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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 our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

Statistics

For	For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.				
n/a	Cor	firmed			
	×	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.			
×		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. 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			
		Our web collection on statistics for biologists contains articles on many of the points above.			

Software and code

Policy information about availability of computer code		
Data collection	n/a	
Data analysis	n/a	

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 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 the data are available from the corresponding authors on request. There are no restrictions on the data availability.

Field-specific reporting

Life sciences study design

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

Sample size	Sample size is estimated using power analysis to achieve over 80 % of power with 4 samples per group. For statistical analysis, cell counts are estimated to follow a negative binomial distribution and adjusted to account for false positives. Differences will be determined using an unpaired t-test. P < 0.05 is considered statistically significant.
Data exclusions	Samples were discarded when exhibit poor immunostaining or deficient clearing
Replication	Several rounds of experiments were performed to assure the accuracy of the results. Measurements of different brains at the same age yielded similar results.
Randomization	Samples were processed simultaneously and measurements were performed once the staining protocols were completed in no particular order. Sample randomization is not a critical point in the analysis of fixed tissue that is not affected by external or internal factors such as circadian rhythms, etc
Blinding	Authors were not blind to the age of animals during the processing of the samples since the size of the brain correlates with the age. However the authors were blind during most of the 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 & experimental systems

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n/a	Involved in the study	n/a	Involved in the study
	X Antibodies		ChIP-seq
	Eukaryotic cell lines		Flow cytometry
	Palaeontology and archaeology		MRI-based neuroimaging
	X Animals and other organisms		
	Human research participants		
	Clinical data		
	Dual use research of concern		

Antibodies

Antibodies used	Primary antibodies: mouse anti-oxytoxin PS38 (kindly provided by Dr. Harold Gainer; NIH); rabbit anti-oxytocin (Millipore, AB911); rabbit anti-RFP (Abcam, ab62341); rat anti-RFP (Chromotek, 5f8-100); rabbit anti-vasopressin (Millipore, PC234L). Secondary antibody: goat anti-mouse Alexa-488 (Invitrogen, A32723); donkey anti-mouse Alexa-647 (Jackson ImmunoResearch, 715-605-150); goat anti-rabbit Alexa-594 (Invitrogen, A11072); donkey anti-rat Alexa-594 (Jackson ImmunoResearch, 712-585-153).
Validation	Immunostaining controls using secondary antibodies (without primary antibodies) were performed to test the specificity of the primary antibodies for OXT and AVP.

Eukaryotic cell lines

Policy information about <u>cell lines</u>				
Cell line source(s)	State the source of each cell line used.			
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 <u>ICLAC</u> register)	Name any commonly misidentified cell lines used in the study and provide a rationale for their use.			

Palaeontology and Archaeology

Specimen 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).

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 confirm	n that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

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

Animals and other organisms

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

Laboratory animals	Experiments were performed in wild type ICR mice bred in-house. Oxytocin-Ires-Cre:Rosa26iDTR/+OXT-Cre (OXT-Cre thereafter) mice were obtained from Jackson Laboratories (strain ID 024234). OXT-Cre mice were bred with a tdTomato reporter line (Ai9, Jackson Laboratories strain ID 007909)
Wild animals	n/a
Field-collected samples	n/a
Ethics oversight	All experiments were performed according to Spanish and European Union regulations regarding animal research (2010/63/EU), and the experimental procedures were approved by the Bioethical Committee at the Instituto de Neurociencias and the Consejo Superior de Investigaciones Científicas (CSIC).

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, 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.
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.

Clinical data

 Policy information about clinical studies

 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 <u>dual use research of concern</u>

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

 Image: Constraint of the security
 Public health

 Image: Constraint of the security
 National security

 Image: Constraint of the security
 Crops and/or livestock

 Image: Constraint of the security
 Ecosystems
- X Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

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

ChIP-seq

Data deposition

Confirm that both raw and final 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.
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-FITC).

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: functional, structural, diffusion, perfusion.
Field strength	Specify in Tesla
Sequence & imaging parameters	Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.
Area of acquisition	State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.
Diffusion MRI Used	Not used
Preprocessing	
Preprocessing software	Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).
Normalization	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.
Normalization template	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.
Noise and artifact removal	Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings	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).	
Effect(s) tested	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: 🗌 Whole brain 🔲 ROI-based 📃 Both		
Statistic type for inference (See <u>Eklund et al. 2016</u>)	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 connectivity Graph analysis Multivariate modeling or predictive analysis 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