nature research

Corresponding author(s): Shuo Chen, Thomas J. McHugh

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 Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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.*
☐	☒	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 statistics 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. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	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 *d*, Pearson's *r*), indicating how they were calculated
☐	☒	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 about availability of computer code

Data collection	Cheetah v 5.7.4 (Neuralynx)
Data analysis	SpikeSort 3D v 2.5.4 (Neuralynx), Matlab 2013a (MathWorks), Excel 2010 (Microsoft), IGORpro (Wavemetrics), Clampfit (Molecular Devices)

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 data availability statement. 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

All data can be obtained 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.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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

Life sciences study design

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

Sample size	No statistical method was used to pre-determine sample sizes, but we chose sample sizes that exceeded or matched those reported in previous studies with similar methodologies; see manuscript references 7,10,11,18,19,28,29,33-36
Data exclusions	Criteria for data exclusion based on lack of viral expression or mistargeting of injection were established prior to the experiments. Otherwise, no systematic data exclusion was performed.
Replication	The reported findings were reproduced across animals in physiology and anatomical experiments, and across cohorts in the behavioral experiments. The total number of animals and neurons is reported for all experiments. For example data show in the main figures the number of replicate is listed in the Statistics and Reproducibility section of the methods, for extended data figures the information appears in the figure legends.
Randomization	For all experiments mice were randomly assigned as to which virus they received, the injection location and protocol used.
Blinding	Experimenters were blind to genotype, region targeted and optical protocol during injections as well as during histological and behavioral analysis.

Reporting for specific materials, systems and methods

Materials & experimental systems

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

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	The novel Cre-expressing transgenic mouse (Csf2rb2-Cre) and plasmids used for custom virus generation are availble from the authors upon reasonable request.

Antibodies

Antibodies used	Anti-Cre recombinase antibody (Millipore, MAB 3120), Anti-ß-galactosidase antibody (Abcam, ab9361), Anti-GFP antibody (Abcam, ab6673), Anti-c-Fos antibody (Sigma-Aldrich, F7799), Anti-c-Fos antibody (Synaptic Systems, 226003), Anti-PCP4 (Santa Cruz, SC-74816, C-15), Anti-GFP (Abcam, ab13970)
Validation	Anti-Cre recombinase antibody (Millipore, MAB 3120): Anti-Cre Recombinase Antibody, clone 2D8 is a well characterized monoclonal antibody. Proven to reliably detect Cre Recombinase, this mAb is validated for use in ELISA, IC, IF, IH & WB and is backed by multiple publications. Anti-ß-galactosidase antibody (Abcam, ab9361): Chicken polyclonal to ß-galactosidase, suitable for: ELISA, IHC-FrFl, ICC, IHC-FoFr, Flow Cyt, ICC/IF, IHC-Fr, WB, IHC-P. Anti-GFP antibody (Abcam, ab6673):Goat polyclonal to GFP, suitable for: WB, IP, ELISA, ICC/IF, IHC-P, IHC-FrFl, IHC-Fr. Anti-c-Fos antibody (Sigma-Aldrich, F7799): Anti-c-Fos is produced in rabbit using a synthetic peptide (FSGFNADYEASSSR-K) corresponding to the N-terminal region of human c-Fos (amino acids 3-16 with a C-terminal added lysine) conjugated to KLH as immunogen. Anti-c-Fos recognizes c-Fos by immunoblotting, (as single or multiple bands at 50-62 kDa) and by immunohistochemtry. Anti-cFos (Synaptic Systems, 226003): Polyclonal rabbit

purified antibody, affinity purified with the immunogen. Albumin and azide were added for stabilization. Applications - WB: 1:250 to 1:500 (AP staining), ICC: 1 : 1000, IHC: 1:1000. Anti-PCP4 antibody (Santa Cruz Biotechnologies, SC-74816), clone C-15: PCP-4 is an affinity purified rabbit polyclonal antibody raised against a peptide mapping at the C-terminus of PCP-4 of human origin. PCP-4 is recommended for detection of PCP-4 of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate), immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500), immunohistochemistry (including paraffin- embedded sections) (starting dilution 1:50, dilution range 1:50-1 :500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000). Anti-GFP antibody (Abcam, ab13970): Chicken polyclonal to GFP, immunogen is recombinant full length protein corresponding to GFP, suitable for IHC-P (1/500-1/1000), WB, (1/5000), IHC.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	293FT used in AAV production (Thermofisher, R70007)
Authentication	AAV production generated from low passage (no more than 15) 293FT cells stocks that were frozen at passage 2 upon receipt.
Mycoplasma contamination	Cell line was not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	N/A

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Csf2rb2-Cre mice, double transgenic Csf2rb2-Cre x Fos- Tg(Fos-tTA, Fos-EGFP*)1Mmay mice, Rosa-NLSLacX reporter mice. All mice used were male between 3 and 5 months of age.
Wild animals	Not used
Field-collected samples	Not used