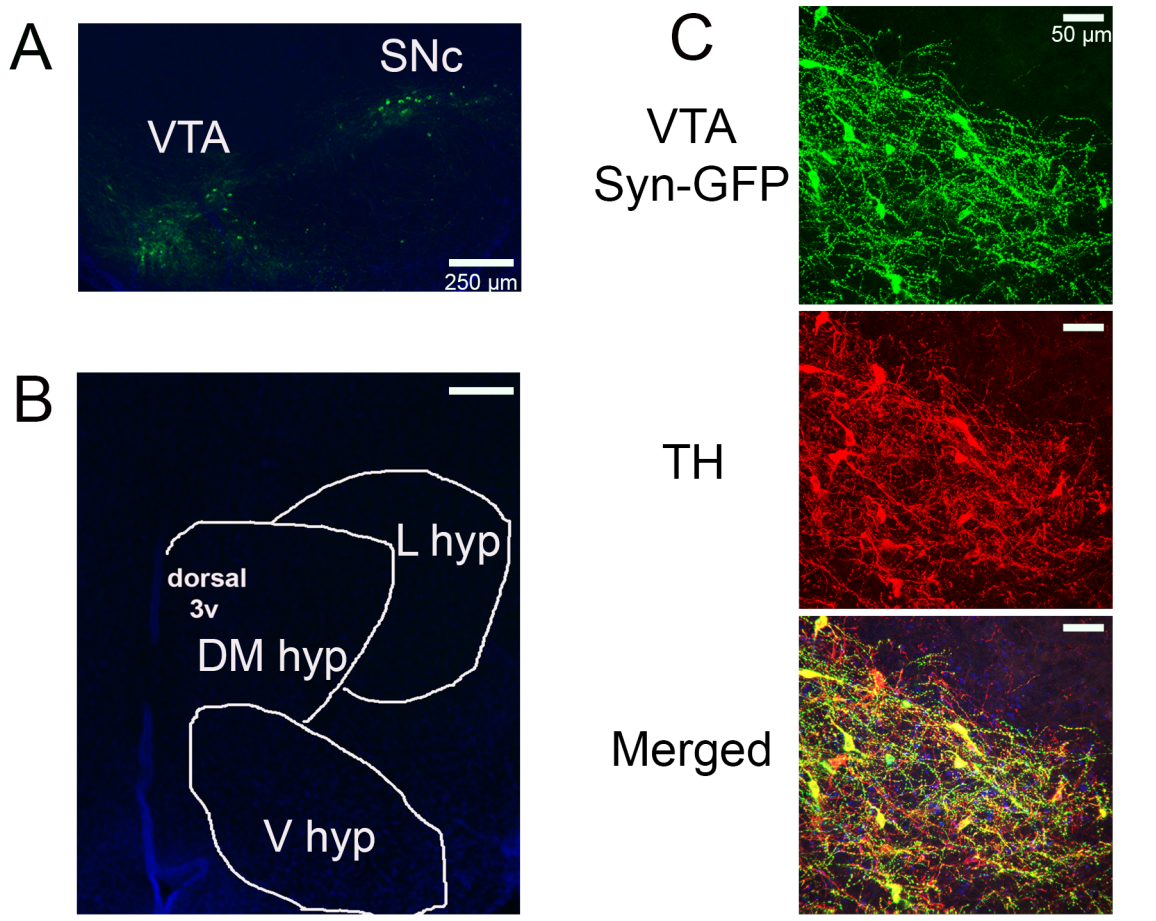

Figure S1 Related to Figure 1. Synaptophysin-GFP virus expression in dopamine projections. Low resolution light images of (A) the midbrain ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) and (B) hypothalamus (hyp) of Slc6a3$^{ires\text{-}cre/+}$ mice injected in the midbrain dopamine centers with the virus expressing synaptophysin-GFP. The results indicate that the injections of recombinant adeno-associated virus containing a doubly-floxed inverted open reading frame encoding synaptophysin-GFP was restricted to the midbrain DA areas and not nearby hypothalamic areas. High magnification images (C) of Syn-GFP (top), TH immunofluorescence (middle), and merged photo of the lateral VTA (bottom). There is a very high overlap between viral vector labeling and TH immunoreactivity (out of 12 GFP positive cell bodies in the current field, 11 co-express TH).

