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Supplemental Information

Newborn mice form lasting CA2-dependent memories of their mothers

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Supplemental figures and table

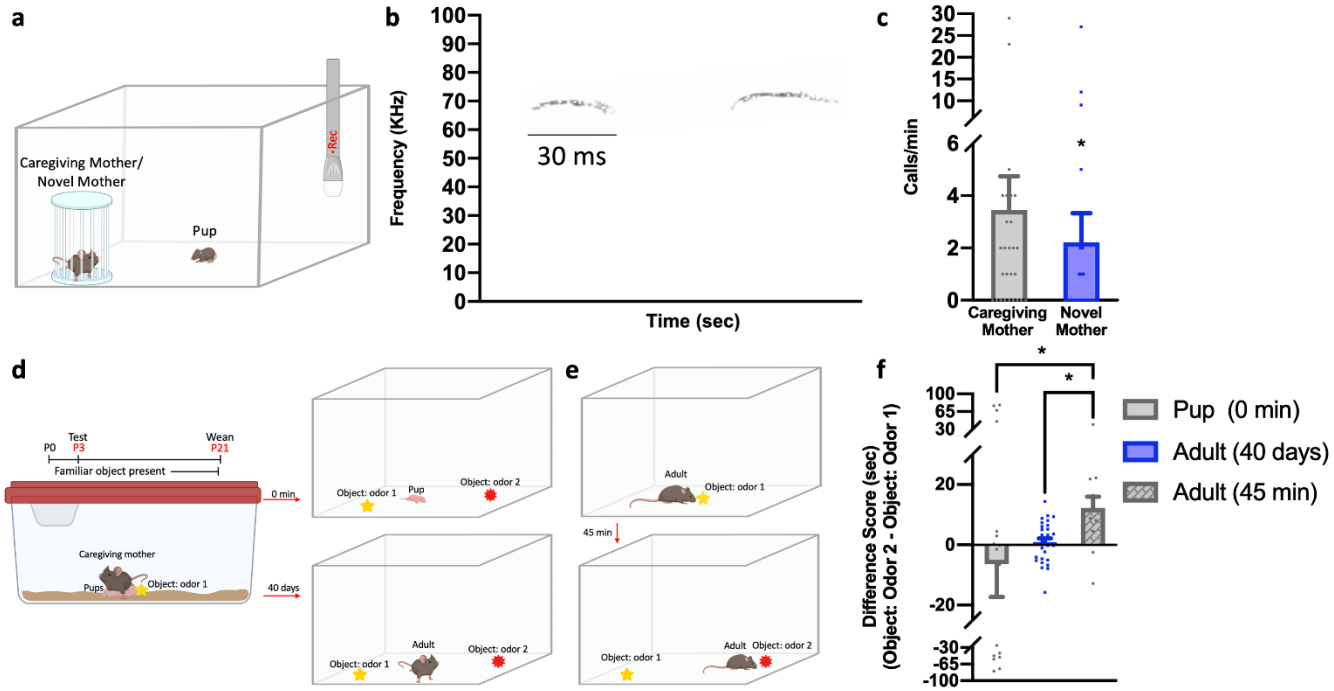

Figure S1: Mouse pups emit more USV calls when in the presence of the caregiving mother compared to a novel mother, and do not display a familiarity preference for objects. Related to Figure 1.

(A) Schematic of pup exposed to a caregiving mother or novel mother while USVs are recorded. (B) Sample pup's USV whistle taken from USV spectrogram. (C) Call numbers are higher when pups are in the vicinity of caregiving mothers versus novel mothers (n=27) (p=0.0249, Wilcoxon matched pairs signed rank test). (D) Schematic of mouse pups reared in a home cage with a distinct odor object (object: odor 1) present until weaning. Familiarity preference for objects was tested on P3 (n=19) by assessing behavior in a two-choice test with object: odor 1 and a novel object: odor 2. A similar test was performed again at P60 (n=29), after mice had been separated from object: odor 1 for 40 days. Objects were counterbalanced across litters. (E) To verify that object memory was intact, mice were exposed to object: odor 1 at P60 and after a brief delay (45 min) tested for novelty preference with a two-choice test with object: odor 1 and object: odor 2 (n=12). (F) Pups showed no preference for the object present in the nest or the novel object. Adults showed no preference for either object after a 40 day interval between exposure to the first object and the test, but show a difference in object investigation with a short interval between object exposure and test ($H(2, 57) = 10.03$, $p=0.0066$, Dunn's post hoc tests: P3 vs P60 (40 days), $p=0.7689$; P3 vs P60 (45 min), $p=0.0051$; P60 (40 days) vs P60 (45 min), $p=0.0500$. * $p < 0.05$ compared to either caregiving mother exposure for the USV experiment or groups within brackets for the object preference experiment; bars represent mean + SEM.

