Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/6/19/eaax7060/DC1

Supplementary Materials for

A persistent alcohol cue memory trace drives relapse to alcohol seeking after prolonged abstinence

Esther Visser, Mariana R. Matos, Rolinka J. van der Loo, Nathan J. Marchant, Taco J. de Vries, August B. Smit, Michel C. van den Oever*

*Corresponding author. Email: michel.vanden.oever@vu.nl

Published 6 May 2020, *Sci. Adv.* **6**, eaax7060 (2020)
DOI: 10.1126/sciadv.aax7060

This PDF file includes:

Figs. S1 to S8

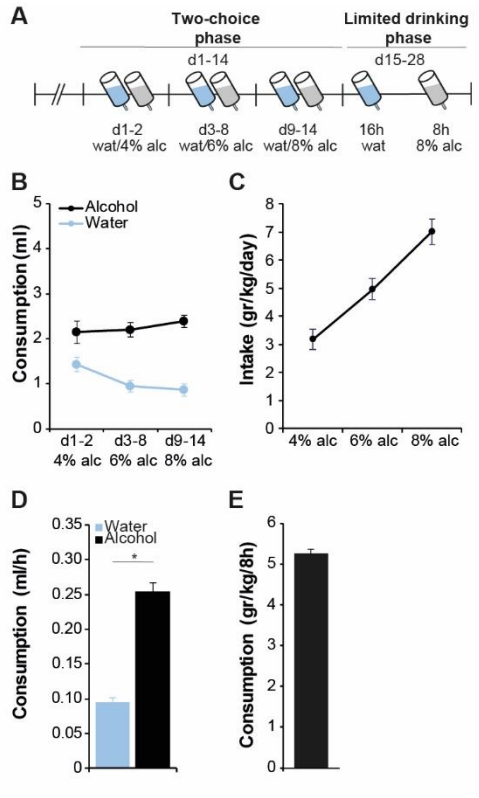

Figure S1. Habituation to alcohol SA in the home cage. (A) Experimental design. Habituation consisted of a two-choice phase and a limited drinking phase. During the two-choice phase, animals had unlimited access to a water bottle and a bottle with increasing concentrations of alcohol. In the limited drinking phase, mice had exclusively access to an 8% alcohol bottle for 8 h during their dark phase and to a water bottle for the remaining hours of the day. **(B)** Mice show a strong preference for alcohol over water during the two-choice phase (ANOVA: Solution $F_{1,21} = 22.16$, $P < 0.001$ and Time x Solution interaction $F_{1.50,31.44} = 3.65$ $P = 0.049$). **(C)** Intake of alcohol increased throughout the two-choice phase, $F_{2,42} = 50.89$, $P < 0.001$. **(D)** During the limited drinking phase, the average intake of alcohol per hour was higher than water $t(21) = -15.01$, $P < 0.001$. **(E)** Alcohol intake during the limited drinking phase in grams per kilogram per 8 hours.