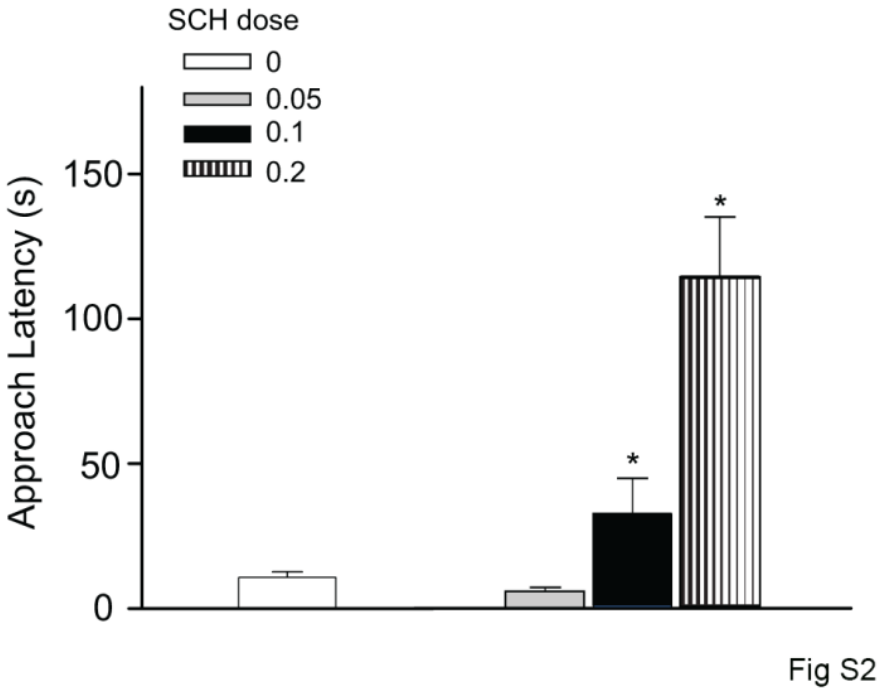

Figure 2 Related to Figure 3. Dose-dependence of SCH 23390 for increased latency to enter the dark chamber before IA training. The approach latency of mice before IA training at 3 SCH doses for the D1-like receptor antagonist. The higher doses of SCH produced a significantly longer approach latency: $F_{(2, 30)} = 12.15$, $p < 0.01$, n = 4-8. The lowest dose, 0.05 mg/kg, did not alter approach latency before training. Therefore, the lowest dose was used for the experiments described in the text to assess contextual aversive learning with the IA paradigm.

