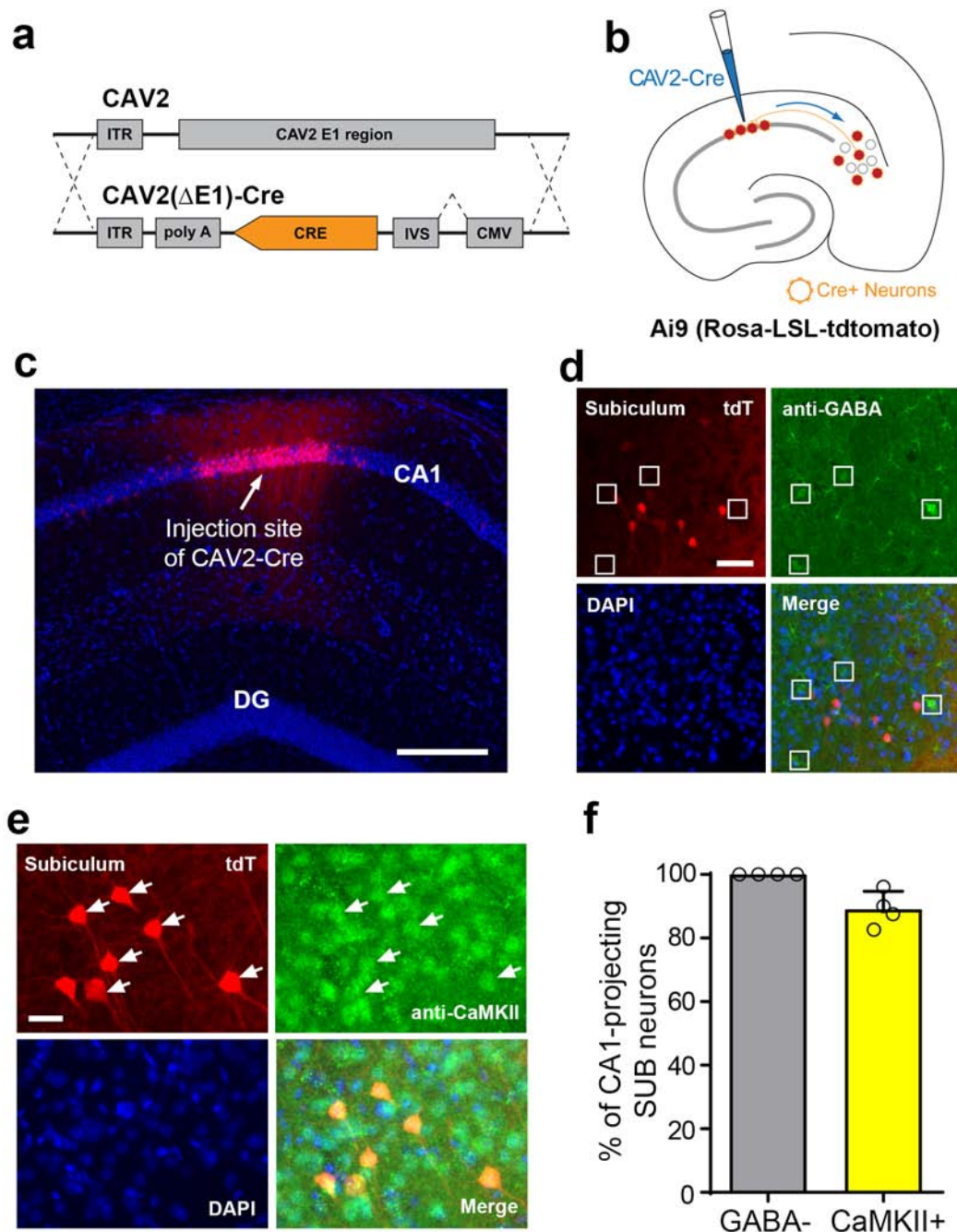


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CA1-projecting subiculum neurons facilitate object-place learning

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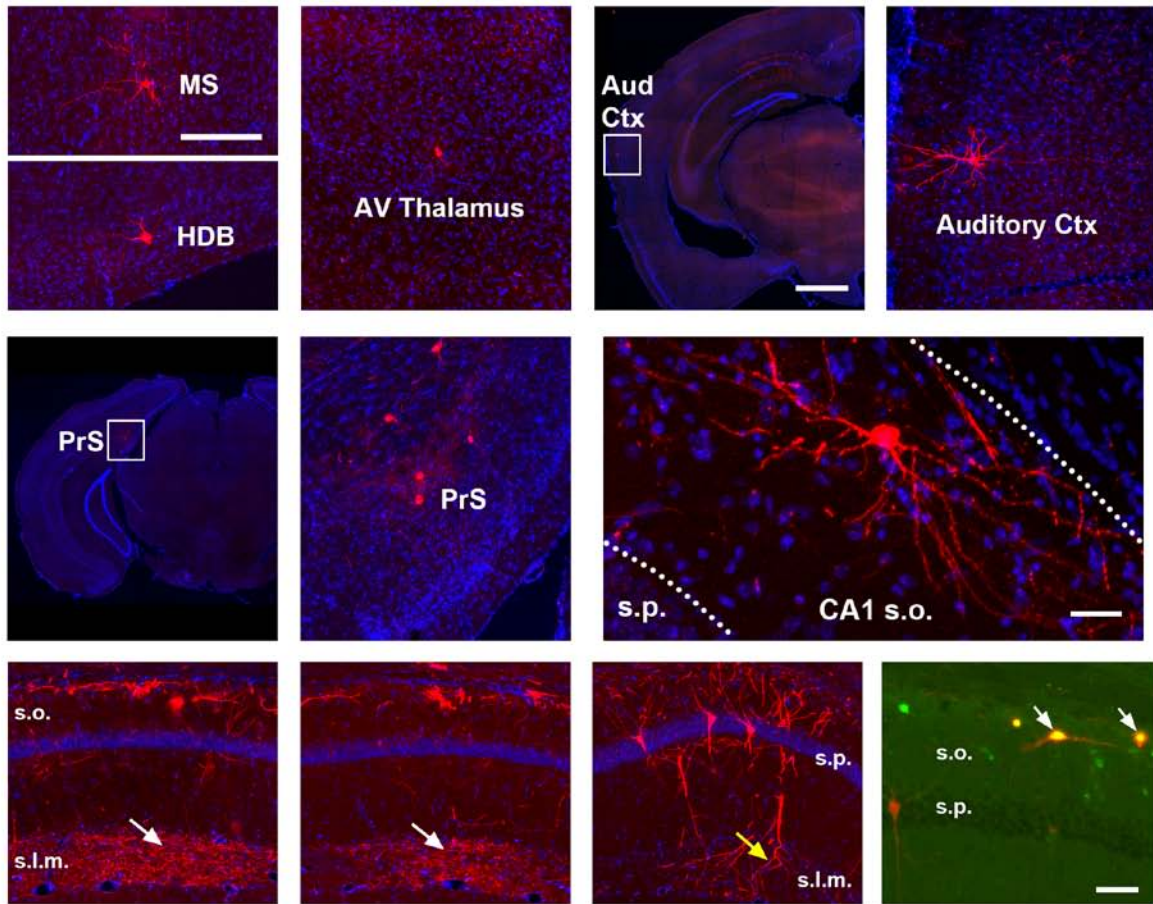
Supplementary Figure 1

Selective targeting of CA1-projecting SUB excitatory neurons by canine adenovirus 2 (CAV2)-mediated retrograde Cre expression.

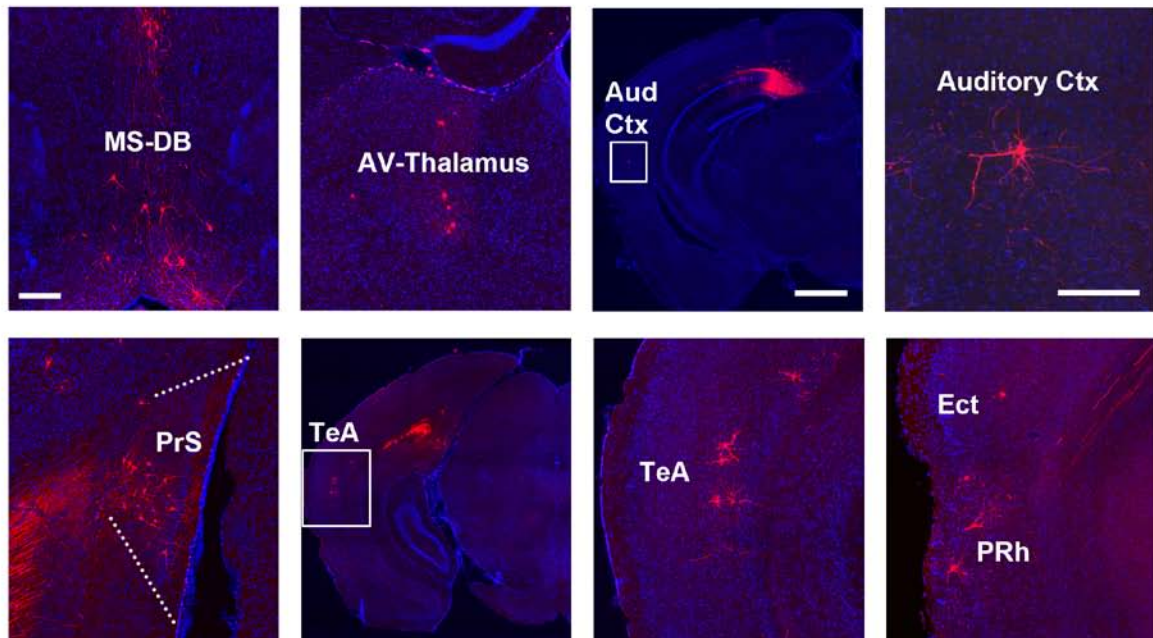
a, Schematic of E1-deleted CAV2 viral vector expressing Cre recombinase. **b**, Schematic illustration of CAV2-Cre injection in the CA1 of an Ai9 Cre reporter mouse (a tdTomato reporter). After injection, CAV2 is able to retrogradely transport to the CA1-projecting SUB neurons and express Cre-recombinase to trigger the expression of tdTomato in the neurons. **c**, An example image of the CAV2-Cre injection site in dorsal CA1 with a viral spread of ~300 μ m. tdTomato expression, shown in red, can be seen in the local infected neurons restricted to the pyramidal cell layer. DAPI staining is blue. Scale bar = 200 μ m. This characterization experiment was independently repeated in 5 mice, each with similar results. **d**, The top left panel shows CAV2-Cre labeled, tdTomato expressing CA1-

