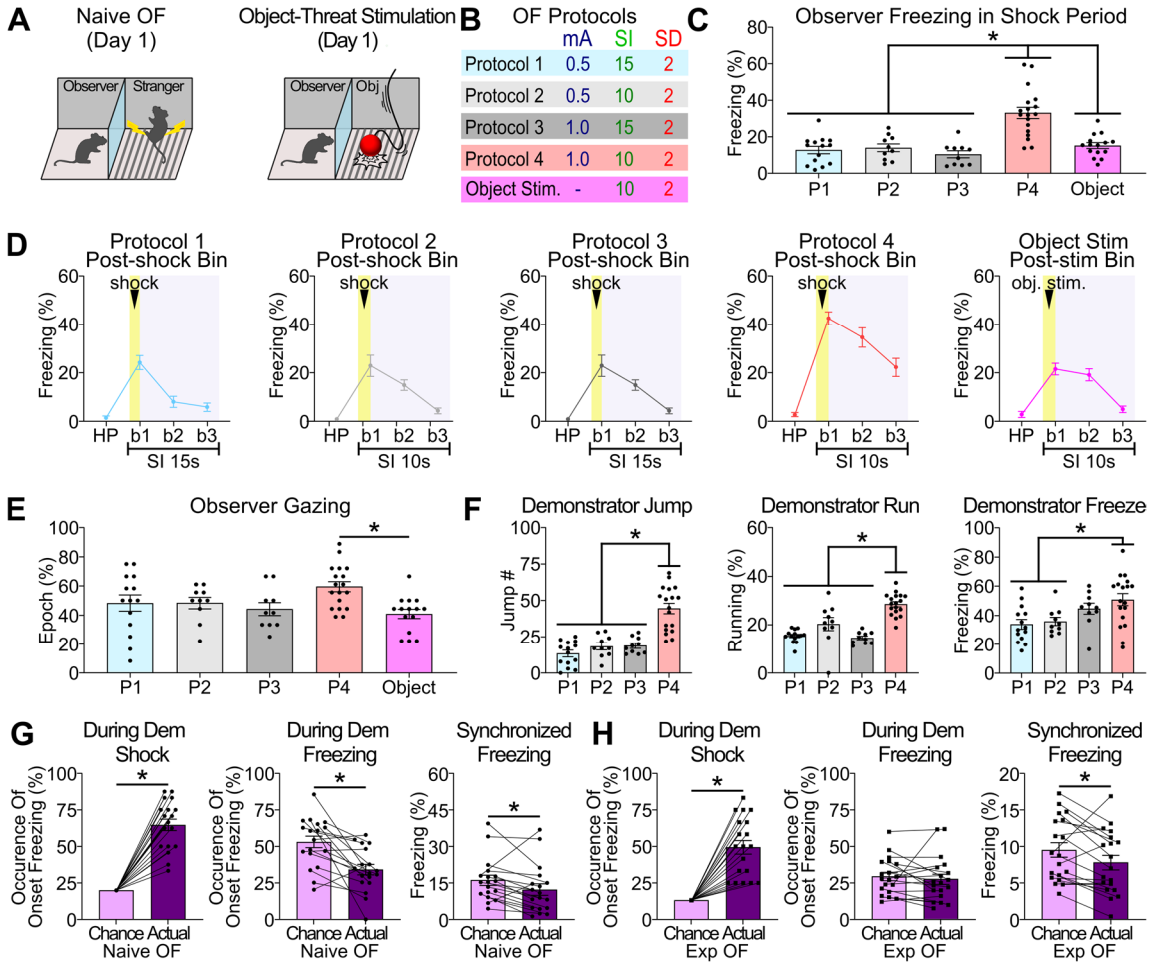

Figure S1. Establishment and validation of Naive and Exp OF. Related to Figure 1.
(A) (Left) Naive observational fear (OF). After a habituation period (HP), different OF protocols (Protocols 1 – 4, listed in (B)) were applied to the demonstrator during the shock period (SP) for 3 minutes. (Right) Object-threat stimulation. After a habituation period, an object was used to simulate visual cues (jumping; object with rope) and auditory cues (banging on grid floor; crash sound waves) with the same shock interval as Protocol 4 for 3 minutes. **(B)** OF protocols. mA; shock intensity, SI; stimulus delivery interval (seconds), SD; stimulus duration (seconds). Object Stim; object-threat stimulation protocol. **(C)** Observer freezing level in SP of OF protocols (P1-P4) or object-threat stimulation (Object). $F_{(4,62)}$ = 16.42, P < 0.0001. P4 vs. all: P < 0.0001. One-way ANOVA with Tukey-Kramer test. N = P1: 14 mice, P2: 10 mice, P3: 10 mice, P4: 18 mice, Obj: 15 mice. **(D)** Observer freezing level in SP of P1-P4 or object-threat stimulation after shock delivery to the demonstrator in post-shock bins (P1-P4) or post-stim bins (Object Stim). Yellow box; shock delivery to the demonstrator or object threat stimulation (0 sec – 2 sec). Gray box; shock period or object stimulation period. N = P1: 14 mice, P2: 10 mice, P3: 10 mice, P4: 18 mice, Obj: 15 mice. **(E)** Observer gazing epochs to demonstrator in SP of P1-P4 or object-threat stimulation. $F_{(4,62)}$ = 3.44, P = 0.02. P4 vs. Obj: P = 0.01. One-way ANOVA with Tukey-Kramer test. N = P1: 14 mice, P2: 10 mice, P3: 10 mice, P4: 18 mice, Object: 15 mice. **(F)** Demonstrator behavior in SP of P1-P4. Jumping (#): $F_{(3,48)}$ = 27.23, P < 0.0001. P4 vs. all: P < 0.0001. Running (%): $F_{(3,48)}$ = 25.25, P < 0.0001. P4 vs. P1 or P3: P < 0.0001, P4 vs. P2: P = 0.001. Freezing (%): $F_{(3,48)}$ = 4.85, P = 0.01. P4 vs. P1: P = 0.01, P4 vs. P2: P = 0.04. One-way ANOVA with Tukey-Kramer test. N = P1: 14 mice, P2: 10 mice, P3: 10 mice, P4: 18 mice. **(G-H)** (Left): Occurrences of onset observer freezing response during demonstrator's shock moment and the chance level. Naive OF: t_{17} = 11.23, P < 0.0001. Exp OF: t_{18} = 7.77, P < 0.0001. (Center) Occurrences of onset observer freezing response during demonstrator freezing response and the chance level. Naive OF: t_{17} = 3.98 P = 0.001 (G). Exp OF: t_{18} = 0.69, P = 0.50 (H). (Right): Synchronized freezing period between observer and demonstrator. Naive OF: t_{17} = 2.20, P = 0.04. Exp OF: t_{18} = 2.36, P = 0.03. Paired t-test (two-sided). N = Naive OF: 18 pairs, Exp OF: 19 pairs. * P < 0.05. Graphs show means ± SEM.

