Role of Dorsal Striatal Histone Deacetylase 5 in Incubation of Methamphetamine Craving

Supplementary Information

Supplemental Methods and Materials

Subjects

We used male Sprague-Dawley rats (Charles River, total n=112), weighing 300-350 g prior to surgery and 325-375 g at the start of the drug self-administration procedure; we maintained the rats under a reverse 12:12 h light/dark cycle with food and water freely available. We kept the rats two per cage prior to surgery and then housed them individually after surgery. We performed the experiments in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8^{th} edition), under the protocols approved by the Animal Care and Use Committee. We excluded 12 rats, due to failure of catheter patency, placement and expression of the viral constructs, and health-related issues.

Intravenous surgery

We anesthetized the rats with either ketamine plus xylazine (80 and 10 mg/kg, i.p., respectively) or with isoflurane gas (5% induction; 2-3% maintenance) and inserted silastic catheters into the rat's jugular vein as previously described (1-3). We injected the rats with buprenorphine (0.1 mg/kg, s.c.) or ketoprofen (2.5 mg/kg, s.c.) after surgery to relieve pain and inflammation; we allowed them to recover 5-7 days before methamphetamine (Meth) self-administration training. During the recovery and training phases, we flushed the catheters every 24-48 h with gentamicin (Butler Schein; 5 mg/mL) dissolved in sterile saline.

Adeno-associated virus (AAV) preparation

We used two sets of viral constructs: the first set includes AAV2-GFP ($4x10^{e12}$ viral particles/ml) and AAV2-mHDAC5 ($5x10^{e12}$ viral particles/ml); the second set includes scAAV1-shLuc ($2.74x10^{e12}$ viral particles/mL) and scAAV1-shHDAC5 (($2.74x10^{e12}$ viral particles/mL).

For AAV2-mHDAC5, we used a novel virus (AAV2-HDAC5 3SA) described in a recent study (4). This virus expresses a dephosphorylated mutant of HDAC5 (S259A/S279A/S498A or 3SA), which concentrates primarily in the nucleus (4). We sub-cloned this full length mutated HDAC5 (mHDAC5) into a pAAV

backbone downstream of a CMV_{ie} promoter, using MluI and BstEII sites. Detailed plasmid maps are available upon request. We used AAV2-GFP (green fluorescence protein) as the control condition. The *in vivo* validation of HDAC5 overexpression by AAV2-mHDAC5 is shown in Fig. 2C and Fig. S1A. We also validated the nuclear localization of mHDAC5 by co-labeling HDAC5 with DAPI (a nuclear stain), as shown in Fig. 2B.

For scAAV1-shHDAC5, the shRNA sequence against HDAC5 (shHDAC5, 5' GAAGGTTCTACAGAGAGCGAGttcaagagaCTCGCTCTCTGTAGAACCTTC 3', lowercase indicates loop domain) is based on a previously published lentiviral vector (5). Briefly, we sub-cloned HDAC5 shRNA expression cassette into a self-complementary AAV backbone that also expresses a nuclear-localized enhanced yellow fluorescent protein (EYFP) reporter from a CMV_{ie} promoter (Addgene 104987). We used a similarly constructed vector that expresses a shRNA against nanoluciferase (shLUC, 5' GCCGAACATGATCGACTATTTttcaagagaAAATAGTCGATCATGTTCGGC 3', lowercase indicates loop domain; Addgene 104986) as the control condition. Viruses were packaged and purified as previously described (6). Detailed plasmid maps are available upon request. The *in vivo* validation of AAV-shHDAC5 is shown in Fig. 3C and S1B.

AAV injections

In Exp. 1-2, we injected AAV-GFP, AAV-mHDAC5, AAV-shLuc or AAV-shHDAC5 bilaterally into the dorsal striatum (DS) and each hemisphere received a total of four injections (0.75 µl/injection), with two injections aiming at the dorsomedial striatum (DMS) and the other two injections aiming at dorsolateral striatum (DLS). We used the following coordinates from Bregma: DMS: AP, 1.2 mm; ML, 2.6 mm (6° angle); DV, -4.0 mm and -5.0 mm. DLS: AP, 1.2 mm; ML, 3.8 mm (6° angle); DV, -5.0 mm and -6.0 mm. These coordinates are based on a previous study (7). We delivered the AAVs by Hamilton syringes (32 gauge) at a rate of 0.375 µl/min. After each injection, we left the injection needle in place for an additional minute to allow diffusion. After the final injection, we filled the drilled hole with bone wax. In Exp. 3, we injected AAV-shLuc or AAV-shHDAC5 bilaterally into rat DMS or DLS (4 independent groups) using the same coordinates as described above; each hemisphere received two injections (0.75 µl/injection).

