# nature portfolio

Corresponding author(s):	Serge Picaud
Last updated by author(s):	27/01/223

# **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>.

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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
	$oxed{\boxtimes}$ 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
$\boxtimes$	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 MC-rack Multichannel System, Phoenix microm imaging software, olympus fluoview FV100

Data analysis Multichannel systems data analysis V 2.6.15, Spyking Circus 0;5, Matlab, GraphPad Prism 9, Fiji ImageJ 1.53q

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 <u>availability of data</u>

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

Some dara are already available on fighshare (https://figshare.com/account/home#/projects/154041) and more are available on demand from the corresponding author

## Human research participants

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

Reporting on sex and gender

Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data where this information has been collected, and consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ecological, evolutionary & environmental sciences

Ethics oversight

Identify the organization(s) that approved the study protocol.

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	f you are not sure,	read the appropriate	e sections before makir	ng your selectior

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>

Behavioural & social sciences

## Life sciences study design

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

Sample size

X Life sciences

Sample sizes were not predetermined but some were increased based on reviewers' demand. They were defined to obtain statistically significant diffeences (evaluated using two-sided unpaired t-test) between the transfected and non-transfected groups.

Data exclusions

No data were excluded for the analysis.

Replication

To verify reproducibility, each stimulation was repeated at least 50 times for each stimulation on several animals. Replications were successfull for each tested preparation

Randomization

Recordings were performed with random allocation between transfected and non-transfected groups. When testing stimuli of different ultrasound pressures, durations and repetitions rates, the stimuli were presented randomly.

Blinding

Blinding was not performed for transfected and non-transfected groups because the fluorescence of the transgene was used to locate the transfected area for electrophysiological recordings and behavior analysis.

# Behavioural & social sciences study design

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

Study description

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

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

## 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).

Disturbance

Describe any disturbance caused by the study and how it was minimized.

# 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 & experimental	systems Methods
n/a Involved in the study	n/a Involved in the study
Antibodies	ChIP-seq
Eukaryotic cell lines	Flow cytometry
Palaeontology and archae	ology MRI-based neuroimaging
Animals and other organis	ms
Clinical data	
Dual use research of conc	ern
Antibodies	
	clonal RBPMS antibodies, NeuN antibody clone 60, polyclonal alexa Flupr secondary antibodies.
Validation The I	RNA binding protein RBPMS is a selective marker of ganglion cells in the mammalian retina.
	iguez, AR; de Sevilla Müller, LP; Brecha, NC ournal of comparative neurology 522 1411-43 2014. Manufacter garantees reactivity in rats and Immunofluorescence
appli	cation of the antibody.
	NeuN Antibody, clone A60 detects level of NeuN and has been published and validated for use in Immunofluorescence in rats.  antibody specifically recognizes the DNA-binding, neuron-specific protein NeuN, which is present in most CNS and PNS neuronal
	ypes of all vertebrates tested.
e 1 - 0 - 0.0	
Eukaryotic cell lines	
Policy information about <u>cell line</u>	es 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 contamination	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 lines (See ICLAC register)	Name any commonly misidentified cell lines used in the study and provide a rationale for their use.
Palaeontology and A	rchaeology
	de provenance information for specimens and describe permits that were obtained for the work (including the name of the
expo	ng authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, rt.
Specimen deposition Indic	ate where the specimens have been deposited to permit free access by other researchers.
they	w dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are
I DI OVI	ded.

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.

Ethics oversight

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Enable the weaponization of a biological agent or toxin

Policy information about <u>st</u> <u>Research</u>	udies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in					
Laboratory animals	Male C57BL/6J mice aged 9 weeks Male P23H rats (9-22 months), Male Long evans rats 2-12 months					
Wild animals	no					
Reporting on sex	Experiments were done on male for ease of housing. No sex difference is expected in the experiments					
Field-collected samples	no					
Ethics oversight  The experimental protocol was approved by the Local Animal Ethics Committee (Committee Charles Darwin n°5, registration number: 9529 and 26889).						
Note that full information on t	he approval of the study protocol must also be provided in the manuscript.					
Policy information about <u>cl</u>	inical studies with the ICMJE guidelines 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	n of concern					
Policy information about <u>d</u>	ual use research of concern					
Hazards						
Could the accidental, delin the manuscript, pose a	berate or reckless misuse of agents or technologies generated in the work, or the application of information presented threat to:					
No Yes						
Public health						
National security						
Crops and/or lives	tock					
Ecosystems  Any other significa	nt area					
Experiments of conce						
•	y of these experiments of concern:					
No Yes	y of these experiments of concern.					
	to render a vaccine ineffective					
	to therapeutically useful antibiotics or antiviral agents					
	nce of a pathogen or render a nonpathogen virulent					
Increase transmiss	ibility of a pathogen					
Alter the host rang	ge of a pathogen					
Enable evasion of	diagnostic/detection modalities					

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Dat			

Γ	Confirm that both raw and fir	al processed data have been	deposited in a public database such as GEO.

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 labels state the marker and fluorochrome used (e.g. CD4-F	FITC	).
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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		e number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial ftrials are blocked) and interval between trials.	
Behavioral performance measures		ber and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used th that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across	
Acquisition			
Imaging type(s) Specify: fu		unctional, structural, diffusion, perfusion.	
Field strength	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 whe	ther a whole brain scan was used OR define the area of acquisition, describing how the region was determined.	
Diffusion MRI Used	Not u	sed	
Preprocessing			
	Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).		
	If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.		
	Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.		
	Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).		
Volume censoring	Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.		
Statistical modeling & inferen	ce		
	Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).		
· ,	Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.		
Specify type of analysis: Who	ole 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 type 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 of Graph analysis Multivariate modeling or pre		s	
Functional and/or effective connectivity		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 predictive analysis		Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.	