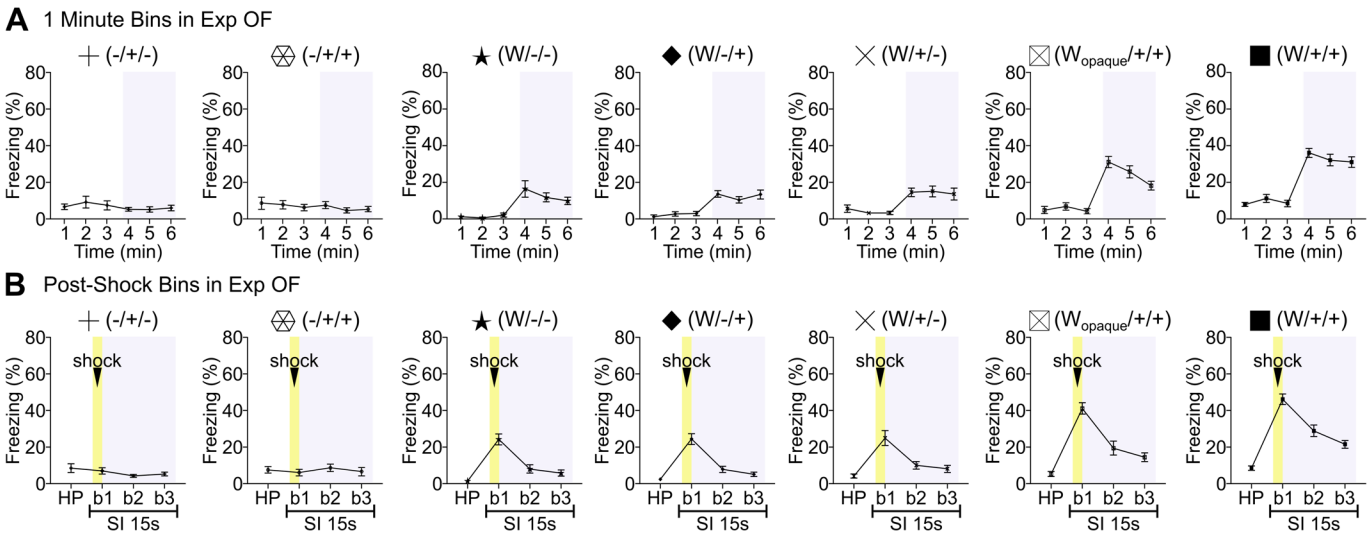

Figure S2. Observer Freezing Behavior during Exp OF and Control Protocols. Related to Figure 1.
(A) Observer freezing levels in the habituation period (1-3 min) and shock period (4-6 min; gray box) of Exp OF or control conditions in 1 min time bins. Groups are as follows: demonstrator shock protocol (W, W_{opaque}, or -) / observer shock experience (+ or -) / social familiarity of demonstrator (+ or -). N = -/+/-: 10, -/+/+: 11, W/-/-: 14, W/-/+: 10, W/+/-: 13, W_{opaque}/+/+: 16, W/+/+: 19. **(B)** Observer freezing levels in post-shock bins in Exp OF. HP; habituation period. SI; shock interval. N = -/+/-: 10, -/+/+: 11, W/-/-: 14, W/-/+: 10, W/+/-: 13, W_{opaque}/+/+: 16, W/+/+: 19.