Apparatus

We trained the rats in self-administration chambers located inside sound-attenuating cabinets and controlled by a Med Associates (Georgia, VT) system. Each chamber has two levers located 8-9 cm above the floor. During self-administration training, presses on the retractable (active) lever activated the infusion pump (which delivered a Meth infusion); presses on the stationary (inactive) lever were not reinforced with drug. For Meth intravenous infusions, we connected each rat's catheter to a liquid swivel (Instech) via polyethylene-50 tubing, protected by a metal spring. We then attached the liquid swivel to a 20-ml syringe via polyethylene-50 tubing and to a 22-gauge modified cannula (Plastics One, VA).

Meth self-administration training

We used a training procedure previously described by Theberge et al. (3), Krasnova et al. (8), and Li et al. (9). We brought the rats to the self-administration room on their first day of training and housed them chronically in the self-administration chambers. We trained the rats to self-administer Meth for 9-h per day (three 3-h sessions, separated by 1 h between sessions) under a fixed-ratio-1 (FR-1) with 20-s timeout reinforcement schedule. We dissolved Meth in saline, and the rats self-administered Meth at a dose of 0.1 mg/kg/infusion over 3.5 s (0.10 ml/infusion). We trained the rats for 10 sessions over a 14-day period (off day every 3rd or 4th day) to prevent loss of body weight during the training phase. [Note: Meth-trained rats lose about 4-8 g after each day of training and regain the lost weight during the off day (3, 8, 9)].

The daily training sessions started at the onset of the dark cycle and began with the extension of the active lever and the illumination of the red house light. The house light remained on for the duration of the each 3-h session. During training, active lever presses led to the delivery of a Meth infusion and a compound 5-s tone-light cue (the tone and light modules were located above the active lever). During the 20-s timeout, we recorded the non-reinforced lever presses. We set 34 infusions as the maximum for each 3-h session to prevent overdose. The red house light was turned off and the active lever retracted after the rats received the maximum infusions or at the end of each 3-h session. The training data from Exp. 1-2 are described in Fig. 1, and the training data from Exp. 3 are described in Fig. S2.

Withdrawal phase

During the withdrawal phase, we housed the rats individually in the animal facility and handled them 3-4 times per week.

Relapse test

We conducted all relapse tests immediately after the onset of the dark cycle. The sessions began with the extension of the active lever and the illumination of the red house light, which remained on for the duration of the session. Active lever presses during testing [the operational measure of drug seeking in incubation of craving studies (10, 11)] resulted in contingent presentations of the tone-light cue, previously paired with Meth infusions, but not the drug.

Immunohistochemistry

After deeply anesthetizing the rats with isoflurane, we perfused them with 4% PFA in PBS. We fixed the extracted brains in 4% PFA in PBS for 1 h before transferring the brains to 30% sucrose in PBS. We sectioned the brains (30 µm) using a Leica cryostat and stored brain slices in cryoprotectant at -20°C. For GFP and HDAC5 immunohistochemistry, we first washed the sections for 10 min in PBS and incubated them with a blocking buffer (2% BSA in PBS with 0.3% Triton-x100) for 1 h at room temperature. Next, we incubated the sections with a directed conjugated antibody against GFP (600-141-215, Dylight 488 conjugated, 1:200, Rockland, PA, RRID: 1961516) and a primary antibody against HDAC5 (ab50001, 1:200, Abcam, UK, RRID: AB_880357) in blocking buffer overnight at room temperature. We washed the sections in PBS 3 times (5 min each) and then incubated them with the secondary antibody Alexa 594-labled anti-mouse (R37121, 1:200, Thermo Fisher Sicentific, MD, RRID: AB_2556549) in blocking buffer for 1 h at room temperature. Finally, we washed the sections in PBS and mounted them on gelatin-coated slides. After air drying the sections, we cover-slipped the sections with Mowiol (Millipore). To demonstrate nuclear localization of mHDAC5, we also cover-slipped some sections with Mowiol containing DAPI.