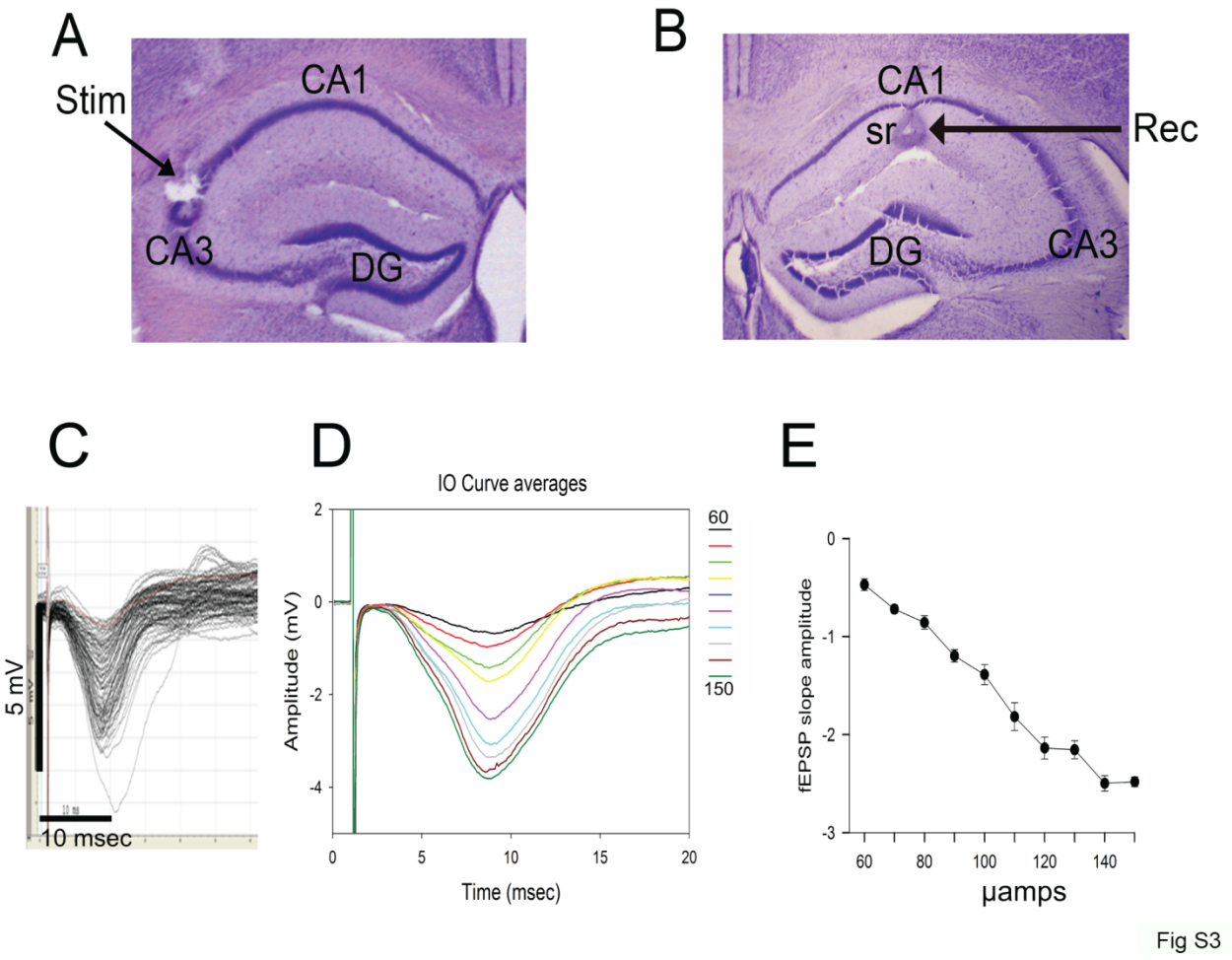

Fig S3

Figure S3 Related to Figure 5. **Post-hoc anatomy of the hippocampus showing stimulus and recording sites.** (A) Example location of the simulating electrode shown by an electrolytic lesion in the left Schaffer collateral near to the CA3 region. (B) Example location of a recording electrode show by an electrolytic lesion in the stratum radiatum layer of the right CA1. (C) Complete set of input/output (I/O) traces from a single electrode given a range of stimuli used to produce the I/O curve. (D) Traces from C averaged in ten sweep bins. fEPSPs were recorded at each current level for 10 stimulations, and the current was increased in 10 µA increments until a maximum slope was achieved. For this experiment, the maximum slope was reached at 150 µA stimulation strength. (E) Plot of the quantified slopes of the fEPSPs at each input current for the electrode recordings displayed in C and D.