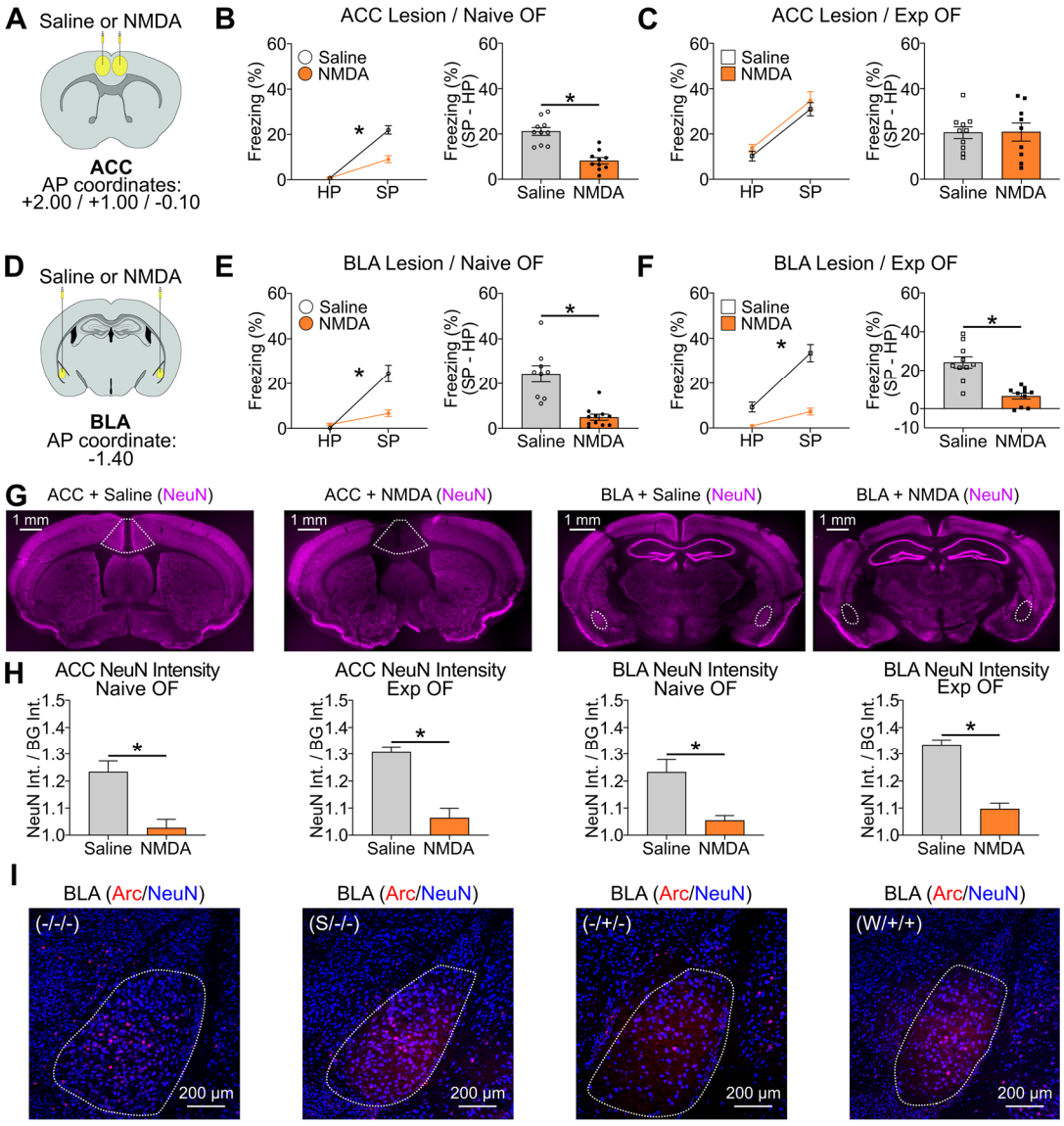

Figure S3. NMDA-induced lesion of ACC or BLA in Naive OF or Exp OF (A-H), and representative images of Arc expression in BLA after Naive or Exp OF (I). Related to Figure 2.
(A, D) NMDA-induced lesion of ACC (A) or BLA (D). **(B-C)** (Left) Observer freezing levels during HP and SP of ACC Saline and NMDA groups in Naive OF (B) or Exp OF (C). Naive OF: $F_{(1,18)}$ = 33.62, P < 0.0001. Exp OF: $F_{(1,17)}$ = 0.003, P = 0.96. Interaction by two-way mixed ANOVA. (Right) Subtraction freezing levels (SP–HP) of ACC Saline and NMDA groups in Naive OF (B) and Exp OF (C). Naive OF: t_{18} = 5.80, P < 0.0001. Exp OF: t_{17} = 0.05, P = 0.96. Unpaired t-test (two-sided). N = ACC in Naive OF; Saline: 10, NMDA:10. ACC in Exp OF; Saline: 10 (12 total. 2 mice removed for HP freezing > 20%), NMDA: 9 (12 total. 3 mice removed for HP freezing > 20%). **(E-F)** (Left) Observer freezing levels during HP and SP of BLA Saline and NMDA groups in Naive OF (E) or Exp OF (F). Naive OF: $F_{(1,18)}$ = 28.44, P < 0.0001. Exp OF: $F_{(1,18)}$ = 28.83, P < 0.0001. Interaction by two-way mixed ANOVA. (Right) Subtraction freezing levels (SP–HP) of BLA Saline and NMDA groups in Naive OF (E) and Exp OF (F). Naive OF: U = 3, P < 0.0001. Exp OF: t_{18} = 5.40, P < 0.0001. Mann-Whitney U-test (two-sided) or unpaired t-test (two-sided). N = BLA in Naive OF; Saline: 9, NMDA:11. BLA in Exp OF; Saline: 10 (13 total. 3 mice removed for HP freezing > 20%), NMDA: 10. **(G)** Coronal sections of ACC or BLA in Saline and NMDA groups. White lines; ACC or BLA region. **(H)** NMDA-induced lesion of ACC or BLA measured by NeuN intensity (normalized by background intensity) in Naive OF or Exp OF. ACC NeuN Intensity in Naive OF: t_{17} = 3.89, P = 0.001. ACC NeuN Intensity in Exp OF: t_{18} = 6.08, P < 0.0001. BLA NeuN Intensity in Naive OF: U = 10, P = 0.003. BLA NeuN Intensity in Exp OF: t_{19} = 8.55, P < 0.0001. Unpaired t-test (two-sided) or Mann-Whitney U-test (two-sided). N = ACC in Naive OF; Saline: 10 mice, NMDA: 9 mice, ACC in Exp OF; Saline: 10 mice, NMDA: 10 mice, BLA in Naive OF; Saline: 10 mice, NMDA: 9 mice, BLA in Exp OF; Saine: 10 mice, NMDA: 11 mice. **(I)** Coronal sections of Arc/NeuN immunohistochemistry in BLA after Naive OF (S/-/-), Exp OF (W/+/+), or control conditions (-/-/- for Naive OF; -/+/- for Exp OF). White lines; BLA region. * P < 0.05. Graphs show means ± SEM.

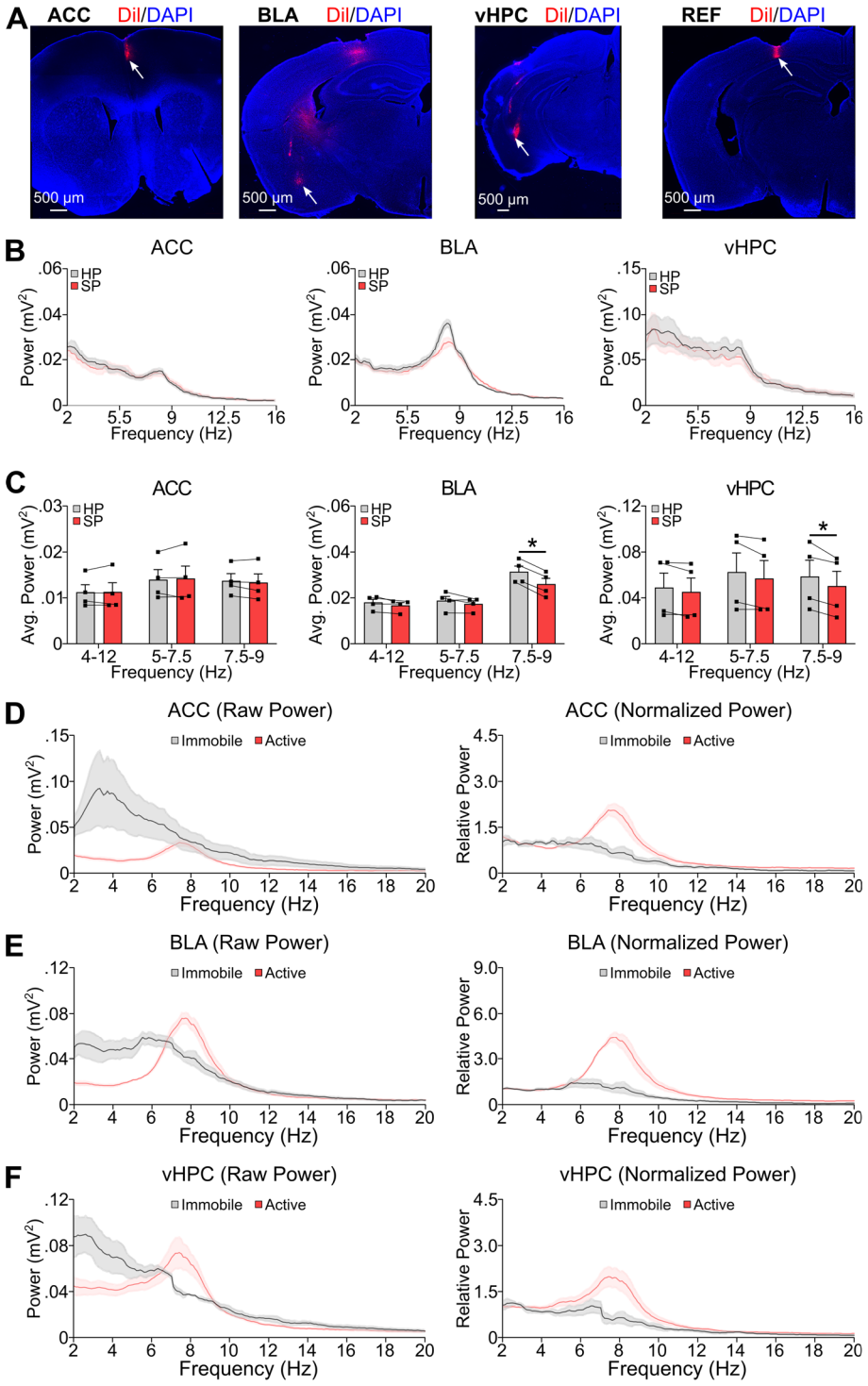

Figure S4. *In vivo* electrophysiology of ACC, BLA, and vHPC during Exp OF. Related to Figure 2.
(A) Coronal sections of electrode placement for ACC (left), BLA (left center), vHPC (right center), and reference (REF, right). DAPI; blue. DiI; red. Arrows indicate implanted electrode tip. **(B)** Averaged power spectrum of local field potentials in ACC (left), BLA (center), and vHPC (right) during habituation period (HP) and shock period (SP) in Exp OF. N = 4 mice. **(C)** Averaged theta 4-12 Hz, 5-7.5 Hz and 7.5-9 Hz power for ACC (left), BLA (center), and vHPC (right) during HP and SP in Exp OF. ACC; 4-12 Hz: t_3 = 0.15, P = 0.89. 5-7.5 Hz: t_3 = 0.40, P = 0.71. 7.5-9 Hz: t_3 = 1.13, P = 0.34. BLA; 4-12 Hz: t_3 = 3.06, P = 0.06. 5-7.5 Hz: t_3 = 1.56, P = 0.22. 7.5-9 Hz: t_3 = 9.78, P = 0.002. vHPC; 4-12 Hz: t_3 = 2.04, P = 0.13. 5-7.5 Hz: t_3 = 2.15, P = 0.12. 7.5-9 Hz: t_3 = 4.00, P = 0.03. Paired t-test (two-sided). N = 4 mice. **(D-F)** Raw power distribution (left) and normalized power distribution (right, raw power / averaged delta (2-4 Hz) power) for ACC (D), BLA (E), and vHPC (F) during the immobile/sleep state (blue, velocity < 0.2 cm/s) or active state (red, velocity > 1.5 cm/s). N = 3 mice. Graphs show means ± SEM.

