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Supplementary information

Microbiota regulate social behaviour via stress response neurons in the brain

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Supplementary information for

Microbiota regulate social behavior via stress response neurons in the brain

Wei-Li Wu[1,2,3*], Mark D. Adame[1], Chia-Wei Liou[2,3], Jacob T. Barlow[1], Tzu-Ting Lai[2], Gil Sharon[1], Catherine E. Schretter[1], Brittany D. Needham[1], Madelyn I. Wang[1], Weiyi Tang[1], James Ousey[4], Yuan-Yuan Lin[2], Tzu-Hsuan Yao[2], Reem Abdel-Haq[1], Keith Beadle[1], Viviana Gradinaru[1], Rustem F. Ismagilov[1,4] & Sarkis K. Mazmanian[1*]

[1]Division of Biology and Biological Engineering, California Institute of Technology, 1200 E. California Boulevard, Pasadena, CA, 91125, USA
[2]Department of Physiology, College of Medicine, National Cheng Kung University (NCKU), 1 University Rd., Tainan 70101, Taiwan
[3]Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University (NCKU), 1 University Rd., Tainan 70101, Taiwan
[4]Division of Chemistry and Chemical Engineering, California Institute of Technology, 1200 E. California Boulevard, Pasadena, CA, 91125, USA

[*]**Correspondence:** wlwu@ncku.edu.tw (W.-L.W.)

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Supplementary information 1

Supplementary information: Statistical tests and exact *P* values for each figure.

Supplementary Video 1: Social interaction test of CNO-injected *Crh^{PVN}* expressing hM3Dq. AAV-hSyn-DIO-hM3Dq-mCherry (hM3Dq) viruses were injected into the PVN of *Crh-ires-Cre* mice to deliver hM3Dq. Shortly after CNO injection, the mouse exhibited the reduction of social behavior toward novel mouse and the increase of non-social behavior.

Supplementary Video 2: Social interaction test of saline-injected *Crh^{PVN}* expressing hM3Dq. AAV-hSyn-DIO-hM3Dq-mCherry (hM3Dq) viruses were injected into the PVN of *Crh-ires-Cre* mice to deliver hM3Dq. The mouse exhibited normal social behavior toward novel mouse after saline (vehicle) injection (same mouse with Supplementary Video 1).

Supplementary information 1

Vasopressin and oxytocin in the PVN have been implicated in social behavior[1]. While SPF and GF mice contain similar numbers of vasopressin expressing neurons, GF mice have fewer oxytocin-expressing neurons in the PVN (Extended Data Fig. 5a-d). Moreover, levels of gene expression for oxytocin, vasopressin, and their receptors in the hypothalamus were similar regardless of microbiome status (Extended Data Fig. 5e), suggesting gut bacterial effects on social behavior are likely not through vasopressin while an oxytocin-dependent pathway cannot be completely ruled out[1].

We labeled neurons in the PVN to visualize the architecture of this brain region linked to the HPA axis. There were no overt changes in the numbers of neurons in the PVN and their projections to the median eminence (ME) in GF mice (Extended Data Fig. 5f-i). Further, we observed no changes in neuronal counts in the PVN following ABX treatment in a reporter mouse (*Crh-ires-Cre;Ai14D*) that enables visualization of corticotrophin-releasing hormone (CRH)-expressing neuron, critical in the stress response of mice (Extended Data Fig. 5j, k). Thus, increased corticosterone levels in GF and ABX mice following social interaction are not due to compensatory effects of PVN neuronal density.

1 Donaldson, Z. R. & Young, L. J. Oxytocin, vasopressin, and the neurogenetics of sociality. *Science* **322**, 900-904 (2008).

Supplementary information: Statistical tests and exact *P* values for each figure.

Fig. 1

1b. Social activity statistics: two-tailed unpaired *t*-test
t=7.893 df=37, $P<0.0001$****

1c. Social activity statistics: two-tailed unpaired *t*-test
t=6.917 df=14, $P<0.0001$****

1d. Social activity statistics: two-tailed unpaired *t*-test
t=4.777 df=50, $P<0.0001$****

1f. c-Fos staining statistics: two-tailed unpaired *t*-test
PVN: t=2.724 df=10, $P=0.0214$*

1g. c-Fos staining statistics: two-tailed unpaired *t*-test
adBNST: t=2.949 df=10, $P=0.0146$*

1h. c-Fos staining statistics: two-tailed unpaired *t*-test
DG: t=3.112 df=10, $P=0.0110$*

1j. c-Fos staining statistics: two-tailed unpaired *t*-test
PVN: t=2.466 df=8, $P=0.0390$*

1k. c-Fos staining statistics: two-tailed unpaired *t*-test
adBNST: t=3.972 df=8, $P=0.0041$**

1l. c-Fos staining statistics: two-tailed unpaired *t*-test
DG: t=2.718 df=8, $P=0.0263$*

1m. Corticosterone statistics: two-tailed unpaired *t*-test
t=3.558 df=29, $P=0.0013$**

1n. Corticosterone statistics: two-tailed unpaired *t*-test
t=2.557 df=14, $P=0.0228$*

1o. Corticosterone statistics: two-tailed unpaired *t*-test
t=3.526 df=58, $P=0.0008$***

Fig. 2

2b. Corticosterone statistics: two-way ANOVA with Bonferroni multiple comparison *post-hoc* test
SPF vs. GF: F (1, 17)=29.46, *P*<0.0001****
CMC vs. MET: F (1, 17)=114.4, *P*<0.0001****
Interaction: F (1, 17)=4.551, *P*=0.0478*

CMC vs. MET
SPF, *P*<0.0001****
GF, *P*<0.0001****

SPF vs. GF
CMC, *P*=0.0001***
MET, *P*=0.0584

2c. Social activity statistics: two-way ANOVA with Bonferroni multiple comparison *post-hoc* test
SPF vs. GF: F (1, 17)=5.625, *P*=0.0298*
CMC vs. MET: F (1, 17)=4.699, *P*=0.0447*
Interaction: F (1, 17)=3.913, *P*=0.0643

CMC vs. MET
SPF: *P*>0.9999
GF: *P*=0.0212*

SPF vs. GF
CMC: *P*=0.0157*
MET: *P*>0.9999

2d. Corticosterone statistics: one-way ANOVA with Bonferroni multiple comparison *post-hoc* test
Main effect: F (2, 37)=21.1, *P*<0.0001****

Control-Sham vs. ABX-Sham: *P*=0.0001***
Control -Sham vs. ABX-ADX: *P*=0.7113
ABX-Sham vs. ABX-ADX: *P*<0.0001****

2e. Social activity statistics: two-way repeated measures ANOVA with mixed effects with Bonferroni multiple comparison *post-hoc* test
Control-Sham vs. ABX-Sham vs. ABX-ADX: F (2, 37)=9.105, *P*=0.0006***
Drugs: F (2.253, 79.61)=9.104, *P*=0.0002***
Interaction: F (6, 106)=6.785, *P*<0.0001****