Image acquisition and HDAC5 immunofluorescence quantification

We acquired fluorescent images of DS with a Zeiss AXIO Imager M2 microscope. We captured the images using iVision (Biovision) at 10X magnification. We measured fluorescent intensity using ImageJ software after subtracting the background (rolling radius=20 pixel). We analyzed two sections/rat between

Bregma +1.70 mm and +1.00 mm in a double-blind manner. For imaging double-labeling of HDAC5 and DAPI, we acquired fluorescent images of DS with Olympus Fluoview FV100 BX61WI upright confocal laser scanning microscope.

RNA extraction, cDNA synthesis and qPCR

We collected and stored DS tissue as described above. For RNA extraction, we homogenized tissue punches in Trizol (Invitrogen) according to the protocol from the manufacturer. We purified RNA with RNeasy Micro columns (Qiagen, Germany). We confirmed the RNA purity by spectroscopy at 260/280 and 260/230>1.8. We reversely transcribed RNA into cDNA using iScript cDNA synthesis (Bio-Rad, CA) and performed quantitative PCR (qPCR) using Taqman assay and Applied Biosystems 7500 systems (Thermo Fisher Scientific). We ran duplicates for each reaction and analyzed the reactions using the ΔΔCt method with glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) as the housekeeping gene. See Table S1 for mRNA primer sequences and description of the genes.

Immunoblotting

We took rats directly from their home cages and performed decapitation after anesthetizing the rats with isoflurane (5%). We obtained a 2-mm coronal section containing striatum using a brain matrix (ASI Instruments). We dissected viral expressing regions in DS with the assistance of fluorescent lamp and filters, as described previously (12). We froze tissue punches in Eppendorf tubes on dry ice and stored tissue at -80°C. To prepare the tissue for subsequent immunoblotting, we quickly sonicated the tissue punches in lysis buffer as previously described (13), and then removed debris by brief centrifugation. We processed all samples (20 μg per lane for AAV-mHDAC5 validation; 30 μg per lane for AAV-shRNA validation) for immunoblotting as previously described (14) and used the following primary antibodies: anti-HDAC5 (sc-133106, 1:500, Santa-Cruz, RRID: AB_2116793) and anti-GAPDH (glyceraldehyde-3-phosphate dehydrogenase, CB1001, 1:10000, Millipore, RRID: AB_2107426). We incubated the blots first with blocking buffer (5% non-fat dry milk in 1xTBS with 0.05% Tween-20) for 1 h at room temperature, and then with primary antibodies diluted in blocking buffer at 4°C overnight. Next, we incubated blots with IRDye 800CW secondary antibodies (anti-mouse IgG, 926-32210, Li-Cor Biosciences, RRID: AB_621842) diluted at 1:2000 in blocking buffer for 1 h at room temperature. We imaged the blots with Odyssey IR fluorescence

scanner (Li-Cor Biosciences) and quantified signals using ImageJ software. We used GAPDH as a loading control.

Statistical analysis

We analyzed the behavioral and molecular data with SPSS (version 20) or Prism GraphPad (version 5) using mixed ANOVAs, ANCOVAs, one-way ANOVA, or t-test, as appropriate. We followed significant interaction or main effects ($p<0.05$) with Fisher PLSD post-hoc tests. For the repeated measures analyses of the training data, we replaced 29 outlier values of inactive lever presses (3 standard deviations above the group mean) with the group mean for a given training day. Additionally, we lost the last hour training data for active and inactive levers on the 3rd training day in Exp. 2, due to computer software malfunction. Therefore, we included the 5-h data instead of the 6-h data for active and inactive levers for this training day. Due to an experimenter error in assigning the correct input to the inactive lever in the Med-PC program, we also lost the data of the inactive lever in the relapse tests of Exp. 3. Therefore, for this experiment, we only present the data of the active lever. For the molecular data, we excluded outlier values (3 standard deviations above the group mean) from the final data presentation and analysis. In Exp. 2, we also set the inclusion criterion of the HDAC5 knockdown by AAV-shHDAC5 (based on *Hdac5* mRNA expression) to be 75% of the control group (AAV-shLUC); we excluded three rats based on this criterion. For the data described in Fig. 3C, Fig. 3F and Fig. S3C, we set the threshold for fold change to be >1.2 or <0.75. We indicated the between- and within-subject factors of the different analysis in the Results section and these statistical comparisons are also listed in Table S2.