projecting SUB neurons. The top right panel shows GABA staining in green. The bottom left and right panels show DAPI stained image and the overlay image. The small white boxes indicate GABA immunopositive neurons that are negative for tdTomato expression. Scale bar = 50 μm . This staining experiment was independently repeated in samples from 4 mice, each with similar results. **e**, CaMKIIa immunostaining on the tdTomato (tdT)-labeled CA1-projecting SUB neurons supports the contention that the CAV2 mediated retrograde labeling is restricted to excitatory SUB neurons. White arrows point to examples of CaMKIIa immuno-positive CA1-projecting SUB neurons. Scale bar = 25 μm . This staining experiment was independently repeated in samples from 4 mice, each with similar results. **f**, Based on our measurements of relatively limited samples (45 cells from 4 different cases), the CAV2-Cre labeled CA1-projecting SUB neurons are devoid of GABA immunoreactivity. Based on the measurements of 97 CAV2-labeled subicular neurons from 4 different mice, 90% of them are CaMKIIa positive. Data are presented as mean $\pm$ SE in the bar graph.

a Monosynaptic inputs to CA1-projecting SUB neurons (from other brain regions)



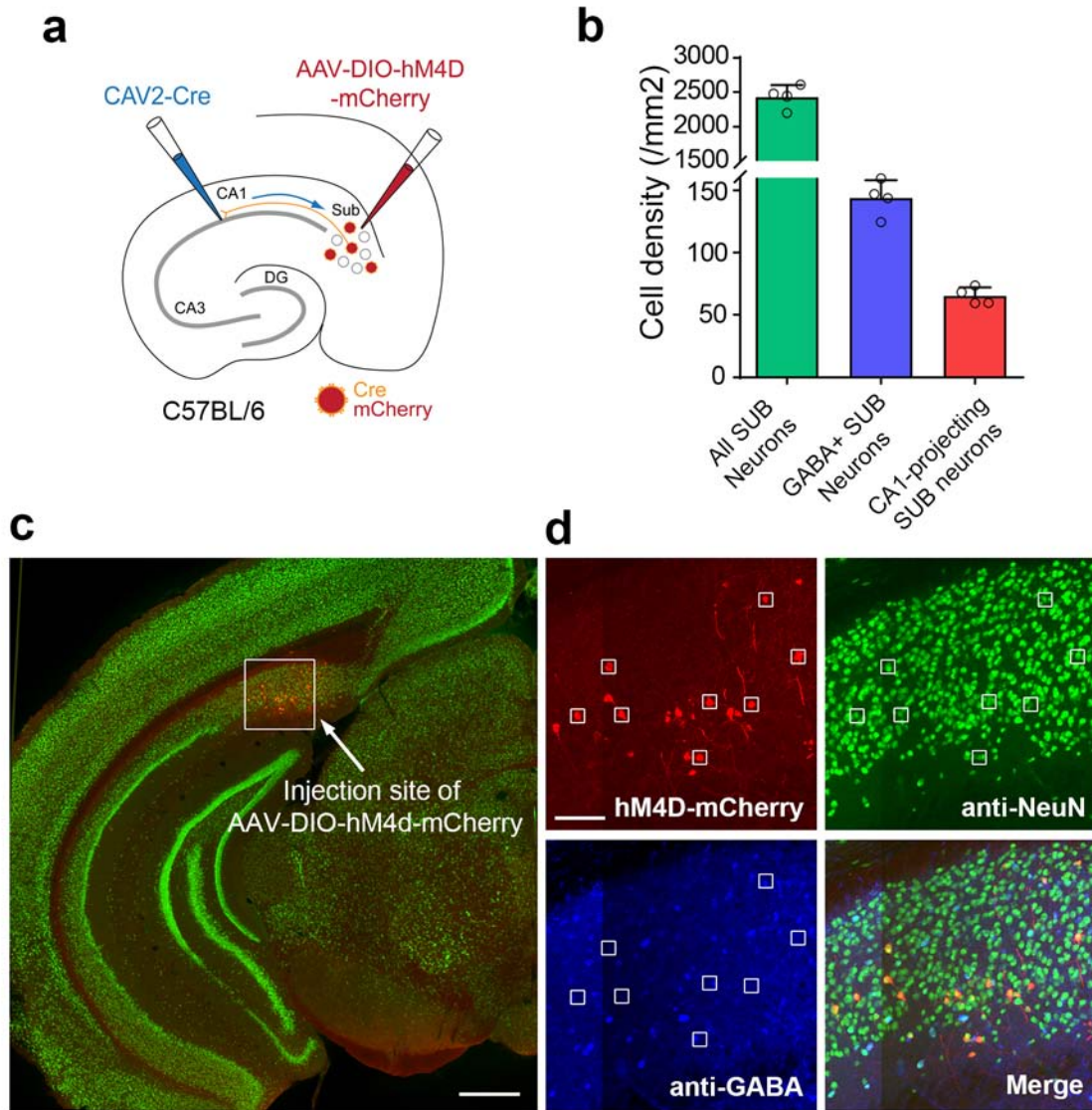
b Monosynaptic inputs to overall excitatory SUB neurons (from other brain regions)



Supplementary Figure 2

Other input mapped brain regions for different SUB neuron types.

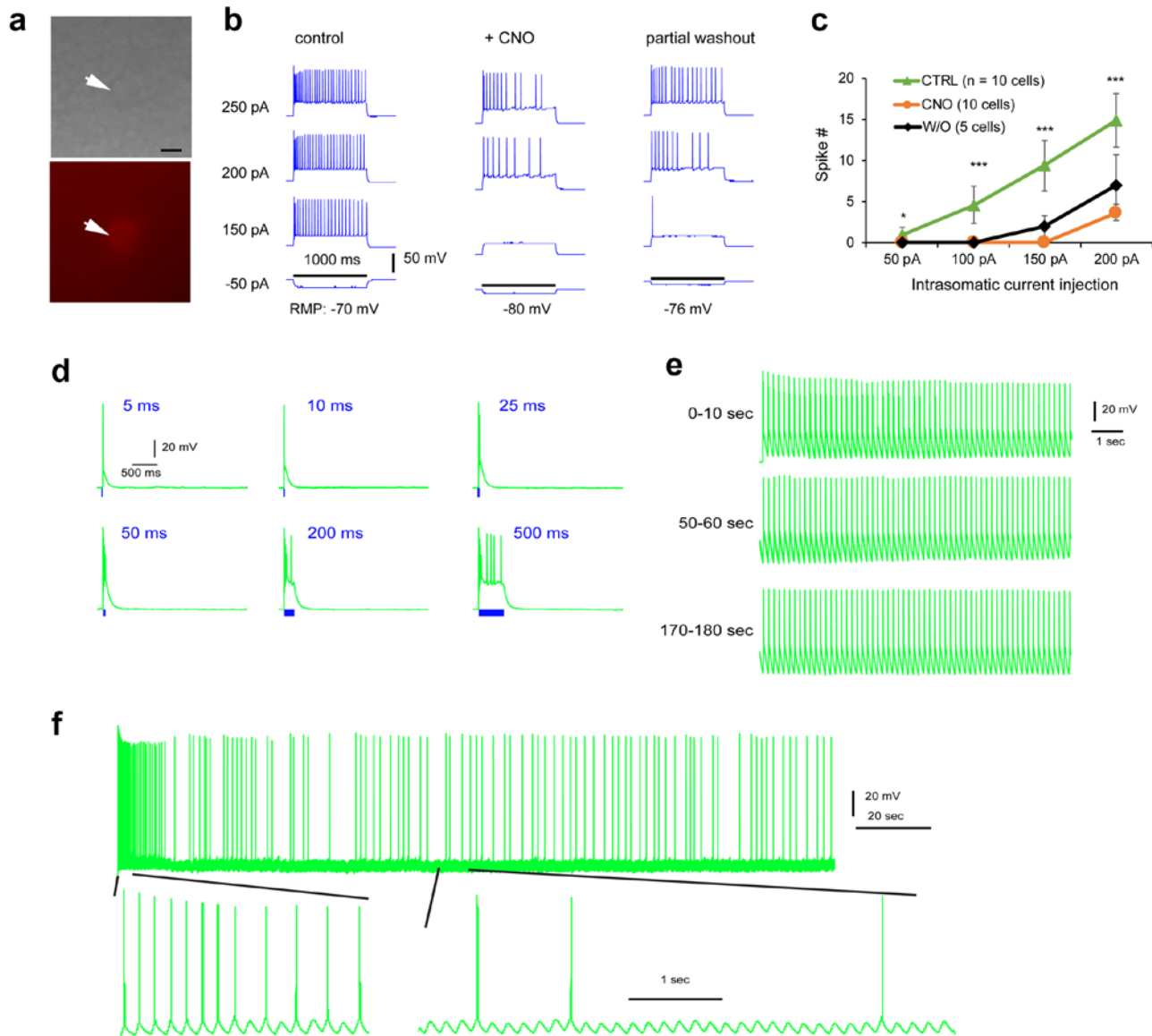
a, Input connection mapping of CA1-projecting SUB excitatory neurons. Input mapped neurons in the medial septum and diagonal band (MS/HDB), anterior ventral thalamus (AV Thalamus), auditory cortex (Aud Ctx), and presubiculum (PrS) are shown. The scale bar (200 μ m) in the MS panel applies to other panels, except the low magnification panels of Aud Ctx and PrS (scale bar = 1 mm) and the last panel of the second row showing an example of labeled putative O-LM cells in the stratum oriens (s.o.) of dorsal hippocampal CA1 (scale bar = 25 μ m). In the bottom row, the first two panels show axonal plexuses (white arrows) in the stratum lacunosum moleculare (s.l.m.) that likely originate from rabies labeled inhibitory neurons in the s.o. layer. We often observed these dense axonal plexuses following rabies tracing of inputs to CA1-projecting SUB neurons. Given that these subiculum neurons receive no input from temporal association cortex, perirhinal cortex, entorhinal or entorhinal cortex, these axonal plexuses in CA1 s.l.m. are unlikely to originate from entorhinal cortex. This experiment was independently repeated in 6 mice, each with similar results obtained. The last panel of the bottom row shows somatostatin (SOM) immunostaining (green) of an example rabies labeled hippocampal section (scale bar = 50 μ m). White arrows point to SOM immuno-positive rabies labeled stratum oriens neurons. Based on the examination of SOM staining of rabies labeled cells in the s.o. layer (22 cells from 4 sections of 2 cases), we found that 73% of the interneurons located in the s.o. layer are immuno-positive for SOM. **b**, Input connection mapping of *Camk2a*-Cre SUB excitatory neurons. Input mapped neurons in MS-DB, AV-Thalamus, auditory cortex, presubiculum, temporal association cortex (TeA), Ectorhinal cortex (Ect), and Perirhinal cortex (PRh) are shown. The scale bar (200 μ m) in the MS-DB panel applies to other panels, except the low magnification panels including Aud Ctx and TeA (scale bar = 1 mm). The scale bar in the enlarged Aud Ctx panel, 200 μ m. This experiment was independently repeated in 5 mice, each with similar results.