1st CMC
Control-Sham vs. ABX-Sham: *P*=0.0032**
Control -Sham vs. ABX-ADX: *P*>0.9999
ABX-Sham vs. ABX-ADX: *P*=0.001**

RU-486

Control-Sham vs. ABX-Sham: *P*=0.823
Control -Sham vs. ABX-ADX: *P*=0.0136*
ABX-Sham vs. ABX-ADX: *P*=0.0814

Metyrapone
Control-Sham vs. ABX-Sham: *P*=0.0242*
Control -Sham vs. ABX-ADX: *P*=0.0808
ABX-Sham vs. ABX-ADX: *P*>0.9999

2nd CMC
Control-Sham vs. ABX-Sham: *P*<0.0001****
Control -Sham vs. ABX-ADX: *P*=0.0917
ABX-Sham vs. ABX-ADX: *P*=0.0001***

Control-Sham
1st CMC vs. RU-486: *P*=0.175
1st CMC vs. Metyrapone: *P*=0.402
1st CMC vs. 2nd CMC: *P*=0.6066
RU-486 vs. Metyrapone: *P*>0.9999
RU-486 vs. 2nd CMC: *P*>0.9999
Metyrapone vs. 2nd CMC: *P*>0.9999

ABX-Sham
1st CMC vs. RU-486: *P*=0.0012**
1st CMC vs. Metyrapone: *P*<0.0001****
1st CMC vs. 2nd CMC: *P*=0.5602
RU-486 vs. Metyrapone: *P*=0.0083**
RU-486 vs. 2nd CMC: *P*=0.0016**
Metyrapone vs. 2nd CMC: *P*=0.0029**

ABX-ADX
1st CMC vs. RU-486: *P*=0.2671
1st CMC vs. Metyrapone: *P*>0.9999
1st CMC vs. 2nd CMC: *P*>0.9999
RU-486 vs. Metyrapone: *P*>0.9999
RU-486 vs. 2nd CMC: *P*=0.3438
Metyrapone vs. 2nd CMC: *P*>0.9999

2f. Social activity statistics: one-way ANOVA with Bonferroni multiple comparison *post-hoc* test
Main effect: F (2, 19)=9.649, *P*=0.0013**

Control-Sham vs. ABX-Sham: *P*=0.0050**
Control -Sham vs. ABX-SDV: *P*=0.0025**
ABX-Sham vs. ABX-SDV: *P*>0.9999

2h. Social activity statistics: two-way repeated measures ANOVA with Bonferroni multiple comparison *post-hoc* test
Subject: F (12, 24) = 2.112, *P*=0.0575
Control vs. *Nr3c1*$^{\Delta DG}$: F (1, 12) = 4.677, *P*=0.0515

Drugs: F (1.654, 19.85) = 10.94, *P*=0.0011**
Interaction: F (2, 24) = 4.281, *P*=0.0257*

Control
1st CMC vs. RU486: *P*=0.0139*
1st CMC vs. 2nd CMC: *P*=0.1229
RU486 vs. 2nd CMC: *P*=0.0090**

Nr3c1$^{\Delta DG}$
1st CMC vs. RU486: *P*>0.9999
1st CMC vs. 2nd CMC: *P*=0.5722
RU486 vs. 2nd CMC: *P*=0.9103

Control vs. *Nr3c1*$^{\Delta DG}$
1st CMC: *P*=0.0063**
RU486: *P*>0.9999
2nd CMC: *P*=0.1650

2i. Corticosterone statistics: two-tailed unpaired *t*-test
t=2.982 df=12, *P*=0.0114*

2j. Social activity statistics: two-way repeated measures ANOVA with Bonferroni multiple comparison *post-hoc* test
Subject: F (12, 24) = 4.700, *P*=0.0006***
Control vs. *Nr3c1*$^{\Delta BNST}$: F (1, 12) = 9.394, *P*=0.0098**
Drugs: F (1.895, 22.74) = 9.236, *P*=0.0013**
Interaction: F (2, 24) = 7.213, *P*=0.0035**

Control
1st CMC vs. RU486: *P*=0.0039**
1st CMC vs. 2nd CMC: *P*=0.1223
RU486 vs. 2nd CMC: *P*=0.0005***

Nr3c1$^{\Delta BNST}$
1st CMC vs. RU486: *P*>0.9999
1st CMC vs. 2nd CMC: *P*>0.9999
RU486 vs. 2nd CMC: *P*>0.9999

Control vs. *Nr3c1*$^{\Delta BNST}$
1st CMC: *P*=0.0648
RU486: *P*>0.9999
2nd CMC: *P*=0.0012**

2k. Corticosterone statistics: two-tailed unpaired *t*-test
t=2.168 df=12, *P*=0.0510

2l. Social activity statistics: two-way repeated measures ANOVA with mixed effects with Bonferroni multiple comparison *post-hoc* test
Control vs. *Nr3c1*$^{\Delta HYPO}$: F (1, 29) = 70.36, *P*<0.0001****
Drugs: F (1.495, 21.67) = 5.905, *P*=0.0143*

Interaction: F (2, 29) = 4.033, P=0.0285*

Control
1st CMC vs. RU486: P=0.0170*
1st CMC vs. 2nd CMC: P>0.9999
RU486 vs. 2nd CMC: P=0.0627

Nr3c1$^{\Delta HYPO}$
1st CMC vs. RU486: P>0.9999
1st CMC vs. 2nd CMC: P=0.1690
RU486 vs. 2nd CMC: P=0.3629

Control vs. *Nr3c1*$^{\Delta HYPO}$
1st CMC: P=0.0012**
RU486: P<0.0001****
2nd CMC: P=0.0486*

2m. Corticosterone statistics: two-tailed unpaired *t*-test
t=5.607 df=9, P=0.0003***

Fig. 3

3a. Hypothalamus *Crh* gene expression statistics: two-way ANOVA with Bonferroni multiple comparison *post-hoc* test
VEH vs. ABX: F (1, 19)=8.675, *P*=0.0083**
Social stimulation vs. No social stimulation: F (1, 19)=13.67, *P*=0.0015**
Interaction: F (1, 19)=4.744, *P*=0.0422*

VEH vs. ABX:
No social stimulation, *P*>0.9999
Social stimulation, *P*=0.0044**

Social stimulation vs. No social stimulation:
VEH, *P*=0.614
ABX, *P*=0.0009***

c. Social activity statistics: two-way repeated measures ANOVA with Bonferroni multiple comparison *post-hoc* test
Subject: F (19, 38) = 1.070, *P*=0.4151
mCherry vs. hM4Di: F (1, 19) = 4.839, *P*=0.0404*
Saline vs. CNO: F (1.654, 31.43) = 2.866, *P*=0.0808
Interaction: F (2, 38) = 9.463, *P*=0.0005***

mCherry vs. hM4Di
1st VEH: *P*>0.9999
CNO: *P*=0.0075**
2nd VEH: *P*>0.9999

ABX mCherry
1st VEH vs. CNO: *P*=0.476
1st VEH vs. 2nd VEH: *P*=0.3592
CNO vs. 2nd VEH: *P*=0.0254*

