

A split attractor design for rapidly writing a navigational goal

Corresponding Author: Dr Katherine Nagel

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Reviewer comments:

Referee #1

(Remarks to the Author)

The manuscript presents a compelling model of how flies maintain a goal direction during odor-driven navigation. It builds on previous studies showing that hΔK exhibits a stable activity bump during odor-triggered straight walking. By combining experiments with computational models, the authors describe a recurrent circuit between PFG and hΔK neurons, along with additional gating mechanisms involving FB tangential neurons, that enables the transfer and retention of heading direction—potentially constituting an olfactory working memory. Synapse-level circuit dynamics of working memory have long been mysterious in systems neuroscience, across species, including primates. So, this work will appeal to a large audience.

The paper is well written, and the mechanisms are described clearly. The combination of experiments and simulations, we felt, was an effective way to test the hypothesis and model presented. We particularly liked the patch-clamp recordings and commend the authors for performing such challenging experiments. The findings are novel and make a significant contribution to the fields of fly navigation and neuroscience.

However, the manuscript would benefit from a clearer delineation between (i) the hypothesis that hΔK neurons encode a persistent memory of odor location and (ii) the alternative that hΔK merely gates PFG activity during straight walking. In addition, the computational analysis relies on several fixed parameters whose influence on the results has not been explored. Addressing these points will substantially strengthen the manuscript.

Major concerns:

1. Memory vs. gating: It is not clear whether hΔK retains a stable memory of heading or if it is simply gated to be active during straight-walking bouts in odor-directed behavior. In imaging experiments, the two possibilities look identical because a retained memory (i.e., the fixed bump location) is indistinguishable from a heading-activity bump during straight turns (the fixed bump location).

- Perhaps the authors could analyze trial sets in which the fly turns during odor presentation. The computational model clearly shows that if the heading changes while the memory is active (i.e., right after odor presentation), the bump location in both PFG and hΔK remains the same regardless of the heading-direction change (Fig. 5c, d).

- Alternatively, the gating hypothesis could be described in the context of the current caveat of the experiments (and the model), and its role in memory could be speculated upon. It is likely that hΔK is retaining memory of heading, but it would be amiss not to present the gating hypothesis as the most parsimonious explanation of the data.

2. Sensitivity analysis of the computational model: The model contains several hard-coded parameters such as the Gaussian connectivity width (σ) and the decay constants of the α -functions. A systematic sensitivity analysis should be performed to demonstrate that the qualitative behavior described in the paper is robust across a biologically plausible range of values. This analysis is important because it shows how the excitatory and inhibitory landscape changes for different parameter values and solidifies the generality of the results.

Minor concerns:

1. Please describe why a specific standard deviation was used for the connectivity Gaussian equation (line 917). Was this obtained from the average number of synapses to neighboring neurons from the connectome? If so, please describe this.
2. In Figures 1j and 1m, comparisons between bump-on and bump-offset were made for bump amplitude and goal deviation, but not for velocity and heading. Using the same comparisons would clarify the presentation.
3. Fig. 3c - It is not clear what the inputs to the model are. We assume that it is the I_x external input current, but this needs to be stated explicitly. In general, we think the model should be described briefly but clearly (around line 174) so that readers can develop intuition without referring to the methods. Currently, the descriptions are not sufficiently clear.
4. Extended Fig. 4a is confusing, and the legend does not help clarify the figure. It requires some rewriting and reworking.
5. Extended Fig. 5a,b: The top panel is confusing as well. Does this indicate that different sinusoidal inputs are given to the computational model?

(Remarks on code availability)

Referee #2

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

(Remarks on code availability)

Referee #3

(Remarks to the Author)

Summary paragraph:

This manuscript by Aaron et al. presents an impressive study that integrates in vivo calcium imaging, electrophysiological recordings, and computational modeling to uncover a novel mechanism for gating olfactory goal memory within a recurrent neural circuit. The authors demonstrate in the model inspired by their data that the interplay between slow excitation and fast inhibition can generate stable yet tunable persistent attractor dynamics, providing a mechanism for memory maintenance. Furthermore, they identify a gating process through which disinhibition allows the network to sustain memory during goal-directed navigation. They also pinpoint a specific group of inhibitory neurons whose activity patterns align with this proposed gating mechanism. Overall, this work provides new insights into how stability and flexibility of attractor dynamics can be modulated at the circuit level to enable goal directed navigation. The manuscript is thoughtfully executed and makes a significant conceptual advance. Below we outline two major concerns and a few minor issues and typo/suggestions. Were these two major concerns to be addressed with the additional data analysis and some of the minor concerns were also addressed we would advocate for publishing this study in Nature.

Major concerns:

1. More detailed quantification of electrophysiological evidence for slow excitation and fast inhibition and their link to model parameter choices: In Fig 2 it would be great if dynamics of excitation and inhibition were quantified to put a time scale to these processes. For example, you could fit exponential rise and decay curves to the filtered voltage traces and report the corresponding time constants (τ). Then in Figure 3, it would be very helpful to understand how closely the “slow” and “fast” PFG models relate to these exact time constants. How were the A_x values in the model picked and how closely to they relate to the electrophysiology measurements? How slow can excitation in the model be and still work? I’m interested and surprised that excitation as slow as seem in Fig 2 & EDF6 (2-3 seconds) is still fast enough to allow for attractor dynamics to occur and for HdeltaK to be able to track PFG/head direction code? Perhaps my intuition is incorrect, but regardless I feel the paper would be improved by defending the parameters choice in Fig 3, and by outlining the range of parameter speeds that can work, and how the model fails outside of those ranges. Related to this point, perhaps moving EDF 4 panel B to the main figure could help other readers with similar questions.
2. Quantification that PFG and HdeltaK activity is stronger during odor tracking after odor exposure: The paper makes the important claim(s) that these cells have “localized bump of activity that was preferential active during odor and persisted after odor offset while the fly maintained its trajectory.” It is statistically true that the population activity of these cells is higher in the wind period after odor as compared to the wind period before odor? It was not obvious that this is true from the data in Fig 1f,g. Maybe I missed it, but I couldn’t find a rigorous test of this, which seems important for this claim.

Minor concerns:

1. Thought-out the paper this network is referred to as being “stable and tunable” but we found this description somewhat vague. Is your exact meaning that the bump can be stable “in location” and tunable in terms of the duration that it can be allowed to persist? I would be helpful to online this more specifically somewhere.

2. I suggest acknowledging earlier that synaptic output from PFG is not via a fast neurotransmitter and outlining the evidence that it is excitatory more explicitly. As currently written, it was confusing to read the whole paper until the discussion with PFG input to HdeltaK referred to as “excitatory” stated as if it was a simple fact (I assumed it was cholinergic). When EDF 6 shows both slow excitation and inhibition and does not seem to prove that this connectivity is monosynaptic. I suggest that the authors handle this topic earlier and explain more explicitly why you think this connectivity is excitatory (known GPCR receptors?) and how strong your evidence is. This request does not require new data, just a different explanation that is “fairer” to the evidence. Related to this point, as a reader I would have been helped by having the PFG Chrimson data (currently in Extended Figure 6) into the main Figure 2.

3. In the results you state that because current injected to HdeltaK doesn’t induce persistent activity that “intrinsic conductance does not contribute to persistent excitation”. This claim seems overstated because it could be that HdeltaK intrinsic properties interact with circuit interactions to create persistence. I recommend rephrasing this statement.

4. Fig 4: Evidence that HdeltaK bump disappearance is not explained by dim indicator expression: I’m concerned that the disappearance of HdeltaK during turning periods could be explained by weaker indicator expression and thus lower SNR in that genotype compared to PFG imaging. Do you have analysis/evidence that rules out this possibility? For example, is the HdeltaK bump amplitude more correlated with straight walking than PFG regardless of bump “presence”? Or, if you clipped the lower bump amplitude values from PFG data set, does the network activity still look different? Related, is the fly walking/turning behavior similar between these two genotypes – to rule out behavioral differences’ underpinning part of such an effect? (I partially ask this final question because I was confused why Fig 4 g vs h did not quantify the same behavioral turn speeds).

5. Can you clarify in the methods the full details of your fly genetic reagents so future experimentalists could replicate your genotypes? It seems important to specify whether all genotypes used in this study were in a w^+ or w^- or heterozygous background for white and to list all relevant Bloomington Drosophila Stock Center (BDSC) numbers and landing sites (attP) even for effector transgenes. Also more details would be important for the exact effectors used (e.g., type of GFP? EGFP? Or CD8GFP? which tdTomato variant/transgene, and was CsChrimson constructs also included with a fluorescent tag? And if so which one?).

6. A claim of the study is that persistent attractor dynamics are gated via dynamic inhibition mediated by FB5V neurons. While the imaging data convincingly show FB5V activity patterns consistent with gating, the evidence remains correlative. We do not believe that additional experiments are required for publication but just wanted to point out that a loss-of-function experiment—silencing FB5V activity for example—would provide critical causal validation and substantially strengthen this conclusion. Given that other inhibitory inputs to hAK (such as FB6M) also project to PFG and therefore are unlikely to provide selective gating, FB5V appears to be the most promising candidate for a causal manipulation. Even a discussion acknowledging the feasibility or limitations of such an experiment would add valuable perspective.

7. Figure 5a: The authors state that only PFG—but not hAK—receives heading input from the compass system. However, hemibrain connectome indicates that hAK neurons extend processes into the EB and receive substantial input from PENb neurons. While this observation does not fundamentally contradict the main conclusions, it should probably be acknowledged and discussed in the text.

8. Line 261 – PFG is said to have a gain less than one, so does EPG in the literature. I recommend mentioning this as well since it is consistent with it just tracking EPG.

9. Extended Figure 8 shows that bump dynamics in both PFG and hAK correlate more strongly with heading than with traveling direction. This is somewhat surprising given that these neurons receive substantially more synaptic input from hAB neurons (encoding traveling direction) than from EPG or $\Delta 7$ neurons (encoding heading). The authors should discuss possible explanations for this discrepancy and speculate on its functional significance. For example, is this because there is no visual optic flow (as in Lyu et al 2021) to signal travel direction to the fly? Or do you have reason to believe that these cells are truly getting more functional input from EPG and Delta7?

Typo/ very minor clarity feedback:

1. Line 56/57 – this sentence reads like there might be a typo.

2. Line 86 – please define the term “recurrent half-center”

3. Line 94: The reference should be to Figure 1a rather than 1b, since 1a does not depict either PFG or hAK neurons.

4. Line 109 – we found the phrase “behavioral persistence times” confusing at first reading, recommend expanding

5. Line 116/117 – We found the phrase “bump activity” in this sentence confusing, do you mean the existence of the bump?

6. 2.Figures 1j and 1m: The gray and colored traces are aligned to different metrics (bump offset vs. center of bump period). This distinction should be clearly indicated on the x-axis.

7. Line 176 by “two weights” do you mean two constants or scaling factor?

8. Line 198 should “region” but regime?

9. Line 258 typo: “.)”

10. Extended Figure 1d: Can you add explanatory text or legend for the asterisk? (currently unexplained; likely indicating trial onset?).

11. Extended Figure 1e: Can you clarify in the text or legend whether the heading data are phase-nulled, which would explain the observed peak at 0°.

12. Figure 3b: It could be helpful to both x- and y-axes on the connectivity matrix.

13. For Extended Figure 4a a more descriptive legends identifying the nodes and components in the network schematic would helpful to a reader to improve interpretability.

14. Two-Photon calcium imaging parameters: Can the imaging method section state which scanning mode was used? galvo–galvo or galvo–resonant?

15. Line 755: “digitize to 10 Kz” should probably read “digitized at 10 kHz.”

16. Lines 759–760: The units for membrane potential is missing (mV).

17. Lines 768–769: I didn’t understand this sentence in the method: “While we only report the results of light pulse stimuli in this paper, the main electrophysiology findings hold across all optogenetic stimuli”. What do you mean by “all optogenetic stimuli.” Does this refer to different temporal patterns? different light intensities, or stimulation of different input types?

(Remarks on code availability)

Modeling code is posted and can “run”. We did not check it for content. Only modeling code is currently online, not the analysis code.

Referee #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

(Remarks on code availability)

The modeling code is provided. We could run the code and reproduce the figure they showed in the manuscript.

