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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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	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 *Give P values as exact values whenever suitable.*
☒	☐	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	SlideBook (Intelligent Imaging, Inc.) and Zen (Zeiss) software were used to acquire images.
Data analysis	Fiji, Matlab (Mathworks, Inc.)

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 raw or processed data will be made available 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

Life sciences study design

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

Sample size	For calcium imaging experiments and immunohistochemistry experiments, n=3 was chosen as the minimum replicate number, and sample size was determined by the number of animals that were expressing the calcium indicator and were appropriately staged for developmental imaging. For counting presynaptic sites, n = 20 was chosen as the minimum replicate number, and sample size was determined by the number of cells that had positive signal in both imaging channels and could be resolved from neighboring cells. These sample sizes were determined to be sufficient due to low observed variability between samples.
Data exclusions	Data were not excluded from analysis.
Replication	All experiments were performed with technical replicates, and all replications were successful.
Randomization	Organisms within a given genotype were selected randomly from bottles and staged for developmental time. Organisms with appropriate developmental age were selected for subsequent experiments.
Blinding	For counting presynaptic sites, the analyzer was blinded to genotype while counting puncta. For other histology or calcium imaging experiments, blinding was not possible. However, quantifications were performed using a computational pipeline equally applied to all conditions and replicates.

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

Antibodies

Antibodies used	Mouse monoclonal anti-V5 (Novus Biologicals #NBP2-52703-0.2mg, 1:150), rat monoclonal anti-FLAG (DYKDDDDK) (Novus Biologicals #NBP1-06712, 1:100), mouse monoclonal anti-c-MYC (Developmental Studies Hybridoma Bank (DSHB) #9E10-concentrate, 1:100), rabbit polyclonal anti-dsRed (Clontech #632496, 1:125), chicken anti-GFP (Abcam #ab13970, 1:1000), rabbit monoclonal anti-HA (Cell Signaling Technology #3724, 1:300), rat monoclonal anti-Ncad (DSHB #DN-Ex #8-c, 1:100), mouse monoclonal anti-Elav (DHSB #Elav-9F8A9, 1:100), mouse monoclonal anti-Repo (DHSB #8D12 anti-Repo, 1:100), mouse monoclonal anti-Chat (DHSB #ChAT4B1, 1:20), mouse monoclonal anti-Pdf (DHSB #PDF C7, 1:1000), rabbit polyclonal anti-DVGLUT (1:1000), rabbit polyclonal anti-DVGAT (1:200), mouse monoclonal anti-TH (Immunostar #22941, 1:200), rabbit polyclonal anti-5-HT (Immunostar #20080, 1:1000), rabbit polyclonal anti-DH31 (1:1000), rabbit polyclonal anti-DH44 (1:1000), rabbit polyclonal anti-SIFamide (1:1000). Secondary antibodies and dilutions used in this study were as follows: Alexa 488 donkey polyclonal anti-chicken (Jackson ImmunoResearch # 703-545-155, 1:400), Alexa 488 donkey polyclonal anti-mouse (Jackson ImmunoResearch #715-545-151, 1:400), Alexa 568 donkey polyclonal anti-rabbit (Invitrogen #A10042, 1:400), Alexa 647 donkey polyclonal anti-rat (Jackson ImmunoResearch #712-605-153, 1:400).
Validation	Antibodies against epitope tags, anti-NCad, anti-Elav, and anti-Repo have been previously validated in Drosophila (e.g. Chen et al., 2014). Primary antibodies against neuropeptides and neurotransmitters have been previously validated (see the following publications: R. W. Daniels et al., J Neurosci 2004, H. Fei et al., J Exp Biol 2010, D. Park, J. A. Veenstra, J. H. Park, P. H. Taghert, PLoS One 2008, P. Cabrero et al., J Exp Biol 2002, S. Terhzaz et al., Biochem Biophys Res Commun 2007.)

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Drosophila melanogaster (female unless otherwise noted) were used for experiments. Genotypes for each experiment can be found in TableS1.
Wild animals	N/A.
Field-collected samples	N/A.
Ethics oversight	No ethical oversight committee was required for use of Drosophila samples.

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