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SUPPLEMENTARY INFORMATION

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Memory formation in the absence of experience

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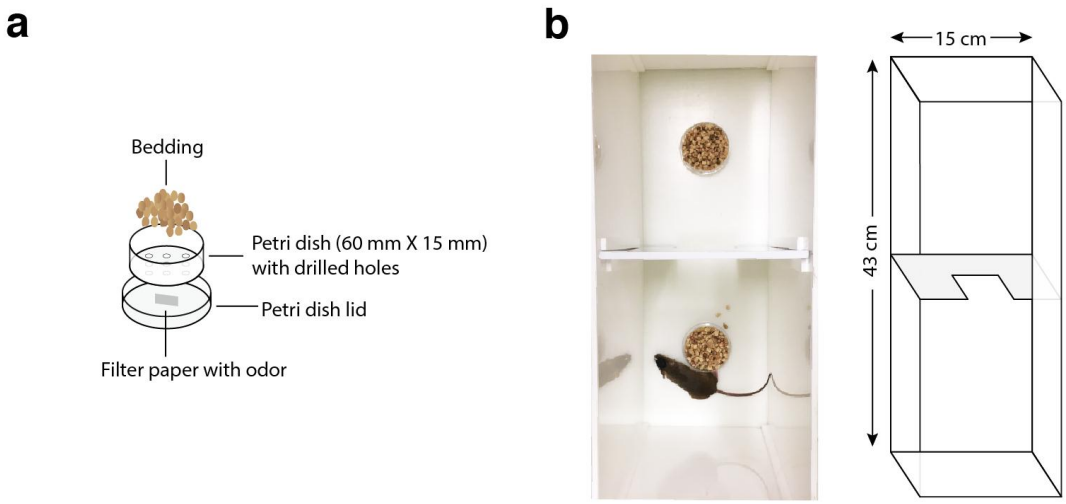
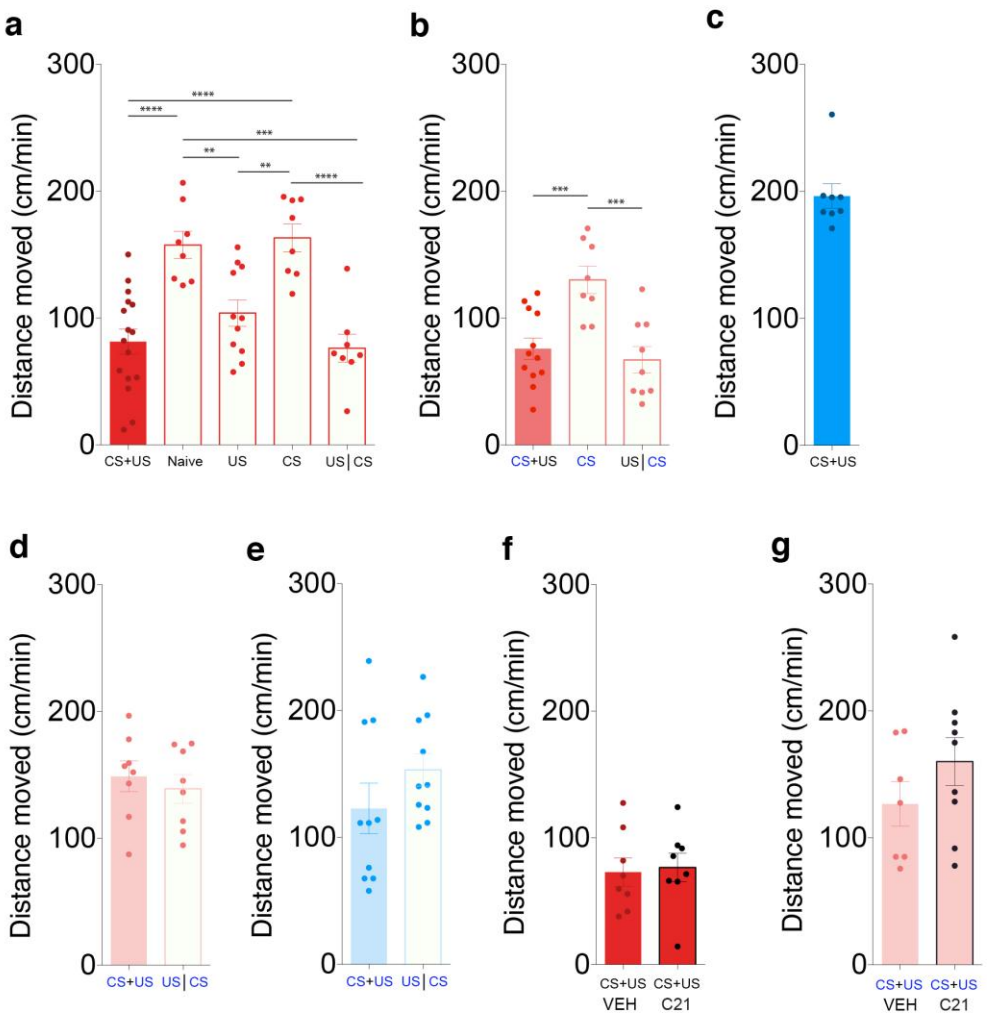


Supplementary Figure 1

Testing apparatus.