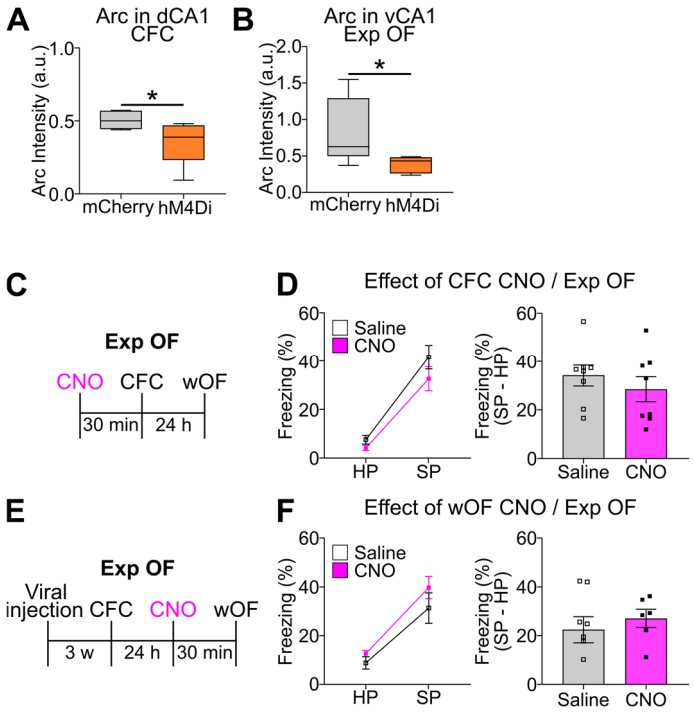

Figure S5. Verification of chemogenetic inhibition of neural activity *in vivo* (A-B) and no effect of CNO injection without hM4Di expression on Exp OF (C-F). Related to Figure 3.

(A-B) Verification of chemogenetic inhibition of neural activity in dCA1 and vCA1 *in vivo.* Intensity of Arc expression (normalized by background intensity) after CNO injection. Mice were injected with CNO 30 minutes prior to CFC (for dCA1, A) or Exp OF (for vCA1, B), and were perfused 1 hour after. Tissue was immunostained for Arc and NeuN. ROIs were selected using the ImageJ software around the cell layer of dHPC or vHPC CA1. The ratio of the Arc channel intensity to background channel intensity (arbitrary units, a.u.) was calculated and compared between experimental and control groups for dCA1 or vCA1. Arc expression was reduced in hM4Di-infected mice in dCA1 after CFC (A) or in vCA1 after Exp OF (B). Arc in dCA1: t_{10} = 2.37, P = 0.04. Unpaired t-test (two-sided). Arc in vCA1: U = 4, P = 0.02. Mann-Whitney U-test (two-sided). N = dCA1/vCA1; mCherry: 6 mice, hM4Di: 6 mice. **(C-F)** CNO injection without hM4Di expression does not affect Exp OF. **(C)** Schedule for CNO injection during CFC in Exp OF. **(D)** (Left) Observer freezing levels during HP and SP of Saline and CNO groups. $F_{(1,14)}$ = 0.71, P = 0.42. Interaction with two-way mixed ANOVA. (Right) Subtraction freezing levels (SP–HP) for Saline and CNO groups. t_{14} = 0.83, P = 0.42. Unpaired t-test (two-sided). N = Saline: 8 mice, CNO: 8 mice. **(E)** Schedule for CNO injection during wOF in Exp OF. **(F)** (Left) Observer freezing levels during HP and SP of Saline (hM4Di-injected) and CNO (non hM4Di-injected) groups. $F_{(1,12)}$ = 0.42, P = 0.53. Interaction with two-way mixed ANOVA. (Right) Subtraction freezing levels (SP–HP) for Saline and CNO groups. t_{12} = 0.65, P = 0.53. Unpaired t-test (two-sided). N = Saline: 8 mice (10 total. 2 mice were removed for HP freezing > 20%), CNO: 6 mice. * P < 0.05. Graphs show means ± SEM, except A-B, which are presented as box plots.

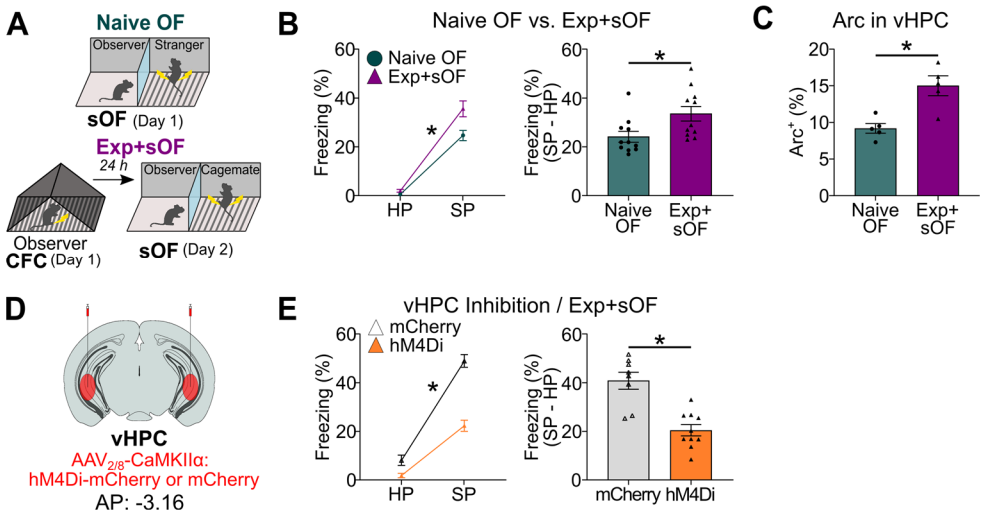

Figure S6. Prior experience enhances observer freezing response during OF even with the strong OF (sOF) protocol, and it requires vHPC activity. Related to Figure 3.

(A) Schedule for Naive OF (top) and experience-dependent enhancement of OF with sOF protocol delivered to the demonstrator (Exp+sOF, bottom). **(B)** (Left) Observer freezing levels during HP and SP of Naive OF and Exp+sOF groups. $F_{(1,20)}$ = 6.40, P = 0.02. Interaction by two-way mixed ANOVA. (Right) Subtraction freezing levels (SP–HP) for Naive OF and Exp+sOF groups. t_{20} = 2.53, P = 0.02. Unpaired t-test (two-sided). N = Naive OF: 11 mice, Exp+sOF: 11 mice. **(C)** Percentages of Arc^+ neurons in vHPC in Naive OF or Exp+sOF. t_8 = 3.89, P = 0.01. Unpaired t-test (two-sided). N = Naive OF: 5, Exp+sOF: 5. **(D)** AAV injection into vHPC. **(E)** (Left) Observer freezing levels during HP and SP of mCherry and hM4Di groups in Exp+sOF. $F_{(1,16)}$ = 24.97, P = 0.0001. Interaction with two-way mixed ANOVA. (Right) Subtraction freezing levels (SP–HP) for mCherry and hM4Di groups in Exp+sOF. t_{16} = 5.00, P = 0.0001. Unpaired t-test (two-sided). N = mCherry: 8 mice, hM4Di: 10 mice. * P < 0.05. Graphs show means ± SEM.