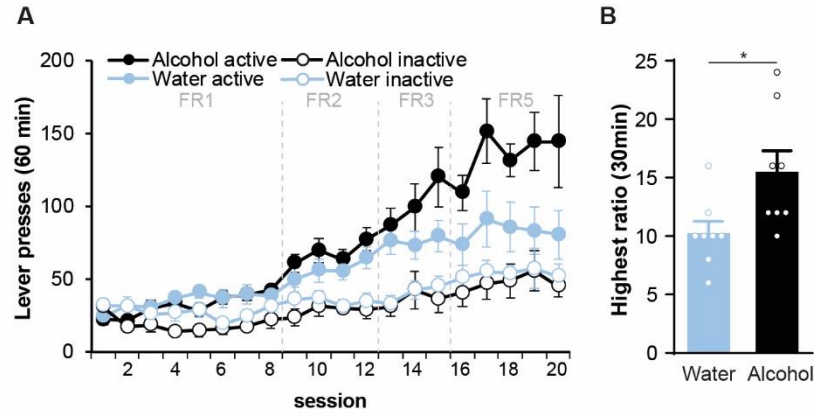

Figure S2. Mice are more motivated to lever press for alcohol than water. (A) Animals were trained in operant chambers to either lever press for 10 µl 8% alcohol (*N* = 8) or 10 µl water (*N = 8*) paired with the presentation of a discrete cue-light. ANOVA confirmed a Session x Lever x Group interaction ($F_{19,266}$ = 2.11, *P* = 0.005) indicating a difference in lever pressing behaviour between groups over sessions. Animals that received alcohol showed a stronger increase in active lever presses over sessions than animals that received water. ANOVA revealed a Session x Group interaction ($F_{19,266}$ = 3.00, *P* < 0.001) and a between Group difference ($F_{1,14}$ = 5.08, *P* = 0.041). Inactive lever presses were not different between groups and sessions (Session x Group: $F_{19,266}$ = 0.26, *P* = 1.00; Group: $F_{1,14}$ = 0.70, *P* =0.42). **(B)** Progressive Ratio test. The number of active lever presses required to obtain a reward increased by 2 for each successive reward. Due to technical problems, we were able to analyse the data of the first 30 min of the test only. Nonetheless, alcohol mice reached a higher ratio within 30 min than animals in the water group (*t*(14) = 2.53, **P* = 0.024), indicating that mice exhibited a higher motivational drive for alcohol.

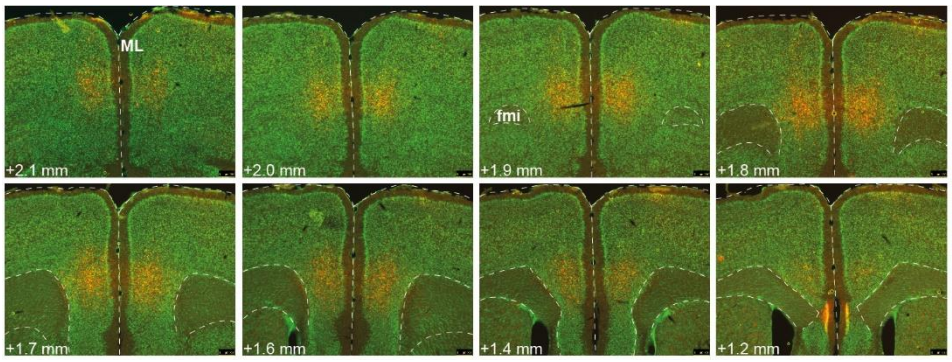

Figure S3. Example of hM4Di-mCherry expression in the mPFC along the anterior-posterior axis. A mixture of AAV-*Fos*::CreER^{T2} and AAV-*hSyn*::DIO-hM4Di-mCherry was injected into the prelimbic cortex of the mPFC. Neurons expressing hM4Di-mCherry were observed along the entire anterior-posterior axis of the prelimbic cortex and to some extend in the anterior cingulate cortex. Coordinates relative to Bregma (based on the Paxinos and Franklin mouse brain atlas are depicted in the bottom left of each image. Scale bar = 250 µm. ML = midline, fmi = forceps minor of the corpus callosum.

