Selective, Retrieval-Independent Disruption of Methamphetamine-Associated Memory by Actin Depolymerization

Supplemental Information

Supplemental Methods and Materials

Stereotaxic Surgery

Rats were anesthetized with dexmedetomidine (0.25 mg/kg, IM) and ketamine (0.5 mg/kg, IM) and implanted bilaterally with 26G guide cannulae (Plastics One) 2mm above the basolateral amygdala complex (BLC) (lateral and basolateral nuclei; AP -2.9 mm, ML ±5 mm relative to bregma; DV -6.7 mm from skull, [1]). Immediately after surgery, buprenex (0.05 ml, SC) was administered. Mice were anesthetized with isoflurane gas and implanted bilaterally with 26G guide cannulae implanted 1 mm above the BLC (AP -1.5 mm, ML ±2.8 mm relative to bregma; DV -3.7 mm from skull). Metacam (1.5 mg/ml, 1-2 drops orally) was administered immediately after surgery.

Jugular Catheter Surgery

For self-administration experiments, rats were implanted with chronic indwelling jugular catheters 48 hrs after food training. Catheters were constructed in house with steel cannulae with screw-type connectors bent to 90°, silastic tubing, Prolite monofilament mesh, and dental cement. The end of the catheter was inserted into the right jugular vein and secured. The catheter ran subcutaneously and exited on the back, posterior to the shoulder blades. Catheters were flushed once daily for 5 days with 100 μl of antibiotic solution (10 mg/ml cefazolin in heparinized saline [70 U/ml]) and 100 μl of low dose heparinized saline (10 U/ml) to ensure patency. Patency was confirmed with 100 μl of propofol (10 mg/ml).

Drug Infusion Rates

Intra-BLC infusions were delivered at a rate of 0.25 μl/min over 2 min for rats and 0.15 μl/min over 2 min for mice. Infusers were left in place for 1 min to allow for diffusion of the

drug. At the completion of behavioral procedures, 40 mm sections were stained with cresyl violet to verify placement of the infusion needle tips (Fig. S1A). To further verify placement of infusion needle tips and spread, 10 mM of Alexa Fluor 488 dye was infused into the BLC at a rate of 0.25 ml/min over 2 min (Fig. S1B). The animals were transcardially perfused with PBS and 4% PFA 30 min following infusion. Brains were postfixed overnight and placed in 30% sucrose for one week. The BLC was sliced in 50 µm sections, mounted on slides and coverslipped with Prolong Gold with DAPI.

Behavioral Procedures

Conditioned Place Preference (CPP)

CPP for a food reward (Kellogg's Froot Loops™) was performed as described, with the following modifications [2]. Rats were food restricted to 90% of their ad libitum weight prior to training. For two days before training rats were given several Froot Loops in their homecage prior to their daily ration of rat chow. During training, animals were given 50 Froot Loops in the CS+ chamber and nothing in the CS- chamber. This was repeated over 14 days, with 7 CS+ and 7 CS- pairings.

Thy1-GFP(m) mice were used to determine spine density. CPP was performed as described above, with the following exceptions. On the pretest day, mice were allowed to freely explore the apparatus for 30 min. The first 15 min served as a habituation period to allow the mice to acclimate to the novel context without inducing latent inhibition and the second 15 min period served as the animals' pretest. Mice assigned to METH CPP were trained over three consecutive days with twice daily training sessions, such that mice received both METH (2 mg/kg) and saline each day, with time of day for METH treatment counterbalanced within groups. Mice assigned to Saline CPP were trained over three consecutive days with twice daily training sessions, both of which were paired with a saline injection. Testing was limited to 7 min for the dendritic spine density experiment to minimize the potential impact of extinction learning on METH CPP-induced spine changes within the BLC.

Auditory Fear Conditioning

Rats were first habituated to the training context (modified Noldus Phenotypers) for a total of 12 min over three exposures in one day. For training, rats explored the chamber for 2 min before receiving 3 pairings of a 30 s auditory tone (6 kHz, 85 dB) that coterminated with a foot shock (2 s, 0.5 mA). For testing conducted 48 hrs later, rats were allowed to explore the novel context for 3 min, followed by 3 min of tone presentation.

Context-Induced Reinstatement of METH Seeking

Prior to catheter/guide cannula implantation and the start of training, all rats were food trained in a 20 hr overnight session to lever press on a fixed ratio (FR) 1 for 45 mg grain pellets (Bioserv) in operant conditioning chambers (Coulbourn Instruments) equipped with two retractable levers and a food dispenser positioned between the levers. Pressing the lever positioned to the right of the dispenser (active lever) resulted in delivery of a food pellet, whereas pressing the left (inactive) lever had no programmed consequences. No contextual cues were present during food training. Surgery was performed 48 hrs after reaching criteria of 100 lever presses for pellets.

METH self-administration training was conducted during 2 hr sessions for 14 consecutive days in Context A. Context A consisted of a solid blue Plexiglas floor, two house lights and an orange scent. Animals were weighed and received a 0.1 ml IV infusion of heparin (70 U/ml) prior to the start of each training session. Animals were trained to press the active lever on an FR1 schedule for METH reinforcement (0.02 mg in a 0.05 ml infusion), followed by a 20 s time-out period. Lever presses on the inactive lever were not reinforced. Animals moved onto the extinction phase after achieving an average of 10 METH infusions per day over 14 consecutive days. After each training session, animals received an infusion of 100 ml of antibiotic solution (10 mg/ml cefazolin in heparinized saline [70 U/ml]) and 100 ml of low dose heparinized saline (10 U/ml) to ensure patency. Animals received 20 g of food pellets after each behavioral session to maintain their weight. Extinction training consisted of 2 hr sessions free of reinforcement conducted in Context B, which had a stainless steel mesh floor, a pink wall, no house lights, a vanilla scent and a piece of Plexiglas with carriage bolt heads slanted against the left wall. Extinction criteria was set for individual animals at 2 consecutive days where active

lever presses were less than 25% of their average lever presses over the final three days of training. Animals then underwent 1 hr of reinstatement testing in Context A for 2 consecutive days. Fifteen min prior to reinstatement day 1 (R1), animals received an intra-BLC infusion of LatA or vehicle.