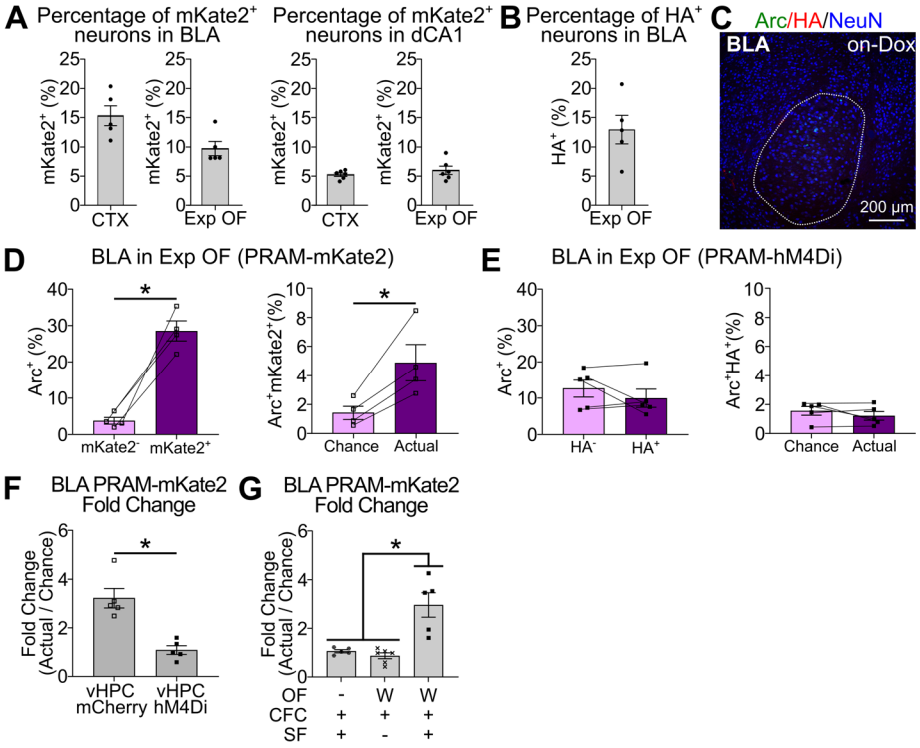

Figure S7. Verification of the robust activity marking (RAM) system (A-E) and fold change analysis in different behavior groups (F-G). Related to Figure 4.

(A) Percentages of mKate2$^+$ BLA or dCA1 neurons for each behavioral condition in Figure 4G-H, 4J-K. N = BLA CTX: 5, BLA Exp OF: 5, dCA1 CTX: 6, dCA1 Exp OF: 6. **(B)** Percentages of HA$^+$ BLA neurons in Figure 5D. N = 5 mice. **(C)** Coronal section of BLA in the on-Dox condition. White lines; BLA region. **(D)** Effect of CNO injection 30 minutes prior to Exp OF on the reactivation of Day 1 CFC-activated cells in mKate2 BLA mice. (Left) Percentages of Arc positive mKate2$^+$ or mKate2$^-$ neurons in BLA in Exp OF: $t_3 = 8.87$, P = 0.003. Paired t-test (two-sided). (Right) Actual and chance levels of Arc$^+$mKate2$^+$ BLA neurons: $t_3 = 4.21$, P = 0.02. N = 4 mice. Paired t-test (two-sided). **(E)** Effect of CNO injection 30 minutes prior to Exp OF on the reactivation of Day 1 CFC cells in hM4Di BLA mice. (Left) Percentages of Arc positive HA$^+$ or HA$^-$ neurons in BLA in Exp OF: $t_4 = 1.05$, P = 0.35. Paired t-test (two-sided). (Right) Actual and chance levels of Arc$^+$HA$^+$ BLA neurons: $t_4 = 1.12$, P = 0.32. N = 5 mice. Paired t-test (two-sided). **(F)** Fold change (Actual/Chance) analysis of Arc$^+$mKate2$^+$ neurons in Exp OF/mCherry in vHPC (from Figure 6B) and Exp OF/hM4Di in vHPC (from Figure 6C) groups: $t_8 = 4.87$, P = 0.001. Unpaired t-test (two-sided). N = Exp OF/mCherry in vHPC: 5 mice, Exp OF/hM4Di in vHPC: 5 mice. **(G)** Fold change (Actual/Chance) analysis of Arc$^+$mKate2$^+$ neurons in No Demonstrator Shock (-/+/+, from Figure 6D), Stranger Demonstrator (W/+/-, from Figure 6E), and Exp OF (from Figure 4H) groups: $F_{(2,13)} = 16.04$, P = 0.0003, Exp OF (W/+/+) vs. Stranger Demonstrator (W/+/-): P = 0.0004, Exp OF (W/+/+) vs. No Demonstrator Shock (-/+/+): P = 0.001. One-way ANOVA with Tukey-Kramer test. N = No Demonstrator Shock (-/+/+): 5 mice, Stranger Demonstrator (W/+/-): 6 mice, Exp OF (W/+/+): 5 mice. * P < 0.05. Graphs show means ± SEM.

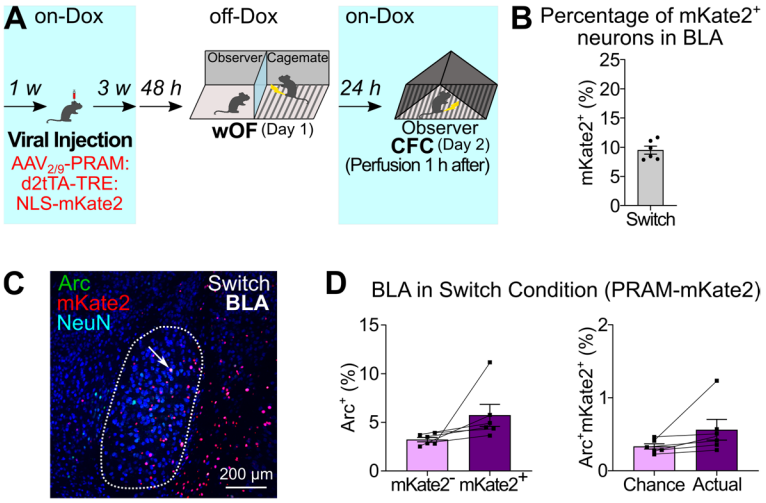

Figure S8. Overlapping neural ensemble activation during both CFC and Exp OF is CFC experience-dependent. Related to Figure 4.

(A) Schedule for the Switch condition. The Switch condition tests whether the reactivation of BLA engram cells encoding CFC experience during Exp OF is indeed CFC experience-dependent, or whether BLA innately contains a subset of neurons that can be activated during both own and other's fear experience. Mice are maintained on the doxycycline diet (on-Dox) for 1 week and are then injected with $AAV_{2/9}$-PRAM:d2tTA-TRE:NLS-mKate2 targeting BLA. After waiting 3 weeks for viral expression, mice are removed from the dox diet (off-Dox) for 48 hours before Day 1 of behavior testing. On Day 1, observers are exposed to OF with a cagemate demonstrator. After a 3 minute habituation period, the weak OF protocol (wOF, one 0.5 mA shock delivered every 15 seconds) is applied to the demonstrator for 3 minutes. After OF, mice are immediately put back on-Dox. On Day 2, observers are exposed to CFC in a different context and are perfused 1 hour later. **(B)** Percentages of $mKate2^+$ BLA neurons in the Switch condition. N = 6 mice. **(C)** Coronal section of BLA. White lines; BLA region. Arrow; $mKate2^+$(red)Arc^+(green) neuron. **(D)** (Left) Percentages of Arc^+ neurons in $mKate2^-$ or $mKate2^+$ neurons in Switch condition: $t_5 = 2.03$, P = 0.10. Paired t-test (two-sided). N = 6 mice. (Right) Actual and the chance levels of $Arc^+mKate2^+$ BLA neurons: $t_5 = 1.86$, P = 0.12. Paired t-test (two-sided). Graphs show means ± SEM. In the Switch condition, there is no significant overlap in BLA neural activity between Day 1 wOF with a cagemate demonstrator and Day 2 CFC. This result indicates that the reactivation of BLA fear engram cells during Exp OF is CFC experience-dependent.