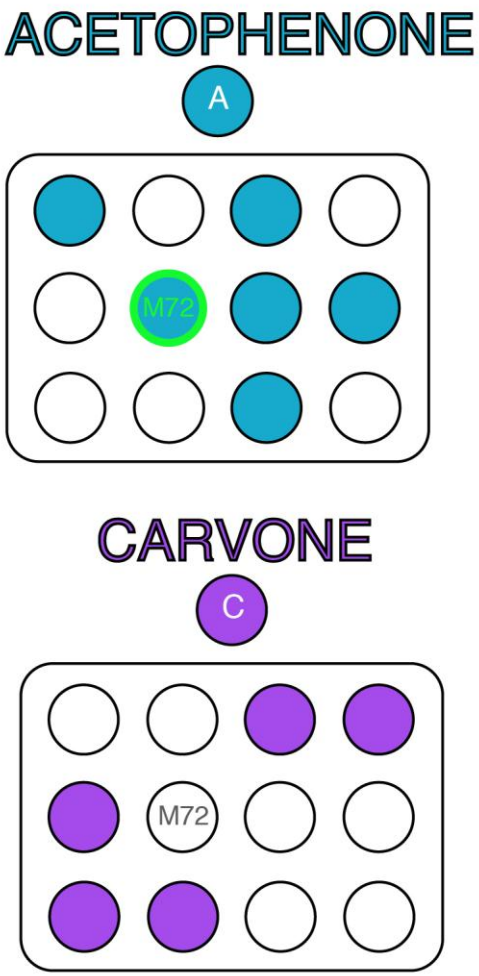
A, Odor preference was assessed in a rectangular box. Filter paper containing acetophenone or carvone was placed in the lid of a Petri dish. This was covered by a Petri dish (with 9 drilled holes), and then bedding. **B**, Photograph and schematic of test apparatus.



Supplementary Figure 2

Distance travelled in test sessions.

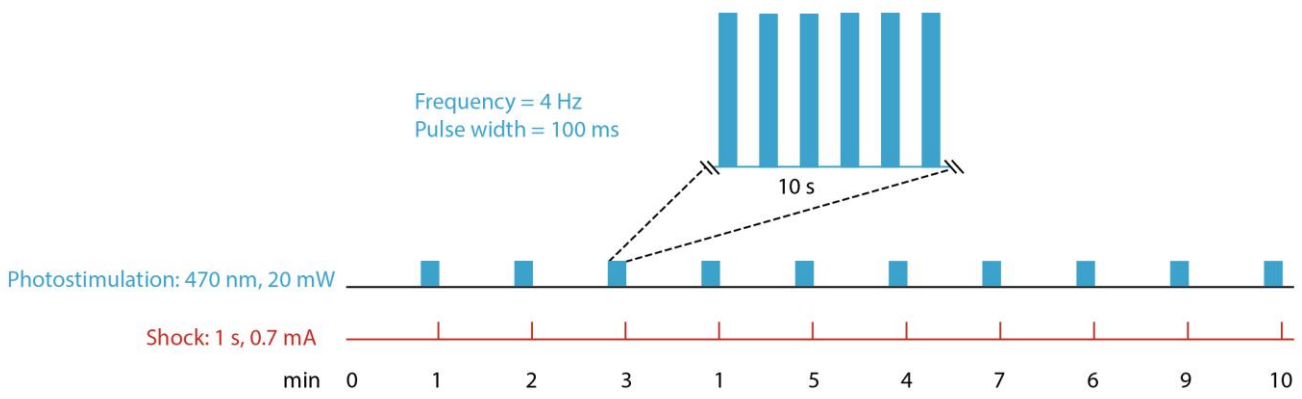
These graphs depict the distance travelled during the test session in each of the experiments. **a**, Acetophenone paired with shock (ANOVA, $F_{4,46} = 13.10$, $P < 0.0001$). (The data shown here corresponds to groups shown in Fig. 1b,c: CS + US group, $n = 15$ mice; naïve/home cage, $n = 8$ mice; US only, $n = 15$ mice; CS only, $n = 8$ mice; US|CS group, $n = 12$ mice). **b**, M72 photostimulation paired shock (ANOVA, $F_{2,26} = 10.86$, $P = 0.0004$). (The data here corresponds to groups shown in Fig. 1f, g: CS + US group, $n = 18$ mice; CS only, $n = 8$ mice; US|CS group, $n = 8$ mice). **c**, Acetophenone paired with food (corresponds to data shown in Supplementary Fig. 6, $n = 12$). **d**, M72 photostimulation paired with LHb stimulation (2 tailed t-test: $t_{14} = 0.59$, $P = 0.56$). (The data shown here corresponds to groups shown in Fig. 2d, e: CS + US, $n = 12$ mice; US|CS, $n = 8$ mice). **e**, M72 photostimulation paired with LDT stimulation (2 tailed t-test: $t_{18} = 1.29$, $P = 0.21$). (The data shown here corresponds to groups shown in Fig. 2i, j: CS + US, $n = 10$ mice; US|CS, $n = 10$ mice). **f**, Acetophenone paired with shock (2 tailed t-test: $t_{14} = 0.24$, $P = 0.81$) (real memory condition in BLA silencing experiment, corresponding to groups shown in Fig. 4d, e). **g**. M72 photostimulation paired with LHb photostimulation (2 tailed t-test: $t_{14} = 1.26$, $P = 0.23$) (real memory condition in BLA silencing experiment, corresponding to groups shown in Fig. 4i, j). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ ****$P < 0.0001$ by Newman-Keuls. Error bars = s.e.m.