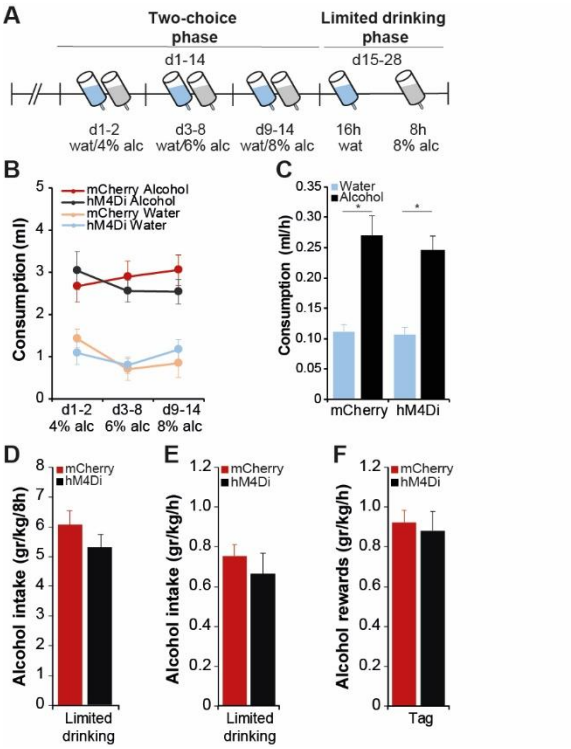

Figure S4. Alcohol habituation in mice that received viral-TRAP. (A) Experimental design of the habituation period. Groups: mCherry (N = 7), hM4Di-mCherry (N = 8). **(B)** Two-choice phase. Both groups showed a strong preference for the alcohol bottle (ANOVA Solution: $F_{1,13}$ = 26.757, P < 0.001), with no differences between groups. **(C)** Limited choice phase. Both groups consumed more alcohol than water (ANOVA Solution: $F_{1,13}$ = 216.817, *P < 0.001). **(D+E)** Alcohol intake during the limited drinking phase was similar in both groups. Graphs show alcohol intake over the entire drinking period (D) and per hour (E) (t(13) = 1.11 P = 0.29). **(F)** The amount of alcohol earned during the tag session did not differ between groups (t(13) = 0.29 P = 0.78).

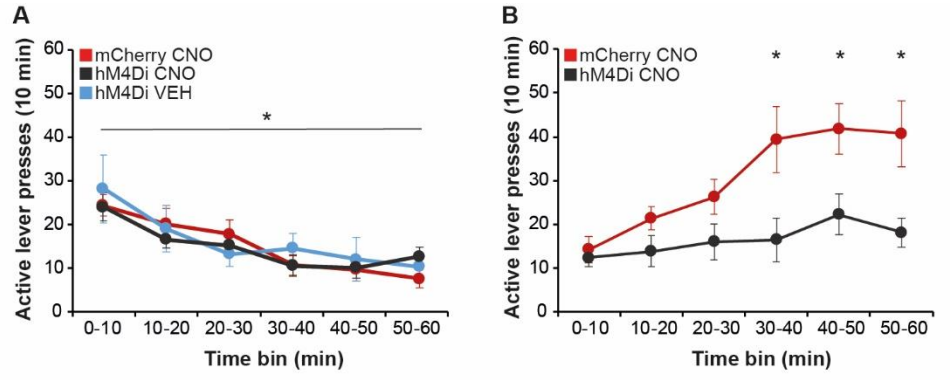

Figure S5. Active lever presses during the context- and cue-induced alcohol seeking tests. (A) All groups (mCherry (N = 7), hM4Di CNO (N = 8), hM4Di VEH (N = 6)from Fig. 2) gradually reduced their active lever presses during the context-induced alcohol seeking test ($F_{3.10,55.82}$ = 14.43, P <0.001), with no differences between the groups (no Group x Bin interaction or between-group effects). **(B)** For the reinstatement test, we observed a significant Time Bin x Group interaction ($F_{3.41,78.31}$ = 2.89, P = 0.035). Whereas mCherry animals (N = 11) showed a strong increase in active lever presses ($F_{2.79,27.93}$ = 5.97, P = 0.003), responses of the hM4Di group (N = 14) remained stable during the session ($F_{5,65}$ = 1.69, P = 0.15). * P < 0.05.