Referee #5

(Remarks to the Author)

In the manuscript “Disinhibition of a recurrent attractor gates a persistent goal signal for navigation,” Lanz et al. discovered a neural circuitry of the insect navigation system in the central complex that establishes a working memory for directional information toward an odor cue. This allows the fly to reliably navigate toward the odor stimulus, even when the odor stimulus disappears. The authors focused especially on two neuron types, hΔK and PFG, in the fly’s central complex, which are strongly and reciprocally connected with each other. Using a virtual reality setting in which the animal’s heading defined the direction of a wind/odor stimulus, combined with two-photon imaging of the population of hΔK and PFG neurons, the authors revealed that both types of neurons respond with a bump of activity in a subdivision of the central complex (the fan-shaped body) when the animal is presented with an odor stimulus. This activity bump remains stable as long as the animal maintains the same goal direction, even after odor offset. Additional patch-clamp recordings showed that maintenance of the activity bump might result from slow excitations via tangential (FB6A and ExR3) neurons and strong, fast inhibitions from additional tangential (FB6M and FB5V) neurons. The authors then used modeling to propose that slow recurrent excitations (compared with fast ones) are key to generating a clear activity bump after stimulus offset that also enables a wide range of “tunability” of this bump after stimulus offset. Intriguingly, the authors then revealed that the way this bump of activity is maintained differs between the hΔK and PFG neurons: while both hΔK and PFG neurons exhibit an activity bump when the animal is heading in the goal direction, during turns, the bump in the hΔK neurons disappears, whereas in PFGs, the activity bump tracks the animal’s heading direction. This finding is consistent with the input of the PFG neurons from compass cells such as Δ7 and EPG. This feature is achieved through a gating mechanism: FB5V and FB6M neurons constantly inhibit hΔK (and FB6M also inhibits PFG) neurons. However, when presented with wind/odor cues during navigation, this inhibition via the FB5V and FB6M neurons is suppressed, resulting in the goal-directed coding observed in the hΔK and PFG network. In general, I am quite enthusiastic about the data. The experiments are well performed, the results are intriguing, and they provide deep insights into the neural mechanisms of navigation, especially goal-directed navigation based on working memory. In my opinion, the story is complete. The major criticism I have, and that I believe needs to be carefully addressed by the authors, is that, in its current state, the manuscript is difficult to understand, especially for non-experts. I understand that the authors attempt to frame the paper around the neural substrate of working memory, but there is a substantial amount of information missing that would (1) allow the reader to understand the relevance of the paper in the context of the insect

navigation system, i.e., the central complex, and (2) make this paper accessible to the broad readership of Nature.

Please find below my points, which I hope will help make this excellent contribution even better:

Major point:

As mentioned above, the manuscript lacks relevant information in the introduction that would frame the paper in the context of the central complex. In my opinion, lines 55-89 are partly redundant with the information given in the abstract and can be shortened. This would free up some space to add additional background information:

- Throughout the manuscript, the authors use the terms fan-shaped body, ellipsoid body, and protocerebral bridge. I assume that not every reader will be familiar with these brain areas. Thus, please mention the relevant brain regions of the central complex (ideally also show them as a subfigure in Fig. 1) at the beginning.
 - Similarly, it is important to mention that, for example, the fan-shaped body consists of vertical columns and that the neurons studied here exhibit a regular connectivity pattern in the FSB. Otherwise, readers will not understand Fig. 1d (columns). The authors should further introduce EPG and $\Delta 7$ neurons in the introduction and provide some information about how the heading information is encoded in the fly's CX, i.e. compass neurons track the fly's heading relative to its environment as an activity bump in the EB (and two bumps in the PB) that shift position when the animal executes turns.
 - Due to the connectivity pattern, these two bumps result in one bump in the FSB. Previous research has shown that the heading-direction information is used in the FSB to compute an allocentric travelling direction. (I know this is mentioned somewhere in the manuscript, but it needs to be clearly stated; otherwise, it will be unclear why you make the comparison in Extended Data Fig. 8c.)
 - It would be very helpful if the authors showed the anatomy of these neurons. Most readers will not be familiar with PFG (Protocerebral Bridge-Fan-Shaped Body-Gall) neurons, and presenting their anatomy (as well as that of the other neurons), at least as supplementary material, would be helpful.
- This is, in my opinion, the minimum additional information needed to appreciate the results presented in this manuscript. Just to be clear: I am not stating this information because I believe the authors do not know about it - they are leading experts in the insect central complex and are fully aware of this – but my goal is to emphasize to them what information I felt was missing.
- The authors do not mention anything about the position of the bump in the fan-shaped body columns. What is the variance in the position of the h Δ K and PFG neuron bumps across the tested flies? Is there an offset, like that shown for the EPG bump, which could be expected?
 - Apparently, there is not a 1:1 connectivity pattern between the 31 h Δ K and the 18 PFG neurons. How exactly are these two networks connected to each other? Is there a bias in the network? How many h Δ K neurons branch per fan-shaped body column?

Additional points:

- Line 98: We recorded NEURAL activity...(otherwise it could also refer to the behavioral activity).
- Lines 100 – 102: The authors might be correct in how they explain the experimental paradigm (wind closed-loop, odor open-loop), but to me, it reads as if the stimuli were not presented via the same stimulus apparatus (as shown in Fig. 1C). Why is the odor stimulus not also presented in closed loop, given that it is delivered to the animal via the closed-loop wind stimulus? Do the authors not believe that the odor stimulus is used to define the direction? I understand their point - that the wind stimulus was set in a closed-loop, not the odor cue - but this caused some confusion for me.
- Given the nature of an odor stimulus, odor cues will remain at the center of the arena even after stimulus offset, if they are not actively vacuumed away. Can the authors exclude the possibility that the effects presented here (i.e., neural activity persisting after stimulus offset) are not a result of residual volatiles remaining in the center of the arena?
- Line 267: Fig. 5j,k should be Fig. 4j,k.
- Lines 312: Please specify already here which tangential neurons you are referring to.
- Line 318: 18 neurons per brain hemisphere or in total (i.e. 9 per brain hemisphere)? If in total, could the authors also indicate the inputs from the contralateral side in Fig 6 a and j.
- Lines 319-322: I understand the authors' conclusion here, but it might be helpful if the authors explicitly mentioned before in the text what they would expect to find based on their model regarding the neural tuning of FB5V neurons.
- Figures 1i,l: Given that the authors also plot the behavior of the animals here, I do not believe it is ideal to call the x-axes "bump persistence post-odor (s)." In my opinion, the x-axis should be "time after odor offset (s)"
- Figures 1h and k: In the figure with the individual trails, it looks like there is a strong inhibition after the bump disappears. Any explanation for this? In addition, given that these plots are based on repeated measurements, I was wondering if the authors noticed any history-dependent /learning effects? Does the bump persistence after stimulus offset increase/decrease with repeated odor presentation?
- Line 732: Wind and compensated AIRFLOW...
- Line 790: Better: For imaging, the brains were...
- Extended fig. 1d: What do the asterisks indicate?
- Extended fig. 1f: Relative to what are the circular diagrams plotted? Is 0° the direction of the wind stimulus? Why is there a bias of long vectors towards 0° in the baseline plot? What does the black line represent? Are they significantly walking towards 0°? This can be tested using circular statistics (e.g. via a Moore's modified Rayleigh test).
- Extended fig. 1g: Remove the "0.1" between the top diagrams 1 and 2. I assume the upwind velocity is related to the animal's walking speed towards the wind/odor stimulus? This needs to be made clear in the figure.

(Remarks on code availability)

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)

The authors have sufficiently addressed major concern 2 and all minor concerns.

We appreciate the work put into addressing major concern 1 and are grateful the authors took our feedback seriously, but feel the concern has not been adequately addressed.

From the computational model, we are convinced that hΔK has the potential to hold a memory of odor location, but do not think this can be concluded from the current experiments. The primary reasons are:

1. Evidence for hΔK's effect on PFG is correlational. The authors provide multiple types of correlational evidence — hΔK and PFG are active with a constant bump during straight walking (Fig. 4a–h), PFG activity follows heading based on amplitude (Fig. 4i–k), and hΔK receives angular velocity-based inhibitory inputs. To establish that hΔK directly influences PFG, a causal link is needed — specifically, whether artificially shifting the bump location in hΔK would induce high-amplitude activity in PFG.
2. Evidence for hΔK's ability to hold memory is also correlational, based on Kathman et al., which showed that hΔK activity does not change when wind direction is rotated. To conclude that hΔK stores a memory of odor location in guiding navigation, a direct demonstration is needed showing that a specific heading is written into hΔK in response to a shifted odor location. That is, when the odor shifts, the fly should turn, but that evidence is missing (or we couldn't find it). In yet other words, the reason of storing that info via recurrent network has not been clear: it is unclear how this information is 'used' for navigation as implied or if it is just stored and discarded.

We recognize the substantial effort that went into this manuscript and want to emphasize that we are not asking the authors to obtain new data. However, we believe that acknowledging and discussing these caveats would improve the paper's clarity and sharpen its conclusions.

We also have concerns about the new analysis in Fig. 4i–k:

1. Could the authors provide a detailed description of how rotational gain was measured, including how measurement noise and scaling are handled? A rotational gain of 0.5 could arise from a 0.5° bump shift per 1° heading change or from a 25° bump shift per 50° heading change — these are not equivalent, as bump tracking precision differs substantially between the two scenarios.
2. Could the authors add distribution plots of heading changes and bump location changes across different bump amplitudes?
3. The temporal filtering approaches for behavioral and imaging data differ — the bump position appears more heavily filtered than the heading data — which may introduce noise into rotational gain calculations. We recommend using the same cutoff frequencies for both signals, particularly if sampling rates differ between imaging and behavioral recordings.
4. The spread around the regression line is large, and the fit appears strongly influenced by a small number of data points. We recommend:
 - Clarifying the p-value for the linear model and how it was computed.
 - Reporting R^2 to indicate variance explained, as Pearson's coefficient is sensitive to outliers and insufficient as a standalone measure.
 - Adding uncertainty bounds around the fit line via bootstrapping to assess whether different samplings of the data would yield substantially different slopes

(Remarks on code availability)

Referee #2

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

(Remarks on code availability)

Referee #3

(Remarks to the Author)

This manuscript by Aaron et al. presents an impressive study that integrates in vivo calcium imaging, electrophysiological recordings, and computational modeling to uncover a novel mechanism for gating olfactory goal memory within a recurrent neural circuit. The authors demonstrate in the model inspired by their data that the interplay between slow excitation and fast inhibition can generate stable yet tunable persistent attractor dynamics, providing a mechanism for memory maintenance. Furthermore, they identify a gating process through which disinhibition allows the network to sustain memory during goal-directed navigation. They also pinpoint a specific group of inhibitory neurons whose activity patterns align with this proposed gating mechanism. Overall, this work provides new insights into how stability and flexibility of attractor dynamics can be modulated at the circuit level to enable goal directed navigation. The manuscript is thoughtfully executed and makes a significant conceptual advance.

Following this revision, we were very pleased to see the updates to this manuscript address all major concerns and overall, we think the manuscript is ready for publication. Congratulations!

The new analysis in EDF2 addresses our concerns about evidence for increased bump amplitude following odor and the addition of the PID measurement is nice. We only have one minor statistics point (below) as well as a few display suggestions:

Minor statistics notes:

The additional experiments added to Figure 4 really strengthened the paper. However, for Figure 4j (left) we were slightly worried how much the 2 lower outliers were driving the r of -0.4 value. It may be advisable to do some form of regression diagnostics (e.g. Cook's distance) to rule out this concern for other readers. We are similarly a little worried if the ExR3 effect in Fig 2h, I may also be driven by outliers. Were those numbers really normally distributed to justify a paired t-test over a Wilcoxon signed-rank test? Since the ExR3 claim is not central to the main points of the paper perhaps it would be safe to tone down this statement slightly if a non-parametric test doesn't give a significance p-value.

Display/phrasing suggestions that are not critical:

In EDF2: I found having binary red/blue = "no bump", as opposed to "bump" confusion at first, I expected the opposite because a higher blue or red value above is a larger bump. Suggest flipping that color scheme.

Figure 1g -base and wind and base and odor labels on the plot are too close together to be seen as distinct

Figure 1 legend "raw DF/F" I would suggest cutting "raw" since it's not F...

Genotype table:

gal4 -> Gal4

GFP -> mCD8::GFP (to match table 2 to avoid confusion)

I assume from your genotypes that all flies are $w+/w+?$. I would be great to note that explicitly. I know that + or blank already indicates $w+$, but because other authors sometimes don't specify leaving ambiguous it would be nice to be written out. Or if flies are $w-$ please indicate.