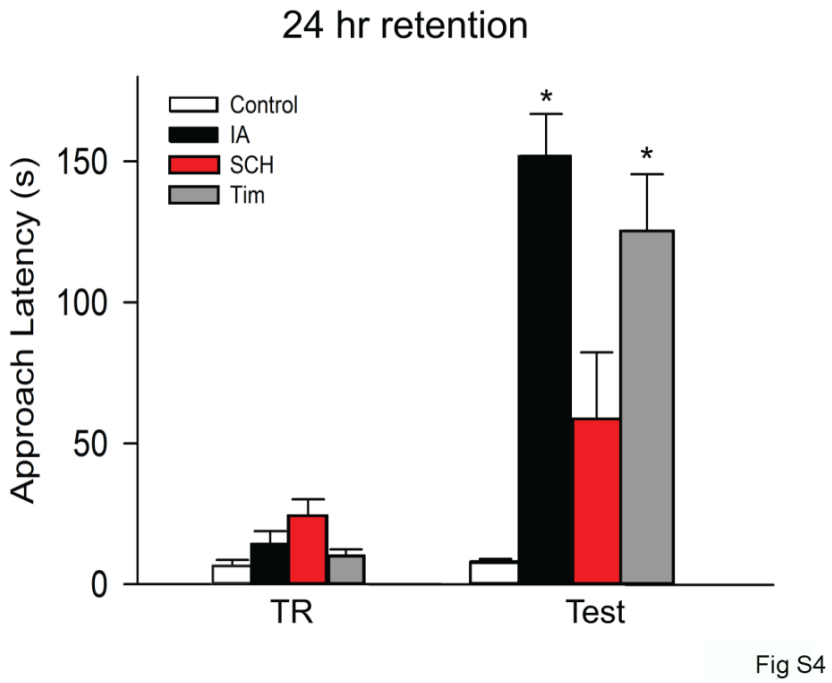

Figure S4, Related to Figures 5 and 6. Behavioral results from electrode implanted animals are comparable to the synaptic results under the same conditions. The latency to approach the dark side (shocked side) was measured after 24 h retention following IA training. The approach latencies are comparable before the shock (TR, left bars). The test group mice were treated as walk through saline controls (no shock, white bars), with saline and IA (black bar, 147.1 ± 19.9 s, n = 9), SCH + IA (61.2 ± 30.9 s, n = 7, red), or timolol + IA (131.2 ± 20. 16 s, n = 5, gray). IA training in saline treated animals produced a significant increase in approach latency at 24 hr retention: $t_{(8)} = 9.6$, $p < 0.05$ (black bar). Mice receiving SCH injections did not have a significant increase in the approach latency: $t_{(6)} = 1.8$; $p = 0.12$ (red bar). Tim injected mice significantly increased their approach latency at 24 hr: $t_{(4)} = 7.84$, $p < 0.05$ (grey bar).