Supplementary Figure 3

Schematic showing responses of the representative glomerular array (circles) to acetophenone and carvone.

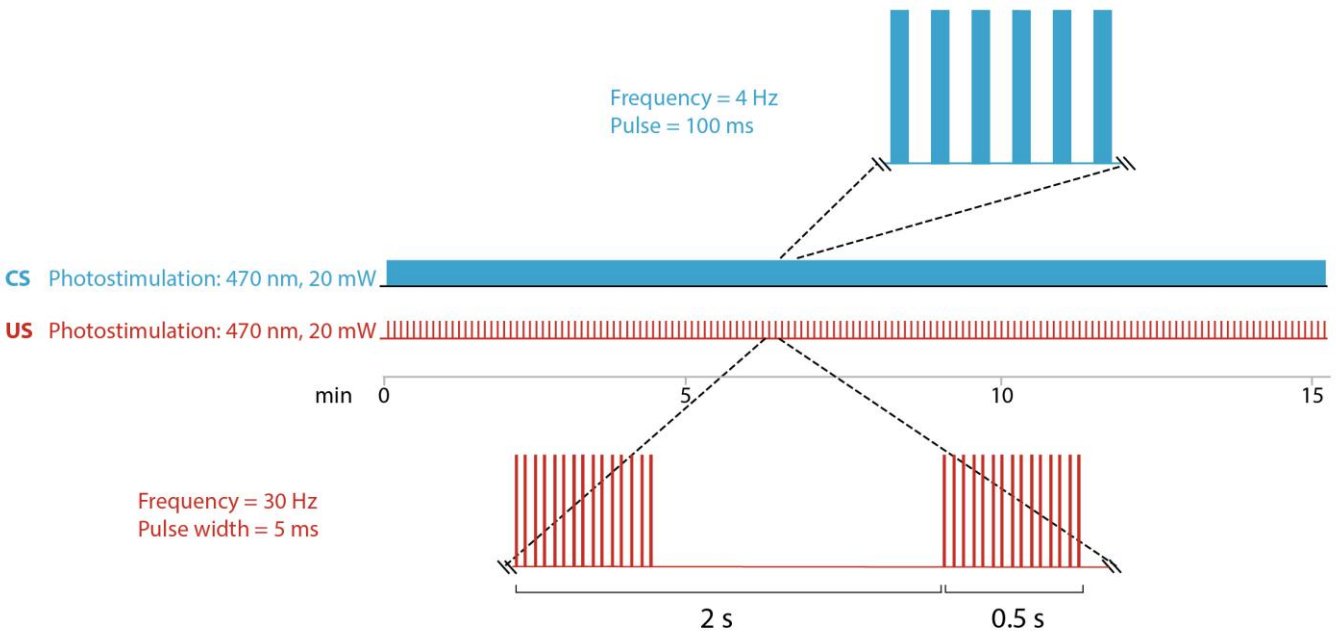
Acetophenone activates the M72 glomerulus (and other glomeruli). Carvone activates other glomeruli, but not the M72 glomerulus.