(Remarks on code availability)

Referee #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

(Remarks on code availability)

Referee #5

(Remarks to the Author)

In the revised manuscript, the authors have responded to/addressed all my comments. After a thorough evaluation, I would like to commend the authors for their comprehensive and thoughtful response to my comments and for an excellent paper. The revisions have significantly strengthened the paper, and I appreciate the clarity and rigor with which the authors addressed each of my points.

I have no further comments to add and believe the manuscript is now suitable for publication in its current form.

(Remarks on code availability)

Version 2:

Reviewer comments:

Referee #1

(Remarks to the Author)

My concerns have been addressed. Thanks for the improved manuscript.

(Remarks on code availability)

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Lanz et al. 2026: Disinhibition of a recurrent attractor gates a persistent goal signal for navigation.

Response to Reviewers

Referees' comments:

Referee #1 (Remarks to the Author):

The manuscript presents a compelling model of how flies maintain a goal direction during odor-driven navigation. It builds on previous studies showing that hΔK exhibits a stable activity bump during odor-triggered straight walking. By combining experiments with computational models, the authors describe a recurrent circuit between PFG and hΔK neurons, along with additional gating mechanisms involving FB tangential neurons, that enables the transfer and retention of heading direction—potentially constituting an olfactory working memory. Synapse-level circuit dynamics of working memory have long been mysterious in systems neuroscience, across species, including primates. So, this work will appeal to a large audience.

The paper is well written, and the mechanisms are described clearly. The combination of experiments and simulations, we felt, was an effective way to test the hypothesis and model presented. We particularly liked the patch-clamp recordings and commend the authors for performing such challenging experiments. The findings are novel and make a significant contribution to the fields of fly navigation and neuroscience.

We thank the Reviewers for their positive comments on the significance and appeal of our manuscript! We are particularly appreciative for the major review suggestions, which we think greatly improve our paper.

However, the manuscript would benefit from a clearer delineation between (i) the hypothesis that hΔK neurons encode a persistent memory of odor location and (ii) the alternative that hΔK merely gates PFG activity during straight walking. In addition, the computational analysis relies on several fixed parameters whose influence on the results has not been explored. Addressing these points will substantially strengthen the manuscript.

Major concerns:

1. Memory vs. gating: It is not clear whether hΔK retains a stable memory of heading or if it is simply gated to be active during straight-walking bouts in odor-directed behavior. In imaging experiments, the two possibilities look identical because a retained memory (i.e., the fixed bump location) is indistinguishable from a heading-activity bump during straight turns (the fixed bump location).
 - Perhaps the authors could analyze trial sets in which the fly turns during odor presentation. The computational model clearly shows that if the heading changes while the memory is active (i.e., right after odor presentation), the bump location in both PFG and hΔK remains the same regardless of the heading-direction change (Fig. 5c, d).
 - Alternatively, the gating hypothesis could be described in the context of the current caveat of the experiments (and the model), and its role in memory could be speculated upon. It is likely that hΔK is retaining memory of heading, but it would be amiss not to present the gating hypothesis as the most parsimonious explanation of the data.

We thank the Reviewer for raising this important question. The reviewer is correct that our model predicts that if heading changes during an active memory, the bump location should not change. Our model further predicts that the presence of a memory should be signaled by the amplitude of the bump in PFG neurons, with higher amplitude representing the memory state and lower amplitude representing a follower state. We therefore sought to test this hypothesis.

We first ran a new experiment to test if the mobility of PFG activity depends on the ongoing bump amplitude. In this experiment, we shifted the wind stimulus by 90° in presence or absence of odor. We then calculated a rotational gain, defined as the total change in bump position divided by the total change in heading, where a gain of 1 indicates perfect tracking and a gain of 0 indicates a perfectly stable bump. We found that PFG bump stability depended strongly on bump amplitude, as predicted by our model: low amplitude PFG bumps shifted with heading, while high amplitude PFG bumps remained stationary. Across trials, rotational gain was negatively correlated with bump amplitude, arguing that PFG can alter its function from tracking heading to maintaining a stable bump position as a function of amplitude.

A second prediction of our gating model is that PFG bump position should stabilize following odor presentation. We tested this idea in our original dataset by calculating rotation gain over time (using a sliding window to calculate rotation

gain over chunks of time). We found that PFG bumps did in fact stabilize following odor onset, as indicated by a drop in rotation gain.

In our previous manuscript (Kathman et al. 2024), we performed similar wind-shift experiment and found that the hΔK bump almost never rotated with the wind shift or any compensatory changes in heading. Here we compare our findings with hΔK to our findings with PFG and find that hΔK bumps show low rotational gain at all times, also consistent with our model. All of these results have been added to Figure 4.

Taken together, we think these new experiments and analyses support our original hypothesis that PFG can switch between a follower model and a memory mode that differ in how much the bump moves with a change in heading.

2. Sensitivity analysis of the computational model: The model contains several hard-coded parameters such as the Gaussian connectivity width (σ) and the decay constants of the α -functions. A systematic sensitivity analysis should be performed to demonstrate that the qualitative behavior described in the paper is robust across a biologically plausible range of values. This analysis is important because it shows how the excitatory and inhibitory landscape changes for different parameter values and solidifies the generality of the results.

We thank the reviewer for this excellent suggestion. The hard-coded parameters mentioned – Gaussian connectivity width and the decay constants of the α -functions – were handpicked in the original submission. In this revision, we have fit these parameters to real data and performed sensitivity analyses on both parameters:

We fit the Gaussian connectivity to the connectivity between hΔK and PFG neurons in the hemibrain connectome. We also performed a sensitivity analysis to determine how the bump width depends on the connectivity width. This analysis found that the bump width scales with the connectivity width, following an exponential plateau, and that our best fit model generates bumps that match our calcium imaging data. The fits and sensitivity analysis are both now shown in Ext. Data Fig. 5.

We fit the decay constants of the α -functions to our synaptic physiology data. For the fast synapses, we fit the decay constant to the voltage responses of hΔK neurons following FB6M stimulation. For the slow synapses, we fit the decay constant to the voltage responses of hΔK neurons following FB6A/FB6D (65C03-Gal4) stimulation in the presence of nicotinic acetylcholine receptor blockers (IMI and curare). As neurons labeled by 65C03-gal4 and hΔK neurons express acetylcholine, these blockers should abolish both the fast synaptic signaling from 65C03-gal4 and recurrent signaling through hΔK neurons, leaving only the slow synaptic signaling from 65C03-gal4. We used these data to fit the slow synaptic time constant because our physiology data from PFG stimulation was more variable across cells. The PFG experiments were also not conducted with acetylcholine blockers, so the time course of the PFG synaptic input cannot be isolated from any slow changes emerging from hΔK recurrent signaling. In addition to these fits, we performed a sensitivity analysis with our simple two-unit model to determine how the slow network decay depends on the decay rates for excitation and inhibition. This analysis shows that slow network decay is tightly linked to the excitation decay rate, but relatively insensitive to inhibition decay rate. These fits are now shown in Ext. Data Fig. 5 and the sensitivity analysis is in Ext. Data Fig. 7.

We re-ran all of our simulations in all main (3 and 5) and extended figures (5-9) using the new fits for Gaussian connectivity and α -function decay constants.

Minor concerns:

1. Please describe why a specific standard deviation was used for the connectivity Gaussian equation (line 917). Was this obtained from the average number of synapses to neighboring neurons from the connectome? If so, please describe this.

We now fit this standard deviation to data from the hemibrain connectome, and re-ran our model using the revised values. In Ext. Data Fig. 5, we now explore how the choice of this parameter affects that width of the predicted calcium bump and show that a larger standard deviation generates wider bumps.

2. In Figures 1j and 1m, comparisons between bump-on and bump-offset were made for bump amplitude and goal deviation, but not for velocity and heading. Using the same comparisons would clarify the presentation.

Thanks for this suggestion. We now show the same comparison for forward velocity and P(upwind orientation). This comparison reveals that the drops in forward velocity and P(upwind orientation) with bump offset were relatively weak compared to bump-on data. This is understandable because the new walking directions following bump offsets in hΔK and PFG neurons can also be upwind orientated, as shown in the examples in Figure 1d,e. We have updated Figure 1 and the text to reflect this small difference.

3. Fig. 3c - It is not clear what the inputs to the model are. We assume that it is the I_x external input current, but this needs to be stated explicitly. In general, we think the model should be described briefly but clearly (around line 174) so that readers can develop intuition without referring to the methods. Currently, the descriptions are not sufficiently clear.

Thanks for this feedback. We have substantially revised the Results text (now line 195-225) to clarify the structure of the model, the way external input is provided, and how both the recurrent synaptic weights and synaptic time constants were fit.

We have also added a clarification in the Methods to state that “ I_x was used to set the input patterns to each population”.

4. Extended Fig. 4a is confusing, and the legend does not help clarify the figure. It requires some rewriting and reworking.

We have removed Ext. Data Fig. 4a. We hope the schematics in Figure 3 and the descriptions in the results are sufficient for understanding the main ideas behind the model.

5. Extended Fig. 5a,b: The top panel is confusing as well. Does this indicate that different sinusoidal inputs are given to the computational model?

This figure is now in Ext. Data Fig. 6a,b. The reviewer is correct; in the top panel we performed four different simulations with the inputs for each simulation corresponding to the four sinusoids. We have changed the colours of the sinusoids and the simulations to make this clearer. We have also updated the legend text for this figure to clarify what was done.

Referee #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #3 (Remarks to the Author):

Summary paragraph:

This manuscript by Aaron et al. presents an impressive study that integrates in vivo calcium imaging, electrophysiological recordings, and computational modeling to uncover a novel mechanism for gating olfactory goal memory within a recurrent neural circuit. The authors demonstrate in the model inspired by their data that the interplay between slow excitation and fast inhibition can generate stable yet tunable persistent attractor dynamics, providing a mechanism for memory maintenance. Furthermore, they identify a gating process through which disinhibition allows the network to sustain memory during goal-directed navigation. They also pinpoint a specific group of inhibitory neurons whose activity patterns align with this proposed gating mechanism. Overall, this work provides new insights into how stability and flexibility of attractor dynamics can be modulated at the circuit level to enable goal directed navigation. The manuscript is thoughtfully executed and makes a significant conceptual advance. Below we outline two major concerns and a few minor issues and typo/suggestions. Were these two major concerns to be addressed with the additional data analysis and some of the minor concerns were also addressed we would advocate for publishing this study in Nature.

We thank the reviewers for their positive comments on the significance and appeal of our manuscript! In addition to their thoughtful major/minor concerns, we also want to thank the reviewers for providing in-depth feedback on typos and figure design.

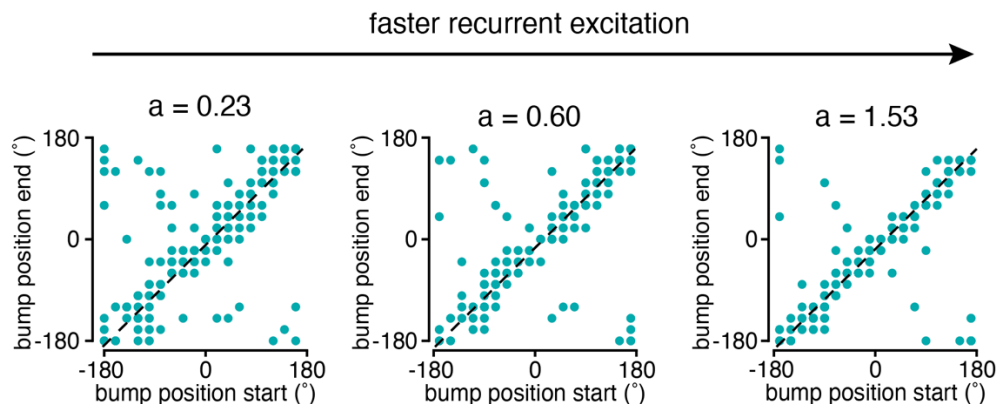
Major concerns:

1. More detailed quantification of electrophysiological evidence for slow excitation and fast inhibition and their link to model parameter choices: In Fig 2 it would be great if dynamics of excitation and inhibition were quantified to put a time scale to these processes. For example, you could fit exponential rise and decay curves to the filtered voltage traces and report the corresponding time constants (τ). Then in Figure 3, it would be very helpful to understand how closely the “slow” and “fast” PFG models relate to these exact time constants. How were the A_x values in the model picked and how closely do they relate to the electrophysiology measurements? How slow can excitation in the model be and still work? I’m interested and surprised that excitation as slow as seen in Fig 2 & EDF6 (2-3 seconds) is still fast enough to allow for attractor dynamics to occur and for HdeltaK to be able to track PFG/head direction code? Perhaps my intuition is incorrect, but regardless I feel the paper would be improved by defending the parameters choice in Fig 3, and by outlining the range of parameter speeds that can work, and how the model fails outside of those ranges. Related to this point, perhaps moving EDF 4 panel B to the main figure could help other readers with similar questions.

