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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

Single nuclei capture was performed on 10X Genomics Single-Cell 3' system with target capture of 8000 nuclei per sample. The 10x nuclei capture and library preparation protocol was performed according to the manufacturer's recommended protocol (10X Genomics). Sequencing reads were aligned to mouse reference genome mm10 with CellRanger (version 6.0.2). The generated gene expression matrix files arising from two batches of experiments were further analyzed using Seurat v4 package.

Immunohistochemistry were performed on cyro-microtome brain slice sections. Fluorescent signals were acquired using a confocal laser scanning microscope (Olympus, FLUOVIEW IX83-FV3000). Fluorescence in situ hybridization (FISH) was performed according to the manufacture's protocol using RNAscope multiplex fluorescent reagent kit and appropriately designed probes (Advanced Cell Diagnostics, USA).

Adult mice were anesthetized with pentobarbital sodium solution (50 mg/kg) via an intraperitoneal (i.p.) injection. Mice were placed on a stereotaxic frame (RWD Life Science, 68046) and anesthesia were maintained with 1%-1.5% isoflurane during surgery. Injection of AAV virus was performed with a manual viral syringe pump connected to glass micropipette with a 10-50 μm diameter tip (Drummond Scientific Company, Wiretrol II). Mice were randomly assigned to experimental and control groups before virus injection.

Calcium signals were recorded using a dual-color fiber photometry system (Thinkertech, Nanjing Bioscience Inc.). The laser power at the tip of the optical fiber was adjusted to be 30 μW for 488 nm excitation channel and 10 μW for the control channel (570 nm) to decrease laser bleaching. GCamP6s channels were used to respond the changes in calcium activity of ZI neurons, and mCherry fluorescent signals were used to exclude disturbances caused by the external environment and artificial factors. Averaged traces of Ca2+ fluorescent signal changes and heatmap were plotted using the MATLAB program provided by ThinkerTech Nanjing Bioscience.

For optogenetic manipulation during the behavioral assessments, pulses of 465 nm laser stimulation was set by laser stimulator (INPER-B1-465, Inper, Hangzhou., China). Before behavioral tests, mice were habituated to the environments for at least 30 min after connection to a laser stimulator. The laser power at the tip of the optical fiber was adjusted to 5-10 mW for the optogenetic stimulation (10 ms pulses at 20 Hz).

The EEG and EMG signals were recorded and amplified using PowerLab 4 data acquisition unit (35PL3504/P, AD. Instruments, Australia). NREM, REM and wake states were analyzed for each 5-s epoch by LabChart8 (wake: desynchronized EEG and high EMG activity; NREM sleep: synchronized EEG with high power at 0.5–4 Hz and low EMG activity; REM sleep: desynchronized EEG with high power at theta frequencies (6–9 Hz) and low EMG activity).

A 350 mm × 350 mm square box was used for the self-grooming test. Mice were placed in the chamber to acclimate for at least 10 min before the behavioral tests. For the optogenetic manipulation, mice were placed in the center of the apparatus and the behavioral assay was performed with a 3-min Laser OFF, 3-minute Laser ON and 3-min Laser OFF administration pattern. The total time spent in self-grooming was measured.

In the open-field test, mice were placed in a 450 mm × 450 mm square box to acclimate for at least 30 min before the behavioral tests. For the optogenetic manipulation, mice were placed in the center of the apparatus and a 12-minutes behavioral assay was performed with a 3-min Laser OFF and 3-minute Laser ON administration pattern. The open field was cleaned with 75% ethanol between each trial and the moving traces were tracked by ANY-Maze software. Total distance traveled and time spent in the center zone were analyzed.

Data analysis

Clampfit 10.7 (Molecular Device)
ANY-Maze 6.3 (Stoelting)
Matlab (R2017b. MathWorks)
ImageJ (Fiji. National Institutes of Health)
Prism 7 (Graphpad)
LabChart 8 (AD. Instruments)

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 the relevant data, resources and reagents are available and will be fulfilled by the corresponding authors upon reasonable request. Source data in this study are provided in the Source Data file. The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformation, Beijing Institute of Genomics, Chinese Academy of Sciences that are publicly accessible at https://ngdc.cncb.ac.cn/gsa. (GSA: CRA017157)

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	This research did not involve any human-derived materials.
Reporting on race, ethnicity, or other socially relevant groupings	This research did not involve any human-derived materials.
Population characteristics	This research did not involve any human-derived materials.
Recruitment	This research did not involve any human-derived materials.
Ethics oversight	This research did not involve any human-derived materials.

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	Samples sizes were selected based on previous experience from related research and literature (Liu et al., 2017;Dong et al., 2019; Ahmadlou M et al., 2021; Yu et al., 2022; Schroeder A et al., 2023).
Data exclusions	In single nucleus RNA sequencing analysis, the Low abundant genes (expressed in less than 3 cells) and cells of potentially low quality (nFeature (number of detected genes) < 1000 or nFeature > 6,000, or counts > 25,000, or percentage of mitochondrial genes > 5%) were removed from downstream analysis. In fiber photometry and optogenetic behavioral experiments, mice with no fluorescent reporter expression, or the fluorescent reporter expression was outside the target region, or the optic fiber was misplaced were excluded from the dataset.
Replication	Two independent cohort of micro-dissected zona incerta brain slice sections were harvested for single-nuclei RNA sequencing analysis. Histology and immunofluorescent staining data were quantified from 9 brain slices of 3 mice for each group. All the behavioral test were conducted in more than 4 mice for each group.
Randomization	Littermate mice were split into random groups before AAV viral injection. For optogenetic stimulation during EEG/EMG recording, the interval of optogenetic stimulation was randomized within 10 - 15 minutes.
Blinding	The behavioral experiments and data analyses were conducted blindly.

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	Guinea pig polyclonal anti-Cre recombinase (1:500, Synaptic Systems 257004, Germany) Mouse anti-Calretinin (1:100, Swant CR6B3, Switzerland) Goat anti- Guinea pig 647 (1:1000, Invitrogen A-21450, USA) Donkey anti- Mouse 555 (1:1000, Invitrogen A-31570, USA)
Validation	The primary and secondary antibodies used in this study were validated by the manufacturers and were used in previous studies (Kim W et al., 2023; Ambrosi P et al., 2022).

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	C57BL/6J (JAX# Stock No. 000664) and transgenic GAD67-GFP mice (JAX# Stock No. 007677) were obtained from Jackson Laboratories. Transgenic mice Ecel1-Cre and Pde11a-Cre (C57/BL6 background) were generated by using CRISPR/Cas9 technology at the Shanghai Model Organisms Center (China) or Biocytogen biotechnology company. The Gad67-GFP mice at postnatal day 40 (P40) were used for single-nuclei RNA sequencing. Adult Ecel1-Cre and Pde11a-Cre mice (>8 months) were used for EEG/EMG recordings and behavioral tests. Adult C57BL/6J mice were used for immunofluorescent staining.
Wild animals	The study did not involve wild animals.
Reporting on sex	Male mice were used for EEG/EMG recordings and behavioral analysis. Both sexes of mice were used for virus mediated circuit tracing, immunohistochemistry or fluorescence in situ hybridization (FISH) experiments.

Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Mice were housed and treated according to the guidelines of the Zhejiang University (ZJU) Laboratory Animal Care and Use Committee and were approved by the Animal Advisory Committee at ZJU (ZJU20210231).

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