

Reporting Summary

Nature Research 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 Research 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 (n) 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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P 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 d , Pearson's r), 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 Zen v1.1.2.0 was used to acquire all calcium imaging data. No other code was used to collect data.

Data analysis Zen v1.1.2.0 was used to define regions-of-interest (ROIs) for the analysis of all calcium imaging data. Matlab (v. R2017a) was used to process the calcium imaging data with a double-exponential fitting (expfit2 function) to correct for photobleaching. Code is available from corresponding author upon request. Graphpad Prism v8.4.0 was used to perform all statistical analyses. Neuprint database used for electron microscopy analyses is available online (<https://neuprint.janelia.org/>).

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 Research [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 list of figures that have associated raw data
- A description of any restrictions on data availability

All data, code and statistics used in this study are included as Supplementary Information and/or Source Data. They are also all available from the corresponding author upon request.

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](https://www.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 statistical methods were used to predetermine sample size. We chose sample sizes that are comparable to similar experiments conducted by others in the field.
Data exclusions	For calcium imaging experiments, it was decided prior to conducting the experiments that flies that did not respond to bright light stimulus at the end of the experiment would be excluded from analysis. No behavioral data were excluded.
Replication	Replication was extensive throughout the manuscript. Key experimental results were repeated independently at least twice. For each genotype/condition for all calcium imaging and behavioral experiment datasets, data was collected on at least two different days. Data collected on different days did not vary considerably.
Randomization	Where appropriate, individual flies or groups of flies (for calcium imaging / functional connectivity and behavioral experiments, respectively) were randomly assigned to experimental groups. Electron microscopy analyses were conducted on publicly available data from one female fly.
Blinding	Investigators were blinded to group allocation during data analysis of calcium imaging and behavioral experiments. Investigators were not blinded during data collection for logistical reasons.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	For all experiments, 3-10-day old <i>Drosophila melanogaster</i> flies were used. For behavioral experiments, mixed-sex groups of flies were used. For calcium imaging experiments, female flies were used. For electron microscopy analyses, the data is from a female fly.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve animals collected from the field.
Ethics oversight	No ethical approval or guidance was required for the study protocol, as is common in experiments involving <i>Drosophila</i> flies.

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