nature portfolio

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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 (*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	To collect behavioral, histological and electrophysiological data, we used software packages and custom code (detailed in the Material & Methods section) written in Matlab 2015b (or more recent versions), Python 3.6.0 (or more recent versions), Octave, OpenGL, Patchmaster v2 x 32, and Fiji 2.1.0 (or more recent versions), and the following open source software programs and plugins: QuPath 0.2.3, SpikeGLX V20230101-phase30, PylonRecorder2, Bonsai V2.6.2, and BonVision V0.11.0, Psychtoolbox.
Data analysis	To analyse the data, we used custom code written in Matlab 2015b (or more recent versions), Python 3.6.0 (or more recent versions), Julia 1.11 and R Studio 1.1.383 (or more recent versions). The following packages/plugins were used: TrakEM2 (ImageJ plugin), StarDist (ImageJ plugin), R package lme4 and emmeans, Kilosort2, phy2, scikit-learn package (Python), DABEST (Python package), DeepLabCut V2.1.9 or newer, Fitmaster, Synaptosoft.

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

The source data necessary to replicate key panels of figures 1-5 together with relevant code can be found here: https://doi.org/10.5061/dryad.q2bvq83xc

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 methods were used to predetermine sample size. Sample sizes were set to match, or exceed, similar studies in the field. For behavioural studies we used cohorts of 14-116 animals; comparable experiments in mice have used 28 animals (https://doi.org/10.1016/j.cub.2016.06.006) or 10 animals (https://doi.org/10.1016/j.cub.2013.08.015). For c-Fos studies we used 21-66 animals; in published studies quantifying c-Fos in the context of innate behaviours, groups of 6-10 animals were used (https://doi.org/10.1038/s41586-018-0078-2). For Neuropixels recordings in behaving mice, we used 3-6 animals; comparable studies analysed 135 units obtained from 4 animals (https://doi.org/10.1073/pnas.2500321122) and we have previously published recordings from 6-8 animals in difficult to target regions (https://doi.org/10.7554/eLife.50697). Our optogenetic experiments were performed in cohorts of 5-13 animals; similar experiments targeted at modulating colliculus-mediated behaviours have been performed with groups of 7-8 animals (https://doi.org/10.1038/s41467-024-46460-z).
Data exclusions	For the optogenetics and electrophysiology experiment, animals were excluded if post-hoc immunohistochemistry showed no or little YFP staining in dPAG and/or incorrect fiber/electrode placement. We excluded optogenetic trials for which the animal moved less than 10 cm/s on average during the 0.5 s before laser trigger. For the smFISH-IHC analysis, we removed 7 sections that had poor staining intensity. We removed trials during the sweep-looming experiment when animals retreated to the hut during the sweeping stimulus (P. maniculatus, N=1; P. polionotus, N=3; P. leucopus, N=8), such that all animals were exposed to the subsequent looming stimulus. We removed trials for which animals did not show evidence of detecting the stimulus during the experiment to test for the effect of the presence/absence of a hut on the looming response (P. maniculatus, N=1/0; P. polionotus, N=1/2). All data exclusion criteria are explained in the Methods.
Replication	We performed all experiments successfully multiple times. In the Methods section "Animal Usage", a detailed description of the number of animals per experiment can be found. In the sections describing each experimental protocol, inclusion and exclusion criteria are described. Each included animal and/or recorded cell represents a replication, which is represented by displaying the individual data points or data traces for all types of experiments.
Randomization	The behavioral and electrophysiological experiments were based on observations, and no experimental groups were used. For the c-Fos, optogenetics and chemogenetics experiments, animals were randomly assigned to experimental groups (experimental vs. control).
Blinding	Investigators were not blind to experimental conditions. Most analyses in this study rely on quantitative and automated methods to analyse

Blinding	data. To determine if mice had detected a visual stimuli in Extended Data Figure 2, two independent observers manually scored videos, and only videos were included for which both observers determined that mice detected visual stimuli (for details, see Methods).

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	rabbit anti-c-Fos antibody (Synaptic Systems, 226003), donkey anti-rabbit Alexa 647 antibody (Invitrogen, A31573), donkey anti-chicken Alexa 488 (Immuno Jackson, 703-545-155), chicken anti-GFP (Thermo Fisher, A-10262), HRP-labeled goat anti-rabbit antibody (PerkinElmer, NEF812001EA), rabbit anti-GFP (Thermo Fisher, A-11122)
Validation	The rabbit anti-c-Fos antibody was validated in tissue with known induction/expression patterns, and compared to alternative c-Fos antibodies. The manufacturer states that this antibody binds to human, mouse, rat, monkey, dog, pig and cow protein, and was validated by Western blot and immunohistochemistry. The chicken anti-GFP and rabbit anti-GFP antibodies were validated by the manufacturer, using relative expression analysis in Western blot.

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	Peromyscus maniculatus bairdii (BW), Peromyscus polionotus subgriseus (PO), Peromyscus leucopus (LL), Mus musculus (C57Bl/6j). All animals were older than 8 weeks at time of testing.
Wild animals	No wild animals were used in this study.
Reporting on sex	Our study included both male and female mice, and approximately even numbers of males and females were used in all experiments. Sex was determined at weaning through inspection of the anogenital area. All behaviors and effects of optogenetic and chemogenetic manipulation were observed in both sexes, so that we decided to pool data across sexes. Sex was not considered further.
Field-collected samples	No field collected samples were used in this study.
Ethics oversight	Institutional Animal Care and Use Committee (IACUC) of Harvard University, and Animal Ethics Committee of KU Leuven.

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