) are accounted for, the analyses show how the algorithm is plausible in the face of ORN timing variability.

We look forward to attempting this difficult experiment in the future and thank the reviewer for the suggestion.

As the authors note, the other additions to the model (60:40 mixing and temporal filtering) do not largely affect the ability of the HRC model to extract direction, but these details may become more important when considering other ways that a neural implementation will differ from the mathematical model, such as the introduction of noise at each stage of neural transmission. It would be useful if the authors can provide intuition about how the HRC reliably detects timing differences smaller or on the same order as its tau, as this is not obvious to a non-specialist reader.

The HRC response peaks when the input delays are comparable to the internal delay of the model. However, for input delays larger and smaller than the internal delay, the model’s response falls off rather gradually, so that it is directionally responsive over quite a large dynamic range of input delays. In particular, the HRC response is typically proportional to the input delay for small input delays (see Figure 4b), so with reasonably signal to noise, the internal delay will not prevent the model from sensitively detecting even quite short input delays. To provide intuition to the reader we have added two sentences at the end of first and second paragraph of the section “ORN timing enables motion sensing”.

From a biological perspective, the overarching open question is the significance of odor-evoked antennal correlations for odor-guided navigation by animals in natural conditions, when other salient cues (for instance, wind direction) are available. This is a very challenging hypothesis to prove, one that the data in the manuscript do not directly address. We think it can reasonably be considered beyond the scope of a first report, but the language in the manuscript should show constraint and reflect the current state of the evidence, in particular, in the title and and the last sentence of the abstract. The title as currently written makes it sound as if odor motion sensing is the primary mechanism by which complex plumes are navigated, which of course is certainly not the case. If relevance/applications to robotic navigation are to be invoked, the authors should also include a discussion of the current state-of-the-art for VOC sensors in terms of the detection range of odor velocities supported by their current spatiotemporal resolution, and a comparison of whether gradient sensing or odor motion sensing is more easily implemented. This would provide useful information for helping engineers to prioritize their work in this domain.

We have lightened the language in the abstract and have changed the word “enables” to “enhances” in the title. Regarding robots, we added right before the discussion a phrase about recent developments in the use of mm-sized VOC sensors in miniature robots to detect odor signal transients and pointed readers to our discussion in Methods and Extended Data Figure 10 of the relationship between odor motion detection, the distance between the two sensors, and the statistics of the turbulent air flow.

Referee #3 (Remarks to the Author):

I thank the authors for their thorough and thoughtful responses to my questions. The revised manuscript is very strong, and I appreciate the authors' addition of several points, including new analyses and discussion points. I think the observations reported here are of broad interest to the scientific community and would be happy to see them published in Nature.

- Bing Brunton

We are glad that Dr. Brunton finds the revised manuscript to be very strong and thank her for her feedback.