# nature portfolio

Corresponding author(s):	Yan Zhang, Xin-Feng Liu, Zhi Zhang

Last updated by author(s): Jun 28, 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>.

<u> </u>				
St	'a'	119	:†1	CS

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.
$\boxtimes$	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
$\boxtimes$	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 high gaists contains articles on many of the points above

#### Software and code

Policy information about availability of computer code

Data collection

Fiber photometry:Thinkertech (Nanjing, China)

 $Whole cell \ recording: patch-clamp \ electrophysiology \ data \ were \ analyzed \ with \ Clamp fit \ pClamp \ 10.0 \ software \ using \ MultiClamp \ 700B$ 

(Molecular Devices) and Digidata 1550B (Molecular Devices)

Von Frey test:von Frey filaments (North Coast Medical)

Hargreaves test:Hargreave's Apparatus (Model390; IITC Life Science Inc, Woodland Hills, CA)

Fluorescence imaging data:Olympus FV3000 microscope

Data analysis

GraphPad Prism v.8.0.1

Olympus FV10-ASW 4.0a Viewer

ImageJ 1.48

Clampfit software v.10.0 MATLAB software R2017a

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

Source data are provided with this paper. There are no restrictions on data availability in the manuscript.

#### 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
	and a field a short constant and the con

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 r	naking your selection.

Ecological, evolutionary & environmental sciences

Behavioural & social sciences For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

## Life sciences study design

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

No sample size calculation was performed. Our sample sizes are similar to those reported in previous publications Sample size (PMID: 31451801; PMID: 33686297; PMID: 34663957)

Mice that were incorrect for viral injection or optic fiber placement were excluded from this study. Data exclusions

All attempts at replication were successful. Behavioral experiments were replicated multiples times with independent mice, and data were Replication analyzed by two different experimenters blind to the group allocation of the samples. Numbers of replicates (n) are indicated in the figure

legends.

Randomization

X Life sciences

All animal were randomly assigned to different groups.

Blinding All investigators were blinded to group allocation during data collection and analysis.

## 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.

ς.	
ピ	
3	
$\geq$	
2	
ŭ	

Materials & experim	ental systems	Methods
n/a Involved in the stud	у	n/a Involved in the study
Antibodies		ChIP-seq
Eukaryotic cell line	25	Flow cytometry
Palaeontology and	d archaeology	MRI-based neuroimaging
Animals and other	organisms	
Clinical data		
Dual use research	of concern	
Plants		
Antibodies		
Anti-TPH2 (1:500, rabbit, Abcam, Cat# ab111828), Anti-VGluT3 (1:500, rabbit, Synaptic Systems, Cat# 135203) Anti-GABA (1:500, rabbit, Sigma, Cat# A2052) Anti-TH (1:1000, rabbit, Emd millipore, Cat# AB152) Goat anti-rabbit Alexa Fluor 488 (1:500, Jackson ImmunoResea Goat anti-rabbit Alexa Fluor 594 (1:500, Jackson ImmunoResea Goat anti-rabbit Alexa Fluor 647 (1:500, Jackson ImmunoResea Goat anti-r		Synaptic Systems, Cat# 135203)
		647 (1:500, Jackson ImmunoResearch Labs, Cat# 111-585-003)
Validation Anti-TPH:RRID:AB_10862137		7
vandation	Anti-Vglut3:RRID:AB_887886	
	Anti-GABA:RRID:AB_477652	!
	Anti-TH:RRID:AB_390204	
Goat anti-rabbit Alexa Fluor Goat anti-rabbit Alexa Fluor		
		594:RRID:AB 2338059

### Animals and other research organisms

Field-collected samples

Policy information about <u>studies involving animals</u>; <u>ARRIVE guidelines</u> recommended for reporting animal research, and <u>Sex and Gender in Research</u>

In all experiments, male and female mice aged 8-10 weeks were used. C57BL/6J mice were purchased from Beijing Vital River Laboratory Animal Technology, VGluT3-Cre and Ai14 (RCL-tdTOM) mice were purchased from Jackson Laboratories, DAT-Cre and GAD2-Cre mice were gifts from GQ Bi and Z Zhang, respectively. Mice were kept on a 12-h light/dark cycle (lights on at 7am) at a stable temperature of 23 ± 1 °C and a consistent humidity of 50 ± 5%. Food and water were freely available.

Wild animals

No wild animals were used.

We included both male and female mice in our study and reported them differently in the figures. We did not find any sex differences in any of our results, therefore we pooled data from different sexes together with equal representation.

Ethics oversight All animal protocols were approved by the Animal Care and Use Committee of the University of Science and Technology of China.

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

No sample was collected from the field.