

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	All custom Python and Matlab code for data collection is available on Github at https://github.com/nagellab/Lanzetal2025 Fly trajectories on the walking ball were tracked using Fictrac version 2.00
Data analysis	All custom Python and Matlab code for data analysis is available on Github at https://github.com/nagellab/Lanzetal2025 Data analysis was performed in Matlab R2024b Connectomic analysis and computational modeling were performed using Python 3.7.7

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All data collected during this study are available at [10.5281/zenodo.19687299](https://doi.org/10.5281/zenodo.19687299)

In addition, the following databases were used in this study:

- Hemibrain connectome, hemibrain:v1.2.1, <http://neuprint.janelia.org>
- FAFB connectome, FAFB v783, <https://codex.flywire.ai>
- Male CNS connectome, male-cns:v0.9m, <http://neuprint.janelia.org>

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes for imaging and behavioral experiments were based on Kathman et al. 2026. Sample size electrophysiology were based on Suver et al. 2019 and Currier et al. 2020.
Data exclusions	For imaging experiments, flies were only included for further analysis if they reliably oriented upwind on at least 50% of trials, which was identified by a convergence of heading values towards the upwind direction (0°) and an increase in mean upwind velocity around wind or odor onset. For electrophysiology experiments, cells were only included for analysis if they showed visible spiking activity, defined as rapid deflections above 10 mV. Other than these criteria, all data and replications were included in the manuscript.
Replication	Imaging experiments in behaving flies were performed in at least 6 flies per genotype. Electrophysiology experiments were performed 5-15 cells (generally one per fly) for each genotype.
Randomization	All stimuli for behavior and imaging were presented in random order within a block of trials. All comparisons were based on genotype or pharmacological treatment and all flies of a given genotype or treatment were included in each sample.
Blinding	All analyses for behavior and imaging data were automated and applied equally to all genotypes make genotype blinding unnecessary. Blinding was often impossible because genotype could be determined visually from marker phenotypes indicating the presence or absence of balancers, or cell type could be determined visually during imaging or electrophysiology. The same code was applied to all genotypes to produce the analysis, therefore the experimenter's knowledge of the genotype was irrelevant to the outcome of the experiment.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

chicken anti-GFP Fisher Scientific RRID:AB_1074893
 mouse anti-nc82 DSHB RRID:AB_2314866
 rabbit anti-DsRed Clontech 632496
 Alexa488-conjugated goat anti-chicken Fisher Scientific RRID:AB_2534096
 Alexa633-conjugated goat anti-mouse Fisher Scientific RRID:AB_2535719
 Alexa568-conjugated goat anti-rabbit Fisher Scientific RRID:AB_2315774
 Alexa568-conjugated streptavidin Fisher Scientific RRID:AB_2576217

Validation

Antibodies were validated by staining driver lines with known expression. Antibodies were used only to identify the anatomy of labeled neurons and not to identify molecular species or localization. GFP antibody has been used in WB, IHC, and ICC/IF in at least 17 confirmed publications according to ThermoFisher Scientific: <https://www.thermofisher.com/antibody/product/GFP-Antibody-Polyclonal/PA1-9533>. nc82 antibody has been cited 1201 times according to DSHB: <https://dshb.biology.uiowa.edu/nc82>. ds-Red antibody has been cited 2618 times according to Takara Bio (previously Clontech): <https://www.takarabio.com/products/antibodies-and-elisa/fluorescent-protein-antibodies/red-fluorescent-protein-antibodies?srsltid=AfmBOop1hxSp7GD1OPh1oBI0ynhl9cXuk1yLLAfzN4G1gnFE95m-pwWU>.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

Drosophila melanogaster, aged 1-14 days. Genotypes described in detail in Methods

Wild animals

n/a

Reporting on sex

All experiments were performed in female flies.

Field-collected samples

n/a

Ethics oversight

Only invertebrate organisms were used in this study, therefore no animal protocol was required.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a