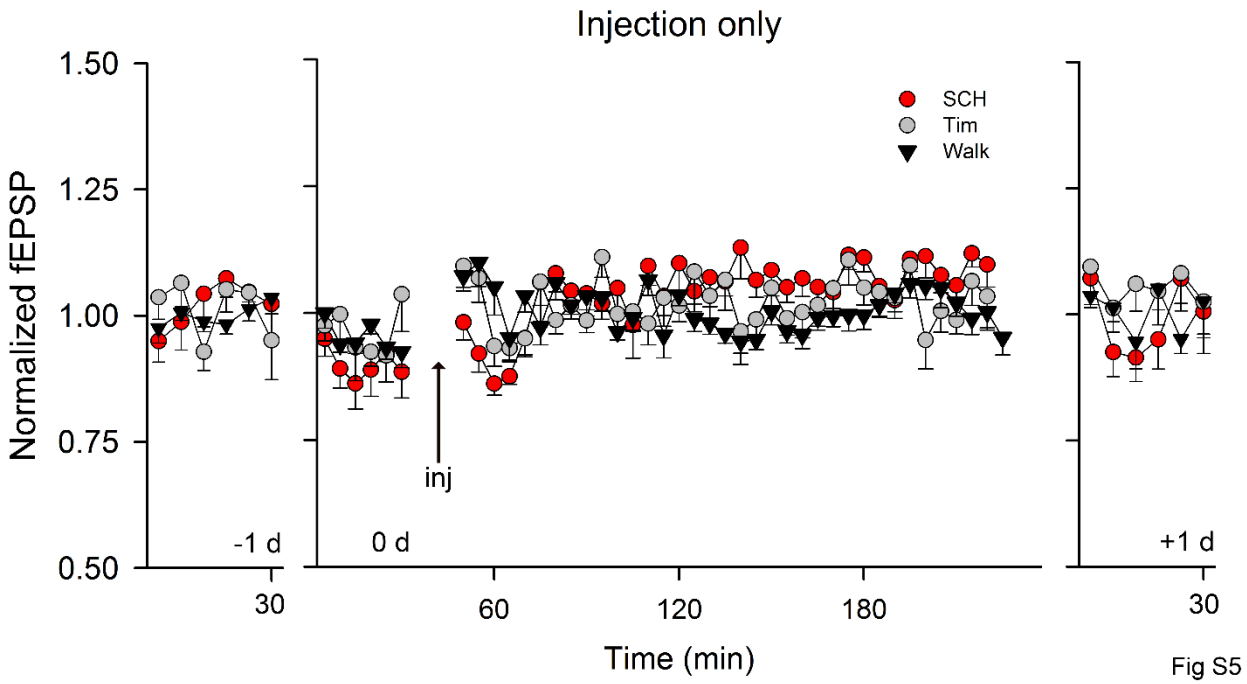

Figure S5, Related to Figure 6. Injections of SCH and Timolol in the IA absence of training did not change subsequent fEPSPs. One week after training and testing, a new I/O curve and baseline fEPSPs were recorded from implanted mice. For reference, walk through data recorded from control animals in Figure 4 is graphed here in black triangles. Injections (i.p.) of SCH (red circles) did not change the slope of the fEPSP relative to baseline: $F_{(41,\ 246)} = 1.03$, n = 7, $p > 0.05$. Timolol (gray triangles) in the absence of training also did not change the slope of the fEPSP relative to baseline: $F_{(41,164)} = 0.94$, n = 5, $p > 0.05$.

Supplemental Experimental Procedures

Tyrosine hydroxylase and dopamine transporter immunohistochemistry. We bilaterally injected the midbrain of *Slc6a3*$^{ires\text{-}cre/+}$ mice (Backman et al., 2006) with 1 µl (6.0 x 10^12 gc/mL) *AAV-DJ8-EF1α-DIO-Synaptophysin-GFP* or *AAV-DJ8-EF1α-DIO-Synaptophysin-Ruby-Red* (Grimm et al., 2008) aiming to express GFP in DA neurons at sites encoding synaptophysin. We started with the following parent plasmid from addgene Plasmid 27235: *pFCK(1.3)GFP-SynaptophysW* (Dittgen et al., 2004). Synaptophysin-GFP was excised using *Not*I and *Bsr*GI and shuttled into a custom chloramphenicol resistant shuttle plasmid that contained the MCS *Spe*I-*Not*I-*Bsr*GI-*Sca*I-*Xho*I- *Spe*I. From there, Synaptophysin-GFP was excised using *Spe*I and cloned into the pAAV-flex vector. Clones were verified for flex orientation by restriction digest and transfection (Arenkiel, 2011; Arenkiel and Ehlers, 2009). We injected synaptophysin:GFP in the midbrain (± 3.64 A/P, ± 0.5 M/L, ± 4.0 D/V) of mice expressing *Slc6a3*$^{ires\text{-}cre/+}$ or WT littermates, and after two weeks fixed the brain and cut slices (40 µm) for immunohistochemistry for TH using a rabbit polyclonal antibody (Abcam). Sections were analyzed on a confocal microscope and images were analyzed for green fluorescence using ImageJ (http://rsbweb.nih.gov/ij/). Metamorph (Molecular Devices) was used to analyze co-localization.

***Ex vivo* hippocampal slices and patch clamp recordings.** We tested the effects of IA training by measuring synaptic AMPA/NMDA-receptor current ratios from *ex vivo* hippocampal slices. Researchers conducting the IA training provided mice to those conducting the electrophysiology without revealing the training conditions. Mice were

transcardially perfused with ice-cold oxygenated sucrose-based solution was used before cutting horizontal slices (270 μm thick) (Yang et al., 2011).