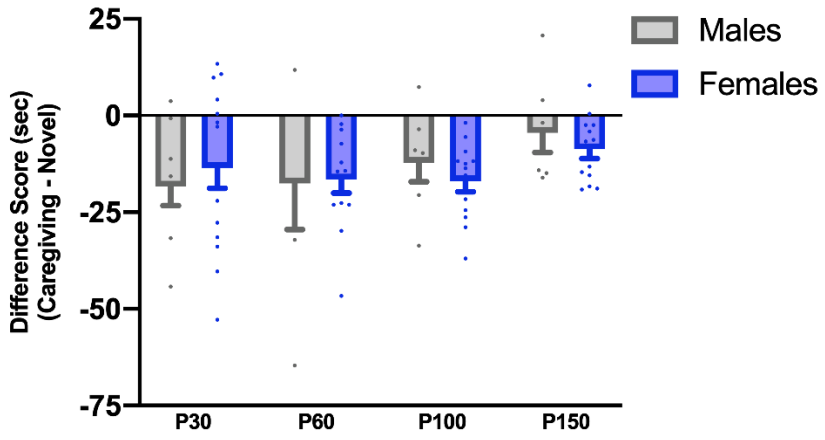

Figure S2: Postweaning males and females display similar preference for a novel mother over the caregiving mother. Related to Figure 1.

Postweaning males and females spend more time investigating a novel mother compared to the caregiving mother at all ages examined. No significant sex differences were observed at any time point ($F(3, 76) = 0.4063$, $p=0.7489$, post hoc tests: P30 males (n=9) vs females (n=15), $p=0.9268$; P60 males (n=7) vs females (n=13), $p=0.9999$; P100 males (n=7) vs females (n=13), $p=0.9524$; P150 males (n=7) vs females (n=13), $p=0.9670$; bars represent mean + SEM.

