# nature portfolio

Corresponding author(s):	Yang Dan
Last updated by author(s):	Sep 7, 2023

## **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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

_				
5	tа	ŤΙ	ıςt	ics

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. <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>
$\boxtimes$	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
$\boxtimes$	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 <u>availability of computer code</u>

Data collection

Confocal images were taken with Zen 2010 software. Sleep recordings were done with OpenEx software suite (v2.20) (Tucker-Davis Technologies). Two-photon imaging data were collected with ScanImage 3.8, an open source software. Light sheet imaging was done by LifeCanvas Technologies with SmartSPIM light sheet microscope using SmartSPIM's acquisition software (please check LifeCanvas Technologies website for more details).

Data analysis

Softwares and scripts used are described in the Methods section and listed below as well.

Data analysis: Confocal images were processed with ImageJ (v 2.3.0), and manual cell counting was done with a custom-written graphical user interface programmed in MATLAB software (MATLAB, R2019b). Brain state classification was done semi-automatically using a custom-written graphical user interface programmed in MATLAB (as reported in our previous publications). Two-photon imaging data were processed with Inscopix Data Processing software (v 1.3.1), Advanced Normalization Tools (ANTs) toolkits, ImageJ (v 2.3.0), and MATLAB. Statistics were done with GraphPad Prism 9.2.0. Axon boutons were automatically detected by DeepBouton.

Data representation: Figures were prepared with GraphPad Prism 9.2.0, Adobe InDesign 2021, and Adobe Illustrator 2021. The movie was prepared with Adobe Premiere Pro 2022. The 3D rendering of example images were generated with Imaris software (BITPLANE).

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 dataset used for figures are available as supplementary information.

#### Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation), and sexual orientation</u> and <u>race, ethnicity and racism</u>.

Reporting on sex and gender	NO human research participants in the study.
Reporting on race, ethnicity, or other socially relevant groupings	NO human research participants in the study.
Population characteristics	NO human research participants in the study.
Recruitment	NO human research participants in the study.
Ethics oversight	NO human research participants in the study.

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 be	ow that is the best fit for your research. I	you are not sure, read the appropriate sections before making your selection.
X Life sciences	Behavioural & social sciences	Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <a href="mailto:nature.com/documents/nr-reporting-summary-flat.pdf">nature.com/documents/nr-reporting-summary-flat.pdf</a>

### Life sciences study design

Blinding

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

Sample size

No statistical methods were used to predetermine the sample sizes. Sample sizes were determined based on previous studies (e.g., Liu et al., 'A common hub for sleep and motor control in the substantia nigra', Science, 2020 ) and lab experience.

Data exclusions Only a few sessions with drug application causing large motion (based on landmarks in the imaging window) were excluded. Otherwise, no data were excluded.

Replication Each experiment was done with multiple mice and each mouse was tested for multiple sessions. All attempts at replication of experiments were successful, as evident by the individual data points reported throughout the figures.

Randomization Mice of specific genotype were randomly assigned to experimental and control groups. Each mouse of a specific genotype was subjected to both control and experimental treatment.

Investigators were not blinded for group allocations since each mouse of a specific genotype was subjected to both control and experimental treatment. For the NE imaging data, the behavioral data and the imaging data were processed independently by two researchers.

## 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 & experime	ental systems	Methods
n/a Involved in the study		n/a Involved in the study
Antibodies  Eukaryotic cell lines		ChIP-seq
		Flow cytometry
Palaeontology and	archaeology	MRI-based neuroimaging
Animals and other	organisms	
Clinical data		
Dual use research o	of concern	
Plants		
Antibodies		
Antibodies used	Information of all antibodie	es used were given in the Methods section and listed below as well.
	Primary antibodies:	4 000 11 11 1/40704 0 1101 11 7 1 1 1
		1:200 dilution) (#3724, Cell Signaling Technology); es (1:1500 dilution) (#234009, Synaptic Systems)
		1:200 dilution) (ab5076, Abcam);
		es (1:500 dilution) (AS-55043A, AnaSpec);
		es (1:300 dilution) (M11217, Life Technologies) tibodies (1:500 dilution)((#400006, Synaptic Systems)
		(1:1000 dilution) ((#019-19741, Fujifilm Wako)
	Secondary antibodies:	
	anti-goat IgG-Alexa Fluor 59	94 (1:500 dilution)(A-11058, Invitrogen);
		488 (1:500 dilution)(A-21206, Invitrogen); SuperBoost Kit (B40943, Invitrogen);
		7 (1:500 dilution)(A21449, Invitrogen);
	Biotin-SP-conjugated anti-r	rat (1:500 dilution)(712-065-153, Jackson ImmunoResearch);
Alexa Fluor 594-conjugated streptavidin (1:1000 dilution) (016-580-084, Jackson ImmunoResearch) anti-rabbit IgG-Alexa Fluor 546 (1:500 dilution) (A10040, Invitrogen) anti-chiken IgG-Alexa Fluor 488 (1:500 dilution) (703-545-155, Jackson ImmunoResearch)		
		SuperBoost Kit with Poly-HRP-conjugated anti-rabbit secondary antibody (B40922, Thermo Fisher Scientific)
Validation	All antibodies were validate	ed for immunostaining by the manufactures on their websites or by previous studies. For example:
manufacture's website)		(#3724, Cell Signaling Technology) (validated with HA-transfected and untransfected cells on the
		es (#234009, Synaptic Systems): reacts with: rat, mouse, , human, ape. Other species not tested yet.
		ab5076, Abcam): reacts with: Rat, Human; predicted to work with: Pig, Macaque monkey
		es (AS-55043A, AnaSpec): reactivity with mouse es (M11217, Life Technologies): validated with mcherry transfected cells on the manufacture's website
· ·		tibodies ((#400006, Synaptic Systems): reacts with mouse. Other species not tested yet.
	rabbit anti-lba1 antibodies	((#019-19741, Fujifilm Wako): react with Human, Mouse, Rat
Animals and othe	er research organ	nisms
Policy information about st	<u>:udies involving animals</u> ; <u>A</u>	ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in

<u>Research</u>

Laboratory animals	All mouse lines were purchased from Jackson Laboratory or Mutant Mouse Resource and Research Center (MMRRC) and maintained on a C57BL/6J background. The following lines were used in this study (Jackson stock number or MMRRC number in parenthesis): Tmem119-2A-CreERT2 (031820), RCL-GCaMP6s (028866), R26-LSL-Gi-DREADD (026219), R26-LSL-Gq-DREADD (026220)., Dbh-Cre (036778-UCD) . 2-6 month-old mice of both sexes were used in this study.
Wild animals	This study did not involve wild animals.
Reporting on sex	Both male and female mice were used in this study.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	All procedures were approved by Animal Care and Use Committees of the University of California, Berkeley and were done in
241103 0 4 61 318114	accordance with federal regulations and guidelines on animal experimentation.

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