Supplementary Figure 3

Characterization of CAV2-Cre labeled and DREADDs targeted CA1-projecting SUB neurons.

a, Targeted expression of DREADDs in CA1-projecting SUB neurons with dual injection of CAV2-Cre in CA1 and AAV2-DIO-hM4D-mCherry injection in SUB. **b**, Cell density quantification of overall subicular neurons (NeuN stained neurons), GABA+ subicular neurons, and CA1-projecting subicular neurons. The CAV2-Cre labeled CA1-projecting SUB neurons (from a single CAV2-Cre injection in CA1) account for $2.7\% \pm 0.1\%$ (mean $\pm$ SE, pooled from 4 cases) of the overall neuron population in the subiculum. **c-d**, An example section image shows the expression of hM4D-mCherry (red) in CA1 projecting SUB neurons. NeuN staining is shown in green. Scale bar = 500 μ m. **d**, An enlarged view of the white square area in **c** with GABA and NeuN immunostaining. Scale bar = 100 μ m. Essentially all CAV2-Cre labeled CA1-projecting SUB neurons are excitatory neurons. Based on the measurements of 241 CAV2-labeled subicular neurons from 4 different mice, 98% of them are GABA negative. The experiments were independently repeated in 4 mice, each with similar results.

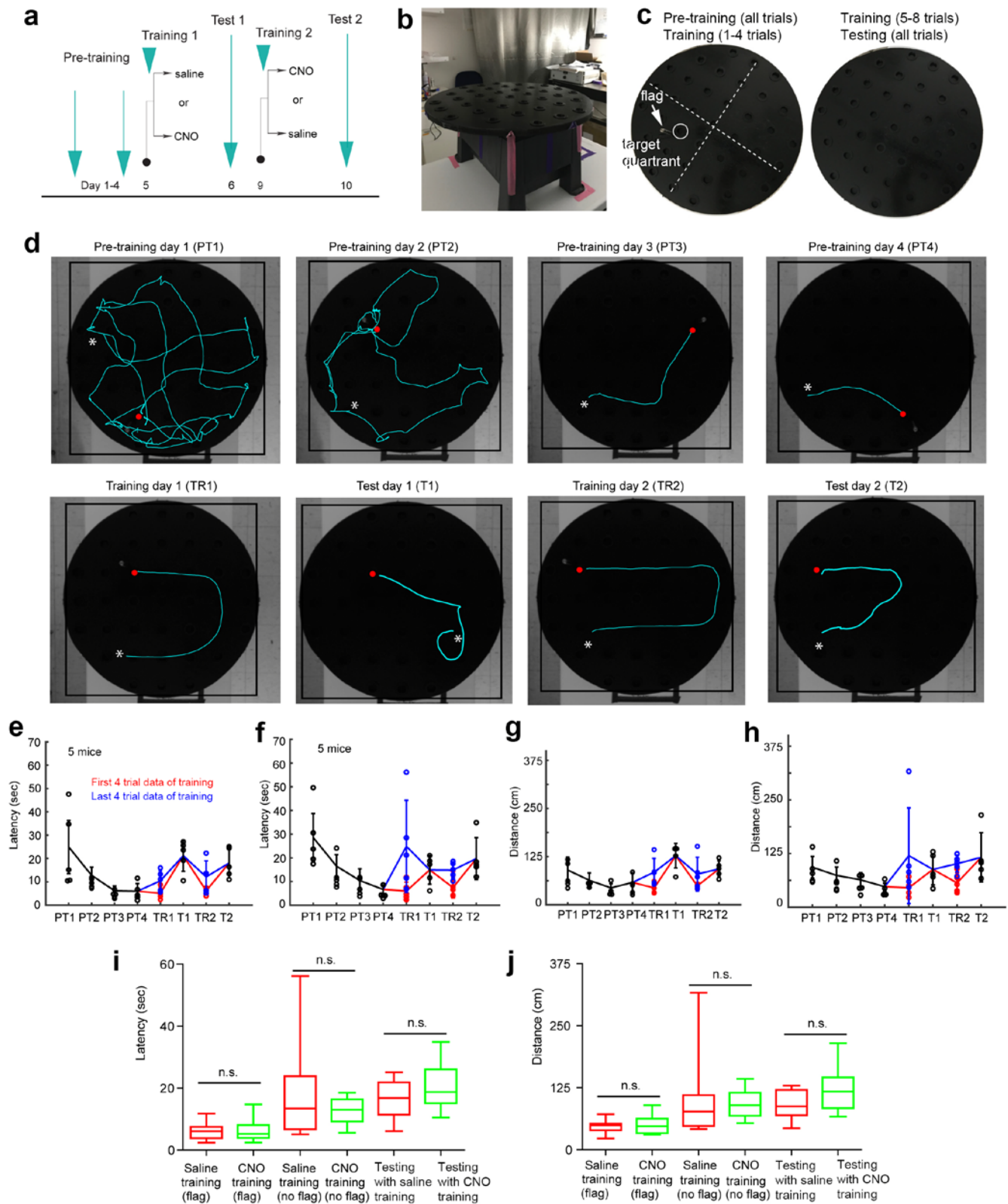


Supplementary Figure 4

***In vitro* validation of DREADDs-mediated SUB neuronal inactivation, and *in vitro* optogenetic stimulation of SUB excitatory neurons.**

a-c. SUB excitatory neuronal inactivation through the hM4D inhibitory DREADDs system. Four Camk2a-Cre mice were injected with AAV2-DIO-hM4D-mCherry injection in the subiculum; brain slices were made for slice electrophysiology. **a.** An example hM4D-mCherry expressing neuron was targeted based upon their red fluorescence (see the arrowheads). Scale bar, 10 μ m. **b.** The 5 μ M CNO application hyperpolarized the cell's resting membrane potential (RMP), and suppressed action potential firing by intrasomatic current injections. The average hyperpolarization of hM4D-expressing SUB excitatory neuronal resting membrane potentials was -5.3 ± 1.2 mV (mean $\pm$ SE, n = 10 cells), while there was no significant effect for the resting membrane potentials of control, non-hM4D expressing cells (0.86 ± 2.2 mV, n = 4 cells). **c.** Group data show that the CNO application exerts strong inhibition of evoked spiking activities of hM4D expressing SUB neurons. Overall, intrasomatic current injection at 50-100 pA induced suprathreshold spikes at

control (CTRL, n = 10 cells), while ~200 pA current injection were required to evoke spiking with CNO application to hM4D expressing neurons (n = 10 cells). The average numbers of spikes at control and during the CNO application differ significantly (*, $p < 0.05$; ***, $p < 0.001$). The data are presented as mean $\pm$ SE in the error bar plot; a two-tailed, paired t-test was used for each comparison. We also include the partial washout (W/O) data from 5 cells in the plot. **d-f.** *in vitro* tests of optogenetic stimulation of SUB excitatory neurons. Four Camk2a-Cre mice were injected with AAV1-DIO-ChR2-YFP injection in the subiculum; brain slices were made for slice electrophysiology. **d.** Example responses of a ChR2-expressing SUB neuron to different durations of 473 nm blue laser stimulation, applying a matched light intensity of *in vivo* behavioral experiments. The blue tick or short line beneath the response trace indicated the duration. The examination was repeated for at least 10 cells, each with similar results. **e.** The segments of the response trace of an example cell illustrate faithful spiking responses to laser stimulation (6 Hz, 50 ms) for 3 minutes. Eight of the 15 recorded cells belong to this group. **f.** In comparison, another example cell responded less reliably with spikes (but with clear modulation of subthreshold membrane potentials) with the same laser stimulation. Seven of the 15 recorded cells belong to this group. Overall, these data support effective optogenetic stimulation of SUB neurons *in vivo* with the application of the same stimulation protocol.