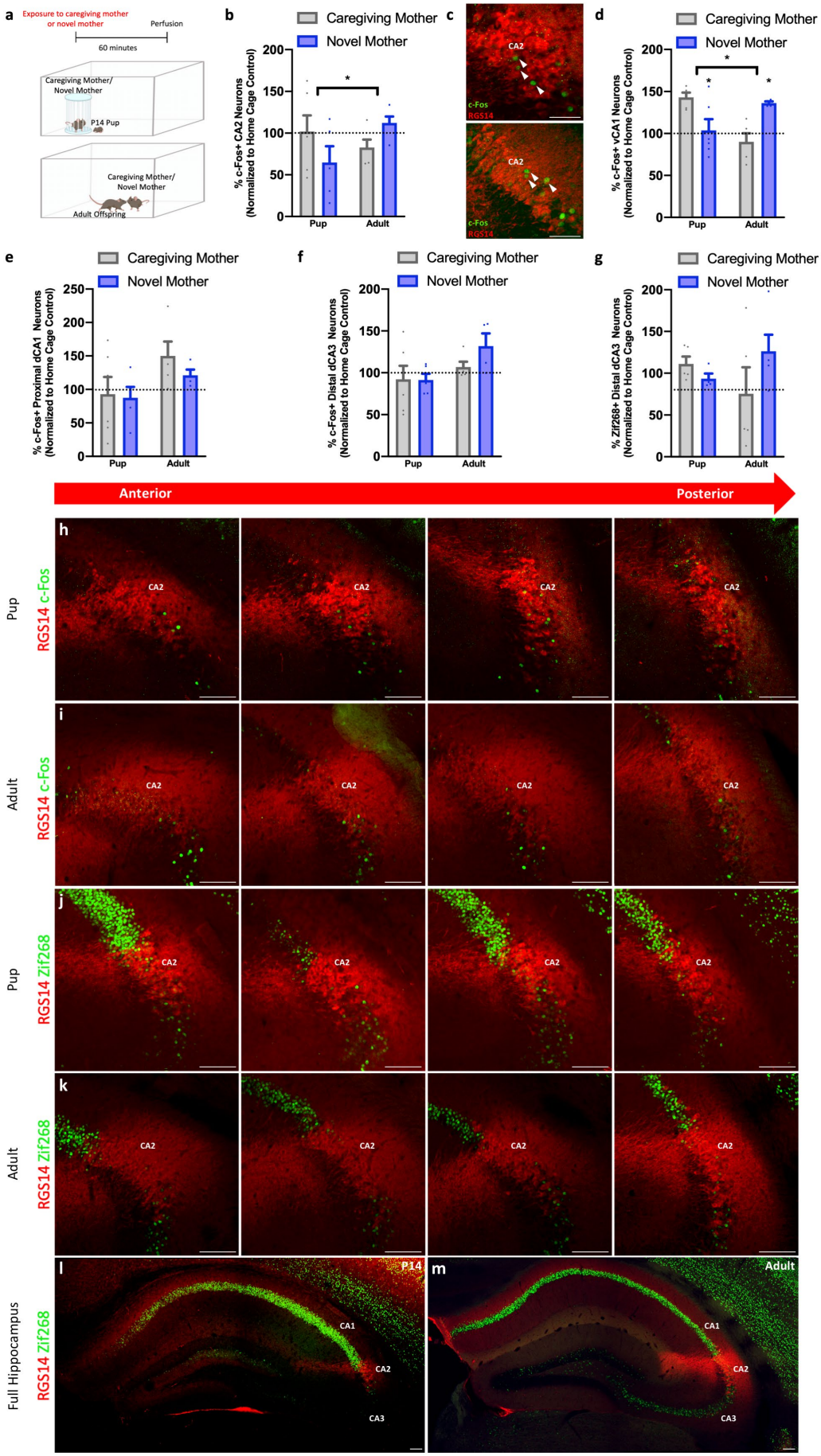
a
Exposure to caregiving mother or novel mother
Perfusion
60 minutes
Caregiving Mother/ Novel Mother
P14 Pup
Caregiving Mother/ Novel Mother
Adult Offspring
b
Caregiving Mother
Novel Mother
% c-Fos+ CA2 Neurons (Normalized to Home Cage Control)
Pup
Adult
c
CA2
c-Fos
RGS14
d
% c-Fos+ vCA1 Neurons (Normalized to Home Cage Control)
e
% c-Fos+ Proximal dCA1 Neurons (Normalized to Home Cage Control)
f
% c-Fos+ Distal dCA3 Neurons (Normalized to Home Cage Control)
g
% Zif268+ Distal dCA3 Neurons (Normalized to Home Cage Control)
Anterior
Posterior
h
Pup RGS14 c-Fos
i
Adult RGS14 c-Fos
j
Pup RGS14 Zif268
k
Adult RGS14 Zif268
l
P14
CA1
CA2
CA3
m
Adult
Full Hippocampus RGS14 Zif268

