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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	Two-photon images were collected using ScanImage (v5.4); EEG/EMG signals were collected using Biological Data Acquisition and Analysis System (BL-420, v2.1, Chengdu TME tech. Ltd.).
Data analysis	Calcium analyses were performed using CaImAn in Python version (v1.9.12; Github.com); dendritic spine and confocal images were analyzed using NIH ImageJ software (FIJI 1.53s); statistical analyses were performed using GraphPad Prism software (v8.4).

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

All data are available in the main text or the supplementary materials.

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 statistical methods were used to pre-determine sample sizes, but sample sizes for this study are similar to those reported in previous publications (PMIDs: 28671692, 34292944, 35561213, 36103825)
Data exclusions	Imaging with excessive movement were removed from data analyses.
Replication	For two-photon imaging, EEG/EMG recordings, behavior tests, and confocal imaging, almost all experiments were replicated in three or more animals from more than two litters (typically three). Results in Extended Data Fig. 7m, n were replicated in two animals from two litters. For histology experiments, results were replicated in more than three brain sections per mouse, and results from different animals were consistent.
Randomization	Animals were randomly assigned to treatment groups.
Blinding	The experimenters were blinded to treatment groups such as surgery (sham vs. SNI), virus injection (empty vs. functional), and pharmacology (e.g., lidocaine vs. saline). Calcium and acetylcholine imaging data analyses were conducted using an automated analysis pipeline, which avoids the potential bias of manual analyses.

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	Primary antibodies: mouse monoclonal anti-NeuN (clone A60) (1:500; MAB377; Millipore), rabbit polyclonal anti-GFP (1:1,500; ab6556; Abcam), mouse monoclonal anti-mCherry (clone GT844) (1:500; SAB2702295; SigmaAldrich), mouse monoclonal anti-CGRP (clone 4901) (1:400; C7113; SigmaAldrich), goat polyclonal anti-ChAT (1:400; AB144P; Millipore), mouse monoclonal anti-ChAT (clone CL3169) (1:200; MA5-31382; Invitrogen), rabbit polyclonal anti-VAChT (1:300; 139103; Synaptic Systems), mouse monoclonal anti-VGAT (clone 117G4) (1:300; 131011; Synaptic Systems). Secondary antibodies: donkey anti-rabbit Alexa Fluor 488 (1:500; A21206; Invitrogen), donkey anti-rabbit CF488A (1:500; 20015; Biotium), donkey anti-rabbit CF543 (1:500; 20308; Biotium), donkey anti-rabbit Alexa Fluor 647 (1:500; A31573; Invitrogen), donkey anti-mouse Alexa Fluor 488 (1:500; A21202; Invitrogen), donkey anti-mouse CF543 (1:500; 20305; Biotium), donkey anti-mouse CF647 (1:500; 20046; Biotium), donkey anti-goat Alexa Fluor 488 (1:500; A11055; Invitrogen), donkey anti-goat CF543 (1:500; 20314; Biotium), donkey anti-goat CF647 (1:500; 20048; Biotium).
Validation	Mouse monoclonal anti-NeuN (clone A60) (MAB377; Millipore): https://www.emdmillipore.com/US/en/product/Anti-NeuN-Antibody-clone-A60,MM_NF-MAB377 Rabbit polyclonal anti-GFP (ab6556; Abcam): https://www.abcam.com/gfp-antibody-ab6556.html Mouse monoclonal anti-mCherry (clone GT844) (SAB2702295; SigmaAldrich): https://www.sigmaaldrich.com/US/en/product/sigma/sab2702295 Mouse monoclonal anti-CGRP (clone 4901): https://www.sigmaaldrich.com/US/en/product/sigma/c7113 Goat polyclonal anti-ChAT: https://elifesciences.org/articles/8352

mouse monoclonal anti-ChAT (clone CL3169): Pang Y et al., Front Cell Dev Biol. (2022) 10: 849854.
Rabbit polyclonal anti-VAChT: https://sysy.com/product/139103
Mouse monoclonal anti-VGAT (clone 117G4): https://sysy.com/product/131011

Animals and other organisms

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

Laboratory animals	Thy1-YFP-H mice (Jackson Laboratory; Stock No: 003782), C57BL/6J (Stock No: 000664), VipIRES-Cre (Stock No: 010908) , VipIRES-FLPo (Stock No: 028578) , SstIRES-Cre (Stock No: 013044) , PvalbT2A-Cre (Stock No: 012358) , PvalbT2A-FLPo (Stock No: 022730), ChATIRES-Cre (Stock No: 006410), Slc17a6IRES-Cre mice (Stock No: 016963), and Rosa26-LSL-Cas9-EGFP mice (Stock No: 026175). Thy1-GCaMP6slow founder line 3 were obtained from Dr. Wenbiao Gan's laboratory at New York University. Mice were group-housed in temperature- and humidity-controlled rooms on a 12-h light-dark cycle (light: ZT0–12; dark: ZT12–24). Two- to 3-month-old animals of both sexes were used for all the experiments.
Wild animals	No wild animals were used in this study.
Field-collected samples	No field collected samples were used in this study.
Ethics oversight	All animal experiments were carried out according to the protocols approved by the Columbia University Institutional Animal Care and Use Committee as consistent with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

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