Supplemental Results (self-administration training in Exp. 1-3)

Experiment 1 (Fig. 1A)

Both groups (AAV-GFP and AAV-mHDAC5) increased their number of Meth infusions over days ($F_{9,189}=36.3$, $p<0.001$). There were no group differences in total drug intake during training ($p>0.05$) but there was a significant interaction between Training Session and Virus Condition ($F_{9,189}=2.6$, $p=0.007$) due to somewhat different patterns of drug intake over days in the two groups. The analysis of lever presses

showed that in both groups active lever but not inactive lever presses increased over days (Lever x Day interaction, $F_{9,189}$=4.5, p<0.001). There were no group differences in lever presses during training (p>0.05).

Experiment 2 (Fig. 1B):

Both groups (AAV-shLUC and AAV-shHDAC5) increased their number of Meth infusions over days ($F_{9,180}$=12.2, p<0.001). There were no group differences in total drug intake during training (p>0.05). The analysis of lever presses showed that in both groups active lever but not inactive lever increased over days (Lever x Day interaction, $F_{9,180}$=4.9, p<0.001). There were no group differences in lever presses during training (p>0.05).

Experiment 3 (Fig. S2):

For DMS or DLS knockdown experiment, both groups (AAV-shLUC and AAV-shHDAC5) increased their number of Meth infusion over days (DMS: $F_{9,198}$=54.4, p<0.001; DLS: $F_{9,189}$=18.3, p<0.001). There were no group differences in total drug intake during training (p>0.05). The analysis of lever presses showed that for both DMS and DLS, the rats increased their active lever but not inactive lever presses over days (DMS: Lever x Day interaction, $F_{9,198}$=8.8, p<0.001; DLS: Lever x Day interaction, $F_{9,189}$=7.6, p<0.001). In the DMS (but not DLS) knockdown experiment, we observed a significant triple interaction of Lever x Training Session x Virus Condition ($F_{9,198}$=2.5, p=0.011), due to increased active lever presses during the last two training sessions in the AAV-shHDAC5 group.

Table S1. Primer/probe sequences for qPCR and description of each gene

Gene	Description	Taqman probe	Forward primer	Reverse primer
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	CTCATGACCACAGTCCA	GACAACTTTGGCATCGTGGAA	CACAGTCTTCTGAGTGGCAGTGA
Hdac1	Histone deacetylase 1	Rn01519308_g1[a]		
Hdac2	Histone deacetylase 2	Rn01193634_g1[a]		
Hdac3	Histone deacetylase 3	Rn00584926_m1[a]		
Hdac4	Histone deacetylase 4	Rn01427040_m1[a]		
Hdac5	Histone deacetylase 5	GCTGAGGCTCCAGGAATTCC[a]	CTCTGGTCCAAAGAAGCATGATGG	CGGCCTCAACCATTCCCTCCCACAGC
Gnb4	Guanine nucleotide-binding protein, beta-4	Rn01755632_m1[a]		
Grin2a	NMDA receptor 2A	Rn00561341_m1[a]		
Kcnk4	Potassium channel, subfamily K, member 4	Rn00587450_m1[a]		
Kcnq5	Potassium channel, subfamily Q, member 5	Rn01512013_m[a]		
Suv39h1	Histone-lysine N-methyltransferase	Rn01528294_g1[a]		
Rgs20	Regulator of G protein signaling 20	Rn01749217_m1[a]		
Rapgef6	Rap guanine nucleotide exchange factor 6	Rn01495533_m1[a]		
Abca5	ATP-binding cassette 5	Rn00597824_m1[a]		
Cuedc1	CUE domain containing 1	Rn01461298_m1[a]		
Tacr1	Tachykinin receptor 1	Rn00562004_m1[a]		

[a]Taqman catalog number.

Table S2. Statistical analysis (SPSS GLM repeated-measures module). Eta^2 = proportion of explained variance.