We thank the reviewers for this excellent suggestion. The decay constants of the α -functions were handpicked in the original submission. We have now fit these parameters to real data from our electrophysiology data. For fast synapses, we fit the decay constant to the voltage responses of h Δ K neurons following FB6M stimulation. The best-fit alpha function matched the onset and offset voltage responses of h Δ K neurons to FB6M activation, but did not capture the slow synaptic depression. For slow synapses, we could not fit alpha functions well to the FB6A/D (65C03-Gal4) or ExR3 (24C07-Gal4) stimulation data because the resulting dynamics likely reflected a mixture of sources including fast acetylcholine signaling, slow synaptic signaling, and network effects (where the recurrent interactions provide an additional effective time constant). We therefore decided to fit our decay constants to the FB6A/D (65C03-Gal4) stimulation data in the presence of nicotinic acetylcholine receptor blockers (IMI and curare). As neurons labeled by 65C03-gal4 and h Δ K neurons express acetylcholine, these blockers should abolish both the fast synaptic signaling from 65C03-gal4 and recurrent signaling through h Δ K neurons, leaving only the slow synaptic signaling from 65C03-gal4. This data was well fit by alpha functions with a slower decay rate.

We re-ran all of our simulations with these new fitted decay rates, and now show the revised simulations in all of our main Figures (3 and 5) and extended Figures (5-9). In addition to these fits, we performed a sensitivity analysis with our simple two-unit model to determine how the slow network decay depends on the decay rates for excitation and inhibition (Ext. Data Fig. 7c,d). This analysis found that slow network decay is tightly linked to the excitation decay rate, but relatively insensitive to inhibition decay rate, and that our fitted synaptic time constants fall well into the regime for generating stable and tunable persistence.

The reviewer brings up a final interesting point. How does the synaptic decay rate influence the ability of the gated model to faithfully store and update heading memories? We re-ran our analysis from Figure 5f, looking at how well the model stores and stabilizes heading directions as the recurrent excitation increases (see Figure below). In these simulations, we used 200 random heading patterns and tested the model on the same set of patterns with $a = 0.23$, 0.6 (close to slow model in paper), or 1.53. The network is good at stabilizing heading representations for all speeds tested, although there is a small improvement at faster speeds. In our current revision, we have not included this simulation because the manuscript is already quite dense. However, we are happy to include it if the reviewer thinks it is informative.



2. Quantification that PFG and HdeltaK activity is stronger during odor tracking after odor exposure: The paper makes the important claim(s) that these cells have “localized bump of activity that was preferential active during odor and persisted after odor offset while the fly maintained its trajectory.” It is statistically true that the population activity of these cells is higher in the wind period after odor as compared to the wind period before odor? It was not obvious that this is true from the data in Fig 1f,g. Maybe I missed it, but I couldn’t find a rigorous test of this, which seems important for this claim.

We thank the reviewer for this suggestion. We now provide additional plots of the time course of bump activity and quantification of bump activity relative to odor stimuli in Ext. Data Fig. 2. While pre- and post-odor activity is comparable for low numbers of odor pulses, bump activity is more likely in the post-odor compared to pre-odor periods when following high odor pulses. We observed a very similar relationship of bump activity to odor in a broader hΔK line in Kathman et al., 2025 (also on BioRxiv).

Minor concerns:

1. Through-out the paper this network is referred to as being “stable and tunable” but we found this description somewhat vague. Is your exact meaning that the bump can be stable “in location” and tunable in terms of the duration that it can be allowed to persist? I would be helpful to online this more specifically somewhere.

Thanks for this feedback. In our revision, we have tried to be more specific with our language. In our model in Fig. 3, we show that slow excitation allows a bump to be formed over a much wider range of global excitation and inhibition strengths, and also allows the duration of persistent activity to be tuned by adjusting these strengths. We have revised our statements around line 236-238.

In addition, we show in Fig. 5 that disinhibition of hΔK switches the PFG bump from following its heading input to maintaining a bump in a stable position. In this revision, we provide additional experimental support for the idea that positional stability of the PFG bump depends on its amplitude. Throughout the text, we have tried to specify when we mean that the position of the bump is stable.

2. I suggest acknowledging earlier that synaptic output from PFG is not via a fast neurotransmitter and outlining the evidence that it is excitatory more explicitly. As currently written, it was confusing to read the whole paper until the discussion with PFG input to HdeltaK referred to as “excitatory” stated as if it was a simple fact (I assumed it was cholinergic). When EDF 6 shows both slow excitation and inhibition and does not seem to prove that this connectivity is monosynaptic. I suggest that the authors handle this topic earlier and explain more explicitly why you think this connectivity is excitatory (known GPCR receptors?) and how strong your evidence is. This request does not require new data, just a different explanation that is “fairer” to the evidence. Related to this point, as a reader I would have been helped by having the PFG Chrimson data (currently in Extended Figure 6) into the main Figure 2.

This is a good suggestion. We now introduce the idea that PFGs express slow neurotransmitters (referencing Wolff et al., 2025) early in the paper around line 105, and again when establishing the motivation for the model (Fig 3, around line 191). We have also moved the PFG activation data to main Figure 2.

3. In the results you state that because current injected to HdeltaK doesn’t induce persistent activity that “intrinsic conductance do not contribute to persistent excitation”. This claim seems overstated because it could be that HdeltaK intrinsic properties interact with circuit interactions to create persistence. I recommend rephrasing this statement.

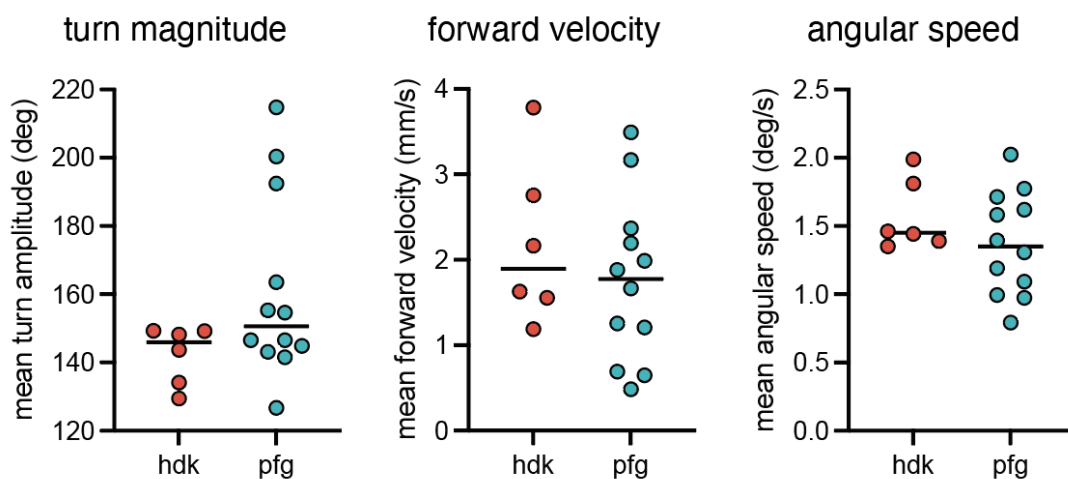
We have clarified our interpretation of this result, saying “We conclude that intrinsic conductances alone are not sufficient to generate persistent excitation in hΔK.”

4. Fig 4: Evidence that HdeltaK bump disappearance is not explained by dim indicator expression: I’m concerned that the disappearance of HdeltaK during turning periods could be explained by weaker indicator expression and thus lower SNR in that genotype compared to PFG imaging. Do you have analysis/evidence that rules out this possibility? For example, is the HdeltaK bump amplitude more correlated with straight walking than PFG regardless of bump “presence”? Or, if you clipped the lower bump amplitude values from PFG data set, does the network activity still look different? Related, is the fly walking/turning behavior similar between these two genotypes – to rule out behavioral differences’ underpinning part

of such an effect? (I partially ask this final question because I was confused why Fig 4 g vs h did not quantify the same behavioral turn speeds).

This is a good point and something we have worried about as well. In this revision we have looked carefully at the rotational gain of the PFG bump, and find that it depends on bump amplitude (Fig. 4i-k). At high bump amplitude the PFG bump shows low rotational gain and does not shift position with changes in heading, while at low bump amplitude, the PFG bump has high rotational gain and readily translates across columns with changes in heading. In contrast, our analysis of hΔK activity (here using a specific hΔK split Gal4 and in our previous manuscript, Kathman et al. 2025, using a broader line VT062617-Gal4), suggests that the hΔK bump almost always has low rotational gain and is fixed in place. The fact that we saw the same type of activity here and in the Kathman manuscript, using two different drivers argues to some extent against this being an effect of a dim indicator. In addition, our modeling suggests that the switch in PFG activity only works if its recurrent partner is really switched off (and so effectively removed from the circuit) during non goal-directed periods. However, you are right that we cannot formally rule out the idea that we observe different activity in hΔK because the SNR in this population is lower. We have revised our discussion of hΔK and PFG differences (around line 321-324) to focus on observations that we think do not depend too heavily on the SNR of the hΔK signal.

As shown below, the behaviour of the hΔK split and PFG split flies was similar. In Figure 4g,h of the manuscript we used different analyses because tracking a circular variable, like bump position, is much easier for continuous representations compared to discrete representations. For a continuous representation, we can easily unwrap changes in bump position and compare to unwrapped changes in heading. This is what we did for PFG neurons, which allowed us to bin the data into small or large turns. In contrast, discrete jumps in bump position can be ambiguous when unwrapped, especially for large changes: for example, does a discrete 135° shift in bump position represent a 135° rightward shift or 225° leftward shift? To avoid this ambiguity, we kept the data in wrapped space, which allowed us to compare hΔK bump shifts and heading shifts in the same encoding space. That is, if the new heading ends up at 135° (regardless of how it got there), then is the new hΔK bump also around 135°?



5. Can you clarify in the methods the full details of your fly genetic reagents so future experimentalist could replicate your genotypes? It seems important to specify whether all genotypes used in this study were in a w^+ or w^- or heterozygous background for white and to list all relevant Bloomington Drosophila Stock Center (BDSC) numbers and landing sites (attP) even for effectors transgenes. Also more details would be important for the exact effectors used (e.g., type of GFP? EGFP? Or CD8GFP? which tdTomato variant/transgene, and was CsChrimson constructs also included with a fluorescent tag? And if so which one?).

Thanks for this. We have now added a Fly Reagent Table (Table 2) listing the precise genetic reagents, sources, and identifiers. The identifiers should point readers towards landing sites and fly backgrounds.

6. A claim of the study is that persistent attractor dynamics are gated via dynamic inhibition mediated by FB5V neurons. While the imaging data convincingly show FB5V activity patterns consistent with gating, the evidence remains correlative. We do not believe that additional experiments are required for publication but just wanted to point out that a

loss-of-function experiment—silencing FB5V activity for example—would provide critical causal validation and substantially strengthen this conclusion. Given that other inhibitory inputs to hΔK (such as FB6M) also project to PFG and therefore are unlikely to provide selective gating, FB5V appears to be the most promising candidate for a causal manipulation. Even a discussion acknowledging the feasibility or limitations of such an experiment would add valuable perspective.

Thanks for this suggestion. We agree that this would be the ideal experiment to directly test our model, and undertook pilot experiments to do this by using VT062617-lexA to image hΔK neurons while use Gal4-UAS reagents to try to silence FB5V. Our pilot imaging revealed that VT062617-lexA was not bright enough for us to detect reliable bump activity. We tried this with two different indicators (lexAOP-GCaMP6f and 7b). Because the strategy of using VT062617-lexA to image hΔK does not look viable at the moment, and we do not currently have a lexA line for either PFG or FB5V, we think this experiment is currently beyond the scope of the present manuscript. We have added a line to the Discussion, noting that this is a key experiment to rigorously test the model in the future (line 482-484).

7. Figure 5a: The authors state that only PFG—but not hΔK—receives heading input from the compass system. However, hemibrain connectome indicates that hΔK neurons extend processes into the EB and receive substantial input from PENb neurons. While this observation does not fundamentally contradict the main conclusions, it should probably be acknowledged and discussed in the text.

We agree, and now include this point when introducing the model in Figure 5:

“In published connectomes, only PFGs receive direct input from EPG and Δ7 neurons that track fly heading (**Fig. 5a, although hΔK does receive input from PEN neurons that encode angular velocity and rotate the heading bump**^{37, 38}), and this input is significantly weaker than the recurrent connections between hΔK and PFGs (Ext. Data Fig. 9a,b).”

