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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	PrairieView was used for two-photon functional imaging. During imaging, fly behavior was visualized by FlyCapture2. Functional responses of ppk23+ soma were also recorded using FlyCapture2. Power measurements for optogenetics were measured using Coherent PowerMax.
Data analysis	Analysis of functional data and processing of anatomic images was performed using ImageJ. Behavioral videos were reviewed using QuickTime. Statistical tests were carried out using PRISM 6 (Graphpad).

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/reviewers upon request. 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

Correspondence and requests for materials and raw data should be addressed to V.R. (ruta@rockefeller.edu).

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	Preliminary experiments were used to assess variance and determine adequate sample sizes in advance of conducting the experiment. Generally, these were n=~15-20 in behavioral assays and n=~6-12 in functional assays. We used similar sample sizes for all experiments where a single variable (e.g. genotype, species, or stimulus) was being compared. See methods sections "Courtship behavior assays and analysis" and "Two-photon functional imaging."
Data exclusions	In preference assays, males that courted for less than 1% of the assay were excluded from analysis. In optogenetic stimulation assays, males were excluded from analysis if post-hoc PCR genotyping failed. For in vivo functional imaging experiments, we did not collect data from flies that did not show locomotor activity, suggesting damage during tethering, or flies whose brains were moving in the Z axis due to unstable tethering. There were no other criteria for exclusion. See methods sections "Courtship behavior assays and analysis" and "Two-photon functional imaging."
Replication	All attempts at replication were successful. Several experiments were carried out repeatedly due to the fact they served as controls for different experimental manipulations. In particular, we carried the following experiments multiple times: behavioral assays for wild-type male species discrimination, in vivo functional responses in the LPC, VNC, P1 and vAB3 neurons, and ex vivo functional responses evoked by vAB3 stimulation. In all cases the results were reliable and robust over the course of the five years it took to complete this study.
Randomization	We used random.org/sequences to randomize the order of all behavior experiments. In order to control for potential variations in experimental conditions across days, we were careful to collect a similar sample size for each variable every day the experiment was conducted. For functional experiments, we interleaved genotypes and female stimuli when applicable. See methods sections "Courtship behavior assays and analysis" and "Two-photon functional imaging."
Blinding	All behavior experiments were conducted with the experimenter blinded to the genotype of any male or female fly that was variable in a given experiment. The experimenter was unblinded only after analysis of the assay. The experimenter was not blinded to the genotype of males used in functional assays. See methods section.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All unique materials are available upon request.

Antibodies

Antibodies used	All antibodies used in this study are commercial and previously validated (see manufacturer's website or reference). We used concentrations based on published protocols. Primary antibodies used were 1:20 Mouse anti-Brp (nc82, Developmental Studies Hybridoma Bank; Buchner, E. Neuron 2006), 1:1000 Sheep anti-GFP (Bio-Rad #4745-1051) and 1:100 rabbit anti-GABA (Catalog #A2052; Sigma, St. Louis, MO).Secondary antibodies used were 1:500 Anti-sheep Alexa Fluor 488, Anti-rabbit Alexa Fluor 546, Anti-mouse Alexa Fluor 647 and Anti-mouse Alexa Fluor 555 (ThermoFischer Scientific). Please see the methods section titled "Immunohistochemistry" for further details.
Validation	*Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.*

Animals and other organisms

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

Laboratory animals	All flies used for behavioral analysis were between 3-6 days old. Flies used for functional analysis were between 3-6 days old. Flies used for immunohistochemistry were 1-2 days old. Experiments that characterized in vivo function or behavior were conducted on male flies in response to either female flies or female pheromones. Images of brains are all males, with the exception of Extended Data Fig. 3d, which shows female anatomy. All ex vivo function experiments were conducted on males. Please refer to Supplemental Table 1 and the methods for further description of research animals.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve samples collected from the field.