Figure S3: Exposure to the caregiving mother compared to a novel mother alters IEG+ cell number in the CA2 and ventral CA1, but not in the dorsal CA1 or dorsal CA3. Related to Figure 1. (A) Timeline and schematic of P14 pup or adult offspring exposure to the caregiving mother or novel mother for immediate early gene experiment. (B) The number of c-Fos+ cells in the CA2 after exposure to the caregiving mother (P14 n=6; adult n=5) or a novel mother (P14 n=5; adult n=5) at P14 or in adulthood followed a similar pattern as that observed for Zif268 (see Figure 1) ($F(1,17) = 4.539$, $p=0.0480$, although post hoc comparisons were not statistically significant, Sidak post hoc tests: novel vs caregiving (P14), $p=0.1964$, novel vs caregiving (adult), $p=0.3760$). (C) Confocal images of c-Fos+ cells (green, arrows) in the RGS14 labeled CA2 region (red) from pup (top) and adult (bottom) exposed to a caregiving mother (pup) or novel mother respectively. (D) The number of c-Fos+ cells in the ventral CA1 was increased after exposure to the caregiving mother (n=5) compared to a novel mother (n=5) in pups and decreased after exposure to the caregiving mother (n=6) compared to a novel mother (n=4) in adults ($F(1,16) = 17.67$, $p=0.0007$, Sidak post hoc tests: novel vs caregiving (P14), $p=0.0217$, novel vs caregiving (adult), $p=0.0148$). (E) No differences were observed in the number of c-Fos+ cells in the proximal dorsal CA1 in pups or adults after exposure to the caregiving mother (P14 n=6; adult n=5) or a novel mother (P14 n=5; adult n=4; $F(1,16) = 0.3133$, $p=0.5834$). (F) No differences were observed in the number of c-Fos+ cells in the distal dorsal CA3 (Caregiving: P14 n=6, adult n=5; Novel: P14 n=5; adult n=4; $F(1,16) = 1.039$, $p=0.3232$). (G) No differences were observed in the number of Zif268+ cells in the distal dorsal CA3 (Caregiving: P14 n=5, adult n=5; Novel: P14 n=4, adult n=5; $F(1,15) = 2.770$, $p=0.1168$). All cell counts were normalized to P14 or adult controls from the same region after home cage exposure. * $p < 0.05$ compared to either groups within brackets or compared to mice exposed to caregiving mothers; bars represent mean + SEM. (H) Confocal images of c-Fos+ cells (green) in the CA2 (labeled with RGS14, red) of a P14 pup at different anterior-posterior levels. (I) Confocal images of c-Fos+ cells (green) in the CA2 (labeled with RGS14, red) of an adult mouse at different anterior-posterior levels. (J) Confocal images of Zif268+ cells (green) in the CA2 (labeled with RGS14, red) of a P14 pup at different anterior-posterior levels. (K) Confocal images of Zif268+ cells (green) in the CA2 (labeled with RGS14, red) of an adult mouse at different anterior-posterior levels. (L) Confocal images of Zif268+ cells (green) in the pup (P14) and (M) adult hippocampus showing the Zif268 staining pattern in the CA fields with CA2 delineated by RGS14 (red). Scale bars equal 200 μm.

