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
	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.
x	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>
x	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
x	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.

Policy information about <u>availability of computer code</u>

Data collection

Software and code

All data collection methods, hardware and software, are described in the Methods section of the manuscript. In summary, MATLAB (R2019b) and Arduino boards were used to control the behavioral task. Neuralynx software was used for data acquisition and spike sorting. ThorCam was used to acquire whisker movement imaging.

Data analysis

Spike sorting and clustering were conducted offline by SpikeSort3D software, first through the KlustaKwik function followed by manual inspection. ImageJ 1.52i was used to pre-process whisker movement imaging and histology data. All data were analyzed using SPSS or custom-written MATLAB (R2019b) scripts. The MatLab scripts used for this study are available at https://figshare.com/articles/dataset/Zhang_Zagha_code_data_NatureComm/22191193.

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

All data used and reported in this study have been deposited in the Figshare database including a "Source Data" file under accession code: https://figshare.com/articles/dataset/Zhang_Zagha_code_data_NatureComm/22191193. Additional data that support the findings of this study are available from the corresponding author E.Z. upon request.

Huma	in res	earch	part	icip	<u>ban</u>	ts
Policy inf	ormatio	n about s	studies	invo	lving	hu

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	w that is the best fit for your research.	If 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 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

Sample sizes were based on previous experience, preliminary data collected for this study, and power analyses.

Data exclusions

Data inclusion criteria are described in detail in the Methods section of the manuscript. In summary, all the behavioral sessions are included;

the recording sessions that are not aligned to principal whisker are excluded from stimulus encoding analyses; the units that are not significantly modulated by opto-tagging are excluded for classification as putative excitatory/inhibitory neurons.

Replication Results were verified between animals; multiple batches of animals were used for this study and the attempts at replication were successful.

Randomization The optogenetic perturbation light stimulus was randomly positioned among four brain regions on each consecutive testing day. The different types of trials were randomly given by MATLAB (R2019b) code. Other than these, no other experiments in this study involves randomization.

Investigators were not blinded to the experimental conditions or mouse genetic backgrounds, yet all data inclusion criteria was controlled by MATLAB (R2019b) code to avoid experimenter bias.

Behavioural & social sciences study design

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

Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).

Research sample

Study description

Blinding

State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.

Sampling strategy

Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.

Data collection

Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.

Timing

Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.

Data exclusions

If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.

Non-participation

State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.

Randomization

If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.

Ecological, evolutionary & environmental sciences study design

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

Study description

Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.

Research sample

Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.

Sampling strategy

Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.

Data collection

Describe the data collection procedure, including who recorded the data and how.

Timing and spatial scale

Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken

Data exclusions

If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.

Reproducibility

Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.

Randomization

Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.

Blinding

Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.

Did the study involve field work?

Yes		No
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Field work, collection and transport

Field conditions

Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).

Location

State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).

Access & import/export

Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).

Ethics oversight

Reporting for specific materials, system	ns and	d methods
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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	ChIP-seq Chip-seq Flow cytometry MRI-based neuroimaging Drganisms		
Antibodies			
Antibodies used	Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.		
Validation	Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.		
Eukaryotic cell lin			
Policy information about <u>ce</u>	ell lines and Sex and Gender in Research		
Cell line source(s)	State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.		
Authentication	Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.		
Mycoplasma contaminati	ion Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.		
Commonly misidentified (See ICLAC register)	lines Name any commonly misidentified cell lines used in the study and provide a rationale for their use.		
Palaeontology an	d Archaeology		
Specimen provenance	men provenance Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.		
Specimen deposition	Indicate where the specimens have been deposited to permit free access by other researchers.		
Dating methods	If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.		
Tick this box to confire	rm that the raw and calibrated dates are available in the paper or in Supplementary Information.		

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance

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

was required and explain why not.

Animals and other research organisms

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>

Both wild type (C57BL/6) and transgenic (VGAT-ChR2) mice were purchased from Jackson Laboratories (014548, 000664) and were subsequently housed and bred in an onsite vivarium. At the beginning of the experiments, all mice were between 6~18 weeks old (age: mean±std: 82±26 days).

Wild animals

Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.

Reporting on sex

Both male and female wild type (C57BL/6) (2 male and 9 female) and transgenic (VGAT-ChR2) (7 male and 6 female) mice were used in this study. Sex differences were not analyzed due to insufficient statistical power.

Field-collected samples

For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

All experimental protocols were approved by the Institutional Animal Care and Use Committee of University of California, Riverside.

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

Clinical data

Ethics oversight

Policy information about <u>clinical studies</u>

All manuscripts should comply with the ICMJEguidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.

Study protocol

Note where the full trial protocol can be accessed OR if not available, explain why.

Data collection

Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.

Outcomes

Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.

Dual use research of concern

Policy information about $\underline{\text{dual use research of concern}}$

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes
	Public health
	National security
	Crops and/or livestock
	Ecosystems
	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes
	Demonstrate how to render a vaccine ineffective
	Confer resistance to therapeutically useful antibiotics or antiviral agent
	Enhance the virulence of a pathogen or render a nonpathogen virulent
	Increase transmissibility of a pathogen
	Alter the host range of a pathogen
	Enable evasion of diagnostic/detection modalities
	Enable the weaponization of a biological agent or toxin
	Any other potentially harmful combination of experiments and agents

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	Confirm that both	raw and final	nrocessed data	have heen	denosited in a	nublic database	such as GEO
	COITHITH CHAL DOCH	i avv aliu iliiai	DIOCESSEU GALA	Have been	uebositeu III a	Dublic database	Such as OLO.

Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission

Provide a list of all files available in the database submission.

Genome browser session (e.g. <u>UCSC</u>)

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

whether they were paired- or single-end.

Antibodies Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot

number.

Peak calling parameters | Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files

used

Data quality Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software

Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

Plots

Confirm that:

The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

All plots are contour plots with outliers or pseudocolor plots.

A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.

Instrument Identify the instrument used for data collection, specifying make and model number.

Software Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a

community repository, provide accession details.

Cell population abundance Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the

samples and how it was determined.

Gating strategy

Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell

population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications	Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.					
Behavioral performance measure		State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).				
Acquisition						
Imaging type(s)	Specify: fu	nctional, structural, diffusion, perfusion.				
Field strength	Specify in	Specify in Tesla				
Sequence & imaging parameters		e pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, ness, orientation and TE/TR/flip angle.				
Area of acquisition State v		hether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.				
Diffusion MRI Used Not		sed				
Preprocessing						
Preprocessing software	Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).					
		rmalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for OR indicate that data were not normalized and explain rationale for lack of normalization.				
		mplate used for normalization/transformation, specifying subject space or group standardized space (e.g. ch, MNI305, ICBM152) OR indicate that the data were not normalized.				
		be your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and logical signals (heart rate, respiration).				
Volume censoring Define your sa		software and/or method and criteria for volume censoring, and state the extent of such censoring.				
Statistical modeling & infere	nce					
		ass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and e.g. fixed, random or mixed effects; drift or auto-correlation).				
Effect(s) tested	Define precise or factorial des	effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA igns were used.				
Specify type of analysis: W	nole brain [ROI-based Both				
Statistic type for inference (See Eklund et al. 2016)	Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.					
Correction	Describe the ty	pe of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).				
Models & analysis						
n/a Involved in the study Functional and/or effective Graph analysis Multivariate modeling or po		s				
Functional and/or effective conn	ectivity	Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).				
Graph analysis		Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).				
Multivariate modeling and predic	ctive analysis	Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.				