# nature portfolio

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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.

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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n/a	Confirmed
	$oxed{\boxtimes}$ 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 d, Pearson's r), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

### Software and code

Policy information about availability of computer code

Data collection

Commercial softwares licensed by microscopy companies was utilized: Olympus Fluoview (version 4.2a), Zeiss Zan 2012(for LSM 710). Digitized analog signals at 10 kHz were obtained with Digidata (version 1550B) and pClamp(version 10.0) software.

Data analysis

Western blotting image analysis was performed with Fiji (version: 2.0.0-rc-69/1.52p). Med Associates Animal Behavior Analysis System (Med Associates Inc.) was used to automatically analyze freezing behavior. We used Mini 60 to analyze the frequency and amplitude of sEPSCs. Graph Pad Prism (version 8.0) was used for statistical analysis.

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  $reviewers. \ We strongly \ encourage \ code \ deposition \ in \ a \ community \ repository \ (e.g. \ GitHub). \ See \ the \ Nature \ Portfolio \ \underline{guidelines \ for \ submitting \ code \ \& \ software} \ for \ further \ information.$ 

#### Data

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. 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

Data availability statement :

The data used to support the findings of this study are available from the corresponding author upon request.

## 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)</u>, <u>and sexual orientation</u> and <u>race</u>, ethnicity and racism.

Reporting on sex and gender	This information was not collected as no human was involved in this study.	
Reporting on race, ethnicity, or other socially relevant groupings	This information was not collected as no human was involved in this study.	
Population characteristics	This information was not collected as no human was involved in this study.	
Recruitment	This information was not collected as no human was involved in this study.	
Ethics oversight	This information was not collected as no human was involved in this 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 below	that is the best fit for your re	esearch. If you are not sure	e, read the appropriate sections before	re making your selection.
X Life sciences	Behavioural & social scient	ences Ecological, e	volutionary & environmental science	es

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

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

Pilot experiments, as well as previous work in our laboratories, were used to predict effect size and indicated n=6-10 per group to be sufficient to detect biologically and statistically significant results. No sample size calculation was performed.

Data exclusions

No data were excluded from the data set.

Replication

The data were repeated on multiple independent animals, all replicates are true biological (rather than technical) replicates. All replication were successful.

Randomization

Animals were randomly allocated to control or treatment groups.

Blinding

The 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.

Materials & experime	ental systems Methods
n/a Involved in the study	n/a Involved in the study
Antibodies	ChIP-seq
Eukaryotic cell lines	s Flow cytometry
Palaeontology and	
Animals and other	
	3.60.110.110
Dual use research of	of concern
Plants	
Antibodies	
Antibodies used	For western blotting, we used anti-rabbit c-Fos antibody (#2250, lot:12 Cell Signaling Technology), anti-mouse β-actin antibody (#AC004, lot:35001000, Abclonal).
	For immunohistochemistry, we used anti-rabbit c-Fos antibody ( #2250, lot:12 Cell Signaling Technology), anti-mouse Vglut2 antibody
	(#ab79157, lot:GR3205581-1, Abcam), anti-mouse GAD67 antibody (#ab26116, lot:GR3357286-2, Abcam), Alexa Fluor594 goat anti-
	mouse antibody (#ab150116, lot:1001006139, Abcam), Alexa Fluor594 goat anti-rabbit antibody (#ab150080, lot:GR3440097-1, Abcam), Alexa Fluor488 goat anti-mouse antibody (#ab150113, lot:GR3419505-1, Abcam), Alexa Fluor488 goat anti-rabbit antibody
	(#ab150077, lot:GR3442008-1, Abcam), Alexa Fluor647 goat anti-mouse antibody (#ab150115, lot:GR3399166-5, Abcam).
\/al: da#: a.a	Validation information of each antihody is as follows, anti-raphit a Fas antihody (Cvi V. Olan V. et al., Call Death Dis. 2024 Jan.
Validation	Validation information of each antibody is as follows: anti-rabbit c-Fos antibody (Gui Y, Qian X, et al., Cell Death Dis. 2024 Jan 17;15(1):61. doi: 10.1038/s41419-024-06451-w). anti-mouse β-actin antibody (Yin Y, Lu JY, et al., Nature. 2020
	Apr;580(7801):147-150. doi: 10.1038/s41586-020-2105-3), anti-mouse Vglut2 antibody (Velasco ER, Florido A, et al., Nat Commun.
	2022 Jul 28;13(1):4374. doi: 10.1038/s41467-022-31442-w), anti-mouse GAD67 antibody (Forsthofer M, Straka H. J Neurol. 2023
	Jan;270(1):57-70. doi: 10.1007/s00415-022-11311-8), Alexa Fluor594 goat anti-mouse antibody and Alexa Fluor488 goat anti-rabbit antibody (Liu TT, Shi X, et al., Cell Death Dis. 2023 Oct 7;14(10):654. doi: 10.1038/s41419-023-06176-2), Alexa Fluor594 goat anti-
	rabbit antibody and Alexa Fluor488 goat anti-mouse antibody (Li L, Chen W, et al., Histol Histopathol. 2024 Jan;39(1):67-77. doi:
	10.14670/HH-18-613), Alexa Fluor647 goat anti-mouse antibody (Chen X, Yao J, et al., Int J Gen Med. 2023 May 8;16:1713-1733. doi:
	10.2147/IJGM.S398908).
Animais and othe	er research organisms
Policy information about <u>s</u> <u>Research</u>	tudies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in
Laboratory animals	C57BL/6J mice were obtained from the Experimental Animal Center of Xuzhou Medical University. Vglut2/Vgat-cre and Vgat-ChR2-
Laboratory aminais	EYFP mice were originally sourced from Jackson Laboratory (USA) and bred at Xuzhou Medical University Laboratory Animal Center.
	Both male and female mice (6 to 8 week-old) were used in the experiments (each group included an equal number of male and
	female mice). Experiments were approved by the Animal Care and Ethics Committee of Xuzhou Medical University and conformed to the National Research Council's Guide for the Care and Use of Laboratory Animals.
Wild animals	No wild animals were used in this study.
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Reporting on sex	Both male and female mice were used in the study, and no difference was observed between the sexes, therefore we combine them
	together for statistical analysis.
Field-collected samples	The study involved no samples collected from the field.
Ethics oversight	The study complied with the Declaration of Basel and was approved by the Animal Care and Use Committee of Xuzhou Medical University (approval number: 202112A255).
Note that full information on	the approval of the study protocol must also be provided in the manuscript.
Plants	
Canalataalia	This information was not collected as no plant was involved in this study.
Seed stocks	This information was not collected as no plant was involved in this study.
Novel plant =====t	This information was not collected as no plant was involved in this study.
Novel plant genotypes	This information was not collected as no plant was involved in this study.
Authentication	This information was not collected as no plant was involved in this study.