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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>.

Statistical parameters

		atistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, mai Methods section).
n/a	Cor	nfirmed
	\boxtimes	The $\underline{\text{exact sample size}}$ (n) for each experimental group/condition, given as a discrete number and unit of measurement
	\boxtimes	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	\boxtimes	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
	\boxtimes	A full description of the statistics including <u>central tendency</u> (e.g. means) or other basic estimates (e.g. regression coefficient) AND <u>variation</u> (e.g. standard deviation) or associated <u>estimates of uncertainty</u> (e.g. confidence intervals)
	\boxtimes	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
\times		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
_		Clearly defined error bars

Our web collection on <u>statistics for biologists</u> may be useful.

Software and code

Policy information about availability of computer code

State explicitly what error bars represent (e.g. SD, SE, CI)

Data collection

Logitech Webcam Software was used to log video data. Biopac Acknowledge (version 5) was used to log cystometry and EMG data. Mightex BioLED Controller software was used to generate LED patterns for photostimulation and photoinhibition. Nikon Elements (version 4) was used to collect all confocal imaging data. Hamamatsu HCImage was used to log photometry data. DSI Ponemah software was used to log corpus spongiosum wireless pressure recordings. Molecular Devices pClamp software was used to log patch clamp physiology data.

Data analysis

Adobe After Effects (version CS5) was used to trim videos, and urine marks were subsequently analyzed using custom MATLAB software (version 2014b). Noldus Ethovision XT was used to automatically track mice and determine distance traveled and odor sniffing periods. Molecular Devices Clampfit and Origin Lab OriginPro software was used to analyze patch clamp physiology data. MATLAB was also used to compute all statistics and plot all data.

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

Analysis code is available in the Supplementary Software file or online at: github.com/stowerslab/smuf. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Field-specific reporting					
Please select the b	est 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				
or a reference copy of	the document with all sections, see <u>nature.com/authors/policies/ReportingSummary-flat.pdf</u>				
_ife scier	nces study design				
	sclose on these points even when the disclosure is negative.				
Sample size	For most experiments we did not have pre-specified effect size and used sample sizes consistent with other studies in the field. We used the effect size from preliminary wild-type chemogenetic experiments to calculate sample sizes for ESR1-Cre and CRH-Cre chemogenetic experiments (Fig. 7d-e), using the 'sampsizepwr' function in MATLAB (> 6 mice).				
Data exclusions	No individual data points are excluded				
Replication	Certain experiments included criteria for failed replication (e.g. animals did not behave in control conditions or viral injections were incorrectly targeted), in which case the data were not analyzed further:				
	(1) Preliminary behavioral data gathered before any manipulation experiments established a criterion for animals that perform voluntary urination behavior. The number of mice that did not fulfill this criterion is detailed in the Methods "Odor-motivated urination assay" and "Chemogenetic inhibition" sections.				
	(2) Preliminary immunostaining data gathered before any manipulation experiments established the limits of Bar used for determination of injection hits or misses (same criteria for ESR1-Cre and CRH-Cre). The number of mice that did not fulfill this criterion is detailed in Methods				
	"Chemogenetic inhibition" and "Optogenetic stimulation/inhibition" sections. (3) For cystometry and EMG, we only photostimulated mice for which bladder-distension bursting was seen, such that we have a positive control for bursting. The number of mice that did not fulfill this criterion is detailed in the Methods "Electromyography and cystometry" section.				
Randomization	For each experiment, animals were maintained under identical conditions, such that no randomization was used to assign groups.				
Blinding	Data collection and analysis were generally not performed blind to the conditions of the experiments. However, automated data analysis in MATLAB and Ethovision was used to track animal behavior such that no blinding is necessary to ensure behavioral data integrity. Semi-automated analyses similarly assisted cell counting, where the Nissl channel was used to manually define the Bar region-of-interest, rather				

Reporting for specific materials, systems and methods

Materials & experimental systems		Me	Methods	
n/a	Involved in the study	n/a	Involved in the study	
\boxtimes	Unique biological materials	\boxtimes	ChIP-seq	
	Antibodies	\boxtimes	Flow cytometry	
\boxtimes	Eukaryotic cell lines	\boxtimes	MRI-based neuroimaging	
\times	Palaeontology			
	Animals and other organisms			
\times	Human research participants			

than the cell-counting channel.

Antibodies

Antibodies used

For ESR1 immunostaining, we used a previously characterized primary antibody (Santa Cruz catalog# sc-542 / MC-20, lot# A0716, rabbit polyclonal, 100µg/mL diluted 1:500 in 1% BSA / 0.3% PBST, refs. 17,52, & 65). Santa Cruz went out of business during the course of the study, but the antigen for this antibody is the mouse ESR1 C-terminus fragment, which is believed to recognize a specific N-terminus truncated ESR1 isoform, as detailed in ref. 65. We used standard secondary antibodies from ThermoFisher (Alexa-Fluor 488, catalog# A11070, lot# 1812158, or 647, catalog# A21246, lot#1924449 ,anti-rabbit IgG H+L, 2mg/mL diluted 1:2000 in 1% BSA / 0.3% PBST).

Validation

The ESR1 primary antibody has been previously validated in several studies (refs. 17,52, & 65) as well as by the manufacturer (www.scbt.com/scbt/product/eralpha-antibody-mc-20). We performed initial testing in hypothalamic areas with established ESR1 expression. The secondary antibodies have been used successfully in our lab and many other labs with a variety of different primary antibodies and mouse tissues. We also initially used a primary-antibody-negative control to verify specificity and compared the signal-to-noise ratio against other secondary antibody options.

Animals and other organisms

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

Laboratory animals

All animal procedures were conducted in accordance with institutional guidelines and protocols approved by the Institutional Animal Care and Use Committee at The Scripps Research Institute. Mice were group housed at weaning (<5 per cage), single housed for at least 1 week before any testing, and maintained on a 12/12hr light/dark cycle with food and water available ad libitum. All mice were males with a mean age of ~10 weeks when single housed (range 8-12 weeks), and a mean weight of ~27g (range 25-33g). The number of mice used for each experiment is listed below where applicable and in the figure legends. All mouse lines are available at The Jackson Laboratory: CRH-Cre (ref. 51, stock #: 012704), ESR1-Cre (ref. 52, stock #: 017911), Vgat-Cre (stock #: 016962), Vglut2-Cre (stock #: 016963), ROSA-LSL-tdTomato (Ai9, stock #: 007909), ROSA-LSL-ZsGreen (Ai6, stock #: 007906), and BALB/cByJ (stock #: 000651). CRH-Cre and ESR1-Cre mice were backcrossed into the BALB/cByJ background for 3+ generations.

Wild animals

No wild animals were used.

Field-collected samples

No field-collected samples were used.