Supplementary Figure 4

Training protocol pairing M72 photostimulation with shock.

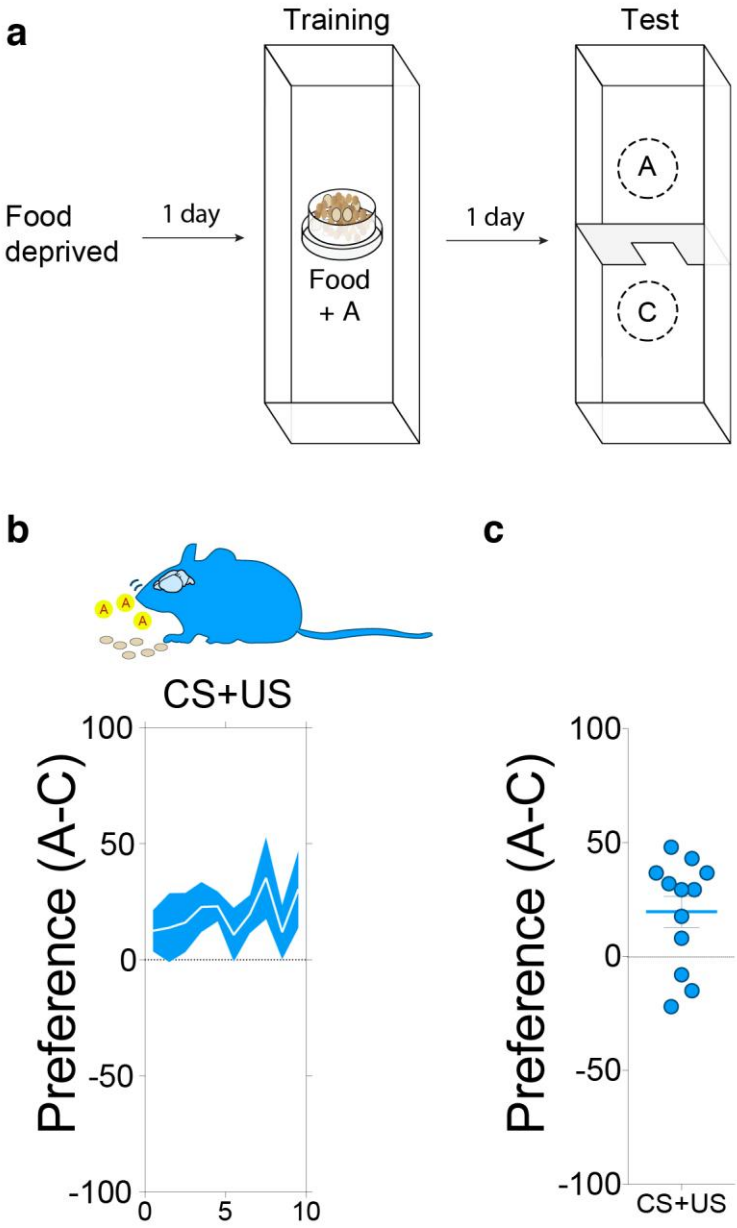
Each train included 40 pulses (pulse width 100 ms) delivered at 4 Hz. During the last 1 s of the train, a 0.7 mA shock was delivered. During training, mice received 10 photostimulation-shock pairs.



Supplementary Figure 5

Training protocol pairing M72 photostimulation (artificial CS) with photostimulation of either LHb-VTA or LDT-VTA projections (artificial USs).

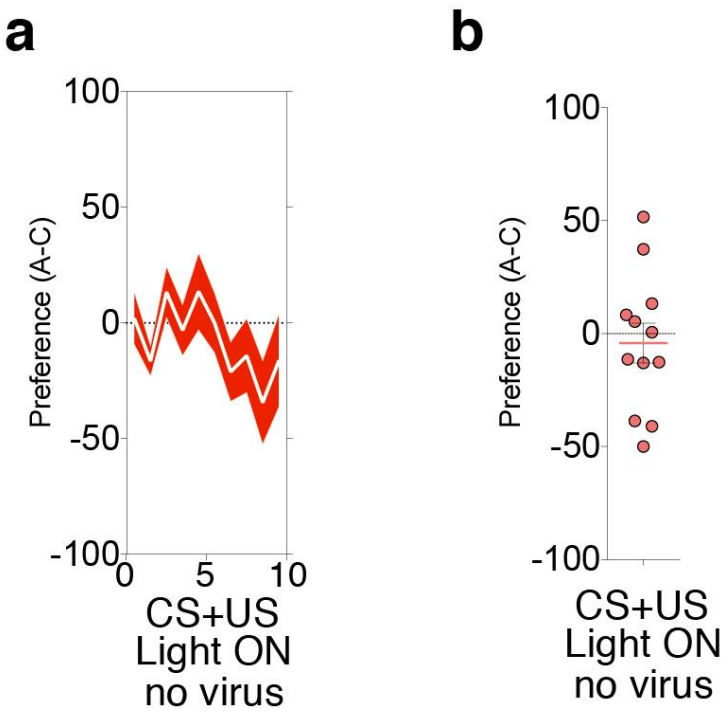
(Top) M72 stimulation occurred continuously through training, and consisted of 100 ms pulses, delivered at 4 Hz. (Bottom) Phasic VTA stimulation consisted of trains (0.5 s duration) of 5 ms pulses delivered at 30 Hz. Inter-train interval was 2 s.