The slices were recorded in well oxygenated ACSF containing the following (in mM): 125 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgSO_4$, and 2 CaCl2, 25 D- (+)-glucose. In order to measure glutamate receptor-mediated currents, picrotoxin (100 μM, Sigma-Aldrich) was routinely included in the ACSF. Patch-clamp recording electrodes (2-3 MΩ) were filled with the following intracellular solution (in mM): 130 cesium methanesulfonate, 10 KCl, 10 HEPES, 1 BAPTA, 3 Na_2ATP, 10 TEA-Cl, 1 GTP, 2 $MgSO_4$, PH 7.3 with CsOH. To record AMPA and NMDA receptor mediated whole-cell currents, neurons were clamped (Axopatch 200B, Axon Instruments) at +40 mV during the recordings. To define AMPA receptor-mediated EPSPs, NMDA receptor mediated currents were inhibited by bath application of DL-APV (100 μM), a NMDA receptor antagonist. To define NMDA receptor-mediated EPSPs, AMPA receptor-mediated EPSPs were digitally subtracted from total the EPSPs. To measure AMPA/NMDA current ratios in dentate gyrus granule cells or CA1 pyramidal neurons, the stimulating electrode was placed on the medial perforant path (Fig. 4A) or the Schaffer collateral path (Fig. 4F) respectively.

Surgery and *in vivo* electrophysiology. Mice were deeply anesthetized and four recording electrodes running anterior to posterior (parallel to the midline) were positioned into the right CA1 region. A concentric bipolar stimulating electrode was implanted in the contralateral (left) Schaffer collateral area (Fig. 5A). Both stimulating and recording electrodes consisted of Teflon-insulated stainless steel wire (50-μm diameter). The final depth of the stimulating and recording electrodes was at a site

eliciting the maximal field potential (fEPSP) slope across the recording electrodes in CA1. Evoked fEPSPs were amplified (x100, AM Systems 1700 amplifier) and filtered at 0.1 to 5 kHz, digitized at 10 kHz and stored on a PC using Clampex 8.2. The fEPSP slope was determined with Clampfit version 9.

Ten days after surgery, mice were handled and habituated by the experimenter and recordings were performed in their home cage, which was placed in the sound attenuating recording chamber (Med Associates, St. Albans VT). On the next day, input-output curves were obtained and baseline responses were recorded for 30 min (1 stimulation every 30 s, 0.1 ms duration pulses). All baseline recordings were carried out with a stimulation intensity that elicited a group average of approximately 40% maximal fEPSP slope across multiple recording electrodes. Baseline recordings were made one day before (-1 d) behavioral conditioning (on 0 d), and a second baseline recording was made prior to conditioning. On the day of conditioning, the baseline responses were recorded for 30 min and animals were injected with either saline, SCH 23390, or Tim 15 min before beginning passive IA training. Baseline fEPSP recordings were resumed 5 min after training, and the recordings were continued for 3 h. The slope measurements were averaged into 5-min time bins (10 sweeps/bin) for each individual electrode and the mean (± SEM) plotted for each group.

For analysis of the field potential data, the slope of the fEPSP was measured for each stimulus evoked postsynaptic response by calculating the depth of the local field potential. The calculated values from every ten consecutive fEPSPs were averaged. The averaged values before any drug treatment or training were normalized to 100%, and defined the baseline. The data groups were analyzed with two-way repeated

measures ANOVA (in SPSS for windows). Then, a Tukey HSD post-hoc test was performed to determine the relationship of significant differences between groups.

Statistical analysis. Paired/unpaired Student's *t* test or one-way ANOVA with Tukey post hoc comparison test were performed for the statistical analysis of the behavioral experiments and AMPA/NMDA ratios, and $p < 0.05$ was considered to be statistically significant. For the *in vitro* experiments, the peak amplitude of the EPSPs was normalized to baseline.