ABX hM4Di
1st VEH vs. CNO: *P*=0.0246*
1st VEH vs. 2nd VEH: *P*=0.3455
CNO vs. 2nd VEH: *P*=0.174

3d. Corticosterone statistics: two-tailed paired *t*-test
mCherry: t=0.1883 df=8, *P*=0.8553
hM4Di: t=2.525 df=9, *P*=0.0325*

3g. c-Fos staining statistics: two-tailed unpaired *t*-test
PVN: t=2.274 df=19, *P*=0.0347*

3i, Corticosterone statistics: two-tailed paired *t*-test
SPF mCherry: t=1.012 df=9, *P*=0.3379
SPF hM3Dq: t=5.015 df=10, *P*=0.0005***

3j. Social activity statistics: two-way repeated measures ANOVA with Bonferroni multiple comparison *post-hoc* test
Subject: F (19, 38) = 4.145, P<0.0001****
mCherry vs. hM3Dq: F (1, 19) = 13.66, P=0.0015**
Saline vs. CNO: F (1.790, 34.00) = 10.82, P=0.0004***
Interaction: F (2, 38) = 26.42, P<0.0001****

mCherry vs. hM3Dq
1st VEH: P=0.7603
CNO: P=0.0001***
2nd VEH: P>0.9999

SPF mCherry
1st VEH vs. CNO: P=0.0827
1st VEH vs. 2nd VEH: P=0.8448
CNO vs. 2nd VEH: P=0.7816

SPF hM3Dq
1st VEH vs. CNO: P=0.0005***
1st VEH vs. 2nd VEH: P=0.366
CNO vs. 2nd VEH: P<0.0001****

3l. CRH (high) infusion social activity statistics: two-tailed paired *t*-test
t=8.175 df=8, P<0.0001****

3m. CRH (low) infusion social activity statistics: two-tailed paired *t*-test
t=3.953 df=6, P=0.0075**

3o. DG glucocorticoid signaling agonism social activity statistics: one-way ANOVA repeated measures with Bonferroni multiple comparison *post-hoc* test
Treatment effect: F (1.532, 9.194) = 70.73, P<0.0001****

VEH vs. CORT: P=0.0002***
VEH vs. DEX: P=0.0002***

3p. BNST glucocorticoid signaling agonism social activity statistics: one-way ANOVA repeated measures with mixed effects with Bonferroni multiple comparison *post-hoc* test
Treatment effect: F (0.5980, 3.588) = 16.57, P=0.0221*

VEH vs. CORT: P=0.0072**
VEH vs. DEX: P=0.0076**

Fig. 4

4b. Social activity statistics: one-way ANOVA with Bonferroni multiple comparison *post-hoc* test
Main effect: F (4, 35)=10.07, *P*<0.0001****

AVNM vs. VNM: *P*>0.9999
AVNM vs. ANM: *P*>0.9999
AVNM vs. AVM: *P*<0.0001****
AVNM vs. AVN: *P*>0.9999

4c. Corticosterone statistics: two-tailed unpaired *t*-test
AVNM vs. AVM: t=2.162 df=14, *P*=0.0484*

4d. Social activity statistics: two-tailed unpaired *t*-test
t=4.988 df=24, *P*<0.0001****

4e. Corticosterone statistics: two-tailed unpaired *t*-test
t=2.482 df=24, *P*=0.0205*

4i. Social activity statistics: two-way repeated measures ANOVA with Bonferroni multiple comparison *post-hoc* test
Subject: F (20, 20) = 2.795, *P*=0.0131*
VEH+Ctrl vs. ABX+Ctrl vs. ABX+E.f.: F (2, 20) = 3.526, *P*=0.0488*
1st trial vs. 2nd trial: F (1, 20) = 5.237, *P*=0.0331
Interaction: F (2, 20) = 4.315, *P*=0.0277*

1st vs. 2nd
VEH+Ctrl: *P*>0.9999
ABX+Ctrl: *P*>0.9999
ABX+E.f.: *P*=0.0056**

1st trial
VEH+Ctrl vs. ABX+Ctrl: *P*=0.2886
VEH+Ctrl vs. ABX+E.f.: *P*=0.832
ABX+Ctrl vs. ABX+E.f.: *P*>0.999

2nd trial
VEH+Ctrl vs. ABX+Ctrl: *P*=0.1004
VEH+Ctrl vs. ABX+E.f.: *P*=0.6402
ABX+Ctrl vs. ABX+E.f.: *P*=0.0047**

4j. Corticosterone statistics: one-way ANOVA with Bonferroni multiple comparison *post-hoc* test
Main effect: F (2, 19)=13.21, *P*=0.0003***

VEH+Ctrl vs. ABX+Ctrl: *P*=0.0015**
VEH vs. ABX+E.f.: *P*>0.9999
ABX+Ctrl vs. ABX+E.f.: *P*=0.0006***

Extended data Fig. 1

1a. Detailed social activity statistics: two-tailed unpaired *t*-test
Anogenital sniff: t=5.552 df=37, *P*<0.0001****
Nose-nose sniff: t=4.773 df=37, *P*<0.0001****
Active approach: t=3.978 df=37, *P*=0.0003***
Push-Crawl: t=2.947 df=37, *P*=0.0055**

1b. Detailed social activity statistics: two-tailed unpaired *t*-test
Anogenital sniff: t=6.452 df=14, *P*<0.0001****
Nose-nose sniff: t=3.585 df=14, *P*=0.0030**
Active approach: t=3.714 df=14, *P*=0.0023**
Push-Crawl: t=1.158 df=14, *P*=0.2662

1c. Social activity statistics: two-way ANOVA with Bonferroni multiple comparison *post-hoc* test
Subject microbiota effect: F (1, 51) = 72.70, *P*<0.0001****
Novel mouse microbiota effect: F (1, 51) = 2.596, *P*=0.1133
Interaction: F (1, 51) = 0.7374, *P*=0.3945

SPF novel mouse: *P*<0.0001****
GF novel mouse: *P*<0.0001****

1d. Detailed social activity statistics: two-tailed unpaired *t*-test
Anogenital sniff: t=3.703 df=50, *P*=0.0005***
Nose-nose sniff: t=3.144 df=50, *P*=0.0028**
Active approach: t=3.792 df=50, *P*=0.0004***
Push-Crawl: t=0.7444 df=50, *P*=0.4601

1e. Social activity statistics: two-tailed unpaired *t*-test
t=0.3865 df=17, *P*=0.7039

1f. Non-social activity statistics: two-tailed unpaired *t*-test
SPF vs.GF subjects (SPF novel mouse): t=0.244 df=37, *P*=0.8086
SPF vs.GF subjects (GF novel mouse): t=1.653 df=14, *P*=0.1205
VEH vs. ABX subjects (SPF novel mouse): t=0.6725 df=50, *P*=0.5044