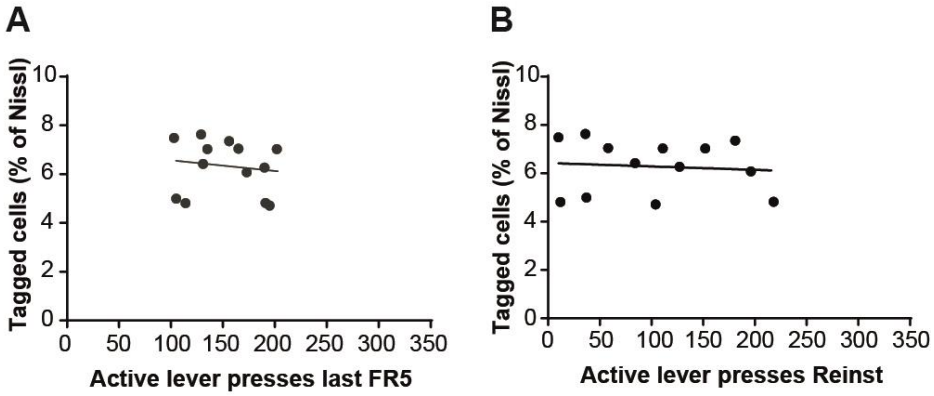

Figure S6. Regression analysis of alcohol SA-tagged cells and active lever pressing. (A) The number of active lever presses during the Tag session did not correlate with the number of hM4Di^{+} tagged cells (r^2 = 0.02, P = 0.64). **(B)** The number of active lever presses during reinstatement did not correlate with the number of hM4Di^{+}-tagged cells (r^2 = 0.008, P = 0.77). Datapoints from Fig. 3D.

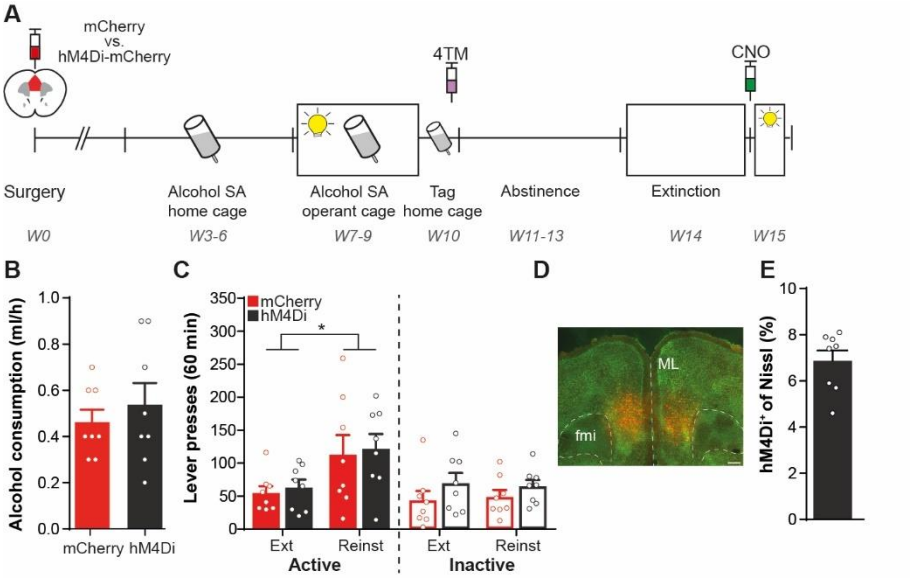

Figure S7. Chemogenetic suppression of a home-cage alcohol consumption-tagged ensemble did not affect cue-induced reinstatement. (A) Experimental design. Groups: mCherry (N = 8), hM4Di-mCherry (N = 8). Animals were trained as previously described, but the activated ensemble was tagged after 60 min of access to an 8% alcohol bottle in the home-cage in absence of specific alcohol-paired cues. **(B)** Alcohol consumption during the Tag session was similar in both groups (U = 36.5, P = 0.65). **(C)** Re-exposure to the alcohol-associated cue evoked reinstatement of alcohol seeking (Active lever presses: $F_{1,14}$ = 19.69, P = 0.001) and suppression of the ensemble did not affect reinstatement (Active lever presses: Session x Group $F_{1,14}$ = 0.001, P = 0.98). *P = 0.001. **(D)** Representative image of hM4Di-mCherry expression in the mPFC. **(E)** Quantification revealed hM4Di-mCherry expression in 6.9 ± 0.5% of mPFC neurons. Scale bar = 250 µm. ML = midline, fmi = forceps minor of the corpus callosum.

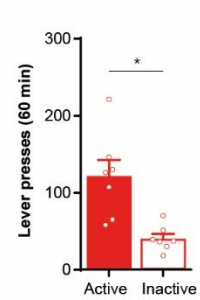

Figure S8. Lever pressing during the second cue-induced sucrose seeking test. Responses of mCherry control animals during a second cue-induced reinstatement test (no CNO treatment) that was performed 24h after the first test (see Fig. 5). Animals still showed a preference for the active lever during the second test. Paired Student *t*-test: $t(6) = 4.54$, $*P = 0.004$.