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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 our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the 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
☐	☒ A statement on 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 statistical parameters 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

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Zeiss Zen version 2.3.69.1018, Ethovision version 3.1.16, Ethovision XT version 15.0.1416
Data analysis	Prism version 9.01 (128), Ethovision version 3.1.16, Ethovision XT version 15.0.1416, ETHOM version 1.0, ImageJ version 2.1.0/1.53c, QIIME2, q2-Deblur 2019.10.

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 and 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 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

Raw data is provided for all figures with individual data points plotted or box and whisker graphs (Figures 1-4 and Extended Data Figures 1-10). The data that support the finding of this study are available from the corresponding author upon request. 16S sequencing data that support the findings of this study have been deposited the Sequence Read Archive (SRA) under BioProject PRJNA632893. We used GreenGenes as our reference database (https://www.nature.com/articles/ismej2011139; https://greengenes.secondgenome.com/) adapted to Qiime2 (https://docs.qiime2.org/2021.2/data-resources/).

Field-specific reporting

Please select the one below that is 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/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	No statistical methods were used to calculate the sample size. Sample size was determined based on prior studies and literature in the field using similar experimental paradigms (Reference: 5, 12, 18, 22).
Data exclusions	No data were excluded except one AVNM mouse used in Extended Data Fig. 2i-k was excluded because of sample contamination.
Replication	All data were successfully replicated and data from multiple experiments were pooled at least from two independent trials. For all mouse behavior test, the replication ranged 5-26 mice; sera corticosterone, the replication ranged 5-30 mice; IHC, the replication ranged 3-11 mice, Gene expression, the replication ranged 3-6 mice.
Randomization	No specific randomization method was used. All mice used were randomly assigned to behavior testing, corticosterone measurement, immunofluorescence quantification, 16S sequencing, microbiota colonization, gene expression, neuronal tracing, adrenalectomy, vagotomy, guide cannula, genetic ablation, drug administrations, and chemogenetic experiments.
Blinding	Investigators were not blind to treatment groups. The experimenters treating the mice were the same as those analyzing the data. The treatment groups had to be clearly identified throughout the study to prevent cross contamination in the cases of gnotobiotic and antibiotic treatment. All statistics were performed in an unbiased manner.

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.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Primary antibodies and their dilutions were: goat anti-c-Fos (1:250; SC-52; Clone 4; Santa Cruz), mouse anti-NeuN (1:1000; MAB377; Clone A60; Millpore Sigma), rabbit anti-oxytocin (1:10,000; 20068; Clone N/A; Immunostar), rabbit anti-vasopressin (1:2000; 20069; Clone N/A; Immunostar), and rabbit anti-Fluorescent Gold (1:1000; AB153-I; Clone N/A; EMD Millpore Sigma). The fluorescence-conjugated secondary antibodies were donkey anti-goat (1:1000; A-32814, A-21082, A11057; ThermoFisher Scientific), donkey anti-rabbit (1:1000; A-21206, A-10042, A-31573; ThermoFisher Scientific), and donkey anti-mouse (1:1000; A-21202, A-10037, A-31571; ThermoFisher Scientific).
Validation	Concentration of antibodies were validated based on manufacturing instructions and serial dilutions. Goat anti-c-Fos (1:250; SC-52; Santa Cruz): https://www.scbt.com/p/c-fos-antibody-4 Mouse anti-NeuN (1:1000; MAB377; Millpore Sigma): https://www.merckmillipore.com/TW/zh/product/Anti-NeuN-Antibody-clone-A60,MM_NF-MAB377?ReferrerURL=https%3A%2F%2Fwww.google.com%2F Rabbit anti-oxytocin (1:10,000; 20068; Immunostar): http://www.immunostar.com/shop/antibody-catalog/oxytocin-antibody/ Rabbit anti-vasopressin (1:2000; 20069; Immunostar) : https://www.immunostar.com/shop/antibody-catalog/vasopressin-antibody/ Rabbit anti-Fluorescent Gold (1:1000; AB153-I; EMD Millpore Sigma): https://www.merckmillipore.com/TW/zh/product/Anti-Fluorescent-Gold-Antibody,MM_NF-AB153-I?ReferrerURL=https%3A%2F%2Fwww.google.com%2F&bd=1

Animals and other organisms

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

Laboratory animals	Wild-type C57BL/6J (00064), Nr3c1f/f (021021; B6.Cg-Nr3c1tm1.1Jda/J), Crh-ires-Cre (012704; B6(Cg)-Crhtm(cre)Zjh/J), and Ai14D (007914; B6.Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J) mice were obtained through Jackson Laboratory. C57BL/6J germ-free (GF) mice were bred in the Gnotobiotic Animal Facility at Caltech. In addition, wild-type C57BL/6JNarl mice used in SDV, brain cannulation, chronic antibiotic intraperitoneal injection, partial colonization experiment, anxiety-like behavior were obtained through National Laboratory Animal Center, Taiwan. All experiments were performed with male animals except for Extended Data Fig. 1j. to avoid the confounding effect of estrous cycle. Female mice were used for breeding. All mice were group housed (2–5 mice per cage) unless specified with a 13 h light/11 h dark cycle (lights on at 06:00) at 21–23 °C and 45-55% relative humidity within a range of 30–70% in ventilated cages (Super Mouse 750TM, Lab Products Inc). All behavior tests were performed at 8-16 weeks of age.
Wild animals	The study did not use wild animals.
Field-collected samples	The study did not involve field-collected samples.
Ethics oversight	All experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals using protocol approved by California Institute of Technology (Caltech) Institutional Animal Care and Use Committee (#1411-16G and #1411-19) and National Cheng Kung University (NCKU) Institutional Animal Care and Use Committee (#107268 and #108224).

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