1g. Non-social activity statistics: two-tailed unpaired *t*-test
Digging: t=0.1678 df=16, *P*=0.8689
Grooming: t=0.7198 df=16, *P*=0.4820
Rearing: t=0.7558 df=16, *P*=0.4608
Total: t=1.422 df=16, *P*=0.1743

1h left. Social activity statistics: one-way repeated measures ANOVA
Main effect: F (1.722, 18.94) = 0.6116, *P*=0.5294

1h middle. Nose-to-nose sniff statistics: one-way repeated measures ANOVA
Main effect: F (1.941, 21.35) = 1.876, *P*=0.1784

1h right. Nose-to-tail sniff statistics: one-way repeated measures ANOVA

Main effect: F (1.675, 18.42) = 0.7903, *P*=0.4477

1i. Social activity statistics: one-way ANOVA
Main effect: F (2, 13) = 0.6098, *P*=0.5583

1j. Distance traveled statistics: one-way ANOVA
Main effect: F (2, 13) = 1.073, *P*=0.3705

1k. Female social activity statistics: two-tailed unpaired *t*-test
SPF vs. GF: t=3.193 df=17, *P*=0.0053**
VEH vs. ABX: t=2.927 df=8, *P*=0.0191*

1l. Social activity statistics: two-way ANOVA with Bonferroni multiple comparison *post-hoc* test
SPF vs. GF: F (1, 40) = 96.47, *P*<0.0001****
Hours post-isolation: F (4, 40) = 0.5102, *P*=0.7285
Interaction: F (4, 40) = 0.6018, *P*=0.6635

SPF vs. GF
4-5 hr: *P*=0.0024**
5-6 hr: *P*<0.0001****
6-7 hr: *P*<0.0001****
7-8 hr: *P*=0.0028**
8-9 hr: *P* =0.0003***

1m. Social activity statistics: one-way ANOVA
Main effect: F (2, 16) = 1.031, *P*=0.379

1n. 3-chamber social test: Distance traveled**:** two-tailed unpaired *t*-test
SPF vs. GF: t=3.249 df=17, *P*=0.0047**

1o. 3-chamber social test: Chamber duration: one-tailed paired *t*-test
SPF: Mouse vs. Object: t=7.706 df=8, *P*<0.0001****
GF: Mouse vs. Object: t=4.787 df=9, *P*=0.0005***

1o. 3-chamber social test: Chamber entries: one-tailed paired *t*-test
SPF: Mouse vs. Object: t=1.148 df=8, *P*=0.1420
GF: Mouse vs. Object: t=1.475 df=9, *P*=0.0872

1p. 3-chamber social test: Distance traveled: two-tailed unpaired *t*-test
VEH vs. ABX: t=1.611 df=18, *P*=0.1246

1q. 3-chamber social test: Chamber duration: one-tailed paired *t*-test
VEH: Mouse vs. Object: t=1.247 df=9, *P*=0.1220
ABX: Mouse vs. Object: t=3.110 df=9, *P*=0.0063**

1q. 3-chamber social test: Chamber entries: one-tailed paired *t*-test
VEH: Mouse vs. Object: t=0.8102 df=9, *P*=0.2194
ABX: Mouse vs. Object: t=4.477 df=9, *P*=0.0008***

1s. c-Fos staining statistics: two-tailed unpaired *t*-test
SPF vs. GF: t=1.804, df=10, *P*=0.1014
VEH vs. ABX: t=1.800, df=8, *P*=0.1095

1t. c-Fos staining statistics: two-tailed unpaired *t*-test
PVN: t=0.8376 df=9, *P*=0.4239
adBNST: t=1.21 df=9, *P*=0.2571
DG: t=1.958 df=9, *P*=0.0818
BLA: t=1.215 df=9, *P*=0.2552

1u. Corticosterone statistics: two-way repeated measures ANOVA with Bonferroni multiple comparison *post-hoc* test
Subject: F (8, 16) = 1.739, *P*=0.1648
VEH vs. ABX: F (1, 8) = 10.42, *P*=0.0121*
Time: F (1.724, 13.79) = 129.2, *P*<0.0001****
Interaction: F (2, 16) = 2.006, *P*=0.1670

VEH vs. ABX
Iso: *P*=0.5775
0 hr, *P*=0.0916
1 hr, *P*=0.1751

Time: VEH
Iso vs. 0 hr: *P*=0.0129*
Iso vs. 1 hr: *P*=0.0027**
0 hr vs. 1 hr: *P*=0.0023**

Time: ABX
Iso vs. 0 hr: *P*=0.0624
Iso vs. 1 hr: *P*=0.0016**
0 hr vs. 1 hr: *P*=0.0062**

1v. Corticosterone statistics: two-way ANOVA with Bonferroni multiple comparison *post-hoc* test
ABX vs. GF: F (1, 27) = 6.006, *P*=0.0210*
Intact microbiota vs. No microbiota: F (1, 27) = 8.229, *P*=0.0079**
Interaction: F (1, 27) = 4.570, *P*=0.0417*

ABX vs. GF
Intact microbiota: *P*>0.9999
No microbiota: *P*=0.0085**

Intact microbiota vs. No microbiota
ABX: *P*>0.9999
GF: *P*=0.0125*

1w. Corticosterone statistics: two-way ANOVA
SPF vs. GF: F (1, 34) = 14.65, *P*=0.0005***
Hours post-isolation: F (4, 34) = 0.6051, *P*=0.6616
Interaction: F (4, 34) = 0.009598, *P*=0.9998

1x. Corticosterone statistics: two-way ANOVA
VEH vs. ABX: F (1, 48) = 12.07, *P*=0.0011**
Hours post-isolation: F (1, 48) = 1.873, *P*=0.1775
Interaction: F (1, 48) = 0.1878, *P*=0.6667

1y. Social activity statistics: one-way ANOVA with Bonferroni multiple comparison *post-hoc* test
Main effect: F (2, 44) = 49.73, *P*<0.0001****

SPF vs. GF: *P*<0.0001****
SPF vs. exGF: *P*=0.0001***
GF vs. exGF: *P*<0.0001****

1z. Corticosterone statistics: one-way ANOVA with Bonferroni multiple comparison *post-hoc* test
Main effect: F (2, 36) = 8.334, *P*=0.0011**

SPF vs. GF: *P*=0.0012**
SPF vs. exGF: *P*>0.9999
GF vs. exGF: *P*=0.0467*

Extended data Fig. 2

2a. Bacterial DNA statistics: two-tailed unpaired *t*-test
SPF vs. GF: t=4.583 df=6, *P*=0.0038**
VEH vs. ABX: t=3.863 df=6, *P*=0.0083**

2b. Bacterial DNA statistics: two-way repeated measures ANOVA with Bonferroni multiple comparison *post-hoc* test
Subject: F (6, 12) = 0.5051, *P*=0.7933
VEH vs. ABX: F (1, 6) = 3452, *P*<0.0001****
Time: F (1.029, 6.177) = 3.720, *P*=0.1002
Interaction: F (2, 12) = 3.72, *P*=0.0553

