natureresearch

Corresponding author(s): Nuo Li

Reporting Summary

Nature Research 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 Research policies, see <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main

Statistical parameters

text	, or l	Methods section).
n/a	Cor	nfirmed
	\boxtimes	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	\square	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	\boxtimes	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
\boxtimes		A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	\boxtimes	A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (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 Give <i>P</i> values as exact values whenever suitable.
\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
	\boxtimes	Clearly defined error bars State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information at	pout availability of computer code
Data collection	Behavioral data were acquired using commercial hardware (Bpod, Sanworks) and software (https://github.com/sanworks/Bpod_Gen2). Electrophysiology data were acquired using Open-Source RHD2000 Interface Software from Intan Technology (version 1.5.2, http:// intantech.com/downloads.html#software). Anatomy data were acquired using a commercial confocal microscope (LSM 700, Carl Zeiss) operated with Zeiss Zen Software (v2.2).
	Anatomy data were acquired using a commercial comocar microscope (LSM 700, Can Zeiss) operated with Zeiss zen software (vz.z).
Data analysis	Behavior and electrophysiology analyses were performed in Matlab using custom scripts and published analytical methods. Anatomy images were processed in FIJI ImageJ software (v.1.46).
	Anatomy data were aligned to the Allen Mouse Common Coordinate Framework using custom Matlab scripts and the B-spline Grid,
	Image and Point based Registration package available on the Matlab FileExchange (https://www.mathworks.com/matlabcentral/
	fileexchange/20057-b-spline-gridimage-and-point-based-registration).
	Custom analysis scripts and data will be deposited on CRCNS.org.
	Analysis scripts and data will also be available from the corresponding author 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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Raw and processed data are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

K Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/authors/policies/ReportingSummary-flat.pdf</u>

Life sciences study design

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

Sample size	The sample sizes are similar to sample sizes used in the field: for behavior, 3 mice or more per condition; for electrophysiology, more than 100 units per brain region.
Data exclusions	In 1 of the fastigial-stimulation mice, the injection of ChR2 virus missed CN and its data were excluded.
Replication	All key results were replicated in multiple mice.
Randomization	Mice were randomly pre-allocated into experimental groups.
Blinding	Unless stated otherwise in specific experiments, the investigators were not blinded to allocation during experiments and outcome assessment. Trial types were randomly determined by a computer program. During spike sorting, experimenters cannot tell the trial type, so experimenters were blind to conditions.

Reporting for specific materials, systems and methods

Materials & experimental systems

Methods

n/a	Involved in the study
\boxtimes	Unique biological materials
	Antibodies
\boxtimes	Eukaryotic cell lines
\boxtimes	Palaeontology
	Animals and other organisms
\boxtimes	Human research participants

n/a	Involved in the study
n 2	

|--|

\ge		Flow	cytometry
-------	--	------	-----------

MRI-based neuroimaging

Antibodies

Antibodies used	Mouse anti-NeuN (Millipore, MAB337, lot 2654334) Chicken anti-GFP (Aves, GFP-1020, lot GFP697986) Rabbit anti-RFP (Rockland, 600-401-379, lot 36815) Mouse anti-vGlut2 (Millipore, MAB5504, lot 2012363)
Validation	Mouse anti-NeuN: the immunofluorescent labeling has been validated with mouse brain slices. (http:// www.merckmillipore.com/NL/en/product/Anti-NeuN-Antibody-clone-A60,MM_NF-MAB377, see also Figure 1 and Extended Data Figure 1 in this study). Chicken anti-GFP: the immunofluorescent labeling has been validated with mouse brain sections. (http://www.aveslab.com/ products/epitope-tag-and-gfp-antibodies/anti-gfp-green-fluorescent-protein-antibodies-2/, see also Extended Data Figure 8 in this study).

Rabbit anti-RFP: the immunofluorescent labeling has been validated with mouse tissues (https://rockland-inc.com/Product.aspx? id=34801, see also Extended Data Figure 8 in this study).

Mouse anti-vGlut2: the immunofluorescent labeling has been validated with mouse brain sections (http:// www.merckmillipore.com/NL/en/product/Anti-Vesicular-Glutamate-Transporter-2-Antibody,MM_NF-MAB5504, see also Extended Data Figure 8 in this study).

Animals and other organisms

Laboratory animals	 This study is based on data from 70 mice (age > P60, both male and female mice, Supplemental Table 1): 13 C57B1/6 mice were used for cerebellar nuclei (CN) ChR2 photo-activation experiments. A subset of these mice (10 mice) wer used for ALM recordings during photo-activation of the CN. 8 C57B1/6 mice were used for CN lesion experiments. 7 L7-cre × Ai32 (Rosa26-LSL-ChR2-EYFP, JAX Stock#012569) mice were used for CN photo-inhibition experiments during behavior. A subset of these mice (4 mice) were used for ALM recordings during CN photo-inhibition. 2 L7-cre × Ai32 mice were used to characterize CN photo-inhibition using electrophysiology. 6 L7-cre × Ai32 mice were used to quantify general effects of CN photo-inhibition on movements. 10 C57B1/6 mice were used for CN recording experiments. 8 VGAT-ChR2-EYFP mice (Jackson laboratory, JAX Stock#014548) were used for CN recordings during ALM photo-inhibition. 4 VGAT-ChR2-EYFP mice were used for behavioral experiments in which both ALM and CN activity were manipulated to test the causal role of ALM activity in behavior following a fastigial perturbation. 12 C57B1/6 mice (not listed in Supplemental Table 1) were used for anatomical tracing.
Wild animals	The study did not involve wild animals