8. Line 261 – PFG is said to have a gain less than one, so does EPG in the literature. I recommend mentioning this as well since it is consistent with it just tracking EPG.

Thanks, we have added a line saying the heading encoding by PFG neurons was “similar to EPG neurons of the heading compass”

9. Extended Figure 8 shows that bump dynamics in both PFG and hΔK correlate more strongly with heading than with traveling direction. This is somewhat surprising given that these neurons receive substantially more synaptic input from hΔB neurons (encoding traveling direction) than from EPG or Δ7 neurons (encoding heading). The authors should discuss possible explanations for this discrepancy and speculate on its functional significance. For example, is this because there is no visual optic flow (as in Lyu et al 2021) to signal travel direction to the fly? Or do you have reason to believe that these cells are truly getting more functional input from EPG and Delta7?

We agree, this network is well positioned to encode either heading directions from EPG neurons or travel directions from hΔB neurons. We might expect different behavior with respect to travel direction because hΔB provides input to both PFG and hΔK, in contrast to compass input. We were also surprised that bump position correlated worse with travel direction than with heading and we think the reviewer’s explanation is a good theory, that travel direction representations may be poor in conditions without optic flow. We have added a comment on this in the Results around line 295.

Typo/ very minor clarity feedback:

We thank the reviewers for all the typo and clarity feedback. They caught many things we missed!

1. Line 56/57 – this sentence reads like there might be a typo.

Fixed.

2. Line 86 – please define the term “recurrent half-center”

We agree this term is confusing. We have removed it from the manuscript.

3. Line 94: The reference should be to Figure 1a rather than 1b, since 1a does not depict either PFG or hΔK neurons.

Fixed.

4. Line 109 – we found the phrase “behavioral persistence times” confusing at first reading, recommend expanding

Changed to “goal-directed walking times”

5. Line 116/117 – We found the phrase “bump activity” in this sentence confusing, do you mean the existence of the bump?

Changed to “The presence of bumps”

6. 2.Figures 1j and 1m: The gray and colored traces are aligned to different metrics (bump offset vs. center of bump period). This distinction should be clearly indicated on the x-axis.

Good idea, we updated the x-axis labels

7. Line 176 by “two weights” do you mean two constants or scaling factor?

Yes. In our revision we have tried to clarify this: " The global strength of excitation and inhibition could be **scaled** through two free parameters."

8. Line 198 should “region” but regime?

Fixed.

9. Line 258 typo: “.)”

Fixed.

10. Extended Figure 1d: Can you add explanatory text or legend for the asterisk? (currently unexplained; likely indicating trial onset?).

Fixed, we added the asterisk to the legend.

11. Extended Figure 1e: Can you clarify in the text or legend whether the heading data are phase-nulled, which would explain the observed peak at 0°.

Yes, 0° is always upwind. We made the figure and caption clearer.

12. Figure 3b: It could be helpful to both x- and y-axes on the connectivity matrix.

Fixed.

13. For Extended Figure 4a a more descriptive legends identifying the nodes and components in the network schematic would helpful to a reader to improve interpretability.

We removed this subpanel because several reviewers did not understand it

14. Two-Photon calcium imaging parameters: Can the imaging method section state which scanning mode was used? galvo–galvo or galvo–resonant?

Added, we used galvo-galvo scanning.

15. Line 755: “digitize to 10 Kz” should probably read “digitized at 10 kHz.”

Fixed.

16. Lines 759–760: The units for membrane potential is missing (mV).

Fixed.

17. Lines 768–769: I didn’t understand this sentence in the method: “While we only report the results of light pulse stimuli in this paper, the main electrophysiology findings hold across all optogenetic stimuli”. What do you mean by “all optogenetic stimuli.” Does this refer to different temporal patterns? different light intensities, or stimulation of different input types?

Yes, we meant that the voltage responses we observed, mainly the fast responses to FB6M/FB5V (plus slow depression) and slow and persistent responses to 65C03/ExR3 were seen across all temporal patterns, albeit much weaker for low power stimuli. We have rewritten this sentence as: “While we only report the results of light pulse stimuli in this paper, the voltage changes we observed – such as fast inhibition or slow persistent excitation - were seen across all optogenetic temporal patterns used.”

Referee #3 (Remarks on code availability):

Modeling code is posted and can “run”. We did not check it for content. Only modeling code is currently online, not the analysis code.

Referee #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #4 (Remarks on code availability):

The modeling code is provided. We could run the code and reproduce the figure they showed in the manuscript.

Referee #5 (Remarks to the Author):

In the manuscript “Disinhibition of a recurrent attractor gates a persistent goal signal for navigation,” Lanz et al. discovered a neural circuitry of the insect navigation system in the central complex that establishes a working memory for directional information toward an odor cue. This allows the fly to reliably navigate toward the odor stimulus, even when the odor stimulus disappears. The authors focused especially on two neuron types, hΔK and PFG, in the fly’s central complex, which are strongly and reciprocally connected with each other. Using a virtual reality setting in which the animal’s heading defined the direction of a wind/odor stimulus, combined with two-photon imaging of the population of hΔK and PFG neurons, the authors revealed that both types of neurons respond with a bump of activity in a subdivision of the central complex (the fan-shaped body) when the animal is presented with an odor stimulus. This activity bump remains stable as long as the animal maintains the same goal direction, even after odor offset. Additional patch-clamp recordings showed that maintenance of the activity bump might result from slow excitations via tangential (FB6A and ExR3) neurons and strong, fast inhibitions from additional tangential (FB6M and FB5V) neurons. The authors then used

modeling to propose that slow recurrent excitations (compared with fast ones) are key to generating a clear activity bump after stimulus offset that also enables a wide range of “tunability” of this bump after stimulus offset. Intriguingly, the authors then revealed that the way this bump of activity is maintained differs between the hΔK and PFG neurons: while both hΔK and PFG neurons exhibit an activity bump when the animal is heading in the goal direction, during turns, the bump in the hΔK neurons disappears, whereas in PFGs, the activity bump tracks the animal’s heading direction. This finding is consistent with the input of the PFG neurons from compass cells such as Δ7 and EPG. This feature is achieved through a gating mechanism: FB5V and FB6M neurons constantly inhibit hΔK (and FB6M also inhibits PFG) neurons. However, when presented with wind/odor cues during navigation, this inhibition via the FB5V and FB6M neurons is suppressed, resulting in the goal-directed coding observed in the hΔK and PFG network.

In general, I am quite enthusiastic about the data. The experiments are well performed, the results are intriguing, and they provide deep insights into the neural mechanisms of navigation, especially goal-directed navigation based on working memory. In my opinion, the story is complete. The major criticism I have, and that I believe needs to be carefully addressed by the authors, is that, in its current state, the manuscript is difficult to understand, especially for non-experts. I understand that the authors attempt to frame the paper around the neural substrate of working memory, but there is a substantial amount of information missing that would (1) allow the reader to understand the relevance of the paper in the context of the insect navigation system, i.e., the central complex, and (2) make this paper accessible to the broad readership of Nature.

We thank the reviewer for their positive comments on the significance and appeal of our manuscript! In this revision, we have made several changes to the figures and text to make the paper easier to understand for a non-fly and more general audience.

Please find below my points, which I hope will help make this excellent contribution even better:

Major point:

As mentioned above, the manuscript lacks relevant information in the introduction that would frame the paper in the context of the central complex. In my opinion, lines 55-89 are partly redundant with the information given in the abstract and can be shortened. This would free up some space to add additional background information:

- Throughout the manuscript, the authors use the terms fan-shaped body, ellipsoid body, and protocerebral bridge. I assume that not every reader will be familiar with these brain areas. Thus, please mention the relevant brain regions of the central complex (ideally also show them as a subfigure in Fig. 1) at the beginning.
- Similarly, it is important to mention that, for example, the fan-shaped body consists of vertical columns and that the neurons studied here exhibit a regular connectivity pattern in the FSB. Otherwise, readers will not understand Fig. 1d (columns). The authors should further introduce EPG and Δ7 neurons in the introduction and provide some information about how the heading information is encoded in the fly's CX, i.e. compass neurons track the fly's heading relative to its environment as an activity bump in the EB (and two bumps in the PB) that shift position when the animal executes turns.
- Due to the connectivity pattern, these two bumps result in one bump in the FSB. Previous research has shown that the heading-direction information is used in the FSB to compute an allocentric travelling direction. (I know this is mentioned somewhere in the manuscript, but it needs to be clearly stated; otherwise, it will be unclear why you make the comparison in Extended Data Fig. 8c.)
- It would be very helpful if the authors showed the anatomy of these neurons. Most readers will not be familiar with PFG (Protocerebral Bridge-Fan-Shaped Body-Gall) neurons, and presenting their anatomy (as well as that of the other neurons), at least as supplementary material, would be helpful.

This is, in my opinion, the minimum additional information needed to appreciate the results presented in this manuscript. Just to be clear: I am not stating this information because I believe the authors do not know about it - they are leading experts in the insect central complex and are fully aware of this – but my goal is to emphasize to them what information I felt was missing.

- Apparently, there is not a 1:1 connectivity pattern between the 31 hΔK and the 18 PFG neurons. How exactly are these two networks connected to each other? Is there a bias in the network? How many hΔK neurons branch per fan-shaped body column?

Thank you for these helpful suggestions! We made several changes to the figures and text to address these suggestions.

- 1) First, before introducing hΔK and PFG neurons, we introduce the idea of representing goal signals as bumps of activity across the columns of the FB:

“Several FB local neuron types have been shown to encode goal direction signals for navigation as positionally stable bumps of activity across the columns of the FB^{22-24,26-28}.”

2) Second, we split Ext Data Fig. 1 into two figures, where the first extended data figure contains useful background information for non-fly readers. Ext. Data Fig. 1a,b now shows the EM morphology of the hΔK population, the PFG population, and a pair of recurrently connected hΔK and PFG neurons. Ext. Data Fig. 1c provides a schematic showing how PFG and hΔK neurons are recurrently connected given that these populations contain different numbers of neurons. Ext. Data Fig. 1d provides a schematic showing the connectivity of EPG neurons --> PFG neurons <--> hΔK neurons, which will hopefully help demonstrate how bump representations in the ellipsoid body are mapped onto PFG and hΔK neurons in the FB. And finally, Ext. Data Fig. 1e provides a schematic showing how FB tangential neurons globally innervate hΔK and PFG neurons.

3) To introduce this anatomy and connectivity, we have added a paragraph in the introduction explaining why we hypothesized the hΔK-PFG network connectivity might function to store heading directions in working memory:

“hΔK neurons are embedded in a recurrent network reminiscent of classic models of working memory (Fig. 1a,b), as indicated by three shared motifs. First, hΔK neurons are tightly recurrently connected with a second group of FB local neurons, called PFGs (Fig. 1b; Ext. Data Fig. 1a-c). This recurrent connectivity is highly structured, with strong recurrence within columns of the FB and little connectivity across columns (Ext. Data Fig. 1c,d,f). Second, PFG neurons receive direct inputs from EB compass neurons that represent heading as a continuously updating bump of activity^{13,29}. These inputs are also spatially structured, such that each column of the FB receives heading inputs tuned to different segments of the world (Fig. 1b; Ext. Data Fig. 1d)8. Finally, hΔK and PFG neurons both receive global inputs from several types of broadly-projecting tangential neurons (Fig 1b; Ext Data Fig. 1e). The similarity of this network to classic working memory models suggests that this architecture might function to store heading representations over time to orchestrate stable goal-directed walking.”

In this version of the introduction and Ext. Data Figure 1, we do not explicitly talk about how the EPG bump is split into two bumps within the protocerebral bridge and then reconverged into one bump in the FB. We think the main idea that reader's need to know is that the heading representation in EPG neurons maps onto the PFG neurons of the FB, such that each section of the FB should represent different parts of heading space. We hope that by introducing the column organization of the FB early, and then laying out how hΔK and PFG populations interact and receive heading representations across these columns, reader's will be better prepared for following our results and model.

- The authors do not mention anything about the position of the bump in the fan-shaped body columns. What is the variance in the position of the hΔK and PFG neuron bumps across the tested flies? Is there an offset, like that shown for the EPG bump, which could be expected?

We expect that hΔK and PFG neurons inherit offsets in their heading representation from EPGs, which have been shown to vary across flies. This question is hard to answer in our dataset for a few reasons. First, both hΔK and PFG neurons frequently exhibit periods where the bump position remains stationary despite changes in heading (Figure 4), which impairs our ability to identify heading receptive fields in these populations. And second, the wind position shifts between trials, starting at a new random position for each trial. Since we did not simultaneously measure EPG signals, we cannot be certain of how heading representations change across trials.

