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).

- 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

- 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 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 | 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 than the cell-counting channel. |

Reporting for specific materials, systems and methods

Materials & experimental systems

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<tr>
<th>n/a</th>
<th>Involved in the study</th>
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<tr>
<td>✗</td>
<td>Unique biological materials</td>
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<td>✗</td>
<td>Antibodies</td>
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Methods

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<td>Flow cytometry</td>
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<td>MRI-based neuroimaging</td>
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**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.