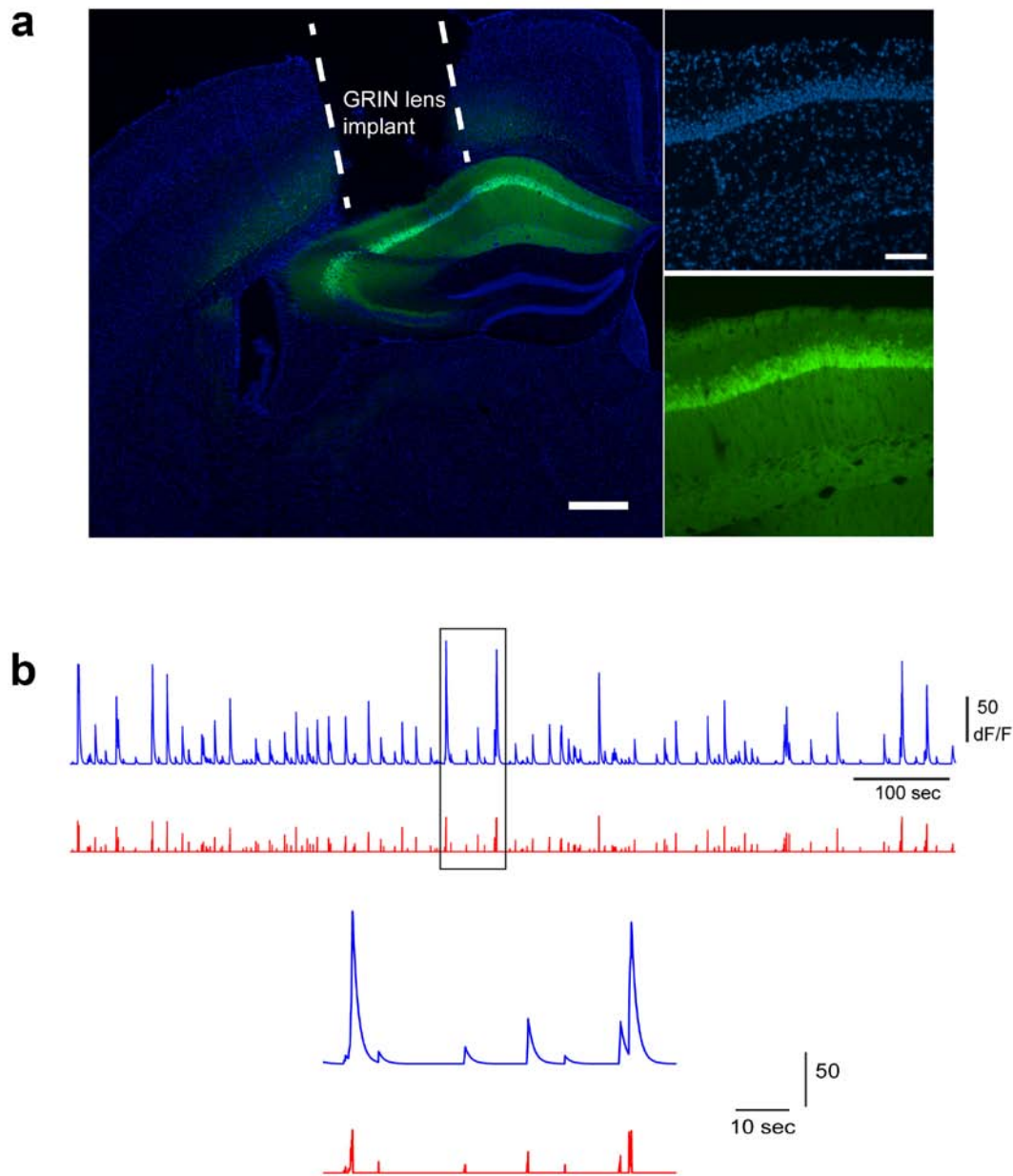


Supplementary Figure 5

DREADD-based inactivation of CA1-projecting SUB neurons does not impact mouse performance on a dry-land version of

Morris water maze test.

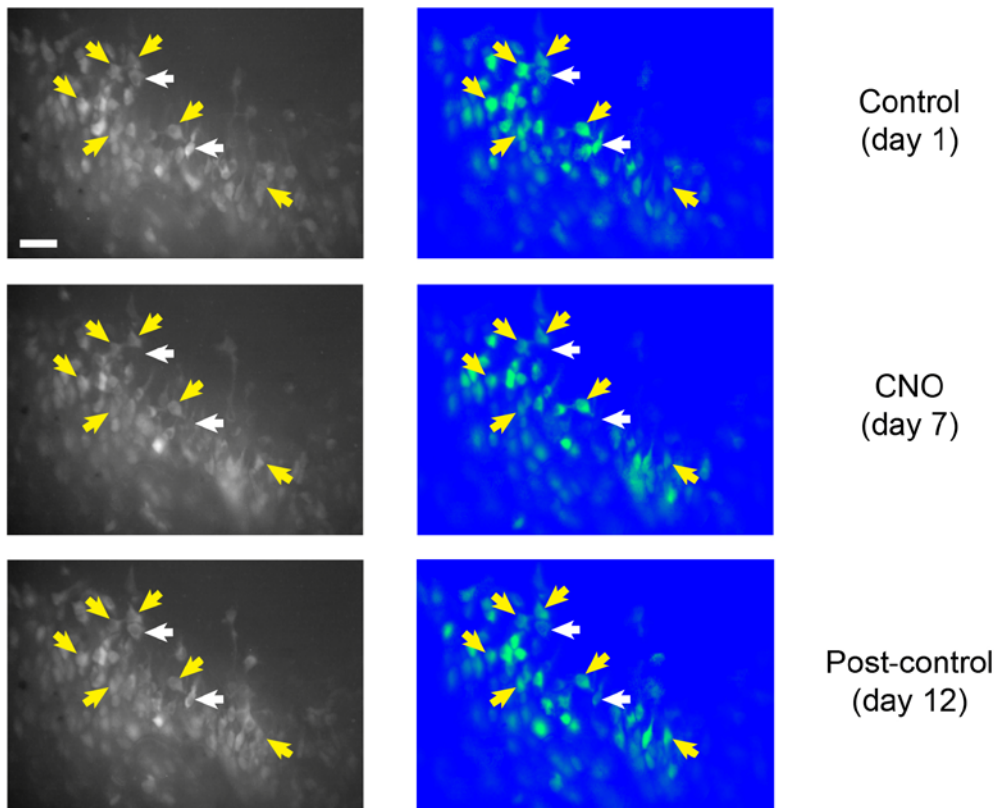
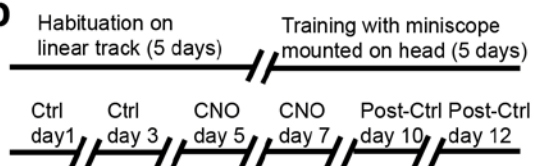
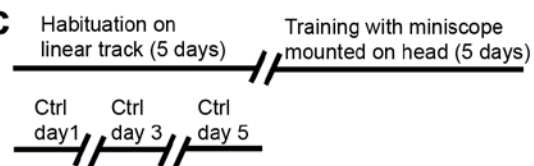
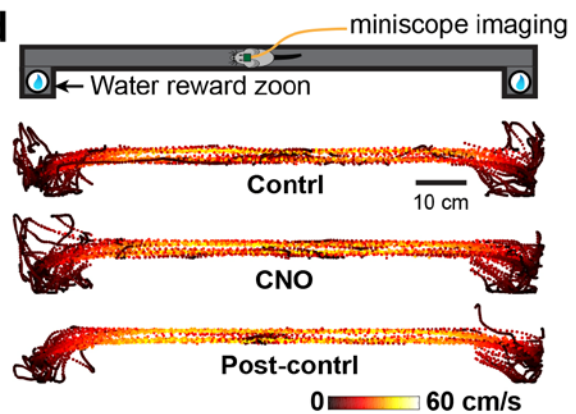
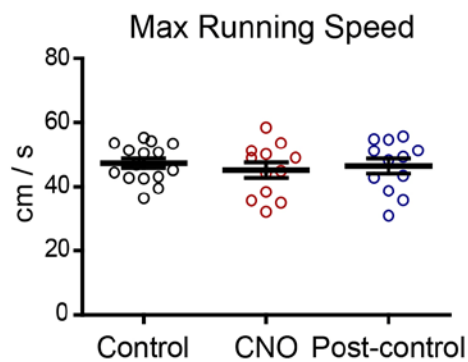
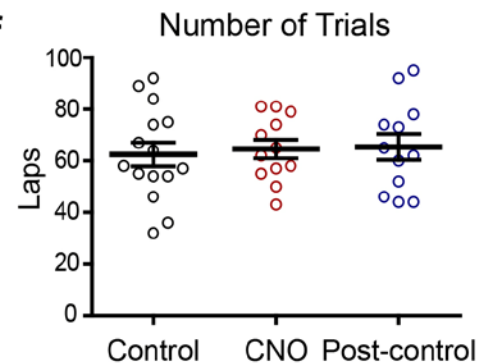
a. A schematic shows the experimental timeline. On the training day (day 5), the cohort of mice (10 mice with bilateral hM4D expression in CA1-projecting SUB neurons) were either injected with CNO or saline (5 mice each) at 45 minutes before training trials. For the retraining session (day 9), the two groups of mice received reversed CNO or saline treatments. **b** shows the experimental apparatus in a room with rich distal cues. **c.** Illustrations of a water cup location (white circle) flagged by a silver metal post in a targeted quadrant (left). Flagged targets were used on pre-training days (1-4) and the first 4 trials for training days (5 and 9). No flag marker was used for the last 4 trials of training days (5 and 9) nor for all 8 trials on testing days (6 and 10). **d.** Video-tracked movement trajectories at different dry-land maze task phases from an example mouse undergoing the task. Plotted examples are from Trial 1 of pre-training days (PT1-4), Trial 2 of training days (TR1 and TR2), Trial 4 of testing day 1 (T1), and Trial 5 of testing day 2 (T2). The mouse release location is indicated by a white asterisk, and the targeted cup location is labeled with a red dot, while the mouse movement trajectories are in cyan. This experiment was independently repeated in 5 mice per group, each with similar results. **e-j.** Latency to find the targeted cup location and distance traveled to find the location are determined to assess performance. Data are presented as mean $\pm$ SD in the error bar plots. **e-f.** Plots of latency for the two groups of mice with different orders of treatments on training days. Data are presented as mean $\pm$ SD, with trial results pooled from all 5 mice. The mice in **e** received CNO on training day 5 and saline on the training day 9, while the mice in **f** received saline on the training day 5 and CNO on training day 9. The data from the first 4 trials of training with a flag (red line) are plotted separately from the data from the last 4 trials of training without a flag (blue). **g-h.** Plots of distance travelled before reaching the target zone for the same two groups of mice with different orders of treatments on training days. **i-j.** Average latency and distance data of the 10 mice at different training and testing conditions. Overall, CNO-hM4D mediated inactivation of CA1-projecting SUB neurons does not affect animal's performance of the dry-land maze task, compared with the saline treatment control. For the box plots, the three box lines from top to bottom represent the 25th, 50th (median), and 75th percentile of data values of the samples. The whiskers extend to the most extreme values within 1.5 times of the interquartile range of the median. The Kruskal-Wallis test was used. Latency time: saline training (flag) vs. CNO training (flag): adjusted $p > 0.9999$; saline training (no flag) vs. CNO training (no flag): adjusted $p > 0.9999$; testing with saline training vs. testing with CNO training: adjusted $p > 0.9999$. Distance: saline training (flag) vs. CNO training (flag): adjusted $p > 0.9999$; saline training (no flag) vs. CNO training (no flag): adjusted $p > 0.9999$; testing with saline training vs. testing with CNO training: adjusted $p > 0.9999$.)