Additional points:

- Line 98: We recorded NEURAL activity...(otherwise it could also refer to the behavioral activity).

Fixed.

- Lines 100 – 102: The authors might be correct in how they explain the experimental paradigm (wind closed-loop, odor open-loop), but to me, it reads as if the stimuli were not presented via the same stimulus apparatus (as shown in Fig. 1C). Why is the odor stimulus not also presented in closed loop, given that it is delivered to the animal via the closed-loop wind stimulus? Do the authors not believe that the odor stimulus is used to define the direction? I understand their point - that the wind stimulus was set in a closed-loop, not the odor cue - but this caused some confusion for me.

We agree that this is confusing. The wind position is in closed loop with the fly's walking behavior to simulate wind from a fixed direction, while odor is presented with experimenter-controlled timing. We have revised the text to clarify these points.

- Given the nature of an odor stimulus, odor cues will remain at the center of the arena even after stimulus offset, if they are not actively vacuumed away. Can the authors exclude the possibility that the effects presented here (i.e., neural activity persisting after stimulus offset) are not a result of residual volatiles remaining in the center of the arena?

Thanks for this question. In this revision, we show photo-ionization detector (PID) measurements of odor concentration at the location of the fly (Ext Data Fig. 2). These recordings illustrate that the odor signal onset and offset kinetics are much faster than the persistence we see in the behaviour or neural activity.

- Line 267: Fig. 5j,k should be Fig. 4j,k.

Fixed.

- Lines 312: Please specify already here which tangential neurons you are referring to.

Added.

- Line 318: 18 neurons per brain hemisphere or in total (i.e. 9 per brain hemisphere)? If in total, could the authors also indicate the inputs from the contralateral side in Fig 6 a and j.

The 18 neurons refers to the total number of neurons, with 9 per hemisphere. We have made this clearer in the text.

- Lines 319-322: I understand the authors' conclusion here, but it might be helpful if the authors explicitly mentioned before in the text what they would expect to find based on their model regarding the neural tuning of FB5V neurons.

We list our predictions in the first paragraph of this section.

- Figures 1i,I: Given that the authors also plot the behavior of the animals here, I do not believe it is ideal to call the x-axes "bump persistence post-odor (s)." In my opinion, the x-axis should be "time after odor offset (s)"

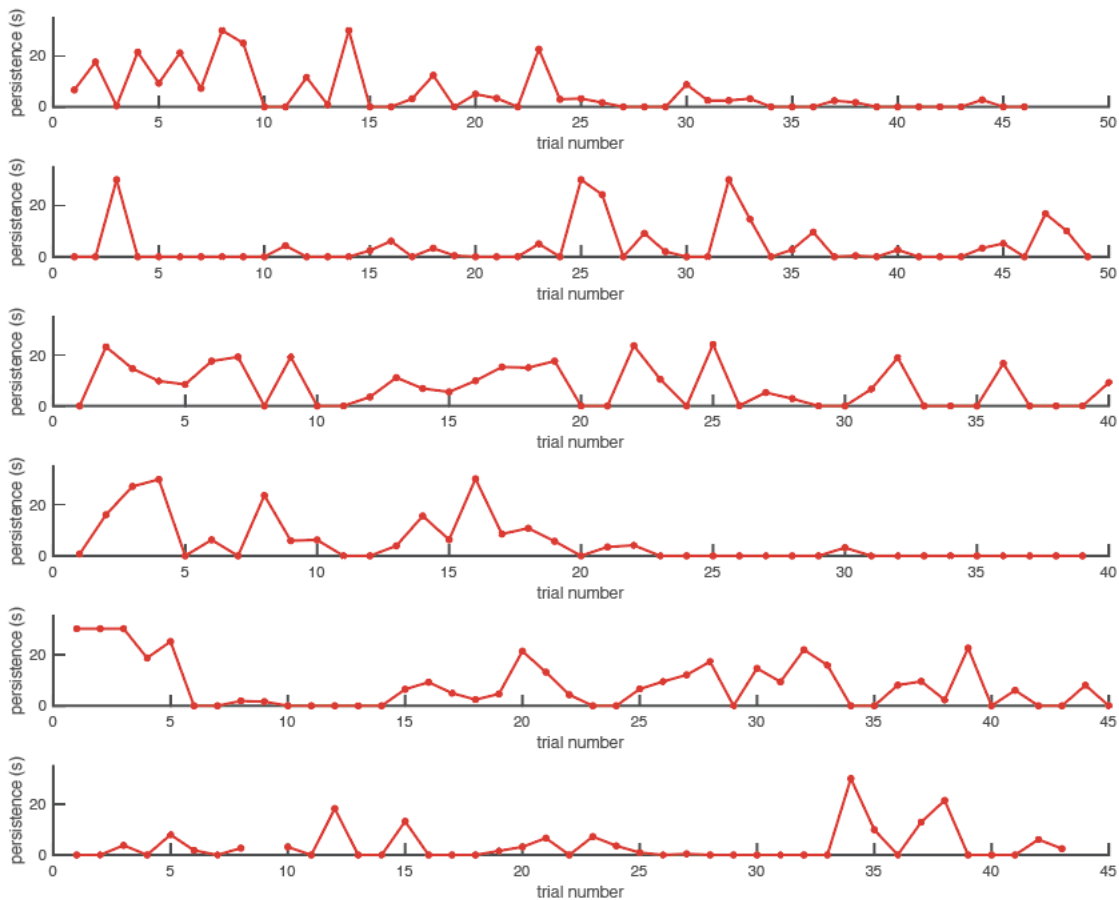
Thanks, we have changed the axis label.

- Figures 1h and k: In the figure with the individual trails, it looks like there is a strong inhibition after the bump disappears. Any explanation for this? In addition, given that these plots are based on repeated measurements, I was wondering if the authors noticed any history-dependent /learning effects? Does the bump persistence after stimulus offset increase/decrease with repeated odor presentation?

Good observation! Flies often turn after they complete their upwind run, either to walk in a new direction or to perform a looping local search behavior. Because we saw that both FB5V and FB6M increase activity during turns, this inhibition is likely related to the turning behavior.

In terms of history-dependent effects, we do not see any obvious history-dependent effects on persistence timing. In the below plot, each row is a different hAK fly with the bump persistence plotted by trial number. There is no gradual increase or decrease in the persistence timing. In a previous paper (Kathman et al., 2025), we found that flies often undergo periods of low or high responsiveness to odor, with each phase lasting many trials at a time. We found that hAK activity is low during periods of low responsiveness and high during periods of high responsiveness, suggesting some type of long-term

modulation of the network. We observe similar runs of high persistence and low persistence in this data set (for example rows 1 and 2 below).



- Line 732: Wind and compensated AIRFLOW...

Fixed.

- Line 790: Better: For imaging, the brains were...

Fixed.

- Extended fig. 1d: What do the asterisks indicate?

Fixed, we added the asterisk to the figure legend. The asterisks indicate trial start.

- Extended fig. 1f: Relative to what are the circular diagrams plotted? Is 0° the direction of the wind stimulus? Why is there a bias of long vectors towards 0° in the baseline plot? What does the black line represent? Are they significantly walking towards 0° ? This can be tested using circular statistics (e.g. via a Moore's modified Rayleigh test).

Thanks for these questions, we have added a clarification to the Figure caption. The black lines on the plots indicate the mean vector for each condition and 0° (upwards) is the upwind direction. The reviewer is correct that there is a slight bias towards 0° in the baseline plot, particularly for the longer run durations. This is not unexpected because we did not

include blank trials in our experiment, so if the flies maintained their walking bout direction for long enough following trial start, then wind and odor were turned on, and the flies already walking upwind would often continue walking upwind. Unsurprisingly, given these results, both the baseline and post-odor vectors passed significance using the Moore's modified Rayleigh test (baseline: $r^* = 3.2262$, $N = 507$; post-odor: $r^* = 8.3238$, $N = 507$).

- Extended fig. 1g: Remove the "0.1" between the top diagrams 1 and 2. I assume the upwind velocity is related to the animal's walking speed towards the wind/odor stimulus? This needs to be made clear in the figure.

We removed the 0.1 typo and added clarification about upwind velocity in the figure caption:

"Mean upwind velocity (**walking aligned with upwind**), hΔK bump amplitude, and hΔK bump probability during baseline (no stimuli), wind, and pulses (1, 4, 7, or 10) of odor."

Response to Reviewers

We are very pleased that the reviewers felt we had addressed most of their major concerns. We appreciate that Reviewers 1 and 2 have some concerns about the analysis methods for the new experiments we added in the previous revision. In the present revision, we thoroughly address each of their concerns, revising our analysis methods to make the sampling rates for heading and bump movement equivalent, and using bootstrapping to judge the validity of the observed correlations. We also appreciate that Reviewer 1 still has some concerns about the relationship of our model to the experimental data, and makes several useful suggestions for future experiments to directly test additional predictions of our model. We address these concerns through text revisions and in our Response below.

Referees' comments:

Referee #1 (Remarks to the Author):

The authors have sufficiently addressed major concern 2 and all minor concerns.

We are very glad to hear that our previous revision addressed major concern 2 and all minor concerns.

We appreciate the work put into addressing major concern 1 and are grateful the authors took our feedback seriously, but feel the concern has not been adequately addressed.

From the computational model, we are convinced that hΔK has the potential to hold a memory of odor location, but do not think this can be concluded from the current experiments. The primary reasons are:

1. Evidence for hΔK's effect on PFG is correlational. The authors provide multiple types of correlational evidence — hΔK and PFG are active with a constant bump during straight walking (Fig. 4a–h), PFG activity follows heading based on amplitude (Fig. 4i–k), and hΔK receives angular velocity-based inhibitory inputs. To establish that hΔK directly influences PFG, a causal link is needed — specifically, whether artificially shifting the bump location in hΔK would induce high-amplitude activity in PFG.
2. Evidence for hΔK's ability to hold memory is also correlational, based on Kathman et al., which showed that hΔK activity does not change when wind direction is rotated. To conclude that hΔK stores a memory of odor location in guiding navigation, a direct demonstration is needed showing that a specific heading is written into hΔK in response to a shifted odor location. That is, when the odor shifts, the fly should turn, but that evidence is missing (or we couldn't find it). In yet other words, the reason of storing that info via recurrent network has not been clear: it is unclear how this information is 'used' for navigation as implied or if it is just stored and discarded.

We recognize the substantial effort that went into this manuscript and want to emphasize that we are not asking the authors to obtain new data. However, we believe that acknowledging and discussing these caveats would improve the paper's clarity and sharpen its conclusions.

We appreciate the Reviewer's thoughtful assessment of our model and the data we show here and in previous papers to support the model. Our claim that the hΔK-PFG circuit supports a short-term goal memory is based on the following pieces of data:

- (1) Sparse activation of hΔK neurons (VT062617-Gal4 driving 15% SPARC>Chrimson) caused flies to run in a reproducible direction with respect to the wind (Matheson et al. 2022). This is the strongest causal evidence that hΔK neurons can encode a goal direction and that patterns of activity in hΔK are

meaningful for behavior. In that paper we observed that each fly, having a fixed pattern of sparse Chrimson expression, adopted a specific run direction relative to wind. Run direction did not correlate with either the location of expression within the fan-shaped body, or its concentration as a “bump”. We believe that the current study helps explain this result, as we show in our model that any patterns of asymmetric expression in hΔK will tend to resolve into a bump of the same width driven by the circuit’s attractor dynamics.

2) In the Kathman et al. 2025 preprint, we show that optogenetic silencing of hΔK neurons (using VT062617-Gal4 and an hΔK split-Gal4 lines) reduces the persistence of upwind runs in freely-walking flies. This experiment provides a second piece of causal evidence that hΔK activity contributes to the maintenance of a goal direction. We made related observations in the Matheson paper where we found that silencing of VT062617-Gal4 neurons led to impaired upwind orientation late in the odor period. We interpret these results to mean that the choice of run direction is made elsewhere but that the hΔK circuit enables the fly to maintain this direction after odor has disappeared, consistent with its observed persistent bump activity. We also show in that preprint that the hΔK bump does not move when the wind is rotated, driving a compensatory turn in the fly, which is consistent with the bump representing a fixed goal rather than representing heading.