VEH vs. ABX
1w: VEH vs. ABX: *P*=0.001**
2w: VEH vs. ABX: *P*=0.0001***
3w: VEH vs. ABX: *P*<0.0001****

2c. Bacterial DNA statistics: two-way ANOVA with Bonferroni multiple comparison *post-hoc* test
Subject: F (6, 12) = 1.672, *P*=0.2112
VEH vs. ABX: F (1, 6) = 106.3, *P*<0.0001****
Time: F (1.630, 9.779) = 35.21, *P*<0.0001****
Interaction: F (2, 12) = 18.71, *P*=0.0002

VEH vs. ABX
1w: VEH vs. ABX: *P*=0.2237
2w: VEH vs. ABX: *P*=0.0179*
3w: VEH vs. ABX: *P*<0.0001****

Time_Vehicle
1w vs. 2w: *P*=0.6526
1w vs. 3w: *P*=0.002**
2w vs. 3w: *P*=0.0636

Time_ABX
1w vs. 2w: *P*=0.0398*
1w vs. 3w: *P*=0.0078**
2w vs. 3w: *P*>0.9999

2g. Observed species: Kruskal-Wallis
AVNM vs Negative control, H=0.16819572, *P*=0.68172033, q=0.68172033
AVNM vs Vehicle, H=10.9600516, *P*=0.00093097***, q=0.00279292
Negative control vs Vehicle, H=4.61472603, *P*=0.03169856*, q=0.04754784

2h. Faith's phylogenetic diversity: Kruskal-Wallis
AVNM vs Negative control, H=0.375, *P*=0.54029138, q=0.54029138
AVNM vs Vehicle, H=10.5992647, *P*=0.00113133**, q=0.00339398
Negative control vs Vehicle, H=6, *P*=0.01430588*, q=0.02145882

2i. Unweighted UniFrac: PERMANOVA
AVNM vs Negative control, Pseudo-F=1.19256034, *P*=0.2637, q=0.2637
AVNM vs Vehicle, Pseudo-F=11.5191588, *P*=0.0002***, q=0.0006
Negative control vs Vehicle, Pseudo-F=11.2825915, *P*=0.0071 **, q=0.01065

2j. Weighted UniFrac: PERMANOVA
AVNM vs Negative control, Pseudo-F=1.01514874, *P*=0.3274, q=0.3274
AVNM vs Vehicle, Pseudo-F=15.3627766, *P*=0.0005***, q=0.0015
Negative control vs Vehicle, Pseudo-F=18.4648706, *P*=0.0055 **, q=0.00825

Extended data Fig. 3

3a. Open-field (distance moved in 15 min) statistics: two-tailed unpaired *t*-test
t=0.2091 df=15, P=0.8371

3b. Open-field (center time in 15 min) statistics: two-tailed unpaired *t*-test
t=2.907 df=15, P=0.0108*

3c. Light-Dark box (light time in 10 min) statistics: two-tailed unpaired *t*-test
t=2.184 df=16, P=0.0442*

3d. Elevated plus maze (open arm time in 5 min) statistics: two-tailed unpaired *t*-test
t=2.451 df=16, P=0.0261*

3e. Open-field (distance moved in 15 min) statistics: two-tailed unpaired *t*-test
t=0.9382 df=18, P=0.3606

3f. Open-field (center time in 15 min) statistics: two-tailed unpaired *t*-test
t=0.9860 df=18, P=0.3372

3g. Light-Dark box (light time in 10 min) statistics: two-tailed unpaired *t*-test
t=0.07870 df=18, P=0.9381

3h. Elevated plus maze (open arm time in 5 min) statistics: two-tailed unpaired *t*-test
t=0.1605 df=18, P=0.8743

3i. open-field (distance moved in 60 min) statistics: two-way repeated measures ANOVA
Subject: F (28, 140) = 2.010, P=0.0044**
VEH vs. ABX: F (1, 28) = 0.2481, P=0.6223
Time: F (1.234, 34.55) = 3.521, P=0.0609
Interaction: F (5, 140) = 0.3179, P=0.9015

3j. water consumption statistics: two-way repeated measures ANOVA
Subject: F (8, 32) = 10.49, P<0.0001****
SPF vs. GF: F (1, 8) = 0.1007, P=0.7591
Time: F (1.915, 15.32) = 634.1, P<0.0001****
Interaction: F (4, 32) = 0.9299, P=0.4590

3k. olfactory habituation/dishabituation statistics: two-way repeated measures ANOVA with Bonferroni multiple comparison *post-hoc* test
Subject: F (19, 266) = 3.863, P<0.0001****
SPF vs. GF: F (1, 19) = 13.13, P=0.0018**
Odors: F (4.431, 84.18) = 36.67, P<0.0001****
Interaction: F (14, 266) = 5.652, P<0.0001****

SPF vs. GF
Water 1: P>0.9999
Water 2: P>0.9999
Water 3: P>0.9999
Almond 1: P>0.9999

Almond 2: *P*=0.3966
Almond 3: *P*>0.9999
Banana 1: *P*=0.5646
Banana 2: *P*>0.9999
Banana 3: *P*>0.9999
B6 Cage 1-1: *P*=0.0172*
B6 Cage 1-2: *P*=0.0402*
B6 Cage 1-3: *P*=0.2028
BTBR Cage 1-1: *P*>0.9999
BTBR Cage 1-2: *P*>0.9999
BTBR Cage 1-3: *P*=0.8881

3l. olfactory habituation/dishabituation (soiled cage) statistics: two-way repeated measures ANOVA with Bonferroni multiple comparison *post-hoc* test
Subject: F (19, 95) = 3.975, *P*<0.0001****
SPF vs. GF: F (1, 19) = 0.4843, *P*=0.4949
Odors: F (4.010, 76.19) = 34.26, *P*<0.0001****
Interaction: F (5, 95) = 5.166, *P*=0.0003***

SPF
B6 Cage 1-1 vs. B6 Cage 1-2: *P*=0.0020**
B6 Cage 1-1 vs. B6 Cage 1-3: *P*=0.0002***
B6 Cage 1-1 vs. BTBR Cage 1-1: *P*>0.9999
BTBR Cage 1-1 vs. BTBR Cage 1-2: *P*=0.0002***
BTBR Cage 1-1 vs. BTBR Cage 1-3: *P*<0.0001****

GF
B6 Cage 1-1 vs. B6 Cage 1-2: *P*=0.0120*
B6 Cage 1-1 vs. B6 Cage 1-3: *P*=0.0397*
B6 Cage 1-1 vs. BTBR Cage 1-1: *P*=0.0143*
BTBR Cage 1-1 vs. BTBR Cage 1-2: *P*<0.0001****
BTBR Cage 1-1 vs. BTBR Cage 1-3: *P*<0.0001****