Supplementary Figure 6

Histology of the GRIN lens placement, and illustration of extraction of neural activity from calcium signals.

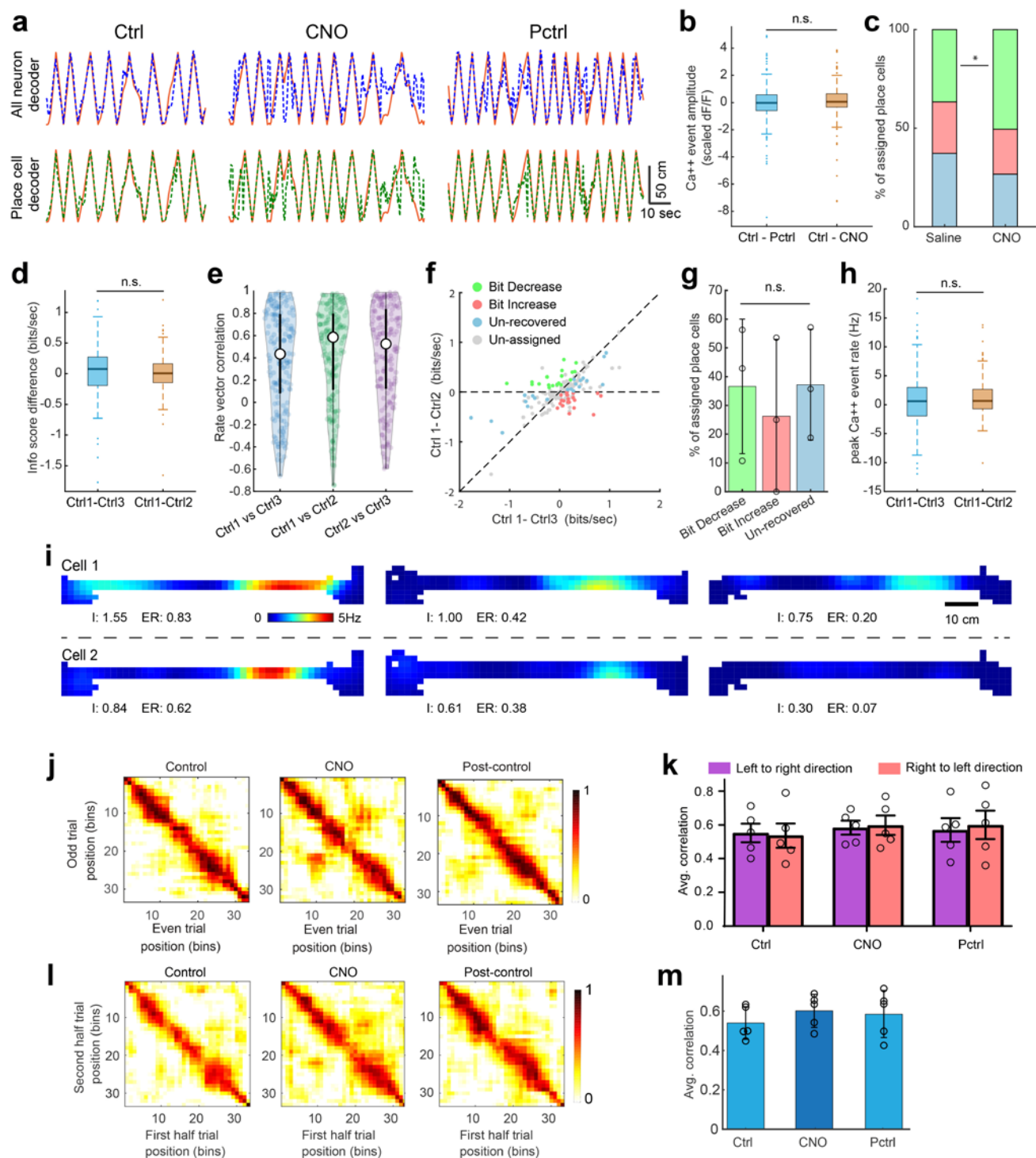
a. The left image panel shows a coronal brain section from an experiment mouse with the dashed lines indicating the boundaries of a GRIN lens implant. The DAPI staining is blue, and GCaMP6f expression is green. Right panels show an enlarged region of CA1 beneath the lens. Overall normal-looking structure of remaining tissue and robust GCaMP6f expression in stratum pyramidale provide support for our imaging of healthy CA1 place cells. Scale bars, 250 and 50 μm . **b.** The upper traces show the temporal trace of denoised calcium signal activity (blue line) from an example cell and the corresponding deconvolved spiking activity (red line). We use a metric similar to the scaled version of dF/F to indicate signal amplitudes. Lower traces show the expanded segment of both traces indicated in the black box above. We use deconvolved spiking activity for downstream analysis and apply a threshold to deconvolved spiking activity across all the sessions to identify effective calcium events. The experiments were independently repeated in 14 mice, each with similar results.

a**b****c****d****e****f**

Supplementary Figure 7

Imaging stability and mouse behavioral performance during *in vivo* GCaMP6f-based calcium imaging.

a, Left column: Three panels show maximum intensity projected images of CA1 neurons expressing GCaMP6f of control, CNO, and post-control sessions acquired from the same mouse during 15 min linear track running on the indicated days, respectively. Scale bar, 25 μ m. Right column: Three panels show the corresponding neurons extracted using the CNMF-E algorithm from the across-session concatenated video data (see the Methods). The yellow arrows track robustly activated example neurons across all sessions, while the white arrows point to the cells (two examples) that are identified during control and post-control session maps but do not show clear activation in the CNO session map. **b**, The experiment timeline of linear track running and imaging with CNO inactivation. **c**, The experiment timeline of linear track running and imaging with 3 consecutive saline injection control sessions. **d**, Three examples of dotted plots show animal running trajectories with color coded speed information. Brighter colors indicate higher speed. There is no significant difference of animal's running speeds across different conditions. **e**, Comparison of animal's maximum running speeds across control (15 sessions), CNO (12 sessions), and post-control (12 sessions) sessions from 6 mice. One-way ANOVA was used, $p = 0.075$. **f**, Comparison of animal's running laps on the linear track across control, CNO, and post-control sessions (as in **e**) from 6 mice. One-way ANOVA was used, $p = 0.89$. Data are presented as mean $\pm$ SE.