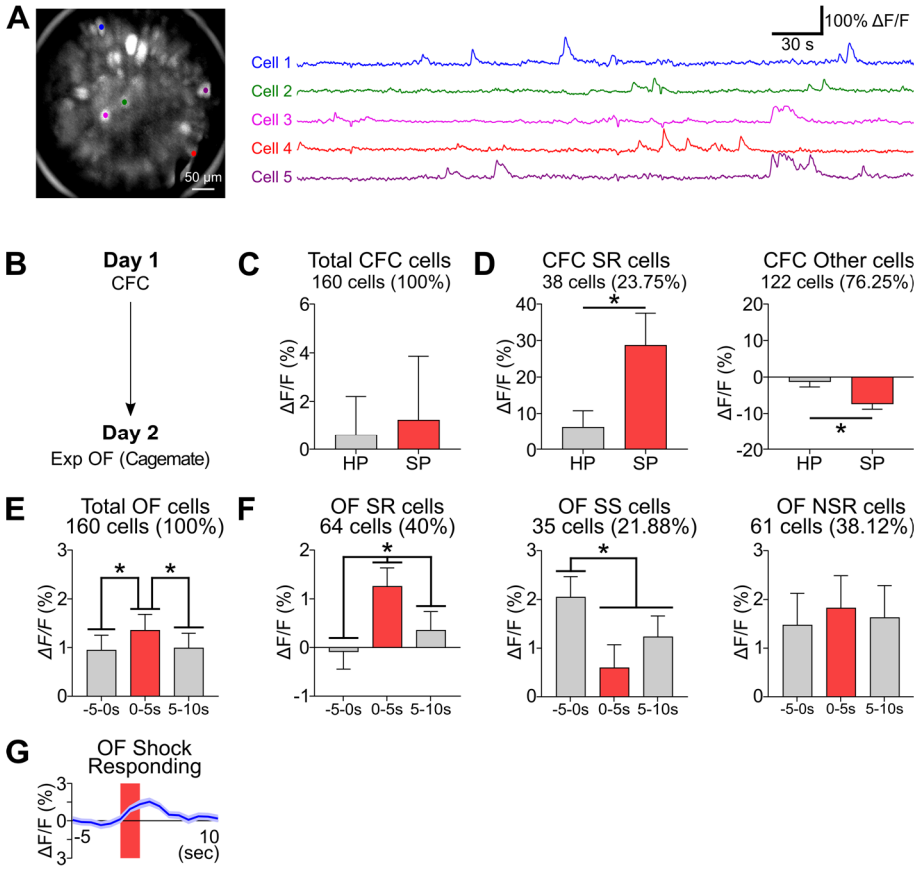

Figure S9. Longitudinal, *in vivo* Ca2^{+} imaging of BLA neurons during CFC on Day 1 and Exp OF on Day 2. Related to Figure 5.

(A) Stacked image acquired through microendoscope in Exp OF (left). Colored cells correspond to example traces of relevant fluorescence changes (right, *ΔF/F* %). **(B)** Recording schedule for *in vivo* Ca2^{+} imaging of BLA neurons. **(C)** Averaged calcium activity (*ΔF/F* %) in the habituation period (HP) and shock period (SP) during CFC. t_{159} = 0.28, P = 0.78. n = 160 cells. Paired t-test (two-sided). N = 5 mice. **(D)** Averaged calcium activity (*ΔF/F* %) in the HP and SP during CFC for Shock-Responding (SR, left) and Other (shock-suppressed and non-shock responding, right) cells. SR cells: t_{37} = 3.03, P = 0.005. n = 38 cells. Other cells: t_{121} = 4.92, P < 0.0001. n = 122 cells. Paired t-test (two-sided). N = 5 mice. **(E)** Averaged calcium activity (*ΔF/F* %) in -5-0s, 0-5s, and 5-10s period after demonstrator shock delivery during Exp OF. $F_{(2,318)}$ = 6.43, P = 0.003, -5-0s vs. 0-5s: P = 0.01, 0-5s vs. 5-10s: P = 0.03. n = 160 cells. Repeated measures one-way ANOVA with Tukey-Kramer test. N = 5 mice. **(F)** Averaged calcium activity (*ΔF/F* %) in -5-0s, 0-5s and 5-10s period after demonstrator shock delivery during Exp OF for SR cells (left), Shock-Suppressed (SS) cells (center), and Non-Shock Responding (NSR) cells (right). SR cells: $F_{(2,126)}$ = 27.38, P < 0.0001, -5-0s vs. 0-5s: P < 0.0001, -5-0s vs. 5-10s: P = 0.01, 0-5s vs. 5-10s: P = 0.001. n = 64 cells. SS cells: $F_{(2,68)}$ = 19.36, P < 0.0001, -5-0s vs. 0-5s: P < 0.0001, -5-0s vs. 5-10s: P = 0.003. n = 35 cells. NSR cells: $F_{(2,120)}$ = 2.98, P = 0.06. n = 61 cells. Repeated measures one-way ANOVA with Tukey-Kramer test. N = 5 mice. **(G)** Averaged calcium activity (*ΔF/F* %) in OF SR cells (n = 64 cells) during Exp OF. * P < 0.05. Graphs show means ± SEM.

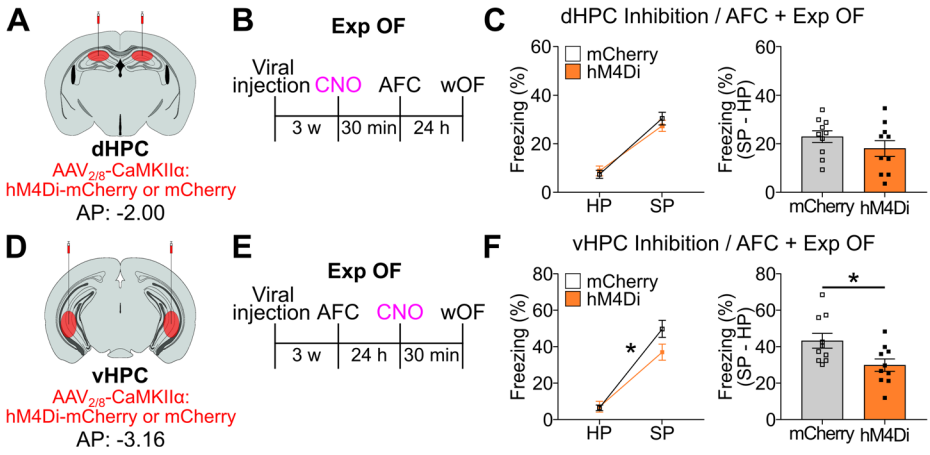

Figure S10. Role of dHPC and vHPC in Exp OF after auditory fear conditioning. Related to Figure 6.
(A, D) Injection strategy for dHPC (A) and vHPC (D). **(B, E)** Experimental schedule for chemogenetic inhibition of dHPC (B) and vHPC (E) in auditory fear conditioning (AFC) + Exp OF. **(C, F)** (Left) Observer freezing levels during habituation period (HP) and shock period (SP) of mCherry and hM4Di groups in dHPC (C) and vHPC (F). dHPC: $F_{(1,17)}$ = 1.05, P = 0.32. vHPC: $F_{(1,18)}$ = 6.40, P = 0.02. Interaction by two-way mixed ANOVA. (Right) Subtraction freezing levels in dHPC (C) and vHPC (F). dHPC: t_{17} = 1.02, P = 0.32. vHPC: t_{18} = 2.53, P = 0.02. Unpaired t-test (two-sided). dHPC; mCherry: 10 mice, hM4Di: 9 mice (10 total. 1 mouse removed for HP freezing > 20%), vHPC; mCherry: 10 mice, hM4Di: 10 mice. Unpaired t-test (two-sided). * P < 0.05. Graphs show means ± SEM.

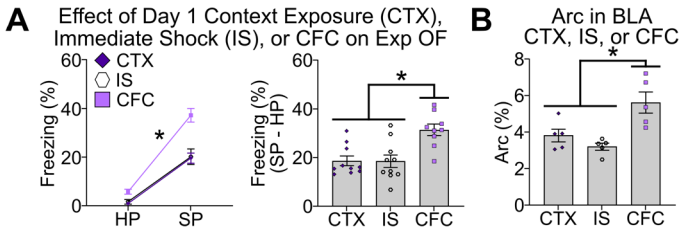

Figure S11. Immediate shock on Day 1 does not produce Exp OF on Day 2, nor does it enhance Arc expression in BLA. Related to Figure 6.