SPF vs. GF
B6 Cage 1-1: *P*>0.9999
B6 Cage 1-2: *P*=0.2822
B6 Cage 1-3: *P*=0.2699
BTBR Cage 1-1: *P*=0.3781
BTBR Cage 1-2: *P*>0.9999
BTBR Cage 1-3: *P*>0.9999

Extended data Fig. 4

4a. qPCR statistics: two-tailed unpaired *t*-test
Arc: t=2.658 df=10*,* *P*=0.0240*
Fos: t=3.785 df=10, *P*=0.0036**
cJun: t=2.922 df=10, *P*=0.0152*
JunB: t=3.32 df=10, *P*=0.0077**
Egr-1: t=2.192 df=10, *P*=0.0532
Egr-2: t=2.475 df=10, *P*=0.0328*
Gadd45b: t=5.072 df=10, *P*=0.0005***
Gadd45g: t=2.309 df=10, *P*=0.0435*
Bdnf: t=2.966 df=10, *P*=0.0142*
Map2: t=0.9036 df=10, *P*=0.3874

4b. qPCR statistics: two-tailed unpaired *t*-test
Arc: t=3.126 df=10, *P*=0.0108*
Fos: t=5.916 df=10, *P*=0.0001***
cJun: t=0.9066 df=10, *P*=0.3859
JunB: t=1.777 df=10, *P*=0.1060
Egr-1: t=4.096 df=10, *P*=0.0022**
Egr-2: t=1.901 df=10, *P*=0.0864
Gadd45b: t=0.1883 df=10, *P*=0.8544
Gadd45g: t=0.8045 df=10, *P*=0.4398
Bdnf: t=1.552 df=10, *P*=0.1517
Map2: t=0.2263 df=10, *P*=0.8255

4c. qPCR statistics: two-tailed unpaired *t*-test
Arc: t=0.7908 df=4, *P*=0.4733
Fos: t=0.8264 df=4, *P*=0.4550
cJun: t=0.544 df=4, *P*=0.6153
JunB: t=0.544 df=4, *P*=0.6154
Egr-1: t=0.9188 df=4, *P*=0.4102
Egr-2: t=1.239 df=4, *P*=0.2830
Gadd45b: t=0.8896 df=4, *P*=0.4240
Gadd45g: t=2.276 df=4, *P*=0.0852
Bdnf: t=0.02943 df=4, *P*=0.9779
Map2: t=0.937 df=4, *P*=0.4018

4d. qPCR statistics: two-tailed unpaired *t*-test
Arc: t=0.2208 df=10, *P*=0.8297
Fos: t=0.6654 df=10,*P*=0.5208
cJun: t=2.254 df=10, *P*=0.0479*
JunB: t=2.649 df=10, *P*=0.0244*
Egr-1: t=2.225 df=10, *P*=0.0502
Egr-2: t=0.6359 df=10, *P*=0.5391
Gadd45b: t=4.087 df=10, *P*=0.0022**
Gadd45g: t=3.34 df=10, *P*=0.0075**
Bdnf: t=3.028 df=10, *P*=0.0127*
Map2: t=1.667 df=10, *P*=0.1266

4f. qPCR statistics: two-tailed unpaired *t*-test
Mrg1b: t=17.68 df=46, *P*<0.0001****
Dsp: t=19.93 df=46, *P*<0.0001****
Tdo2: t=20.25 df=46, *P*<0.0001****

4g. qPCR statistics: two-tailed unpaired *t*-test
Nr3c1: t=1.803 df=10, *P*=0.1016
Nr3c2: t=0.1357 df=10, *P*=0.8947

4h. qPCR statistics: two-tailed unpaired *t*-test
Nr3c1: t=1.612 df=10, *P*=0.1379
Nr3c2: t=0.03036 df=10, *P*=0.9764

4i. qPCR statistics: two-tailed unpaired *t*-test
Crh1: t=0.8771 df=9, *P*=0.4032
Crh2: t=0.4293 df=9, *P*=0.6778
Ucn: t=1.236 df=9, *P*=0.2477
Ucn2: t=2.114 df=9, *P*=0.0636
Ucn3: t=1.975 df=9, *P*=0.0797
Avp: t=0.01786 df=9, *P*=0.9861
Oxt: t=1.598 df=9, *P*=0.1445

4j. qPCR statistics: two-tailed unpaired *t*-test
Nr3c1: t=0.5107 df=10, *P*=0.6206
Nr3c2: t=0.6873 df=10, *P*=0.5075

4k. qPCR statistics: two-tailed unpaired *t*-test
Nr3c1: t=0.7211 df=10, *P*=0.4873
Nr3c2: t=1.856 df=10, *P*=0.0931

4l. qPCR statistics: two-tailed unpaired *t*-test
Crh1: t=0.4691 df=10, *P*=0.6490
Crh2: t=0.9035 df=10, *P*=0.3875
Ucn: t=3.015 df=10, *P*=0.0130*
Ucn2: t=1.599 df=10, *P*=0.1409
Ucn3: t=0.6816 df=10, *P*=0.5110
Avp: t=0.5706 df=10, *P*=0.5809
Oxt: t=0.2315 df=10, *P*=0.8216

Extended data Fig. 5

5c. AVP staining statistics: two-tailed unpaired *t*-test
t=0.8979 df=12, *P*=0.3869

5d. OXT staining statistics: two-tailed unpaired *t*-test
t=3.442 df=12, *P*=0.0049**

5e. qPCR statistics: two-tailed unpaired *t*-test
Avp: t=0.9073 df=6, *P*=0.3992
Avpr1a: t=0.4982 df=6, *P*=0.6361
Avpr1b: t=0.6378 df=6, *P*=0.5471
Avpr2: t=1.38 df=6, *P*=0.2168
Oxt: t=0.09407 df=6, *P*=0.9281
Oxtr: t=1.071 df=6, *P*=0.3252

5g. Fluorogold statistics: two-way repeated measures ANOVA
Subject: F (6, 6) = 0.4886, *P*=0.7977
SPF vs. GF: F (1, 6) = 1.732, *P*=0.2362
Bregma: F (1, 6) = 0.01217, *P*=0.9158
Interaction: F (1, 6) = 0.05600, *P*=0.8208

5i. Fluorogold statistics: two-tailed unpaired *t*-test
t=1.004 df=4, *P*=0.3723

5k. Fluorogold statistics: two-tailed unpaired *t*-test
t=0.7995 df=6, *P*=0.4545

Extended data Fig. 6

6a. Social activity statistics: one-way ANOVA
Main effect: F (2, 13) = 0.5876, *P*=0.5697

6b. Non-social activity statistics: one-way ANOVA
Main effect: F (2, 13) = 0.4003, *P*=0.6781

6c. Non-social activity statistics: two-way ANOVA with Bonferroni multiple comparison *post-hoc* test
SPF vs. GF: F (1, 17) = 0.2421, *P*=0.6290
CMC vs. MET: F (1, 17) = 14.08, *P*=0.0016**
Interaction: F (1, 17) = 0.04764, *P*=0.8298