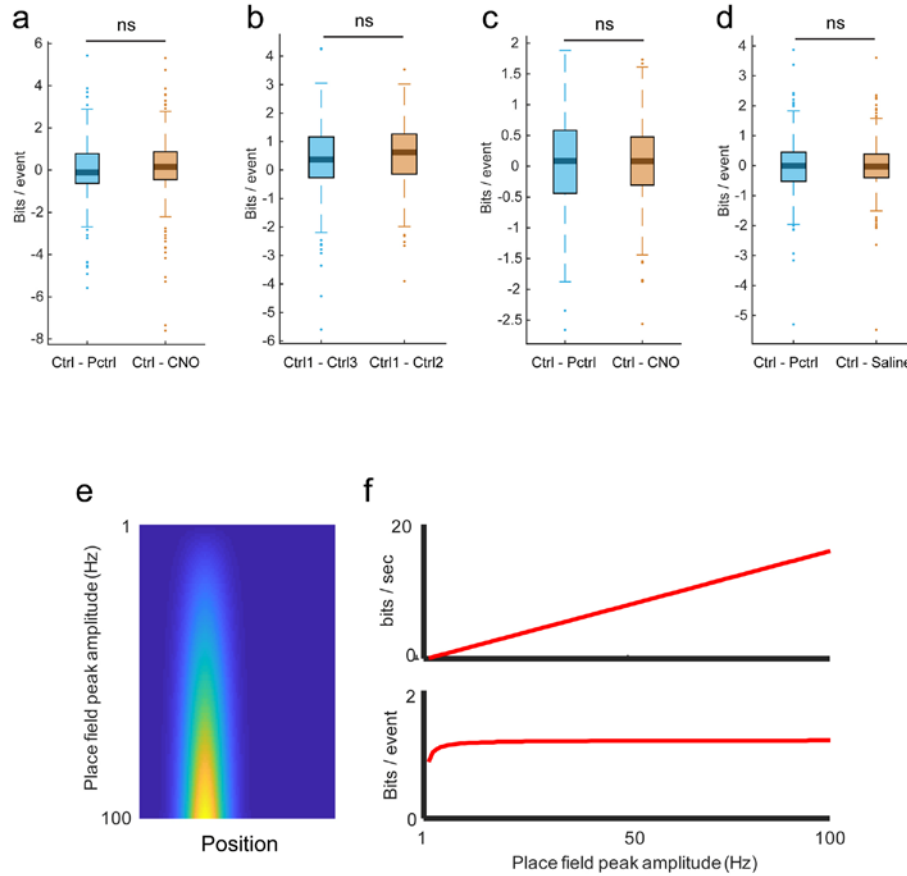


Supplementary Figure 8

Further analysis of linear track inactivation experiments, analysis of CNO-free linear track control experiments, and within-trial stability.

a, Examples of predicted animal positions using a decoder analysis with an artificial neural network model that was trained based on the first control session and then applied to the CNO and Post-control sessions (see Methods). Each column represents different sessions. The orange solid lines are the animal's actual running trajectories; colored dash lines are predicted trajectories based on the

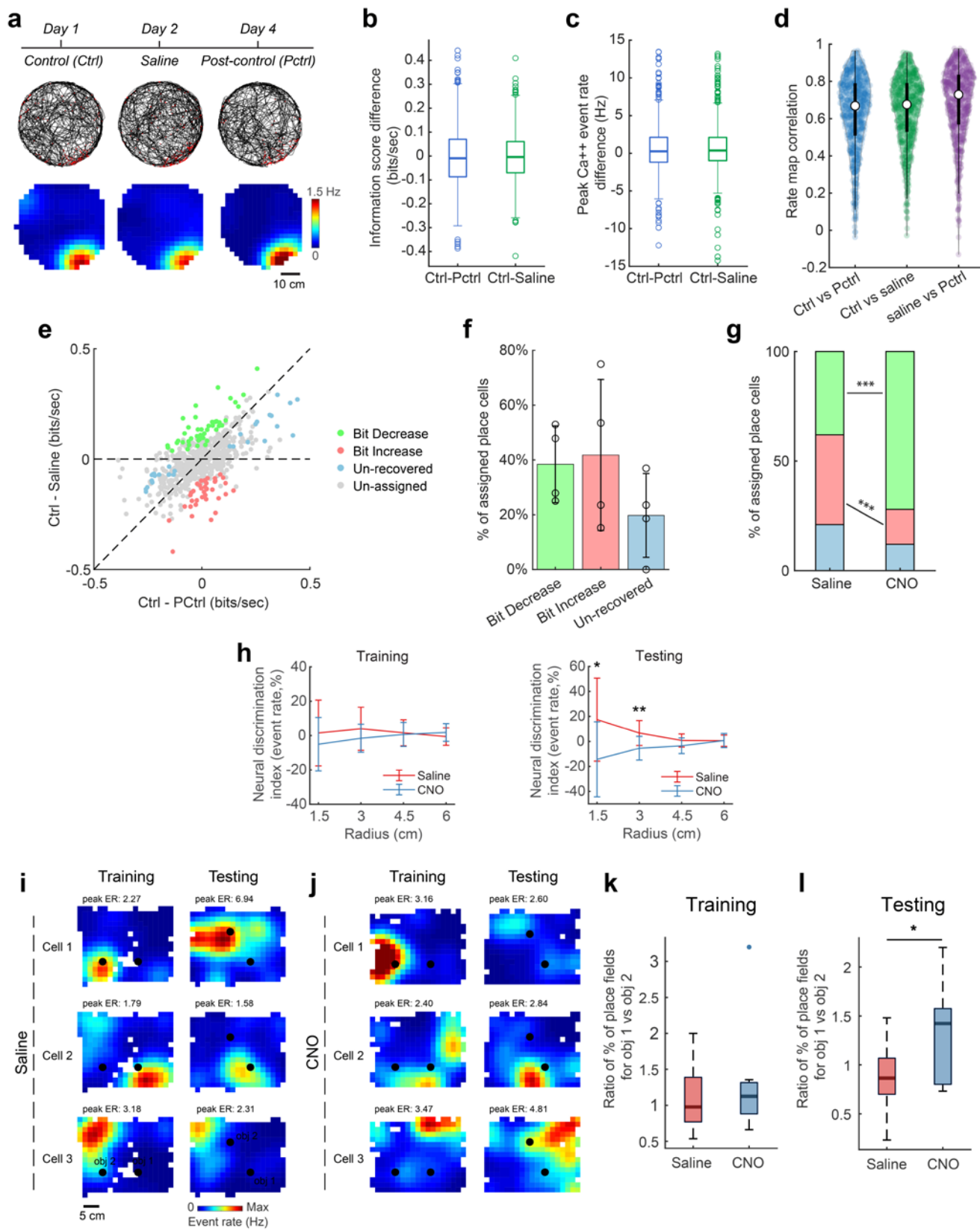
decoder training for all neurons (blue) versus all place cells (green), respectively. Predictions based on neural activities of all the neurons, and place cells from control and post-control sessions are more accurate than the predictions based on neural activities of these cells during the CNO session (see the overall summary in Figure 5k). This experiment was independently repeated using data from 5 mice, each with similar results. **b**, Comparisons of the calcium event amplitude for the CNO experiment (using a metric similar to the scaled dF/F which is used in most calcium imaging literature) between Ctrl and Pctrl (Ctrl – Pctrl, two-tailed t-test against zero, $p = 0.97$), and between Ctrl and CNO (Ctrl – CNO, two-tailed t-test against zero, $p = 0.24$). There is also no significant difference between Ctrl – Pctrl and Ctrl – CNO (two-tailed, paired t-test, $p = 0.24$). $n = 347$ place cells from 6 mice. **c**, The stacked bars represent the average percentage of assigned place cells that fall into each of the categories in the multiple control experiment and the CNO experiment (pooled from individual animals). The chi-square tests of each category between the multiple control ($n = 3$ mice) and CNO experiments ($n = 6$ mice) show significant frequency differences in the bit-decrease group, indicating CNO-induced inactivation effects. Bit-decrease group: $\chi^2 = 5.99$, $p = 0.05$ (*); bit-increase group: $\chi^2 = 1.02$, $p = 0.60$; unrecovered group: $\chi^2 = 4.6$, $p = 0.10$. **d**, Comparison of the difference in spatial information scores (bits/second) in the saline control experiment (see Supplementary Fig. 7c for the control experimental design) between Ctrl1 and Ctrl3 (Ctrl1 – Ctrl3, two-tailed t-test against zero, $p = 0.26$), and between Ctrl1 and Ctrl2 (Ctrl1 – Ctrl2, two-tailed t-test against zero, $p = 0.52$). $n = 174$ place cells from 3 mice. There is also no significant difference between Ctrl1 – Ctrl3 and Ctrl1 – Ctrl2 (two-tailed, paired t-test, $p = 0.40$). **e**, A violin plot showing the distribution of individual place cell's rate vector correlation coefficients for all session combinations in the saline control experiment ($n = 174$ place cells from 3 mice). The white points indicate median values (0.43, 0.58, 0.53), and thin black lines extend to most extreme values within 1.5 times the interquartile range of the median. The filled color width represents a density estimate of the distribution of values along the y axis. **f**, A scatter plot shows recorded place cells from saline control experiment classified into 3 non-overlapping groups: bit-decrease, bit-increase, and un-recovered, based on the statistical significance of differences in information scores (bit/sec) between Ctrl2 and Ctrl1, and between Ctrl2 and Ctrl3. This is determined with the jackknife resampling application for each place cell. Un-assigned place cells did not pass the statistical test after jackknife resampling and were excluded for further categorization analysis. **g**, Of the 58 place cells showing significant differences from 3 mice in the saline control experiment, 37% show decreased information scores in Ctrl2 sessions compared to the Ctrl1 and Ctrl3 sessions (bit-decrease group, green bar), and 26% show increased information scores in Ctrl2 sessions compared to the Ctrl1 and Ctrl3 sessions (bit-increase group, red bar). The remaining ones are in the unrecovered group which accounts for ~ 37% of place cells. Importantly, there is no significant difference in the % of place cells among these three groups ($p = 0.87$, repeated measures ANOVA, $n = 3$ mice). **h**, Comparison of the difference of peak calcium event rates in the saline control experiment across all the 174 place cells recorded from 3 mice, although both Ctrl1 – Ctrl3, and Ctrl1 – Ctrl2 are significantly higher than zero (two-tailed t-test, $p = 0.02$ and 1.6×10^{-6} , respectively), but there is no significant difference between Ctrl1 – Ctrl3 and Ctrl1 – Ctrl2 (two-tailed, paired t-test, $p = 0.15$). **i**, Examples of place cells from the unrecovered group. Each line shows rate maps of control, CNO, and post-control sessions from the same place cell. "I" indicates the spatial information score (bits/second), "ER" indicates the mean calcium event rate. **j**, Examples of cross-position population vector correlation matrices for odd vs even trials when the animal is running from right to left direction on the linear track during control, CNO, and post-control. 1 bin = 2.7 cm. **k**, Average correlation along the diagonal (top left to lower right) of population vectors for odd versus even trials of left to right and right to left running directions, respectively (means are of all same-position correlation values along the upper-left to lower-right diagonals in each, $N = 5$ mice, repeated measures ANOVA with Bonferroni's multiple comparison, $p = 0.66$ and 0.46 , respectively for each direction). **l**, Examples of cross-position population vector correlation matrices for first half vs second half trials when the animal is running on the linear track during control, CNO, and post-control. 1 bin = 2.7 cm. **m**, Average correlation of population vector of first half vs second half trials (means are of all same-position correlation values along the upper-left to lower-right diagonals in each, $N = 5$ mice, repeated measures ANOVA with a Huynh-Feldt adjustment, $p = 0.65$). For the box plots throughout the figure, the three box lines from top to bottom represent the 25th, 50th (median), and 75th percentile of data values of the samples. The whiskers extend to the most extreme values within 1.5 times of the interquartile range of the median. Data are presented as mean $\pm$ SE for all the bar plots.



