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Reporting Summary
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in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

text	text, or Methods section).					
n/a	Confirmed					
	\boxtimes	The 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				
		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				
		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)				
		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 Give <i>P</i> values as exact values whenever suitable.				
	\boxtimes	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings				
	\boxtimes	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				
	\boxtimes	Clearly defined error bars State explicitly what error bars represent (e.g. SD, SE, CI)				

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code							
Data collection	Custom MATLAB(2017 b) code, Lickometer (Dialog Instrument), BioDaq, Med-PC V (Med Associates) was used for data collection.						
Data analysis	Custom MATLAB(2017 b) code, GraphPad Prism 7.02, Leica LAS X, Photoshop CC 2017, Microsoft Excel 2016 were used to analyze data. Code is available from the corresponding author upon reasonable request						

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 <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

Data and code is 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.

K Life sciences

Behavioural & social sciences

es 📃 Ecological, evolutionary & environmental sciences

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

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.						
Sample size	No statistics to determine sample size were used. Sample size is similar to recent papers in behavioural neuroscience.					
Data exclusions	All data were included if virus expression is verified.					
Replication	Essential behavioral and histological results were indepedently replicated by more than two lab members.					
Randomization	No statistical randomization methods were used. However, mice used for data collection were both males and females, at least 6 weeks of age					
Blinding	No blinding was used for data collection. However, we used double-blind test for replicating key results.					

Reporting for specific materials, systems and methods

Materials & experimental systems

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

Methods

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

Antibodies

Antibodies used	goat anti-c-Fos (1:500, Santa Cruz, SC-52G,Lot# K1616), rabbit anti-c-Fos (1:1000, Millipore, ABE457, Lot# 3116957), rabbit anti- GAD65+GAD67 (1:500, Abcam, ab183999, Lot# GR260178-1), chicken anti-GFP (1:1000, Abcam, ab13970, Lot# GR3190550-1), rat anti-mCherry (1:500, Thermo Fisher, M11217,Lot# S1259077), sheep anti-Foxp2 (1:2000, R&D systems, AF5647, Lot# CCUB0109061), and rabbit anti-HSD2 (1:300, proteintech, 14192-1-AP, Lot# 00005269). The following secondary antibodies were purchased from Jackson ImmunoResearch. Cy™3 donkey anti-goat IgG (1:500, 705-165-147), Alexa Fluor® 647 donkey anti- goat IgG (1:500,705-605-147), Alexa Fluor® 488 donkey anti-rabbit IgG (1:500, 711-545-152), Cy™3 donkey anti-rabbit IgG (1:500, 711-165-152), Alexa Fluor® 647 donkey anti-chicken IgY (1:500, 703-545-155), Cy™3 donkey anti-Rat IgG (1:500, 712-165-150), Alexa Fluor® 647 donkey anti-sheep (1:500, 713-605-147).
Validation	All antibodies were purchased from vendors in the USA. These products are normally quality controlled at the company. Furthermore, the antibodies were validated and used in previous studies. Goat anti-c-Fos, Rabbit anti-GAD65+GAD67, rat anti- mCherry, and chicken anti-GFP antibodies were used in Nature 555.7695 (2018): 204. Rabbit anti-c-Fos and sheep anti-Foxp2 antibody were used in Neuron 96.1 (2017): 190-206. Rabbit anti-HSD2 antibody was validated from Endocrine connections (2015): EC-15. Nevertheless, we characterized and validated their signals in the brain regions known to express these genes prior to experiments.

Animals and other organisms

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

Laboratory animals

Animals were both males and females, at least 6 weeks of age. The following mice were purchased from the Jackson Laboratory: C57BL/6J, stock number 00064. Slc17a6-Cre, stock number 016963. Ai75D, stock number 025106, Ai3, stock number 007903.

	HSD2-Cre mice were provided by A. and G. Fejes-Tóth (Dartmouth Medical School). PDYN-GFP mice were provided by D. Kong (Tufts University School of Medicine). PDYN-Cre mice were provided by B. Lowell (Harvard Medical School) and M. Krashes (NIH). Ai110 line was provided by D. Anderson (Caltech).
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve samples collected from the field.