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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 <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

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For	or all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or l	Methods section.
n/a	(a Confirmed	
	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of meas	urement
	A statement on whether measurements were taken from distinct samples or whether the same sample was m	easured 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 compa	risons
	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)	(e.g. regression coefficient
	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 freed <i>Give P values as exact values whenever suitable.</i>	om and P value noted
×	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 outc	omes
×	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

Electrophysiological data was acquired using commercially available software; MC_Rack (MCS GmbH, Germany) or SmartBox GUI (NeuroNexus, MI, USA).

Data analysis

Data analysis was performed using commercially available software; single unit isolation used Offline Sorter (V3; Plexon, TX, USA); Statistical analysis was performed using NeuroExplorer (V5; Nex Technologies) and GraphPad Prism 7.04 (GraphPad Software Inc., CA, USA).

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. 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

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Field-specific reporting

Life sciences study design

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

Sample size

Experimental design throughout was informed by previous studies performed in the investigators laboratories and corresponding power analyses performed using G*power 3.1 (Universitat Kiel, Germany). Sample sizes were set such that effect sizes (d) of 2-2.5 could be identified at power=0.95 and alpha=0.05.

Data exclusions

For analysis of effects of DREADD based manipulations there was a pre-established criteria that data would only be included in the analysis when the mcherry fluorescent tag could be detected in SCN neurons following post-hoc immunohistochemistry (indicating successful viral expression). According to these criteria, 12 mice (n=6 each for Gq and Gi DREADD injections) that did not show successful SCN transfection were not considered for further analysis.

Replication

All attempts at replication were successful.

Randomization

For electrophysiological studies, mice of appropriate genotype were chosen for recording at random and, were relevant, stimuli were interleaved across multiple trials (typically >100) to avoid order effects. For whole-animal physiological monitoring, mice were randomly allocated to receive viral injections delivering Gi or Gq DREADDs. Subsequent testing of responses to DREADD activation at different timepoints was additionally randomised across animals.

Blinding

For electrophysiology, the experimenter was inherently unaware of the identify of neurons while performing single unit isolation and subsequent analyses were performed using purely objective criteria. For whole-animal physiological monitoring on virally transfected animals, it was not possible to blind the experimenter to group allocation during data collection but all subsequent analyses were similarly based on objective measures.

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.

iviateriais & experimental systems		ivietnoas	
n/a Involved in the study		n/a	Involved in the study
	x Antibodies		ChIP-seq
	Eukaryotic cell lines		Flow cytometry
	Palaeontology		MRI-based neuroimaging
	X Animals and other organisms		
	Human research participants		
	Clinical data		

Antibodies

Antibodies used

For GFP immunohistochemistry, Chicken anti GFP primary antibody was used from abcam (ab13970; lot#GR293362-1) along with donkey anti chicken secondary antibody from Jackson Immuno research (703-545-155; lot#134115).

For mCherry immunohistochemistry, rabbit anti DS Red antibody was used from Taka Ra Bio (632475; lot #1509043), along with donkey anti rabbit 555 secondary antibody from thermofisher (A-31572; lot#1945911)

Validation

 $All \ antibodies \ have \ been \ extensively \ used/validated \ previously \ as \ outline \ on \ the \ manufacturers \ websites:$

https://www.abcam.com/gfp-antibody-ab13970.html

https://www.jacksonimmuno.com/catalog/products/703-545-155

https://www.takarabio.com/products/antibodies-and-elisa/fluorescent-protein-antibodies/red-fluorescent-protein-antibodies https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31572

Eukaryotic cell lines

Policy information about cell lines

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 ICLAC register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Palaeontology

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

Animals and other organisms

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

Laboratory animals Experimental animals were generated by crossing VIP-IRES-Cre mice (JAX #010908) to animals bearing Cre-dependent channelrhodopsin2 (ChR2)-EYFP (Ai32; JAX #0102569) or Archaerhodopsin 3-EYFP (Ai40D; The Jackson Laboratory, Strain

021188) constructs.

Male and female mice (age 41-180 days) were used throughout.

Wild animals The study did not involve wild animals.

Field-collected samples The study did not involve field collected samples

Ethics oversight All animals were used in accordance with the Animals, Scientific Procedures, Act of 1986 (UK) and received both University of Manchester ethics committee and UK Home Office approval.

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

Human research participants

Population characteristics

Policy information about studies involving human research participants

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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."

questions and have nothing to dua here, write see above

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

Recruitment

Policy information about clinical studies

All manuscripts should comply with the ICMJEguidelines for publication of clinical research and a completedCONSORT 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.

ChIP-seq			
Data deposition			
Confirm that both raw and	final processed data have been deposited in a public database such as <u>GEO</u> .		
Confirm that you have dep	osited 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 ma	arker and fluorochrome used (e.g. CD4-FITC).		
The axis scales are clearly v	risible. 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	ber 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.		
	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. Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell Gating strategy 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.

		number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial rials are blocked) and interval between trials.		
Behavioral performance measures		er and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across		
Acquisition				
Imaging type(s)	Specify: fund	tional, structural, diffusion, perfusion.		
Field strength	Specify in Te	sla		
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 wheth	er a whole brain scan was used OR define the area of acquisition, describing how the region was determined.		
Diffusion MRI Used	☐ Not use	rd		
Preprocessing				
Preprocessing software		Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).		
		normalized/standardized, describe the approach(es): specify linear or non-linear and define image types asformation 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.			
		r procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and l 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 & inference	9			
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	e brain	ROI-based Both		
Statistic type for inference (See Eklund et al. 2016)	Specify voxe	l-wise or cluster-wise and report all relevant parameters for cluster-wise methods.		
Correction	Describe the Carlo).	type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte		
Models & analysis				
n/a Involved in the study Functional and/or effective cor Graph analysis Multivariate modeling or predi				
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.		