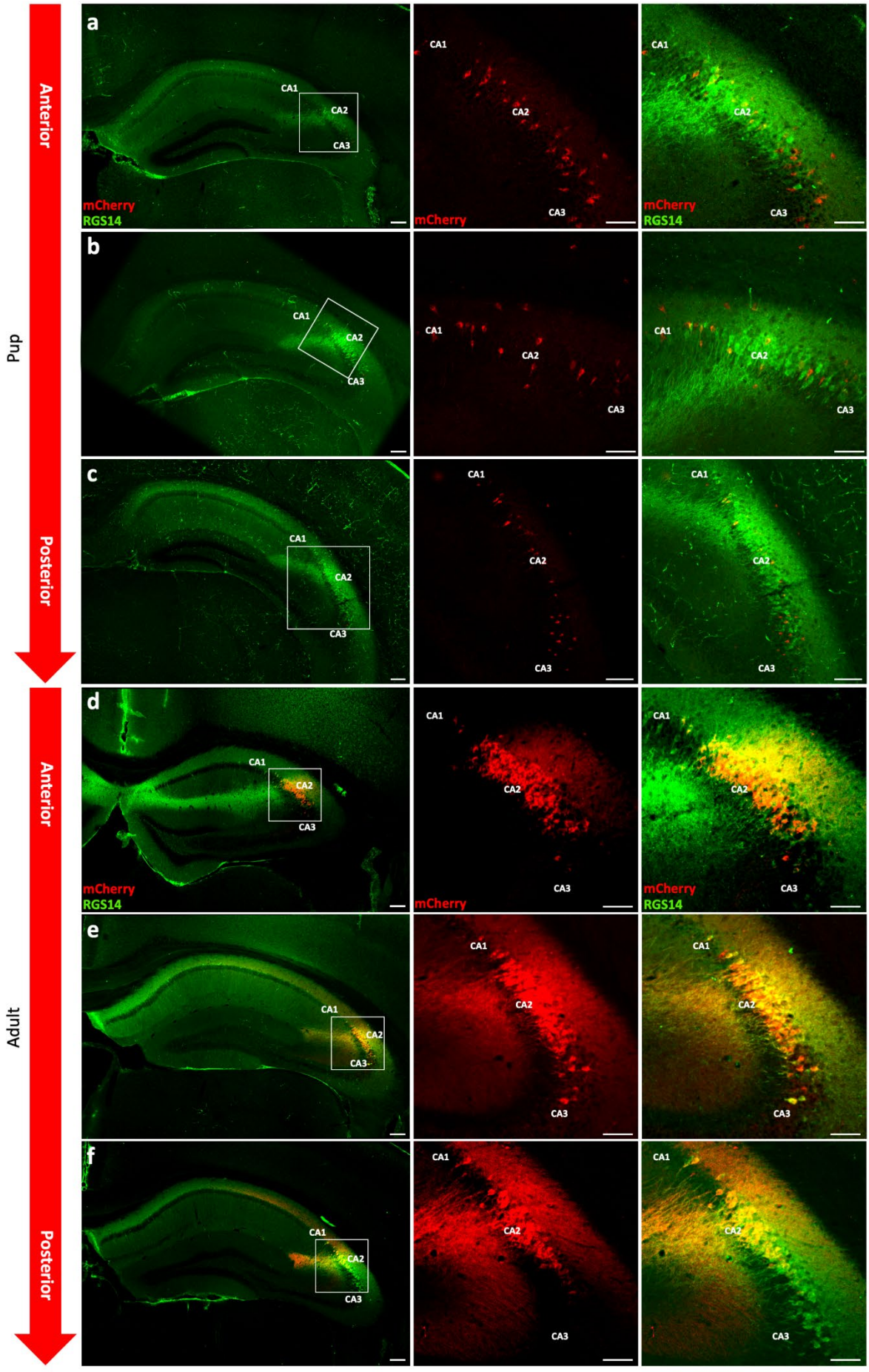
Pup
Anterior
Posterior
Adult
Anterior
Posterior
a
b
c
d
e
f
CA1
CA2
CA3
mCherry
RGS14

Figure S4: **DREADD virus expression is largely confined to the CA2 region in both pups and adults.** Related to Figures 3-5.

(A-C) Confocal images showing mCherry expression after virus infection (red) of the CA2, delineated by RGS14 (green) in a P12 pup (infected with virus on P3, tested behaviorally on P10 and P12 and then perfused on P12) at different anterior-posterior levels. Labeling was moderate and mostly specific to the CA2. (D-F) Confocal images showing mCherry expression after virus infection (red) in the CA2, delineated by RGS14 (green) in an adult mouse at different anterior-posterior levels. Labeling was robust and largely specific to the CA2. Scale bars equal 200 μm.

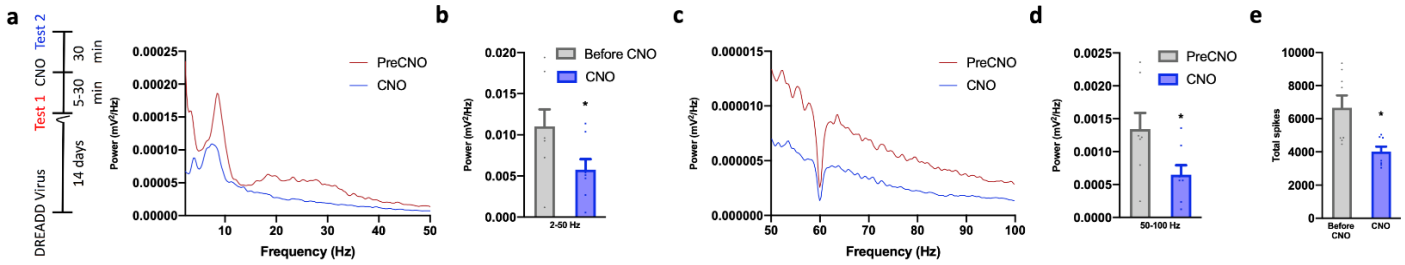

Figure S5: CNO treatment after DREADD infection decreases local field potential (LFP) power in the CA2 of adult mice. Related to Figures 4,5**.**