Spine Density Analysis

Thy1_GFP(m) mice express eGFP in forebrain glutamatergic pyramidal-like neurons under the control of the Thy1 promoter [3]. Forty-eight hrs after CPP training, Thy1-GFP(m) mice received intra-BLC infusions of vehicle or LatA, followed by a preference test. Twenty-four hrs later, the animals were transcardially perfused with PBS and 4% PFA. Brains were postfixed overnight and placed in 30% sucrose for one week. The BLC was sliced in 50 μm sections, mounted on slides and coverslipped with Prolong Gold with DAPI. For 3D-reconstruction of dendrites, high-resolution confocal images of amygdalar neuronal branches were acquired (Olympus). A 4X magnification picture of the whole BLC was taken and a 40X oil immersion lens (N.A. 1.3) - 1.7X scan zoom - was used to take a *z*-stack through a section with a low enough density of dendrites to provide for accurate resolution and analysis of individual dendritic spines. All images were taken from within 3 anterior and/or posterior sections of the cannula track. All *z*-stacks (50-80 images per stack; *z*-distance between two serial images = 0.44 μm) were imported into Image J (NIH) for morphometric analysis. To determine spine density, 10 dendrite sections per animal, ranging from 20-45 μm in length and less than 1 μm in width, were selected for analysis (7 animals per group). Dendrite sections over 1 μm correspond to primary branches, while those under 1 μm are secondary and tertiary. Previous studies have showed that behavioral situations, such as learning and stress, exert a larger effect on high-order branches spine density [4-6]. Accordingly, secondary and tertiary branches were chosen for spine density analysis (Fig. S2). In order to classify dendritic protrusions (spine vs filopodia) filopodia (long thin structures) were identified with the following criteria: ratio of head diameter to neck diameter < 1.2:1 and ratio of length to neck diameter > 3:1. The remaining protrusions were classified as spines.

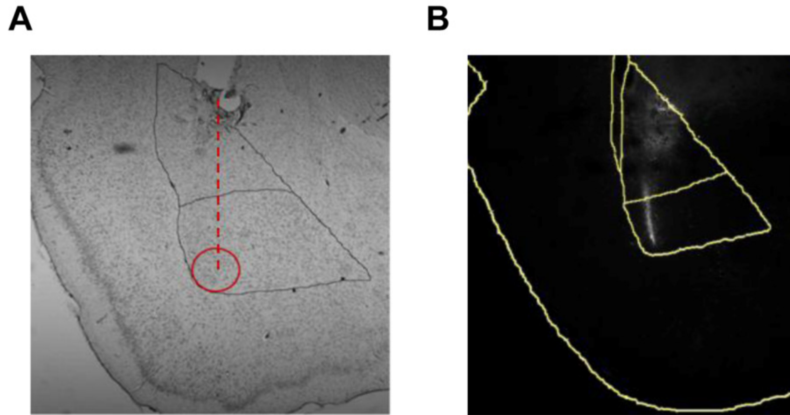

Figure S1. Representative images depicting cannula placements and injection sites in the basolateral amygdala complex (BLC). **(A)** A representative brain slice stained with cresyl violet depicting cannula placement and infusion needle projection (red). **(B)** Intra-BLC infusion of Alexa Fluor 488 dye to show needle tip in ventral BLA with spread throughout dorsal/ventral axis of BLC.

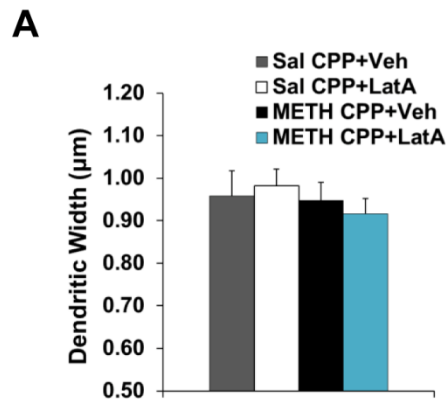

Figure S2. Widths of dendritic sections analyzed for spine density in Fig. 6D-F. Error bars represent SEM. CPP, conditioned place preference; METH, methamphetamine; Sal, saline; Veh, vehicle.

Supplemental References

1. Paxinos G, Watson C (2007): *The rat brain in stereotaxic coordinates. 4 ed.* Academic Press.
2. Rubinow MJ, Hagerbaumer DA, Juraska JM (2009): The food-conditioned place preference task in adolescent, adult and aged rats of both sexes. *Behav Brain Res* 198: 263-266.
3. Feng G, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, Wallace M, *et al.* (2000): Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. *Neuron* 28: 41-51.
4. Maroun M, Ioannides PJ, Bergman KL, Kavushansky A, Holmes A, Wellman CL (2013): Fear extinction deficits following acute stress associate with increased spine density and dendritic retraction in basolateral amygdala neurons. *Eur J Neurosci.*
5. Mitra R, Jadhav S, McEwen BS, Vyas A, Chattarji S (2005): Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. *Proc Natl Acad Sci U S A.* 102: 9371-9376.
6. Vyas A, Jadhav S, Chattarji S (2006): Prolonged behavioral stress enhances synaptic connectivity in the basolateral amygdala. *Neuroscience* 143: 387-393.