3) The present manuscript shows how a heading signal entering PFGs neurons through their compass inputs can be transformed into a short-term memory signal through a gated recurrence mechanism. Our model is consistent with connectivity of hΔK, PFG, and FB5V neurons across published connectomes, the observed dynamics of bump activity in both PFG and hΔK neurons and their dependence on bump amplitude, the dynamics of activity in each population during wind shifts, the dynamics FB5V activity during behavior, and the measured synaptic effects of stimulating FB5V while recording from hΔK.

We agree that additional causal experiments could be done to directly test predictions of our model. Among these, we think the most important would be to manipulate FB5V activity while imaging from hΔK and/or PFG. For example, activating FB5V should suppress hΔK activity and cause the bump in PFG to rotate more freely (increased rotational gain). Conversely, inhibition of FB5V should lead to more prominent bump activity in hΔK and a brighter, less mobile bump in PFG neurons. A discussion of this experiment was requested by Reviewer 2 in the first set of reviews. As we described in our response, we attempted to perform these experiments using VT062617-lexA to image hΔK activity, and Gal4 reagents to manipulate FB5V. We found that the VT062617-lexA line was not bright enough to image hΔK activity using our existing indicators.

A variety of other causal experiments, including the ones suggested here, would allow us to directly test predictions of our model. For example, inhibition of hΔK should have the same effect as activation of FB5V in allowing the PFG bump to move more freely. Writing a bump of activity into hΔK should also cause the bump of activity in PFG to move to a new location, as suggested here. Performing these experiments will require us to make and validate orthogonal expression lines for PFG and FB5V which we do not currently have. We therefore think such experiments are best reserved for a future study. Such a study might also revisit the causal results from the Matheson paper and ask whether we can also generate goal-directed running through laser activation of a specific split-Gal4 line.

In response to these concerns we have made several changes to the manuscript. First, we have removed the word “memory” from the last line of the Abstract (line 28) and replaced the phrase “memory circuit” in line 84 of the Introduction with “attractor circuit.” We also changed the phrase, “Our data and models reveals a circuit motif” (line 95) to “Our data and models **introduce** a circuit motif.”

In addition, we have made several changes in the Discussion to clarify which findings apply to the model, and which are directly supported by experimental evidence.

In line 462-465, we now say:

PFG neurons receive a heading direction signal from the compass system, while hΔK neurons receive inhibition from FB5V. In our model, this allows PFGs to encode the content of memory, while FB5V gating of hΔK controls the timing of memory formation. By separating the input of the network from the gating signal, our model enables rapid gating of recurrent activity.

We have also substantially rewritten the subsequent paragraph (lines 474-491):

Finally we show through modeling that inhibition of one half of such a split attractor network can allow a circuit to rapidly switch between two modes: information following and information storage. Our model— based on precise synapse counts between synaptic partners in this microcircuit— functions similar to a latch in digital logic⁶⁷. When one half of the circuit (hΔK) is strongly inhibited, the recurrent circuit becomes functionally decoupled, and the other half (PFG) can follow their inputs freely. Disinhibition can rapidly turn on the recurrent circuit and lock the current input value into place. This model is consistent with several pieces of experimental data shown here. It reproduces the observed dynamics of both hΔK and PFG neurons during ongoing navigation and wind shifts, as well as the activity of FB5V during odor and turns. Our model is also consistent with causal experiments shown in our previous papers. In Matheson et al. we showed that sparse activation of hΔK neurons (labeled by VT062617-Gal4) could drive movement in a reproducible goal direction, while in Kathman et al. we showed that optogenetic silencing of hΔK neurons reduced the persistence of odor-evoked runs. A rigorous test of our computational model will require additional causal experiments. For example, activation and silencing of FB5V should switch the circuit between information following and information storage, with predictable changes in the amplitude and rotational gain of both PFG and hΔK activity. Writing a bump into hΔK or PFG using laser activation should also produce predictable changes in the activity of the other population and behavior.

We hope that these changes address the reviewer's concern and clarify what we think we have demonstrated here, the relationship between our model and the data supporting it, and which experiments remain to be done to demonstrate that the hΔK-PFG circuit works as our model proposes.

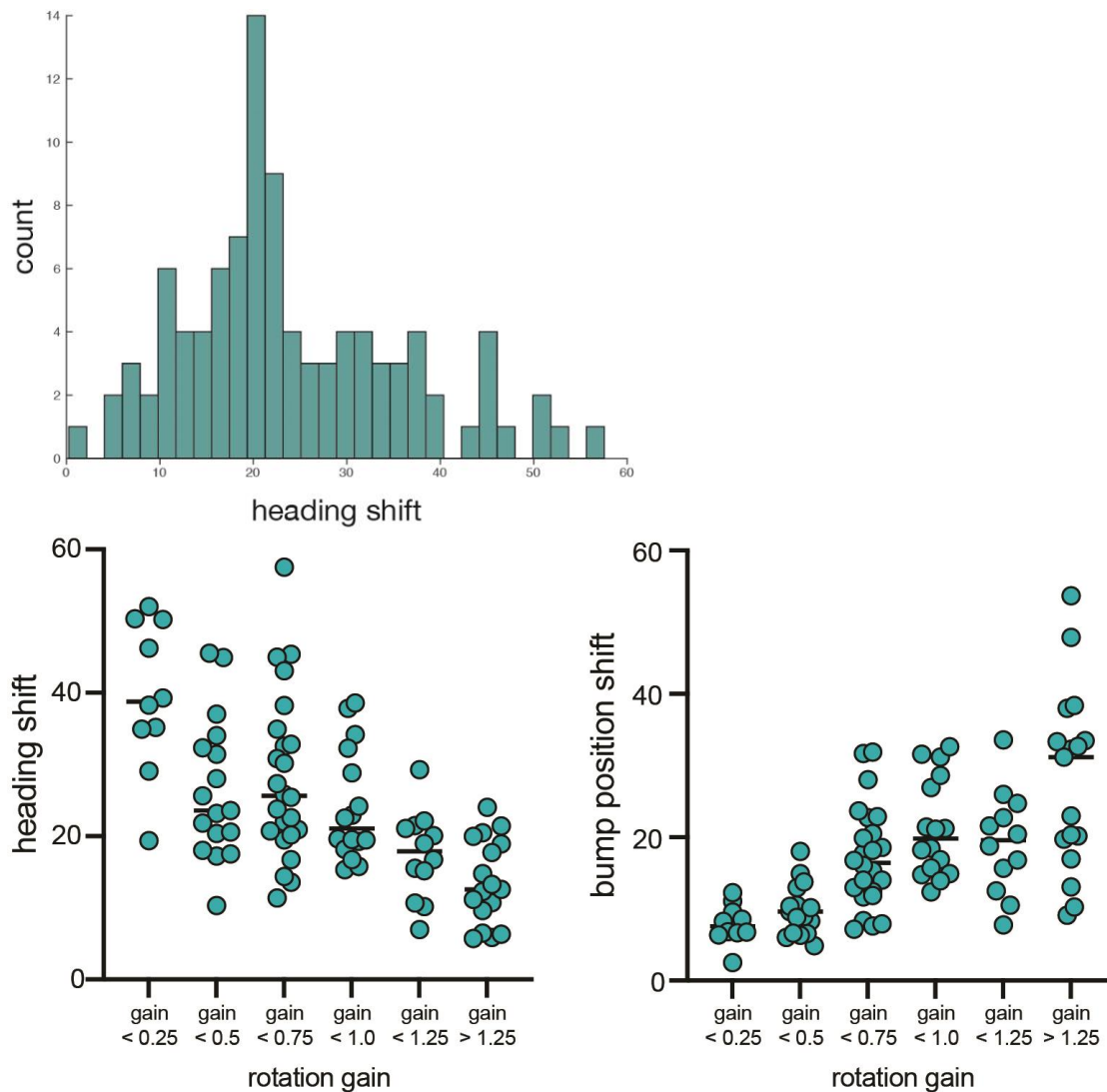
We also have concerns about the new analysis in Fig. 4i–k:

1. Could the authors provide a detailed description of how rotational gain was measured, including how measurement noise and scaling are handled? A rotational gain of 0.5 could arise from a 0.5° bump shift per 1° heading change or from a 25° bump shift per 50° heading change — these are not equivalent, as bump tracking precision differs substantially between the two scenarios.

We thank the reviewer for this question. In this revision we have included a new panel, Ext. Data Fig. 8g, showing the cumulative change in heading and bump position that were used to compute rotational gain for each trial. As you can see from this plot, most of the total heading changes were between 20 and 60 degrees, and the decrease in rotational gain at high amplitudes is largely driven by a reduction in bump

movement when bump amplitude is high. We have also added details to the Methods (line 989-1006) about how rotational gain was calculated and how heading and bump position were selected and processed for this analysis.

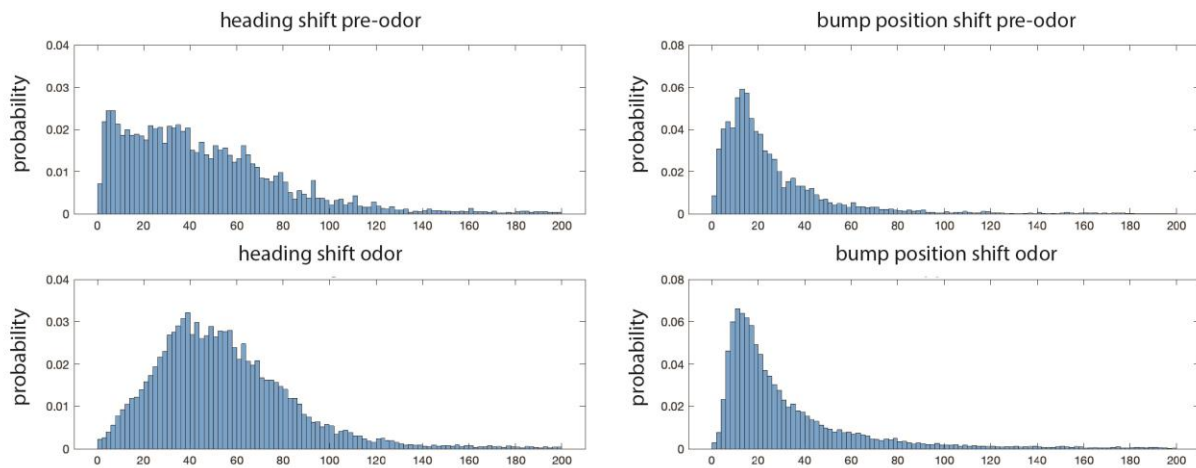
Below, we also plot the distribution of heading shifts across all trials, as well as the heading shift and bump position shift as a function of rotation gain:



The histogram demonstrates that few trials show very small heading changes ($<10^\circ$). The latter plots show that decreases in rotational gain are driven largely by decreases in bump position shift where there are large changes in heading (~ 20 - 50°).

We also plotted distributions of the heading shift and bump position shift for the data used in Fig. 4k, separating the data into pre-odor and odor. In contrast to above, each data point in the histograms corresponds to a single time point (where the shifts were calculated within a 2 s time window aligned to the time point). Again, the heading shifts are primarily above 10 degrees. Using two-sample Kolmogorov-Smirnov tests, we find that the heading shift distribution significantly increases after odor onset whereas the bump position shift distribution significantly decreases after odor onset. This suggests that the drop in

gain we see in Fig. 4k stems from 1) a drop in the bump mobility; and 2) an increase in heading movement without a corresponding increase in bump movement.



2. Could the authors add distribution plots of heading changes and bump location changes across different bump amplitudes?

Thank you for this suggestion. We have now added plots of average heading and bump changes as a function of PFG bump amplitude to Ext. Data Fig. 8g. These data show that the drop in bump position mobility at higher bump amplitudes is true across several bump amplitudes, suggesting the effect is not driven by a few extreme outliers.