(A) On Day 1, observers were exposed to Context A for 3 minutes (context exposure; CTX), were immediately shocked upon entering Context A and then immediately removed from Context A (immediate shock; IS), or were subjected to contextual fear conditioning in Context A for 3 minutes (contextual fear conditioning; CFC). On Day 2, observers from all groups were subjected to wOF with a cagemate demonstrator. (Left) Observer freezing levels during HP and SP of CTX, IS, and CFC groups. $F_{(2,26)}$ = 9.94, P = 0.001. Interaction with two-way mixed ANOVA. (Right) Subtraction freezing levels (SP–HP) for CTX, IS, and CFC groups. $F_{(2,26)}$ = 9.94, P = 0.001. CFC vs. CE: P = 0.002, CFC vs. IS: P = 0.002. One-way ANOVA with Tukey-Kramer test. N = CTX: 10 mice, IS: 10 mice, CFC: 9 mice. **(B)** On Day 1, another group of mice was perfused 1 hour after IS, CTX, or CFC and immunostained for Arc and NeuN in BLA. $F_{(2,12)}$ = 9.44, P = 0.003. CFC vs. CTX: P = 0.02, CFC vs. IS: P = 0.003. One-way ANOVA with Tukey-Kramer test. N = CTX: 5 mice, IS: 5 mice, CFC: 5 mice. * P < 0.05. Graphs show means ± SEM.

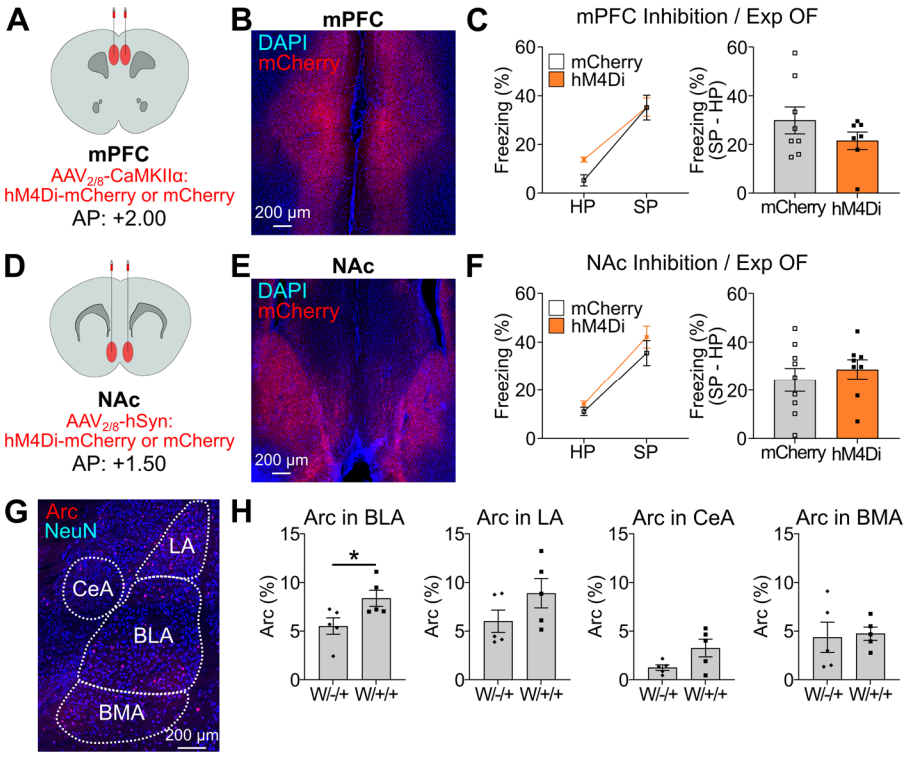

Figure S12. Role downstream vHPC projections in Exp OF. Related to Figure 7.
(A, D) Injection strategy for medial prefrontal cortex (mPFC, A) and nucleus accumbens (NAc, D) in Exp OF. **(B, E)** Coronal sections of mPFC (B) and NAc (E). **(C, F)** Chemogenetic inhibition of mPFC (C) and NAc (F) during Exp OF. (Left) Observer freezing levels during habituation period (HP) and shock period (SP) of mCherry and hM4Di groups in mPFC (C) and NAc (F). mPFC: $F_{(1,13)}$ = 1.51, P = 0.24. NAc: $F_{(1,14)}$ = 0.29, P = 0.60. Interaction by two-way mixed ANOVA. (Right) Subtraction freezing levels in mPFC (C) and NAc (F). mPFC: t_{13} = 1.23, P = 0.24. NAc: t_{14} = 0.54, P = 0.60. Unpaired t-test (two-sided). mPFC; mCherry: 8 mice (9 total. 1 mouse removed for HP freezing > 20%), hM4Di: 7 mice (10 total. 3 mice removed for HP freezing > 20%), NAc; mCherry: 9 mice (10 total. 1 mouse removed for HP freezing > 20%), hM4Di: 7 mice (10 total. 3 mice removed for HP freezing > 20%). **(G)** Coronal section of amygdala subregions, basolateral amygdala (BLA), lateral amygdala (LA), central amygdala (CeA), and basomedial amygdala (BMA). White lines indicate subregion border. **(H)** Percentages of Arc^+ neurons in BLA, LA, CeA, and BMA in Exp OF (W/+/+) or a control condition (W/-/+). BLA: t_8 = 2.39, P = 0.04. LA: t_8 = 1.52, P = 0.17. CeA: t_8 = 2.11, P = 0.07. BMA: t_8 = 0.22, P = 0.83. Unpaired t-test (two-sided). N = W/-/+: 5 mice, W/+/+: 5 mice. * P < 0.05. Graphs show means ± SEM.

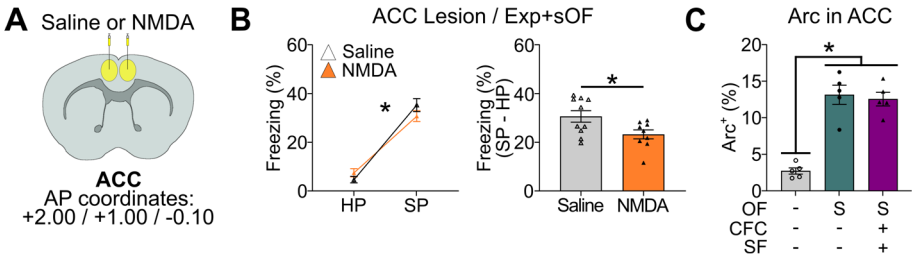

Figure S13. ACC is necessary for OF in the Exp+sOF condition. Related to Figure 7.
(A) NMDA-induced excitotoxic lesion of ACC. **(B)** (Left) Observer freezing levels during HP and SP of Saline and NDMA groups in Exp+sOF. $F_{(1,17)}$ = 5.83, P = 0.03. Interaction with two-way mixed ANOVA. (Right) Subtraction freezing levels (SP–HP) for Saline and NDMA groups in Exp+sOF. t_{17} = 2.42, P = 0.03. Unpaired t-test (two-sided). N = Saline: 10 mice, NMDA: 9 mice. **(C)** While strong OF stimulation increased Arc expression in ACC, prior CFC experience and social familiarity did not further enhance Arc expression in ACC. Percentages of Arc^+ neurons in ACC in a negative control group (-/-/-), Naive OF (S/-/-), and Exp+sOF (S/+/+). $F_{(2,12)}$ = 36.38, P < 0.0001. Negative control group (-/-/-) vs. all: P < 0.0001. One-way ANOVA with Tukey-Kramer test. N = Negative control group (-/-/-): 5 mice, Naive OF (S/-/-): 5 mice, Exp+sOF (S/+/+): 5 mice. * P < 0.05. Graphs show means ± SEM.

