

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).

n/a Confirmed

- ☐ ☒ 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

Behavioral data were collected using the automated video-tracking system ANYmaze (Stoelting, Wood Dale, IL). Images were captured with either a Zeiss Axioplan2 fluorescent microscope and the Axio Vision 4.5 software, or an Olympus IX81 inverted laser scanning confocal microscope and the Fluoview 1000 software. Electrophysiological recordings were performed with Pulse 8.31 (HEKA Elektronik, Lambrecht/Pfalz, Germany); Pulse 8.31 and Igor Pro Software. No custom or open source code was used for data collection.

Data analysis

Statistical analysis was performed with the GraphPad Prism 7 software. Behavioral experiments were analyzed using the automated video-tracking system ANYmaze (Stoelting, Wood Dale, IL). Images were analyzed with ImageJ (<http://rsweb.nih.gov/ij/>) and Adobe Photoshop CS2. Analyses of electrophysiological recordings was performed with Pulse 8.31 (HEKA Elektronik, Lambrecht/Pfalz, Germany); Pulse 8.31 and Igor Pro Software. No custom or open source code was used for data analyses.

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

The data that support the findings of this study are available from the corresponding author upon reasonable request. No custom or open source code was used for data collection and analyses.

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](https://www.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	No statistical method was used to predetermine sample size. Sample sizes were chosen based on published studies and reported power analyses in the field, and are comparable to those in our previous publications. Generally, sample sizes are based on the smallest number required to reach statistical significance in order to reduce unnecessary animal use.
Data exclusions	Mice showing incorrect cannula placement, injection sites or optic fiber placement were excluded from analysis. Plasma corticosterone levels that were not within the detection range (outside the calculated standard curve) were excluded from the experiment. These criterion were pre-established.
Replication	The number of independently replicated experiments is described in the figure legends and text. All replication attempts were successful.
Randomization	Mice were randomly allocated into different experimental groups. In cases of conditional knockout mice and control littermates, mice were assigned to the experimental group on the basis of genotype. No specific randomization method was used. Age-matched littermates were used as controls in all experiments.
Blinding	For behavioral and histological (gene co-expression; tracing) analysis, experimenters were blind to experimental conditions and/or genotype.

Reporting for specific materials, systems and methods

Materials & experimental systems

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

Methods

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

Antibodies

Antibodies used	Anti-tyrosine hydroxylase (#P40101, 1:2000, Pel Freez Biologicals, Rogers, AZ). Anti-synaptophysin (#ab14692, 1:2000, Abcam, Cambridge, UK) Anti-CRH, (1:20000 was kindly provided by Paul E. Sawchenko, Salk Institute, CA. Secondary antibodies were obtained from Invitrogen Life Technologies, Carlsbad, CA. Alexa Fluor 594 goat anti-rabbit IgG (#A11037), Alexa Fluor 647 goat anti-rabbit IgG (#A21244).
Validation	Specificity of the anti-tyrosine hydroxylase (TH) antibody was validated by the manufacturer in rat caudate lysates using western

blot (<http://www.pelfreez-bio.com>) and by us and previous publications in mice (Rao et al, FASEB 2012, 26(5):1921-33; Refojo et al, Science 2011, 333(6051):1903-7). In addition, within the brain slices we tested, TH staining was exclusively observed in dopaminergic neurons of the VTA and SN, which serves as a reasonable control for this antibody.

Specificity of the anti-synaptophysin antibody was validated by the manufacturer in mouse brain tissue lysates using western blot and mouse hippocampal tissue sections using immunohistochemistry (<http://www.abcam.com>). In addition, the antibody was used by multiple other studies for immunohistochemistry in mouse brain slices (e.g. Pan et al, Exp Ther Med 2016, 12(3):1455-1463; Genabai et al, Sci Rep 2017, 7(1):8295; Van Kampen et al, PLoS One 2017, 12(8) e0182896; Hinckelmann et al, Nat Commun 2016, 7:13233; additional references at www.abcam.com).

Anti-CRH, was kindly provided by Paul E. Sawchenko, Salk Institute, CA. Specificity of the anti-CRH antibody has previously been validated (Muglia et al., Nature, 1995; Chen et al., Endocrinology, 2015).

The specificity of the secondary antibodies was validated by the vendors and confirmed by lack of staining in mouse brain slices where the primary antibody was omitted.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	No experimental cell lines were used in this study; however mouse embryonic stem cells were used in order to generate Crhflx mice and Crhr1-ires-Cre mice (described in detail in the Methods section).
Authentication	Morphology, PCR and neomycin selection
Mycoplasma contamination	All tested mouse embryonic cell lines were negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Only mice were used in this study. Crh-ires-Cre (Taniguchi et al, Neuron 2013, , 71:995-1013), Nex-Cre (Goebbels et al, Genesis 2006, 44:611-621) and Camk2a-CreERT2 (Erdmann et al, BMC Neuroscience 2007, 8:63) mice were kindly provided by Josh Huang, Klaus-Armin Nave and Günther Schütz, respectively. Cre-Deleter mice were obtained from TaconicArtemis, Cologne, Germany. Dlx5/6-Flp mice were purchased from Jackson Labraory, (stock no: 010815). Cre and Flp lines that were purchased or obtained from different labs were backcrossed to C57BL/6J for at least 10 generations. Crhr1-ires-Cre mice were generated in this study and kept on a mixed 129S2/SvxC57BL/6J genetic background (for more details refer to the Methods section). RC::FrePe mice were kindly provided by Susan Dymecki (Brust R.D. et al. Cell Reports. 2012, 9:2152-2165 (2012). Bang et al., Eur. J. Neurosci. 2012, 35:85-96). Ai9 (R26CAG::loxP-STOP-loxP-tdTomato, stock no: 007905) and Ai32 (R26CAG::loxP-STOP-loxP-ChR2-EYFP, stock no: 012569) mice were purchased from Jackson Laboratory and were maintained on a C57BL/6J background. Conditional Crh knockout mice (Crhflx) were generated in this study and kept on a mixed 129S2/SvxC57BL/6J genetic background (for more details refer to the Methods section). All experiments were performed in 10-14 week old male mice other than 1) corticosterone measurements in Crhr1-ires-Cre mice, which were performed in female mice (Supplementary Fig. 11d) and 2) VTA-expression of CA(CRHR1), which was performed in 6-9 week old male mice (Fig. 3i-j and Supplementary Fig. 12). More details regarding the genetic mouse models used in this study are provided in the Methods section. All animal experiments and study protocols were approved by the Government of Upper Bavaria, Germany.
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve samples collected from the field.