Supplementary Figure 9

Analysis of bits/event for linear track and open field experiments, and the results of simulation testing bits/second versus bits/event for sensitivity to in-field event rates.

a, Comparison of the difference of spatial information measured by using bits/event (equivalent to bits/spike in computation but using calcium events rather than action potentials/spikes) from DREADDs inactivation experiments on the linear track. Ctrl - Pctrl and Ctrl - CNO are neither significant against zero (two-tailed, one sample t-test, $p = 0.99$ and 0.16 , respectively) nor different from each other (two-tailed, paired t-test, $p = 0.21$, $n = 347$ place cells from 6 mice). **b**, Comparison of the difference of spatial information measured by using bits/event from saline control experiments on the linear track. Although Ctrl - Pctrl and Ctrl - Saline are significantly higher than zero (two-tailed, one sample t-test, $p = 3.02 \times 10^{-5}$ and 7.97×10^{-10} , respectively), they are not different from each other (two-tailed, paired t-test, $p = 0.37$, $n = 174$ place cells from 3 mice). **c**, Comparison of the difference of spatial information measured by using bits/event from DREADDs inactivation experiments in the open field. Ctrl - Pctrl and Ctrl - CNO are neither significant against zero (two-tailed, one sample t-test, $p = 0.18$ and 0.14 , respectively) nor different from each other (two-tailed, paired t-test, $p = 0.90$, $n = 379$ place cells from 4 mice). **d**, Comparison of the difference of spatial information measured by using bits/event from saline control experiments in the open field. Ctrl - Pctrl and Ctrl - Saline are neither significant against zero (two-tailed, one sample t-test, $p = 0.63$ and 0.79 , respectively) nor different from each other (two-tailed, paired t-test, $p = 0.78$, $n = 606$ place cells from 4 mice). For the box plots throughout the figure, the three box lines from top to bottom represent the 25th, 50th (median), and 75th percentile of data values of the samples. The whiskers extend to the most extreme values within 1.5 times of the interquartile range of the median. **e**, 100 firing rate vectors are simulated. For each, a Gaussian-shaped firing field is set against a near-zero firing rate outside the field. Across these vectors (y-axis) the peak in-field firing rate varies from 1 Hz to 100 Hz. Note that the blue-yellow color axis varies from 0-100 Hz to depict the shape and amplitude of the firing fields and that all fields have the same width. **f**, Plots of bits/second (upper graph) and bits/event (lower graph) across the 100 rate vectors varying only in amplitude. Bits/second produces a linear response over a wide range of values while bits/event varies far less once firing rates exceed approximately 5 Hz.



Supplementary Figure 10

Miniscope imaging of CA1 place cell activities in the open field of the control animals and further place field analysis in the OLM experiments

a, Top: tracking data (grey lines) and locations of calcium events (red dots) for a single neuron tracked across sessions. Below: Calcium event rate maps for the same example cell from an open field experiment with saline administration. The corresponding experiment time line is shown at the top. The experiment was independently repeated in 4 mice, each with similar results. **b**, Comparison of the difference of spatial information measured by using bits/sec from saline control experiments in the open field. Ctrl – Pctrl and Ctrl – Saline are neither significant against zero (two-tailed, one sample t-test against zero, $p = 0.24$ and 0.23 , respectively) nor different from each other (two-tailed, paired t-test, $p = 0.81$, $n = 606$ place cells from 4 mice). **c**, Comparison of the difference of peak Calcium event rates from saline control experiments in the open field. Ctrl – Pctrl and Ctrl – Saline are neither significant against zero (two-tailed, one sample t-test against zero, $p = 0.50$ and 0.24 , respectively) nor different from each other (two-tailed, paired t-test, $p = 0.61$). $n = 606$ place cells from 4 mice. **d**, A violin plot showing the distribution of individual place cell's rate map correlation coefficients for all session combinations in the open field saline control experiment ($n = 606$ place cells from 4 mice). The white points indicate median values (0.67, 0.67, 0.73), and thin black lines extend to most extreme values within 1.5 times the interquartile range of the median. The filled color width represents a density estimate of the distribution of values along the y axis. **e**, Recorded CA1 place cells can be classified into 3 non-overlapping groups termed bit-decrease, bit-increase, and un-recovered, based on the statistical significance of differences in information scores (bit/sec) between Saline and Ctrl, and between Saline and Pctrl subsequent to the jackknife resampling method for each place cell. Un-assigned place cells did not pass the statistical test after jackknife resampling and were excluded for further categorization analysis. On the scatter plot, the x-axis is Ctrl – Pctrl (the difference of spatial information scores between Ctrl and Post-ctrl) and the y-axis is Ctrl – Saline (the difference of information scores between Ctrl and Saline). **f**, Of the 110 place cells showing significant differences from 4 mice in the open field saline control experiment, 38% show decreased information scores in Saline sessions compared to the Ctrl and Pctrl sessions (bit-decrease group, green bar), and 41% show increased information scores in Saline sessions compared to the Ctrl and Pctrl sessions (bit-increase group, red bar). The remaining ones are in the unrecovered group which accounts for ~ 20% of place cells. Importantly, there is no significant difference in the % of place cells among these three groups ($p = 0.44$, repeated measures ANOVA). Data are presented as mean $\pm$ SE in the bar plots. **g**, Numbers in the bar graphs represent the average percentages of place cells that fall into each of the categories in the saline experiment and the CNO experiment, pooled from individual animals. The chi-square tests of each category between the saline and CNO groups show significant frequency differences, indicating CNO-induced inactivation effects. Bit-decrease group: $p = 5 \times 10^{-5}$; Bit-increase group: $p = 8 \times 10^{-4}$; unrecovered group: $p = 0.12$. $n = 4$ mice for each group. **h-i**, Further place field analysis of OLM experiments. **h**, The neural discrimination index calculated by varying the size of the radius used to define the field surrounding an object. CNO-treated mice ($n = 11$) do not differ from saline-control mice ($n = 10$) in terms of the neural discrimination index calculated with ensemble Ca⁺⁺ event rates during the training (two-tailed Welch's t-test), but show decreased neural discrimination during the testing when the radius is less than 3 cm ($p = 0.032$ for radius being 1.5cm, $p = 0.0099$ for radius being 3cm, two-tailed Welch's t-test). This neural discrimination index is based on relative event rates and expressed as $(ER_{\text{Object}2} - ER_{\text{Object}1}) / (ER_{\text{Object}2} + ER_{\text{Object}1}) \times 100\%$ wherein $ER_{\text{Object}2}$ and $ER_{\text{Object}1}$ are the ensemble event rates associated with the two objects, respectively. The data are presented as mean $\pm$ SD in the error bar plot. **i**, Place fields for three example cells from a saline control animal are shown here to represent fields that are associated with the moved object, the unmoved object, or a field outside of either. This experiment was independently repeated in 10 mice, each with similar results. **j**, Similar to **i**, place field examples of three cells from a CNO-treated animal. **k**, Box plot of the ratios of the percentage of place cell fields for the unmoved object (obj 1) versus the moved object (obj 2) during the training session. Both ratios of obj 1 / obj 2 place cell fields are roughly at 1 in the saline and CNO animals (two-tailed t-test, $p = 0.646$, $n = 10$ saline animals vs. 11 CNO animals). To calculate the ratio of the percentage of place cell fields between obj 1 and obj 2, for each animal, we first calculated the percentage of place cell fields associated with obj 1 and obj 2 respectively, and then computed their ratio. **l**, Box plot of the ratios of the percentage of place cell fields for the unmoved object (obj 1) versus the moved object (obj 2) during the testing session. The ratios of obj 1 / obj 2 place cell fields are significantly higher in CNO-treated animals than in the saline controls (two-tailed t-test, $p = 0.039$, $n = 10$ saline-control animals vs. 11 CNO-treated animals). For the box plots throughout the figure, the three box lines from top to bottom represent the 25th, 50th (median), and 75th percentile of data values of the samples. The whiskers extend to the most extreme values within 1.5 times of the interquartile range of the median.

