

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	Electron microscopy data were obtained from CATMAID-FAFB (v14), Flywire (v630) and Hemibrain (v1.2.1). Calcium imaging data were collected using Matlab 2013b with ScanImage (v3.8). Confocal images were acquired using the Zeiss ZEN LSM710 microscopy software. Behavioral data acquisition is performed in using previously published, non-comercially available custom-made arenas described in the Methods.
Data analysis	Electron microscopy data were analyzed with custom-written codes using publicly available natverse packages (v0.2.3) in R Studio (v1.1.453). and in Gephi (v0.9.2). Calcium imaging and behavioral data were analyzed using custom-written codes in Matlab R2018a. Confocal images were processed in Fiji/Image J (1.54f). All code and preprocessed data used for the analysis will be publicly available at GitHub: https://github.com/ChiappeLab/Erginkaya_et_al_2025

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](#)

Data generated in this paper are publicly available at Zenodo (DOI: 10.5281/zenodo.14967806). Manually reconstructed FAFB neurons are available at Virtual Fly Brain (<https://fafb.catmaid.virtualflybrain.org/>). Source Data are provided with this paper.

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	No sample size calculations were performed before experiments. Sample sizes were chosen according to standard sample sizes in the field (e.g., Seelig & Jayaraman, Nature, 2015; Lyu et al., Nature, 2021).
Data exclusions	LPTs respond to rotational stimuli along one of the three rotational axes of the head and body. A lack of responses indicates poor preparation quality. For other neurons in the H2-HS network, which we were characterizing for the first time, the physiological preparation involved immobilized flies, preventing behavioral assessments to verify preparation quality. Therefore, we excluded data from flies that showed no detectable calcium responses to any visual stimuli. Additionally, data from freely walking flies with injured limbs were excluded, as such impairments confounded neural manipulations and compromised the proper interpretation of behavioral experiments
Replication	For all physiological experiments, each experiment included 5-10 replications. For most cell-types and experiments we include at least 8 independent flies, and more frequently 10-15. For the behavioral experiments, given the random-based strategy to manipulate neural activity, each fly was an independent experiments, and we repeated these experiments until we reached at least 20 flies for each condition
Randomization	Female flies from the same genotype were chosen randomly from vials where they were group-housed for all experiments. There was no covariate analysis since these flies can be considered similar from the perspective of their genotype and environmental and social experience (we maintained equal density of flies in vials, and we used 3 to 4 day-old flies consistently). In addition, due to the challenges of these experiments, the sample size is limited to allow for an accurate cross-variate analysis. Order of all visual stimuli in calcium imaging experiments were randomized.
Blinding	Experimenters were not blind to the genotype of individual flies. However, data collection and analysis were automated and equivalent across genotypes.

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	Mouse anti-nc82 antibody, DSHB, RRID: AB_2314866. Chicken anti-GFP antibody, Abcam, RRID: AB_300798. Rabbit polyclonal anti-GFP antibody, Thermo Fisher Scientific, RRID: AB_221570. Rabbit anti-GABA antibody, Sigma, RRID: AB_477652. Rabbit anti-DsRed antibody, Abcam, AB356483. Goat AlexaFluor-488 anti-chicken, Invitrogen, RRID: AB_2534096. Goat Alexa-594 anti-rabbit, Invitrogen, RRID: AB_150088. Goat AlexaFluor-633 anti-mouse, Invitrogen, RRID: AB_141431.
Validation	All antibodies used in this study are commercially available and have been applied to the <i>Drosophila</i> nervous system in previous studies. Validation information can be found at manufacturers' websites: mouse nc82 (https://dshb.biology.uiowa.edu/nc82) chicken anti-GFP (https://www.abcam.com/en-us/products/primary-antibodies/gfp-antibody-ab13970) rabbit anti-GFP (https://www.thermofisher.com/antibody/product/GFP-Antibody-Polyclonal/A-11122) rabbit anti-GABA (https://www.sigmaaldrich.com/US/en/product/sigma/a2052) rabbit anti-DsRed (https://www.sigmaaldrich.com/US/en/product/mm/ab356483) Goat Alexa-488 anti-chicken (https://www.thermofisher.com/antibody/product/Goat-anti-Chicken-IgY-H-L-Secondary-Antibody-Polyclonal/A-11039) Goat Alexa-594 anti-rabbit (https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11012) Goat Alexa-633 anti-mouse (https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21050)

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	W1118 Adult <i>Drosophila melanogaster</i> flies were used in all experiments. All experiments were performed with 3-to-4-days old flies.
Wild animals	No wild animals were used in the study.
Reporting on sex	Electron microscopy data were obtained from two individual adult female fly brains and one adult male ventral nerve cord. Female flies were used in all calcium imaging experiments due to their larger body size, providing easier preparation for physiology. Male flies were used in all behavior experiments since they exhibit more continuous locomotion in our experimental conditions.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	In Europe, no ethical approval is required for conducting research using <i>Drosophila melanogaster</i>

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

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.