Supplementary Figure 6

Formation of an odor-reward memory.

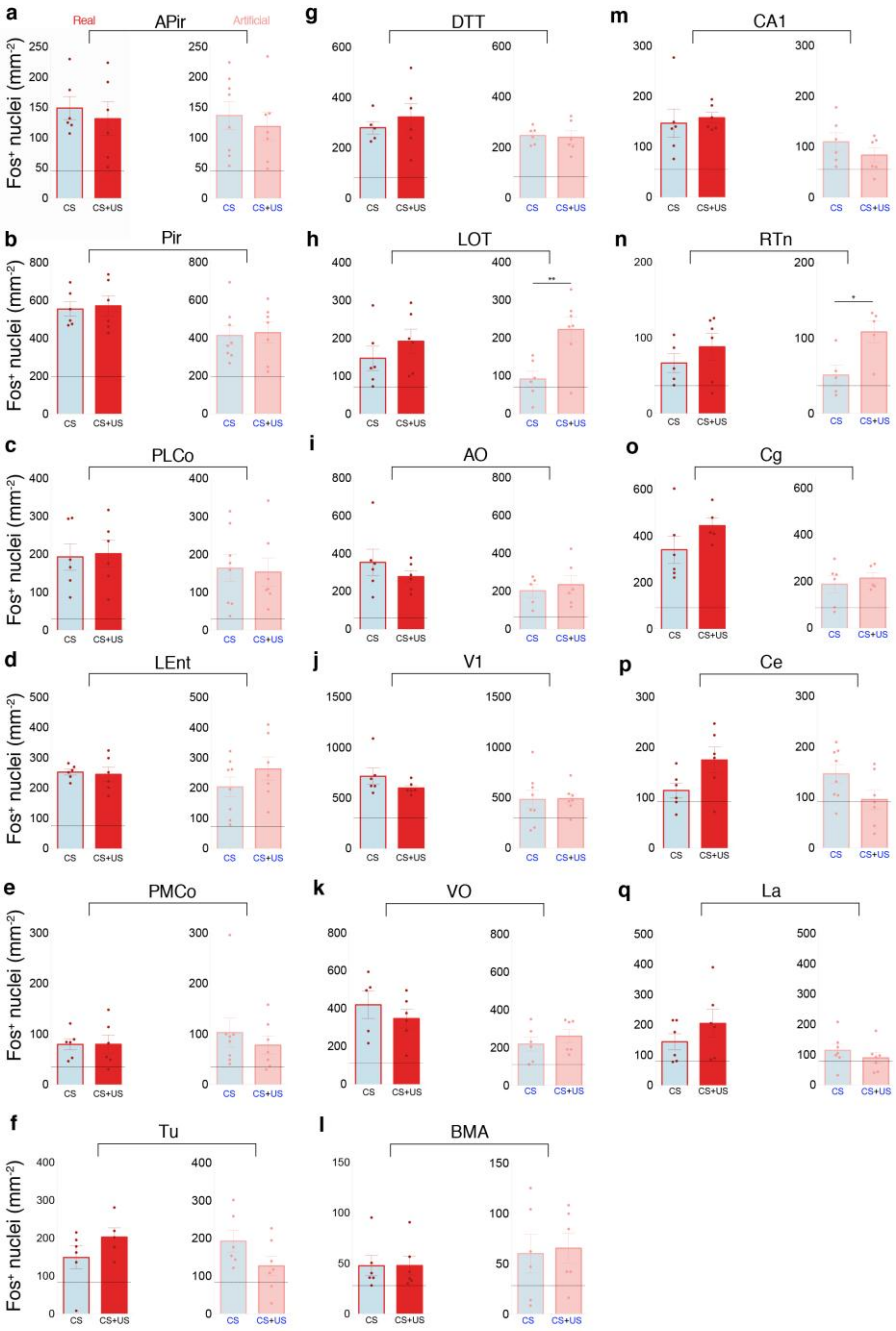
a, Schematic showing apparatus and training protocol. Mice were food-deprived one day prior to training. During training, acetophenone was paired with food, and preference (acetophenone vs. carvone) was evaluated one day later. **b,** In the test, mice (n = 12) spent more time on the acetophenone side of the apparatus (2 tailed, one sample t test vs. zero preference: t_{11} = 2.89, P = 0.015). Shading represents s.e.m. **c**, Summary data showing preference scores for individual mice. Error bar = s.e.m.