Supplementary Table 1a. Quantitative input strengths of Camk2a-Cre SUB excitatory neurons versus CA1-projecting SUB excitatory neurons

Camk2a-Cre excitatory neurons	CA1 S.P.	CA1 S.O.	CA1 S.R.	CA1 S.L.M.	PrS	RSG	Vis Ctx	Au Ctx	TeA	PRh/ Ect	LEn t	mEn t	ACC	MS- DB	Thala- mus
Average CSI	47.72	0.90	0.14	0.17	1.16	0.46	0.74	0.51	0.38	0.39	0.53	0.46	0.09	0.75	0.30
SE	2.69	0.22	0.02	0.05	0.13	0.19	0.22	0.29	0.15	0.08	0.20	0.11	0.03	0.15	0.08
Average %	86.71	1.58	0.26	0.31	2.16	0.81	1.32	0.88	0.68	0.71	0.92	0.85	0.16	1.42	0.54
SE	1.56	0.36	0.03	0.09	0.35	0.30	0.35	0.46	0.26	0.12	0.30	0.22	0.06	21.34	0.15
# of starter	50 ± 8				Total labeled neurons				2681 ± 372				N=5		
CA1- projecting SUB neurons	CA1 S.P.	CA1 S.O.	CA1 S.R.	CA1 S.L.M.	PrS	RSG	Vis Ctx	Au Ctx	TeA	PRh/ Ect	LEn t	mEn t	ACC	MS- DB	Thala- mus
Average CSI	14.90	4.00	0.06	0.08	0.54	0.47	3.27	0.76	0.00	0.00	0.00	0.00	0.00	0.49	0.06
SE	1.25	0.44	0.06	0.08	0.15	0.26	0.55	0.36	0.00	0.00	0.00	0.00	0.00	0.17	0.06
Average %	60.28	16.19	0.21	0.41	2.20	2.01	13.18	2.88	0.00	0.00	0.00	0.00	0.00	1.99	0.21
SE	4.41	1.58	0.21	0.41	0.67	1.20	2.08	1.21	0.00	0.00	0.00	0.00	0.00	0.66	0.21
# of starter	3.2 ± 0.6				Total labeled neurons				77 ± 13				N=6		

Note: The input connection strength index (CSI) is defined as the ratio of the number of presynaptic neurons in a given brain structure versus the number of starter neurons. % is the percentage of the total input, which is defined as the number of labeled neurons in a given brain structure compared to the total number of labeled neurons. The local SUB input quantification is excluded. All data are presented as mean ± SE

Supplementary Table 1b. Statistic comparisons between input measurements of Camk2a-Cre SUB excitatory neuron and CA1-projecting SUB excitatory neuron

CSI	Comparisons of CSI, related to Figure2	Statistic Method	Significance	P value
	CA1 s.p.	t-test	***	9.13 x 10 ⁻⁷
	CA1 s.o.	t-test	***	0.0002
	CA1 s.r.	t-test	ns	0.1873
	CA1 s.l.m	t-test	ns	0.4085
	PrS	t-test	*	0.0156
	RSG	t-test	ns	0.9823
	Visual Ctx	t-test	**	0.0032
	Auditory Ctx	t-test	ns	0.6044
	TeA	t-test	*	0.0239
	PRh/Ect	t-test	***	0.0004
	LEnt	t-test	*	0.0167
	mEnt	t-test	**	0.0013
	ACC	t-test	*	0.0175
	MS-DB	t-test	ns	0.3070
	AV Thalamus	t-test	*	0.0306
%	Comparisons of % of total input	Statistic Method	Significance	P value
	CA1 s.p.	t-test	***	0.0006
	CA1 s.o.	t-test	***	1.75 x 10 ⁻⁵
	CA1 s.r.	t-test	ns	0.8087
	CA1 s.l.m	t-test	ns	0.8440
	PrS	t-test	ns	0.9580
	RSG	t-test	ns	0.4002
	Visual Ctx	t-test	***	0.0006
	Auditory Ctx	t-test	ns	0.1877
	TeA	t-test	*	0.0186
	PRh/Ect	t-test	***	0.0001
	LEnt	t-test	**	0.0083
	mEnt	t-test	**	0.0018
	ACC	t-test	*	0.0240
	MS-DB	t-test	ns	0.4935
	AV Thalamus	t-test	ns	0.2329

Supplementary Table 2a. Data for object-location and novel-object experiments with DREADDs-mediated inactivation of CA1-projecting subicular excitatory neurons

Object-location	Training (10 min)		Testing (5min)	
	Saline	CNO	Saline	CNO
Total Time (s)	20.83	20.67	8.24	7.40
SE	1.03	0.99	0.72	0.49
Discrimination Index (%)	0.51	-0.88	22.31	4.60
SE	2.24	1.04	3.16	2.82

Novel-object	Training (10 min)		Testing (5min)	
	Saline	CNO	Saline	CNO
Total Time (s)	21.27	21.69	7.92	9.70
SE	1.16	1.72	1.05	0.75
Discrimination Index (%)	-0.91	1.02	24.67	21.72
SE	2.24	0.72	5.22	6.30

Supplementary Table 2b. Training and Testing data for object-location memory enhancement experiments with activation of CA1-projecting subicular neurons via optogenetic stimulation

Object-location	Training (3 min)			Testing (5 min)		
	Laser + EGFP ctrl	No laser ctrl	Laser + ChR2	Laser + EGFP ctrl	No laser ctrl	Laser + ChR2
Total Time (s)	4.18	4.19	3.78	5.99	6.14	7.09
SE	0.46	0.42	0.25	0.47	0.75	0.72
Discrimination Index (%)	0.70	1.82	0.15	3.43	2.10	25.25
SE	3.25	2.02	1.95	3.10	4.54	4.69

Supplementary Table 3. Behavioral data of object-location memory experiments concurrent with miniscope imaging

Object-location tasks with miniscope imaging	Training (10 min)		Testing (10min)	
	Saline	CNO	Saline	CNO
Total Time (s)	8.76	9.06	8.70	9.18
SE	1.08	0.86	0.85	0.80
Discrimination Index (%)	-1.32	0.44	21.25	1.86
SE	1.51	1.28	2.58	2.37

Supplementary Table 4. Mouse strains and viral injections for the experiments

Mouse strain	Virus Injection	Target brain region	Experiments performed
Camk2a-Cre; TVA	AAV8-EF1a-FLEX-HB EnvA-SADΔG-mCherry	Subiculum (AP: -3.40 mm)*	Rabies tracing of inputs to subicular excitatory neurons (n = 5)
Ai9 (RCL-tdTomato)	CAV2-Cre	CA1 (AP: -1.94 mm)*	Verification of the efficiency and specificity of CAV2-Cre (n = 4)
C57BL/6	CAV2-Cre AAV8-EF1a-FLEX-HTB EnvA-SADΔG-mCherry	CA1	Rabies tracing of circuit inputs to CA1-projecting subicular excitatory neurons (n = 6)
		Subiculum	
C57BL/6	CAV2-Cre H129DTK-TT (HSV)	CA1 Subiculum	Anterograde HSV tracing of output projections of CA1-projecting subicular neurons (n = 5)
C57BL/6	AAV2/1-CAG-ChR2-Venus	Subiculum	ChR2 photoactivation mediated circuit mapping (n = 5)
C57BL/6	CAV2-Cre AAV2-DIO-hM4D-mCherry	CA1 Subiculum	Inactivation of CA1-projecting subicular neurons during object relocation (n = 21) and novel object recognition (n = 10) tasks, dry-land maze experiments (n = 10)
Camk2a-Cre	AAV2/1-CAG-ChR2-Venus / AAV2-DIO-hM4D-mCherry	Subiculum	In vitro validation of optogenetic stimulation (n = 4) or hM4D-CNO mediated neuronal inhibition (n = 4)
Ai32 (RCL-ChR2(H134R)/EYFP)	rAAV2-retro-hSyn-Cre	CA1	Optogenetic activation of CA1-projecting subicular neurons during object location memory training (n = 18)
C57BL/6	rAAV2-retro-hSyn-EGFP	CA1	GFP control for optogenetic activation during object location memory experiment (n = 8)
C57BL/6	CAV2-Cre AAV1-CamkII-GCaMP6f	CA1 CA1 (AP: -2.04 mm)	Miniscope-based GCaMP6 imaging of CA1 place cells and genetic inactivation of CA1-projecting subicular neurons. Linear track (n = 9), open field (n = 8), object location memory (n = 21)
	AAV2-DIO-hM4D-mCherry	Subiculum	

* Standard anterior-posterior values relative to the bregma (see the Methods) are used for injections in CA1 and the subiculum, except otherwise specified. For all the experiments, mice at 8-12 weeks old (either sex) were used for surgeries and had free access

to food and water in their home-cages before and after surgeries. Please refer to the main text and online methods for more detailed information.