

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	All commercial software to collect the data in this study are described, including confocal microscope (Nikon, NI-SJ) or VS120 virtual microscopy slide scanning system (Olympus) used for tissue staining imaging, virtual reality system (PhenoSys, Germany) used for OKR experiment, SleepSign3-OBi (Version 2.0, Kissei Comtec, Nagano, Japan) used for sleep stage scoring, Medusa system (Bio-Signal Technologies, China) used for sleep recording, fiber photometry system (Thinker Tech Nanjing Biotech) , Apollo system used for in vivo optogenetic-assisted electrophysiological recording (Bio-Signal Technologies) and Inscopix nVoke miniscope (nVista 3.0, USA) used for calcium imaging, . The data is analyzed by Python 3.8 and GraphPad 8.0 Software.
Data analysis	We used python 3.8 for data analysis. Open source toolboxes were used for data analysis: numpy 1.16.5, pandas 0.25.1, statsmodels 0.10.1, matplotlib 3.1.1 were used for data preprocessing and visualization. Networkx 3.0, scipy 1.3.1 and jupyter 1.0.0 were used for clustering analysis. The GraphPad 8.0 Software were also used for statistical testing and data visualization. The eyeball track is analyzed by DeepLabCut algorithm (https://github.com/DeepLabCut/DeepLabCut). Custom codes for endoscopix imaging analysis, eye movements analysis and sleep scoring are available from the corresponding authors upon reasonable request.

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 data are available in the Source Data files accompanying this paper.

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](https://www.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 We did not use statistical methods to pre-determine the sample size. Sample sizes were chosen to be comparable or exceed sample sizes of prior studies in the field (e.g. Dong et al. 2022 Nature Neurosci; Xu et al. 2024 Nature Neurosci).

Data exclusions Mice with no virus expression, where virus expression was outside the target site or where the optic fiber was misplaced were excluded from the data set. For the retrograde ablation experiment, the mice which eye movements were not significantly affected by the neuronal ablation were excluded. In optogenetic experiments specific to REM sleep, trials where the mouse is not in the REM sleep state at the time of stimulation are excluded. Single-cell calcium imaging with weak fluorescence signals or no clearly detectable cell-body shaped ROIs were excluded.

Replication Multiple mice from independent cohorts were used. Sleep recordings with optogenetic stimulation or genetic ablation were repeated twice on separate days for the same animal. Calcium imaging using fiber photometry and endoscopy was performed once or twice for each animal on separate days, each session lasting for 2 hours. Viral tracing and histology images shown in Figures were performed using 2-4 mice. The numbers of animals, recording sessions, or neurons were clearly indicated on the graphs or in figure legends. Example plots were representative for the average result from.

Randomization In this work, mice were randomly assigned to experimental and control groups. For optogenetic open-loop stimulation, the timing of laser stimulation was randomized within a predefined range of intervals (5-17 minutes) between consecutive trials. In the optogenetic stimulation of REM sleep, the laser was applied to randomly selected REM bouts, with stimulation occurring in 50% of these episodes.

Blinding Investigators were not blinded to group allocation during experimentation and outcome assessment. However, batch and automated analyses were used for both control and experimental groups, yielding no subjective bias.

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

Primary antibodies utilized were goat anti-ChAT (Millipore, ab144P, 1:100) (e.g. Wang et al. J Neurophysiol. 2015), rabbit anti-NeuN (Millipore, Cat# ABN78, 1:1000) (e.g. Huang, B., et al. Neuron. 2015), rabbit anti-cleaved caspase-3 (Cell Signaling Technology, Cat# 9661, 1:400) (Dong et al. Elife. 2026), chicken anti-GFP (abcam, Cat# ab13970, 1:800) (Ionescu et al. Nat Neurosci. 2025), rabbit anti-Dsred (Clontech, Cat# 632496, 1:1000) (Hemmi et al. Elife. 2026), rabbit anti-cFos (Millipore, Cat# ABE457, 1:1000) (Isabel F Augur et al. The Journal of neuroscience. 2016), rabbit anti-mCherry (Sicgen, Cat# AB0081-300, 1:400), and rabbit anti-CART (Phoenix Pharmaceuticals, Cat# H-003-62, 1:1000) (Yusaku Iwasaki et al. PLoS One. 2013). Secondary antibodies were purchased from Jackson ImmunoResearch Labs and included Alexa Fluor 647 donkey anti-rabbit IgG (Cat# 711-605-152, 1:800), Alexa Fluor 594 donkey anti-rabbit IgG (Cat# 711-585-152, 1:800), Alexa Fluor 488 donkey anti-rabbit IgG (Cat# 711-545-152, 1:800), Alexa Fluor 488 donkey anti-chicken IgG (Cat# 703-545-155, 1:800), Alexa Fluor 647 donkey anti-goat IgG (Cat# 705-605-003, 1:800), Alexa Fluor 594 donkey anti-goat IgG (Cat# 705-585-003, 1:800), Alexa Fluor 488 donkey anti-goat IgG (Cat# 705-545-003, 1:800).

Validation

All antibodies used in this study were selected based on established literature to ensure specific labeling of target proteins in mice. Prior to the main experiments, a pilot study was conducted to validate antibody effectiveness and specificity.

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

ChAT-Cre transgenic mice (B6;129S6-Chattm2(cre)Low/J; Jax Stock#: 006410; RRID: IMSR_JAX:006410), and Ai14 reporter mice (B6.Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J; Jax Stock#: 007914; RRID: IMSR_JAX:007914) were purchased from Jackson Laboratory. Both lines were subsequently backcrossed onto a C57BL/6 background for further breeding, using wild-type C57BL/6 mice obtained from SLAC Laboratory Animal Co., Ltd. (Shanghai, China). Both male and female mice were used throughout the whole study. All mice were 8-12 weeks old, weighing between 26-30 g, at the start of experimental procedures. All mice were group-housed with ad libitum access to food and water, maintained at constant temperature of 23±2 °C, humidity of 50±10%, on 12h light/dark cycle (light on at 8:30 am).

Wild animals

The study does not involve wild animals.

Reporting on sex

Both male and female mice were used without distinction in this study.

Field-collected samples

No field-collected samples were used in this study

Ethics oversight

All animal experimental procedures were approved by the Animal Experiment and Use Committee of the Shanghai Medical School of Fudan University.

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

Plants

Seed stocks

No seed is used in this study.

Novel plant genotypes

No seed is used in this study.

Authentication

No authentication is required, as this study does not involve the use of any plant material.