Supplementary Figure 7

VTA photostimulation alone does not act as a US during conditioning.

During training, acetophenone was paired with VTA photostimulation in mice (n = 12) not expressing ChR2. **a**, During testing mice did not exhibit conditioned aversion to acetophenone (2 tailed, one sample t test vs. zero preference: t_{11} = 0.47, P = 0.65). Shading represents s.e.m. **b**, Summary data showing preference scores for individual mice. Error bar = s.e.m.

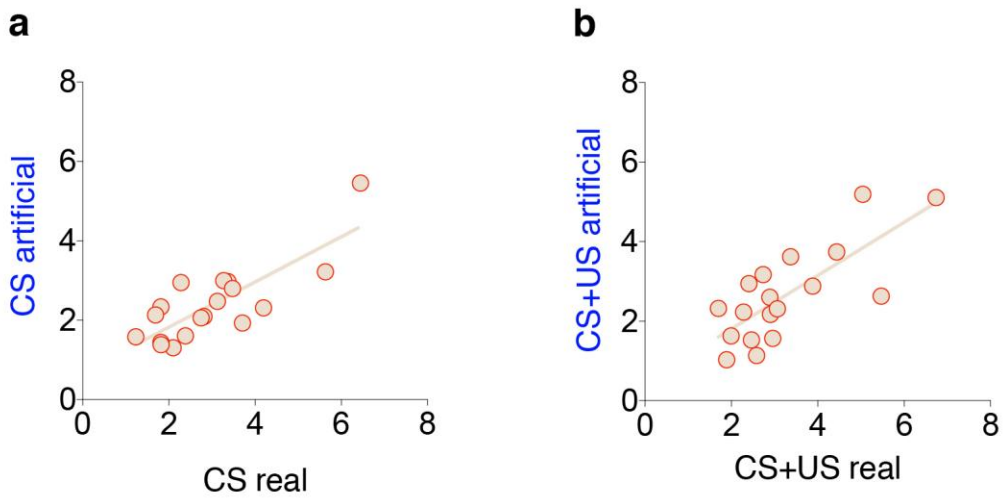


Supplementary Figure 8

Recall induced *c-Fos* expression.

During the training phase mice received either CS alone (i.e., acetophenone [n = 6] or M72 stimulation [n = 8]) or CS + US (i.e., acetophenone paired with shock [n = 6] or M72 stimulation paired with LHb-mVTA stimulation [n = 7]). During testing, the CS only was presented, and *c-Fos* induction assessed in 18 brain regions (see Fig. 3a, b). The numbers of *c-Fos*$^+$ nuclei are shown for mice in the 'real' (left) and 'artificial' (right) memory conditions. Error bars = s.e.m. Groups (CS + US vs. CS only) were compared by unpaired t-tests (2 tailed) in the real and artificial memory conditions. **a**, APir (real): t_{10} = 0.52, P = 0.62; APir (artificial): t_{13} = 0.56, P = 0.59. **b**, Pir