CMC vs. MET:
SPF *P*=0.041*
GF *P*=0.0274*

6d. Non-social activity statistics: two-way repeated measures ANOVA with mixed effects with Bonferroni multiple comparison *post-hoc* test
Control-Sham vs. ABX-Sham vs. ABX-ADX: F (2, 37) = 10.41, *P*=0.0003***
Drugs: F (2.352, 83.11) = 6.284, *P*=0.0017**
Interaction: F (6, 106) = 1.376, *P*=0.2309

1st CMC
Control-Sham vs. ABX-Sham: *P*=0.0034**
Control -Sham vs. ABX-ADX: *P*=0.181
ABX-Sham vs. ABX-ADX: *P*=0.4248

RU-486
Control-Sham vs. ABX-Sham: *P*=0.1261
Control -Sham vs. ABX-ADX: *P*=0.0215*
ABX-Sham vs. ABX-ADX: *P*>0.9999

Metyrapone
Control-Sham vs. ABX-Sham: *P*=0.0006***
Control -Sham vs. ABX-ADX: *P*=0.0145*
ABX-Sham vs. ABX-ADX: *P*>0.9999

2nd CMC
Control-Sham vs. ABX-Sham: *P*>0.9999
Control -Sham vs. ABX-ADX: *P*=0.5615
ABX-Sham vs. ABX-ADX: *P*>0.9999

Control-Sham
1st CMC vs. RU-486: *P*>0.9999
1st CMC vs. Metyrapone: *P*>0.9999
1st CMC vs. 2nd CMC: *P*>0.9999
RU-486 vs. Metyrapone: *P*>0.9999
RU-486 vs. 2nd CMC: *P*>0.9999

Metyrapone vs. 2^{nd} CMC: *P*>0.9999

ABX-Sham
1^{st} CMC vs. RU-486: *P*>0.9999
1^{st} CMC vs. Metyrapone: *P*=0.1455
1^{st} CMC vs. 2^{nd} CMC: *P*=0.3076
RU-486 vs. Metyrapone: *P*=0.1327
RU-486 vs. 2^{nd} CMC: *P*=0.5925
Metyrapone vs. 2^{nd} CMC: *P*=0.0614

ABX-ADX
1^{st} CMC vs. RU-486: *P*=0.5121
1^{st} CMC vs. Metyrapone: *P*=0.3494
1^{st} CMC vs. 2^{nd} CMC: *P*>0.9999
RU-486 vs. Metyrapone: *P*>0.9999
RU-486 vs. 2^{nd} CMC: *P*=0.2175
Metyrapone vs. 2^{nd} CMC: *P*=0.1059

6e. Food intake statistics: two-tailed unpaired *t*-test
SPF naive saline vs. SPF naive CCK-8: t=3.754, df=10, *P*=0.0038**
ABX sham CCK-8 vs. ABX SDV CCK-8: t=3.361, df=9, *P*=0.0084**

6f. Corticosterone statistics: two-tailed unpaired *t*-test
t=0.2007 df=15, *P*=0.8436

6g. c-Fos statistics: two-tailed unpaired *t*-test
adBNST: t=2.373 df=8, *P*=0.0451*
DG: t=1.219 df=8, *P*=0.2577

Extended data Fig. 7

7b. Non-social activity statistics: two-way repeated measures ANOVA with Bonferroni multiple comparison *post-hoc* test
Subject: $F(12, 24) = 1.141$, $P=0.3756$
Control vs. *Nr3c1*$^{\Delta DG}$: $F(1, 12) = 2.817$, $P=0.1191$
Drugs: $F(1.935, 23.22) = 5.473$, $P=0.0119$*
Interaction: $F(2, 24) = 0.02454$, $P=0.9758$

Control
1st CMC vs. RU486: $P>0.9999$
1st CMC vs. 2nd CMC: $P=0.2878$
RU486 vs. 2nd CMC: $P=0.1169$

Nr3c1$^{\Delta DG}$
1st CMC vs. RU486: $P>0.9999$
1st CMC vs. 2nd CMC: $P=0.4122$
RU486 vs. 2nd CMC: $P=0.35$

7c. c-Fos staining statistics: two-tailed unpaired *t*-test
PVN: $t=6.281$, $df=12$, $P<0.0001$****
adBNST: $t=3.877$, $df=12$, $P=0.0022$**
DG: $t=0.03657$, $df=12$, $P=0.9714$

7e. Non-social activity statistics: two-way repeated measures ANOVA with Bonferroni multiple comparison *post-hoc* test
Subject: $F(12, 24) = 3.881$, $P=0.0023$**
Control vs. *Nr3c1*$^{\Delta BNST}$: $F(1, 12) = 5.589$, $P=0.0358$*
Drugs: $F(1.307, 15.68) = 3.063$, $P=0.0915$
Interaction: $F(2, 24) = 1.812$, $P=0.1849$

Control vs. *Nr3c1*$^{\Delta BNST}$
1st CMC: $P=0.331$
RU486: $P=0.7474$
2nd CMC: $P=0.0363$*

7f. c-Fos staining statistics: two-tailed unpaired *t*-test
PVN: $t=7.937$, $df=12$, $P<0.0001$****
adBNST: $t=5.327$, $df=12$, $P=0.0002$***
DG: $t=0.8402$, $df=11$, $P=0.4187$

7h. Non-social activity statistics: two-way repeated measures ANOVA with mixed effect with Bonferroni multiple comparison *post-hoc* test
Control vs. *Nr3c1*$^{\Delta HYPO}$: $F(1, 10) = 4.288$, $P=0.0652$
Drugs: $F(1.711, 16.25) = 3.846$, $P=0.0485$*
Interaction: $F(2, 19) = 0.01390$, $P=0.9862$

Control
1st CMC vs. RU486: $P>0.9999$
1st CMC vs. 2nd CMC: $P=0.9523$

RU486 vs. 2^{nd} CMC: *P*=0.3902

Nr3c1$^{\Delta HYPO}$
1^{st} CMC vs. RU486: *P*>0.9999
1^{st} CMC vs. 2^{nd} CMC: *P*=0.2813
RU486 vs. 2^{nd} CMC: *P*=0.1175

7i. c-Fos staining statistics: two-tailed unpaired *t*-test
PVN: t=1.737, df=9, *P*=0.1163
adBNST: t=2.066, df=9, *P*=0.0688
DG: t=2.415, df=9, *P*=0.0389*

7k. Social activity statistics: two-tailed unpaired *t*-test
t=0.8269 df=8, *P*=0.4323

7l. Non-social activity statistics: two-tailed unpaired *t*-test
t=0.2306 df=8, *P*=0.8234

7m. Corticosterone statistics: two-tailed unpaired *t*-test
t=0.008454 df=8, *P*=0.9935

7n. Social activity statistics: two-tailed unpaired *t*-test
t=2.342 df=8, *P*=0.0473*

7o. Non-social activity statistics: two-tailed unpaired *t*-test
t=0.7509 df=8, *P*=0.4742