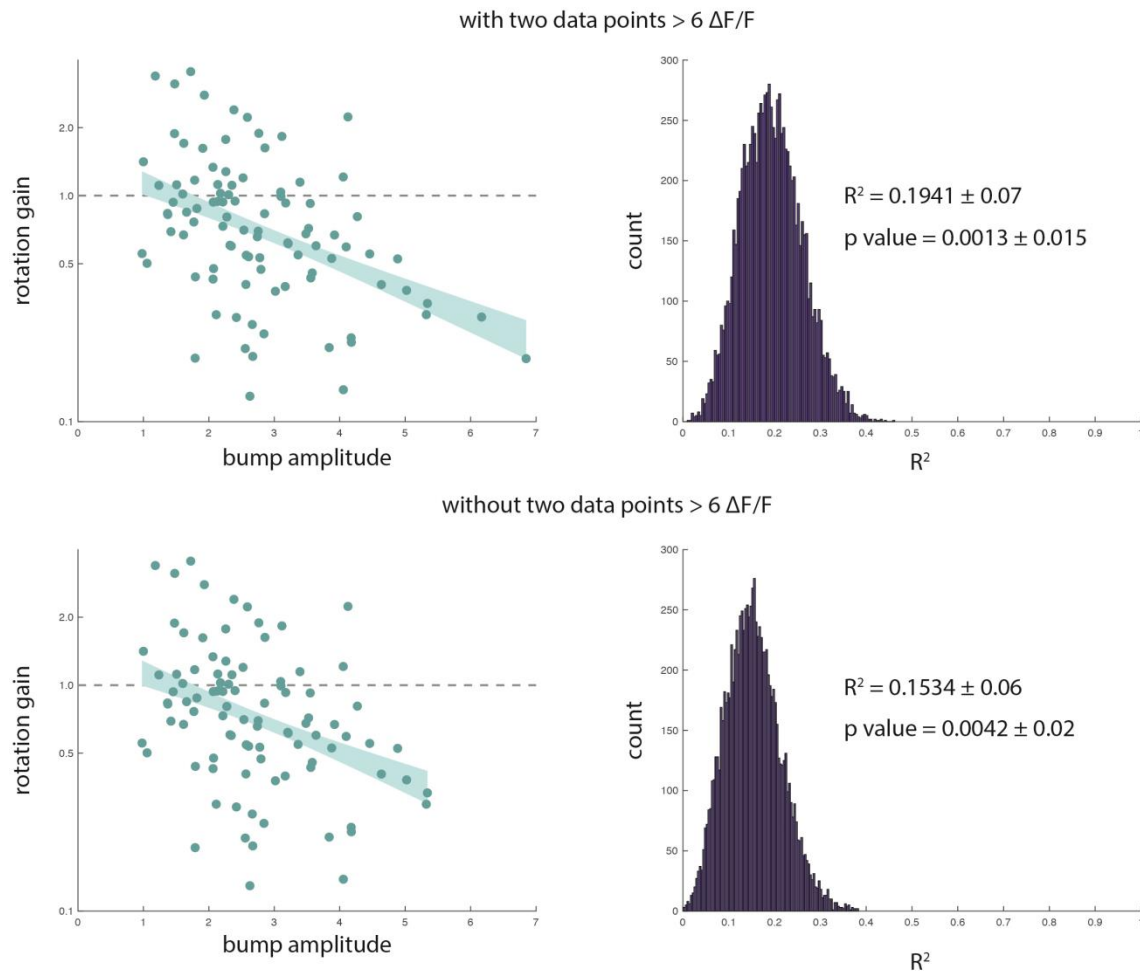
3. The temporal filtering approaches for behavioral and imaging data differ — the bump position appears more heavily filtered than the heading data — which may introduce noise into rotational gain calculations. We recommend using the same cutoff frequencies for both signals, particularly if sampling rates differ between imaging and behavioral recordings.

Thanks for this suggestion. We have now made sure the heading and bump position data are processed in the same manner. We downsampled all data to match the original imaging sampling rate (which is lower than the fictrac behavior sampling rate). We also made sure both heading and bump position data are filtered using the same filters. Specifically, both the bump position data and heading data were filtered by a moving average with a width of 13 samples (sampling rate 100 Hz).

4. The spread around the regression line is large, and the fit appears strongly influenced by a small number of data points. We recommend:

- Clarifying the p-value for the linear model and how it was computed.
- Reporting R^2 to indicate variance explained, as Pearson's coefficient is sensitive to outliers and insufficient as a standalone measure.
- Adding uncertainty bounds around the fit line via bootstrapping to assess whether different samplings of the data would yield substantially different slopes

Thanks for this suggestion. We have now performed bootstrapping (10,000 times) to linearly fit the data in Fig. 4j. Using this method, we were able to add error bars to the linear fit, including the R^2 and p value. Below, we also perform the linear fit after removing the two most extreme data points (the two data points with bump amplitude $> 6 \Delta F/F$). The linear fit is quite similar without these extreme values, although the R^2 is slightly smaller:



Referee #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #3 (Remarks to the Author):

This manuscript by Aaron et al. presents an impressive study that integrates in vivo calcium imaging, electrophysiological recordings, and computational modeling to uncover a novel mechanism for gating olfactory goal memory within a recurrent neural circuit. The authors demonstrate in the model inspired by their data that the interplay between slow excitation and fast inhibition can generate stable yet tunable persistent attractor dynamics, providing a mechanism for memory maintenance. Furthermore, they identify a gating process through which disinhibition allows the network to sustain memory during goal-directed navigation. They also pinpoint a specific group of inhibitory neurons whose activity patterns align with this proposed gating mechanism. Overall, this work provides new insights into how stability and flexibility of attractor dynamics can be modulated at the circuit level to enable goal directed navigation. The manuscript is thoughtfully executed and makes a significant conceptual advance.

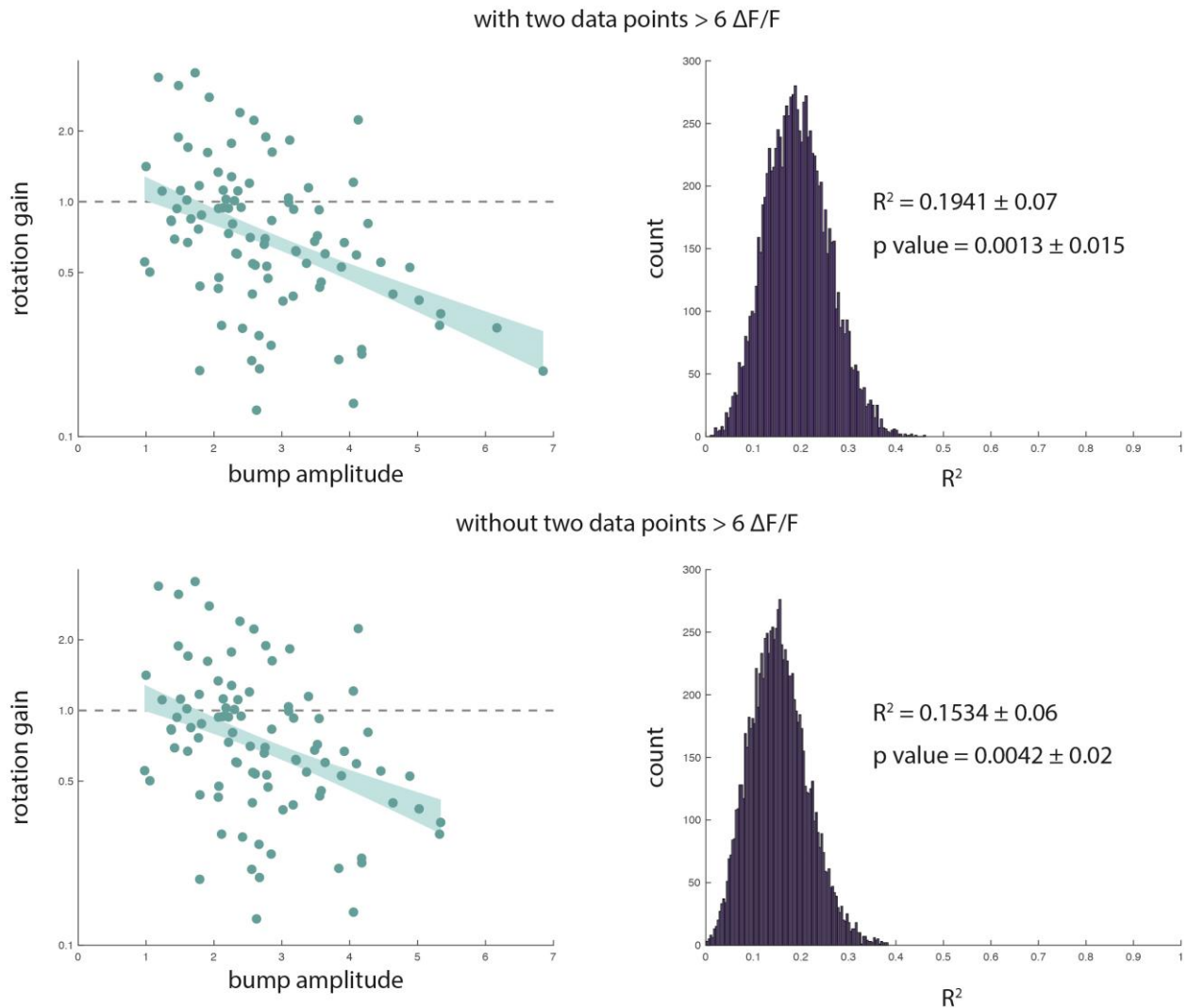
Following this revision, we were very pleased to see the updates to this manuscript address all major concerns and overall, we think the manuscript it is ready for publication. Congratulations!

The new analysis in EDF2 addresses our concerns about evidence for increased bump amplitude following odor and the addition of the PID measurement is nice. We only have one minor statistics point (below) as well as a few display suggestions:

Minor statistics notes:

The additional experiments added to Figure 4 really strengthened the paper. However, for Figure 4j (left) we were slightly worried how much the 2 lower outliers were driving the r of -0.4 value. It may be advisable to do some form of regression diagnostics (e.g. Cook's distance) to rule out this concern for other readers. We are similarly a little worried if the ExR3 effect in Fig 2h,I may also be driven by outliers. Were those numbers really normally distributed to justify a paired t-test over a Wilcoxon signed-rank test? Since the ExR3 claim is not central to the main points of the paper perhaps it would be safe to tone down this statement slightly if a non-parametric test doesn't give a significance p-value.

Thanks for these suggestions. Reviewer #1 had similar concerns. We have done several things to address these concerns. First, we made sure the heading and bump position data were processed in the same manner. We downsampled all data to match the original imaging sampling rate (which is lower than the fictrac behavior sampling rate). We also made sure both heading and bump position data were filtered using the same filters. Second, we performed bootstrapping (10000 times) to linearly fit the data in Fig. 4j. Using this method, we were able to add error bars to the linear fit, including the R^2 and p value. Third, we binned our data by mean bump amplitude and plotted heading and bump position distributions (now Ext. Data Fig. 8g). This data suggests the gains were not influenced by small changes in heading or bump position - most of the heading changes were between 20 and 60 degrees. It also shows that the drop in bump position mobility at higher bump amplitudes is true across several bins, suggesting the effect is not driven by a few extreme outliers. Finally, in an analysis we are not including in the manuscript, we also performed the linear fit after removing the two most extreme data points (the two data points with bump amplitude $> 6 \Delta F/F$). The linear fit is quite similar without these extreme vales, although the R^2 is slightly smaller. Here is the latter analysis:



The ExR3 data was normally distributed according to Shapiro-Wilk test ($p = 0.0963$) and Kolmogorov-Smirnov test ($p = 0.0861$). These are obviously close to not passing significance, but the control vs methysergide comparison was also significant with a Wilcoxon signed-rank test ($p = 0.0207$). Our takeaway is that cells with higher persistence in control trials showed much larger drops in persistence following methysergide application, whereas cells with low initial persistence had weak decreases following methysergide application. We have not altered the results because the statistics were consistent across tests.

Display/phrasing suggestions that are not critical:

In EDF2: I found having binary red/blue = “no bump”, as opposed to “bump” confusion at first, I expected the opposite because a higher blue or red value above is a larger bump. Suggest flipping that color scheme.

Thanks for this suggestion, we have swapped the colors.

Figure 1g -base and wind and base and odor labels on the plot are too close together to be seen as distinct

Fixed.

Figure 1 legend “raw DF/F” I would suggest cutting “raw” since it’s not F...

Fixed.

Genotype table:

gal4 -> Gal4

GFP -> mCD8::GFP (to match table 2 to avoid confusion)

Fixed.

I assume from your genotypes that all flies are w+/w+?. I would be great to note that explicitly. I know that + or blank already indicates w+, but because other authors sometimes don’t specify leaving ambiguous it would be nice to be written out. Or if flies are w- please indicate.

We have added this information to the genotypes table

Referee #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #5 (Remarks to the Author):

In the revised manuscript, the authors have responded to/addressed all my comments. After a thorough evaluation, I would like to commend the authors for their comprehensive and thoughtful response to my comments and for an excellent paper. The revisions have significantly strengthened the paper, and I appreciate the clarity and rigor with which the authors addressed each of my points. I have no further comments to add and believe the manuscript is now suitable for publication in its current form.

Thank you for all of your suggestions in the first round of review. We appreciate your support of our work.

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