(A) Timeline for inhibitory DREADD viral infection and CA2 LFP recording from 2 to 50 Hz. (B) Activating inhibitory DREADDs in CA2 with CNO reduces LFP power (2 to 50 Hz) ($t_{(7)}$=6.020, p = 0.0005, n=8). (C) CA2 LFP recording from 50-100 Hz with 60 Hz notch filter. (D) Activating inhibitory DREADDs in CA2 with CNO reduces LFP power (50 to 100 Hz) ($t_{(7)}$ = 6.010, p = 0.0005, n=8). (E) Activating inhibitory DREADDs in CA2 with CNO reduces total spikes ($t_{(7)}$ = 5.697, p = 0.0007, n=8) Because LFP power is greater at lower frequencies, data are displayed using different y axis ranges. * p < 0.05, bars represent mean + SEM.

Table S1: Post hoc comparisons for statistics in Figures 1, 3, 4 and 5 of the main text

Experiment: Preweaning and postweaning caregiving mother/novel mother preference. Related to Figure 1.

Tukey's HSD test	p value
P3 vs. P6	0.9996
P3 vs. P14	0.9570
P3 vs. P30	0.0005
P3 vs. P60	0.0003
P3 vs. P100	0.0001
P3 vs. P150	0.0142
P6 vs. P14	0.8477
P6 vs. P30	0.0010
P6 vs. P60	0.0006
P6 vs. P100	0.0003
P6 vs. P150	0.0277
P14 vs. P30	0.0013
P14 vs. P60	0.0008
P14 vs. P100	0.0004
P14 vs. P150	0.0116
P30 vs. P60	0.9999
P30 vs. P100	0.9981
P30 vs. P150	0.9850
P60 vs. P100	>0.9999
P60 vs. P150	0.9334
P100 vs. P150	0.8591

Experiment: Preweaning and postweaning caregiving mother/novel mother Zif268 CA2. Related to Figure 2.

Sidak's multiple comparisons	p value
Pup	0.0007
Adult	0.0016

Experiment: DREADD pup caregiving mother/novel mother preference. Related to Figure 3.

Sidak's multiple comparisons	p value
Control virus	
Saline vs CNO	0.6499
DREADD virus	
Saline vs CNO	0.0234

Experiment: DREADD pup caregiving mother/novel mother total investigation times. Related to Figure 3.

Sidak's multiple comparisons	p value
Control virus	
Saline vs CNO	0.9694
DREADD virus	
Saline vs CNO	0.9174

Experiment: DREADD adult caregiving mother/novel mother preference. Related to Figure 4.

Sidak's multiple comparisons	p value
Control virus	
Saline vs CNO	0.6907
DREADD virus	
Saline vs CNO	0.0013

Experiment: DREADD adult caregiving mother/novel mother locomotion. Related to Figure 4.

Sidak's multiple comparisons	p value
Control virus w/caregiving	
Saline vs CNO	>0.9999
Control virus w/novel	
Saline vs CNO	0.9973
DREADD virus w/caregiving	
Saline vs CNO	>0.9999
DREADD virus w/novel	
Saline vs CNO	>0.9999

Experiment: DREADD adult familiar/novel conspecific preference. Related to Figure 5.

Sidak's multiple comparisons	p value
Control virus	
Saline vs CNO	0.6167
DREADD virus	
Saline vs CNO	0.0001

Experiment: DREADD adult sociability. Related to Figure 5.

Sidak's multiple comparisons	p value
Control virus	
Social vs nonsocial	0.0089
DREADD virus	
Social vs nonsocial	<0.0001