7p. Corticosterone statistics: two-tailed unpaired *t*-test
t=0.08905 df=8, *P*=0.9312

7q. Social activity statistics: two-tailed unpaired *t*-test
t=1.461 df=8, *P*=0.1823

7r. Non-social activity statistics: two-tailed unpaired *t*-test
t=1.288 df=8, *P*=0.2339

7s. Corticosterone statistics: two-tailed unpaired *t*-test
t=2.089 df=8, *P*=0.0701

Extended data Fig. 8

8b. Non-social activity statistics: two-way repeated measures ANOVA
Subject: F (19, 38) = 1.287, *P*=0.2477
mCherry vs. hM4Di: F (1, 19) = 0.4935, *P*=0.4909
Saline vs. CNO: F (1.831, 34.80) = 0.4194, *P*=0.6430
Interaction: F (2, 38) = 1.383, *P*=0.2631

8e. c-Fos staining statistics: two-tailed unpaired *t*-test
t=1.063 df=19, *P*=0.3010

8f. c-Fos staining statistics: two-tailed unpaired *t*-test
t=0.5712 df=19, *P*=0.5745

8h. Social activity statistics: two-way repeated measures ANOVA with Bonferroni multiple comparison *post-hoc* test
Subject: F (17, 34) = 2.207, *P*=0.0242*
mCherry vs. hM4Di: F (1, 17) = 0.5406, *P*=0.4722
Saline vs. CNO: F (1.740, 29.58) = 6.540, *P*=0.0060**
Interaction: F (2, 34) = 0.6268, *P*=0.5404

mCherry
1st Vehicle vs. CNO: *P*>0.9999
1st Vehicle vs. 2nd Vehicle: *P*=0.3215
CNO vs. 2nd Vehicle: *P*=0.7152

hM4Dq
1st Vehicle vs. CNO: *P*>0.9999
1st Vehicle vs. 2nd Vehicle: *P*=0.0362*
CNO vs. 2nd Vehicle: *P*=0.0466*

8i Non-social activity statistics: two-way repeated measures ANOVA
Subject: F (17, 34) = 1.263, *P*=0.273
mCherry vs. hM4Di: F (1, 17) = 1.845, *P*=0.1921
Saline vs. CNO: F (1.903, 32.35) = 2.097, *P*=0.1412
Interaction: F (2, 34) = 0.2228, *P*=0.8014

8j. Corticosterone statistics: two-tailed paired *t*-test
t=1.393 df=7, *P*=0.2063

8k. c-Fos staining statistics: two-tailed unpaired *t*-test
PVN: t=0.7245 df=16, *P*=0.4792

8l. c-Fos staining statistics: two-tailed unpaired *t*-test
adBNST: t=1.059 df=16, *P*=0.3054

8o. Non-social activity statistics: two-way repeated measures ANOVA with Bonferroni multiple comparison *post-hoc* test
Subject: F (19, 38) = 1.388, *P*=0.1908
mCherry vs. hM3Dq: F (1, 19) = 30.78, *P*<0.0001****

Saline vs. CNO: F (1.871, 35.56) = 29.65, *P*<0.0001****
Interaction: F (2, 38) = 26.35, *P*<0.0001****

mCherry vs. hM3Dq
1st Vehicle: *P*=0.547
CNO: *P*<0.0001****
2nd Vehicle: *P*>0.9999

mCherry
1st Vehicle vs. CNO: *P*>0.9999
1st Vehicle vs. 2nd Vehicle: *P*=0.3913
CNO vs. 2nd Vehicle: *P*=0.7761

hM3Dq
1st Vehicle vs. CNO: *P*=0.0007***
1st Vehicle vs. 2nd Vehicle: *P*=0.0542
CNO vs. 2nd Vehicle: *P*<0.0001****

8q. Non-social activity statistics: one-way ANOVA with Bonferroni multiple comparison *post-hoc* test
Main effect: F (2, 29) = 5.132, *P*=0.0124*

VEH vs. CRH low: *P*=0.0427*
VEH vs. CRH high: *P*>0.9999
CRH low vs. CRH high: *P*=0.0134*

Extended data Fig. 9

9b. CTB-488 statistics: two-tailed unpaired *t*-test
BNST: t=1.339 df=6, *P*=0.2290
LS: t=1.715 df=6, *P*=0.1373
MeA: t=0.9953 df=6, *P*=0.3580

9e. CTB-488 statistics: two-tailed unpaired *t*-test
BNST: t=1.451 df=5, *P*=0.2064
LS: t=0.8518 df=5, *P*=0.4332
MeA: t=0.8452 df=5, *P*=0.4366

9f. fluorogold statistics: two-tailed unpaired *t*-test
PVN: t=0.1414 df=6, *P*=0.8922
LS: t=0.1688 df=6, *P*=0.8715
MeA: t=0.364 df=6, *P*=0.7284

Extended data Fig. 10

10a. c-Fos staining statistics: two-tailed unpaired *t*-test
t=4.740 df=14, *P*=0.0003***

10e. Social activity statistics: two-tailed unpaired *t*-test
t=2.296 df=21, *P*=0.0321*

10f. *E. faecalis* expression: one-way ANOVA with Bonferroni multiple comparison *post-hoc* test
Main effect: F (2, 6)=29.20, *P*=0.0008***

VEH+Ctrl vs. ABX+Ctrl: *P*>0.9999
VEH+Ctrl vs. ABX+E.f.: *P*=0.0020**
ABX+Ctrl vs. ABX+E.f.: *P*=0.0015**

10g. Social activity statistics: one-way ANOVA with Bonferroni multiple comparison *post-hoc* test
Main effect: F (2, 20)=4.043, *P*=0.0335*

VEH+Ctrl vs. ABX+Ctrl: *P*=0.2543
VEH vs. exABX+E.f.: *P*=0.9303
ABX+Ctrl vs. exABX+E.f.: *P*=0.0336*

10h. Social activity statistics: one-way ANOVA with Bonferroni multiple comparison *post-hoc* test
Main effect: F (2, 24)=7.091, *P*=0.0038**

SPF vs. GF: *P*=0.0280*
GF vs. GF+*E.f.*: *P*>0.9999
SPF vs. GF+*E.f.*: *P*=0.0066**

10i. c-Fos staining statistics: two-tailed unpaired *t*-test
PVN: t=2.672 df=16, *P*=0.0167*

10j. c-Fos staining statistics: two-tailed unpaired *t*-test
adBNST: t=2.144 df=17, *P*=0.0467*

10k. c-Fos staining statistics: two-tailed unpaired *t*-test
DG: t=3.005 df=18, *P*=0.0076**

10l. Corticosterone statistics: one-way ANOVA with Bonferroni multiple comparison *post-hoc* test
Main effect: F (2, 24) = 3.411, *P*=0.0497*

SPF vs. GF, *P*=0.959
SPF vs. GF+E.f., *P*=0.0585
GF vs. GF+E.f., *P*=0.2642