hM_3D_q receptor- cFos co-expression

For co-expression experiments (S3), a c-fos transgene with a GFP linked reporter was used to quantify cfos expression by measuring endogenous fluorescence (*11*).
Mice were injected with CNO and one hour 28 minutes later were perfused and the brains were fixed and stained for HA as decribed under *immunohystochemistry*.

***In vivo* electrophysiology**

For electrophysiological recordings, a total of 12 mice (6 single positive controls and 6 double positive experimental) were used. To mirror the behavioral study, mice were first fear conditioned and returned to their home cage overnight. 24 hours after fear conditioning, the mice were anesthetized with ketamine-medetomidine-atropine and prepped for electrophysiological recording. The head was placed in a stereotaxic frame, the skull was exposed, and a small hole (1 mm in diameter) in the skull was drilled above the left dorsal hippocampus (A, −2 mm from the bregma; L, 1.5 mm from the midline). A bundle of 4 tetrodes comprised of spun 17micron platinum-iridium wire or a 16 channel silicon probe were slowly advanced into the hippocampus and allowed to settle for a period of 10 minutes before recording. Tetrodes were targeted to the CA1 pyramidal layer, while the sites of the multi-channel silicon probe were positioned to span the hippocampal layers. After reaching the desired depth and rest, a 5 or 10 minute baseline period was recorded followed by injection of CNO and another 50+ minutes of recording. During the recording sessions, neurophysiological signals were amplified, bandpass filtered (0.1 Hz to 6 kHz), and acquired continuously at 32 kHz on a 32-channel system. In the case of the silicon probes, the site closest to the CA1 pyramidal layer was analyzed. Data were analyzed using a combination of custom-written MATLAB software and the MATLAB-based toolbox Chronux. Multi-unit spiking activity (MUA) was quantified by first filtering the fully sampled data (200Hz to 6kHz), then setting a threshold of 5 s.d. above the mean and counting all events over the threshold. The mean MUA (in spikes/second) was calculated for 30 to 40 minutes after CNO injection and then compared to 4 minutes of pre-injection baseline using a Wilcoxin signed-rank test.

Supplementary Data

Brain Region	$hM3D^{fos}$ (mean %)	control (mean %)	x fold increase	P value
CA1	40.8	16.2	2.5	0.0129
CA3	23.5	10.5	2.2	0.0188
DG	46.5	2.24	20.8	0.0067
LA	18.5	8.18	2.3	0.0491
BLA	21.9	8.3	2.6	0.0026
CA	24.8	5.8	4.3	0.0074

Table S1. Percent cfos expression in hippocampus and amygdala. Mice from experiment in Fig. 2 were examined 1-hour following the retrieval trial in ctxB and immunostained for cfos expression. Data is presented as a percentage of total cells counted in the corresponding area. hM_3D_q n = 10, control n = 10. Group means were compared by T-test and p-value is indicated.

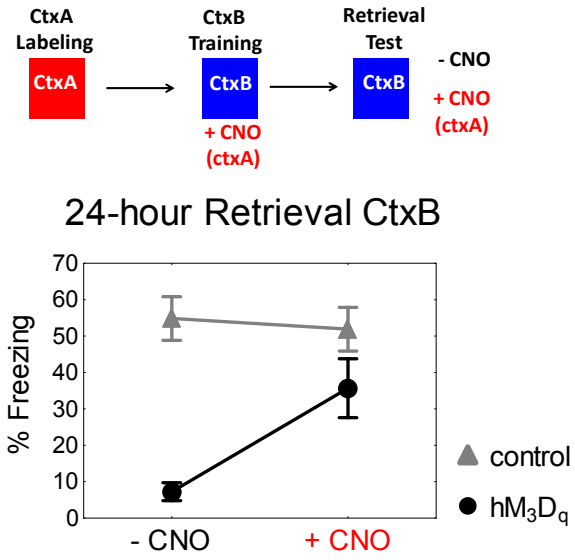

Fig. S1. Using a different context for novel ctxA results in the same effects as shown in experiment 1 (see Fig 3C). $hM3D^{fos}$ mice show impaired 24 hour memory for ctxB that is rescued by injection of CNO. $hM3D^{fos}$ n = 15, control n = 17. Repeated measures ANOVA genotype x CNO interaction $F(1,28) = 5.012$, $p < .05$. Post hoc Fisher's LSD test revealed that $hM3D^{fos}$ mice were freezing significantly less than control mice in the absence of CNO, $p < 0.001$, were statistically similar to controls in the presence of CNO, $p = 0.114$, and showed a significant increase in freezing in ctxB in the presence of CNO compared to ctxB alone, $p < .005$.