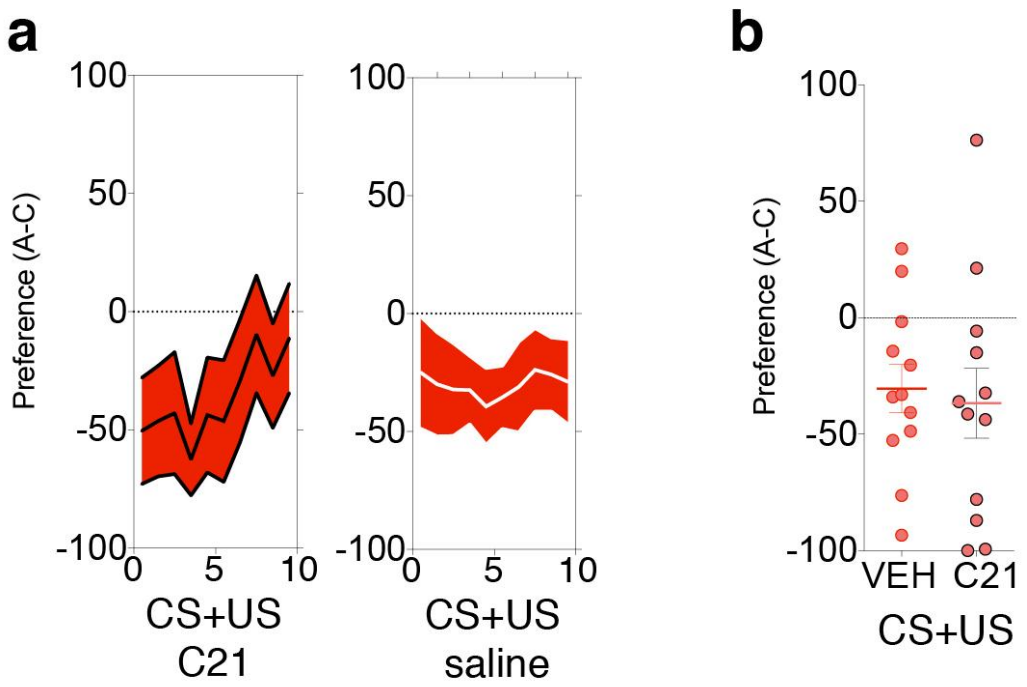
(real): t_{10} = 0.26, P = 0.80; Pir (artificial): t_{13} = 0.20, P = 0.84. **c**, PLCo (real): t_{10} = 0.17, P = 0.86; PLCo (artificial): t_{13} = 0.20, P = 0.84. **d**, LEnt (real): t_{10} = 0.29, P = 0.77; LEnt (artificial): t_{13} = 1.17, P = 0.26. **e**, PMCo (real): t_{10} = 0.00, P = 0.99; PMCo (artificial): t_{13} = 0.71, P = 0.49. **f**, Tu (real): t_{9} = 1.34, P = 0.21; Tu (artificial): t_{11} = 1.70, P = 0.12. **g**, DTT (real): t_{9} = 0.68, P = 0.52; DTT (artificial): t_{10} = 0.25, P = 0.80. **h**, LOT (real): t_{10} = 0.99, P = 0.35; LOT (artificial): t_{11} = 3.25, P = 0.0077. **i**, AO (real): t_{10} = 0.99, P = 0.34; AO (artificial): t_{9} = 0.53, P = 0.61. **j**, V1 (real): t_{10} = 1.41, P = 0.19; V1 (artificial): t_{13} = 0.06, P = 0.95. **k**, VO (real): t_{9} = 0.86, P = 0.41; VO (artificial): t_{10} = 0.81, P = 0.43. **l**, BMA (real): t_{10} = 0.02, P = 0.99; BMA (artificial): t_{10} = 0.22, P = 0.83. **m**, CA1 (real): t_{10} = 0.36, P = 0.73; CA1 (artificial): t_{10} = 1.13, P = 0.29. **n**, RTn (real): t_{9} = 0.95, P = 0.37; RTn (artificial): t_{8} = 2.90, P = 0.020. **o**, Cg (real): t_{9} = 1.43, P = 0.19; Cg (artificial): t_{9} = 0.58, P = 0.58. **p**, Ce (real): t_{10} = 2.05, P = 0.068; Ce (artificial): t_{13} = 1.91, P = 0.079. **q**, La (real): t_{10} = 1.13, P = 0.29; La (artificial): t_{12} = 0.92, P = 0.38. Abbreviations: APir = amygdalopiriform transition area; Pir = piriform cortex; PLCo = posterolateral cortical amygdaloid nucleus; LEnt = lateral entorhinal cortex; PMCo = posteromedial cortical amygdaloid nucleus; Tu = olfactory tubercle; DTT = dorsal tenia tecta; LOT = nucleus of the lateral olfactory tract; AO = anterior olfactory nucleus; V1 = primary visual cortex; VO = ventral orbital cortex; BMA = basomedial amygdaloid nucleus; CA1 = field CA1 of the hippocampus; RTn = rsotromedial tegmental nucleus; Cg = cingulate cortex; Ce = central amygdaloid nucleus; La = lateral amygdaloid nucleus.



Supplementary Figure 9

Regional *c-Fos* expression is highly correlated in real vs artificial conditions.