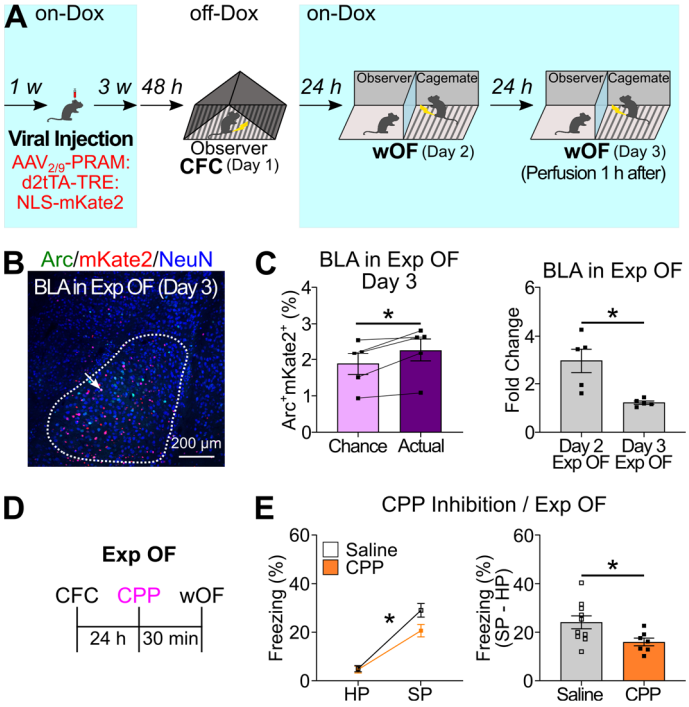

Figure S14. Role of NMDA receptor activation during Exp OF. Related to Figure 8.
(A) Reactivation of CFC-activated neurons in BLA during Day 3 Exp OF. **(B)** Coronal section of BLA in Day 3 Exp OF. **(C)** (Left) Actual percentages and chance levels of $Arc^{+}mKate2^{+}$ neurons in BLA in Day 3 Exp OF. t_4 = 3.26, P = 0.03. Paired t-test (two-sided). N = 5 mice. (Right) Fold change (Actual/Chance) analysis of $Arc^{+}mKate2^{+}$ neurons in BLA in Day 2 Exp OF (from Figure 4H) and BLA in Day 3 Exp OF. U = 0, P = 0.01. Mann-Whitney U-test. N = Day 2 Exp OF: 5 mice, Day 3 Exp OF: 5 mice. **(D)** Experimental schedule for administration of the NMDA-antagonist CPP in Exp OF. **(E)** (Left) Observer freezing levels during habituation period (HP) and shock period (SP) of Saline and CPP groups. $F_{(1,15)}$ = 5.43, P = 0.03. Interaction by two-way mixed ANOVA. (Right) Subtraction freezing levels. t_{15} = 2.33, P = 0.03. Unpaired t-test (two-sided). N = Saline: 10 mice, CPP: 7 mice (8 total. 1 mouse removed for HP freezing > 20%). * P < 0.05. Graphs show means ± SEM.

A) mKate2 (Day 1 CFC) and Arc positivity (Day 2) in Exp OF

	ACC	BLA	dCA1	vCA1
mKate2$^+$	10.74% ± 0.86%	9.73% ± 1.21%	5.99% ± 0.70%	21.19% ± 2.41%
Arc$^+$	13.61% ± 0.83%	10.11% ± 1.60%	23.69% ± 1.12%	8.81% ± 1.16%
mKate2$^+$Arc$^+$	0.68% ± 0.09%	2.76% ± 0.60%	0.92% ± 1.17%	2.24% ± 0.29%
Chance Level of mKate2$^+$Arc$^+$	1.47% ± 0.14%	0.97% ± 0.16%	1.41% ± 0.15%	1.80% ± 0.21%
Chance vs. Actual (P-value)	P = 0.004	P = 0.03	P = 0.02	P = 0.21
Fold Change	0.49 ± 0.08	2.96 ± 0.51 **	0.68 ± 0.10	1.31 ± 0.20
Mice	N = 6	N = 5	N = 6	N = 6

B) mKate2 (Day 1) and Arc positivity in BLA in different OF behavioral conditions

	W/+/+ (Day 2)	W/+/+ (Day 3)	-/+/+	W/+/-
mKate2$^+$	9.73% ± 1.21%	12.76% ± 1.19%	16.52% ± 1.73%	18.73% ± 2.23%
Arc$^+$	10.11% ± 1.60%	7.37% ± 0.97%	6.33% ± 0.32%	8.76% ± 0.99%
mKate2$^+$Arc$^+$	2.76% ± 0.60%	1.14% ± 0.16%	1.09% ± 0.09%	1.43% ± 0.27%
Chance Level of mKate2$^+$Arc$^+$	0.97% ± 0.16%	0.94% ± 0.15%	1.03% ± 0.07%	1.60% ± 0.19%
Chance vs. Actual (P-value)	P = 0.03	P = 0.03	P = 0.41	P = 0.38
Fold Change	2.96 ± 0.51 **	1.22 ± 0.09	1.06 ± 0.06	0.87 ± 0.12
Mice	N = 5	N = 5	N = 5	N = 6

C) mKate2 (Day 1 CFC) and Arc positivity (Day 2) in conditioned context exposure

	BLA	dCA1
mKate2$^+$	15.32% ± 1.70%	5.26% ± 0.30%
Arc$^+$	5.49% ± 0.59%	20.86% ± 0.82%
mKate2$^+$Arc$^+$	1.05% ± 0.13%	1.47% ± 0.08%
Chance Level of mKate2$^+$Arc$^+$	0.83% ± 0.11%	1.10% ± 0.09%
Chance vs. Actual (P-value)	P = 0.04	P = 0.002
Fold Change	1.30 ± 0.12	1.36 ± 0.08
Mice	N = 5	N = 6

D) Arc positivity in Control vs. Exp OF groups

	ACC	BLA	LA	CeA	BMA	dCA1	vCA1	vCA1-BLA
Control Arc$^+$ %	10.73% ± 0.80%	5.53% ± 0.85%	6.03% ± 1.14%	1.26% ± 0.29%	4.38% ± 1.55%	24.76% ± 0.81%	18.97% ± 0.98%	5.88% ± 1.28%
Mice	N = 7	N = 5	N = 5	N = 5	N = 5	N = 4	N = 6	N = 5
Exp OF Arc$^+$ %	9.19% ± 1.34%	8.37% ± 0.83%	8.90% ± 1.50%	3.27% ± 0.90%	4.74% ± 0.68%	23.01% ± 0.89%	16.74% ± 1.68%	10.95% ± 0.95%
Mice	N = 8	N = 5	N = 5	N = 5	N = 5	N = 9	N = 6	N = 5
P-value	P = 0.32	* P = 0.04	P = 0.17	P = 0.07	P = 0.83	P = 0.20	P = 0.24	* P = 0.01

Supplemental Table 2. Summary Table. Related to Figure 8.

(A-C) Percentages of mKate2$^+$ neurons, Arc$^+$ neurons, mKate2$^+$Arc$^+$ neurons in different brain regions and behavioral conditions. "Chance vs. Actual (P-value)" indicates the comparison of the actual percentage of mKate2$^+$Arc$^+$ neurons with the chance level of mKate2$^+$Arc$^+$ neurons. ** in A, B indicates fold change is significantly different ($P < 0.01$) than other groups. Fold change is (Actual percentage / chance level of mKate2$^+$Arc$^+$ neurons). **(D)** Percentages of Arc$^+$ neurons in Exp OF or control condition (W/-/- for ACC; W/+/- for vCA1-BLA; W/-/+ for other brain regions). * indicates $P < 0.05$, significant difference in the percentages of Arc$^+$ neurons for a given region between the control and Exp OF group. Tables show means ± SEM.