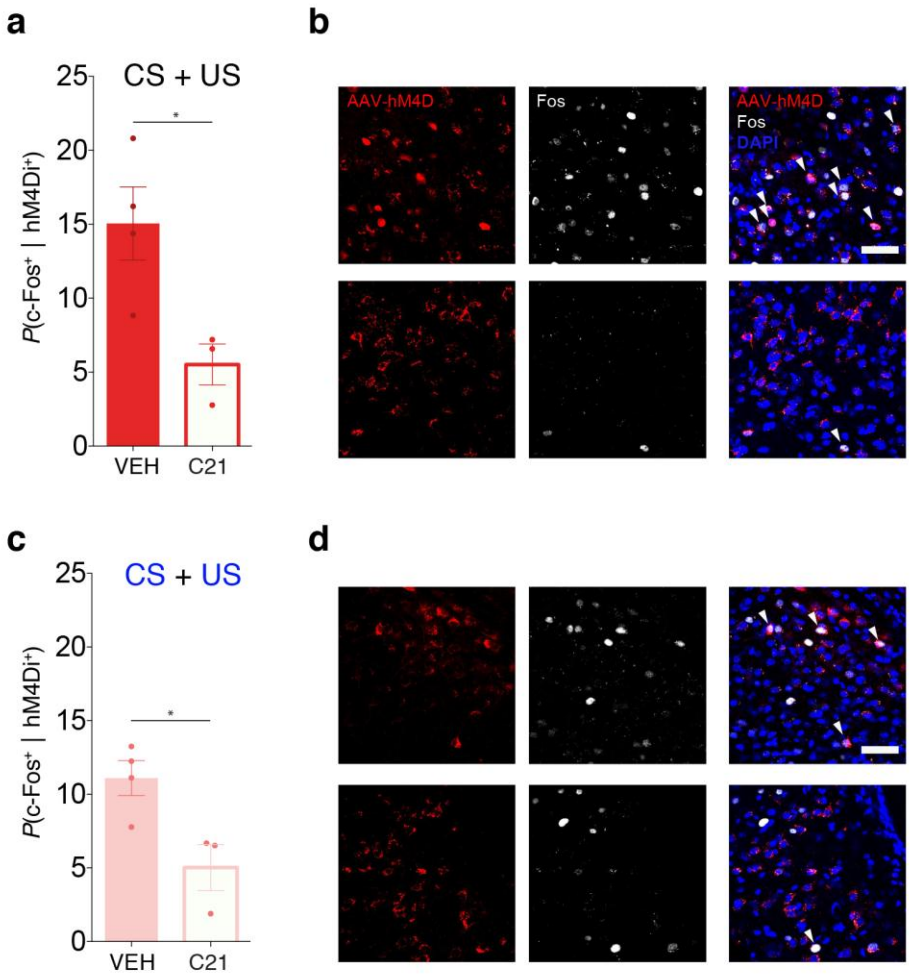
a, CS only condition. Regional (n = 18 regions) *c-Fos* expression is highly correlated in mice in the real vs artificial conditioning groups (Pearson's, r = 0.80, P < 0.0001). **b**, CS + US condition. Regional (n = 18 regions) *c-Fos* expression is highly correlated in mice in the real vs artificial conditioning groups (Pearson's, r = 0.77, P = 0.0002).



Supplementary Figure 10

C21 treatment alone does not affect conditioned odor aversion.

During training, acetophenone was paired with shock in mice not expressing the DREADD, hM4Di. Before testing, mice were treated with VEH (n = 12) or C21 (n = 12). **a**. Both VEH-treated (one sample, 2 tailed t test vs. zero preference: t_{11} = 2.92, P = 0.014) and C21-treated (one sample, 2 tailed t test vs. zero preference: t_{11} = 2.40, P = 0.033) mice avoided acetophenone. Shaded area depicts s.e.m. **b**. Summary data showing preference in VEH- and C21-treated mice did not differ (2 tailed t test, t_{22} = 0.34, P = 0.73). Error bars = s.e.m.



Supplementary Figure 11

C21 treatment reduces recall-induced activation of hM4Di-infected neurons in the BLA.

a, b. During training, acetophenone was paired with shock. During testing, VEH-treated mice (n = 4) or C21-treated mice (n = 3) were presented with the CS (acetophenone) and *c-Fos* was quantified in hM4Di$^+$ neurons in the BLA. C21 treatment reduced *c-Fos* expression (2 tailed t test, t_5 = 3.03, P = 0.029). **c, d**. During training, M72 stimulation was paired with LHb-VTA stimulation. During testing, VEH-treated mice (n = 4) or C21-treated mice (n = 3) were presented with the CS (M72 stimulation) and *c-Fos* was measured in hM4Di$^+$ neurons in the BLA. C21 treatment reduced *c-Fos* expression (2 tailed t test, t_5 = 3.15, $P < 0.05$). In all graphs, error bars = s.e.m. Scale bars (in b and d) = 50 µm.