Figure number	Factor name	F-value	*p*-value	Partial Eta^2
Figure 1A. Self-administration training—HDAC5 overexpression	Infusion:			
	Training day (within)	$F_{9,189}$=36.3	<0.001*	0.63
	Virus condition (between)	$F_{1,21}$=0.6	0.454	0.03
	Virus condition * Training day interaction	$F_{9,189}$=2.6	0.007*	0.11
	Lever Presses:			
	Lever (within)	$F_{1,21}$=62.5	<0.001*	0.75
	Training day (within)	$F_{9,189}$=4.5	<0.001*	0.18
	Virus condition (between)	$F_{1,21}$=2.6	0.121	0.11
	Lever * Training day interaction	$F_{9,189}$=4.5	<0.001*	0.22
	Lever * Training day*Group interaction	$F_{9,189}$=1.0	0.453	0.05
Figure 1B. Self-administration training—HDAC5 knockdown	Infusion:			
	Training day (within)	$F_{9,180}$=12.2	<0.001*	0.38
	Virus condition (between)	$F_{1,20}$=1.9	0.188	0.09
	Virus condition * Training day interaction	$F_{9,180}$=1.6	0.123	0.07
	Lever Presses:			
	Lever (within)	$F_{1, 20}$=35.8	<0.001*	0.64
	Training day (within)	$F_{9,180}$=4.2	<0.001*	0.17
	Virus condition (between)	$F_{1,20}$=0.6	0.445	0.03
	Lever * Training day interaction	$F_{9,180}$=4.9	<0.001*	0.20
	Lever * Training day*Group interaction	$F_{9,180}$=0.5	0.894	0.02
Figure 2C. HDAC5 overexpression—HDAC5 protein expression	Virus condition (between)	t_{21}=4.5	<0.001*	
Figure 2D. HDAC5 overexpression—Relapse test day 2 and 30 (30 min)	Withdrawal day (within)	$F_{1,19}$=42.9	<0.001*	0.69
	Virus condition (between)	$F_{1,19}$=5.6	0.029*	0.23
	Virus condition * Withdrawal day	$F_{1,19}$=3.5	0.075	0.16
Figure 2E. HDAC5 overexpression—Relapse test day 30 (3 h)	Total Responses:			
	Virus condition (between)	$F_{1,20}$=7.3	0.014*	0.27
	Time Course:			
	Session minute (within)	$F_{5,105}$=34.6	<0.001*	0.62
	Virus condition (between)	$F_{1,21}$=5.4	0.030*	0.20
	Virus condition * Session minute	$F_{5,105}$=0.23	0.950	0.01
	Post hocs [Virus condition (between)]:			
	30 min	t_{21}=2.1	0.045*	
	60 min	t_{21}=1.3	0.192	
	90 min	t_{21}=1.5	0.161	
	120 min	t_{21}=1.5	0.159	
	150 min	t_{21}=1.3	0.225	
	180 min	t_{21}=1.0	0.345	
Figure 3C. HDAC5 knockdown—*Hdacs* mRNA expression	Virus condition (between)			
	Hdac1	t_{19}=2.7	0.015*	
	Hdac2	t_{17}=3.1	0.007*	
	Hdac3	t_{18}=2.1	0.047*	
	Hdac4	t_{19}=2.6	0.016*	
	Hdac5	t_{19}=12.7	<0.001*	

Figure number	Factor name	F-value	*p*-value	Partial Eta^2
Figure 3D. HDAC5 knockdown—Relapse test day 2 and 30 (30 min)	Withdrawal day (within) Virus condition (between) Virus condition * Withdrawal day interaction	$F_{1,18}$=19.4 $F_{1,18}$=11.9 $F_{1,18}$=12.0	<0.001* 0.003* 0.003*	0.52 0.40 0.40
Figure 3E. HDAC5 knockdown—Relapse test day 30 (3 h)	Total Responses: Virus condition (between) Time Course: Session minute (within) Virus condition (between) Virus condition * Session minute interaction Post hocs [Virus condition (between)]: 30 min 60 min 90 min 120 min 150 min 180 min	 $F_{1,19}$=7.2 $F_{5,100}$=46.7 $F_{1,20}$=8.7 $F_{5,100}$=5.1 t_{20}=3.3 t_{20}=1.6 t_{20}=2.3 t_{20}=2.5 t_{20}=1.3 t_{20}=1.5	 0.014* <0.001* 0.008 <0.001* 0.003* 0.124 0.035* 0.022* 0.193 0.155	 0.28 0.70 0.30 0.20
Figure 3F. HDAC5 knockdown—Gene expression of potential HDAC5 targets	Virus condition (between) *Gnb4* *Grin2a* *Kcnk4* *Kcnq5* *Suv39h1* *RGS20* *RapGEF6* *Abca5* *Cuedc1* *Tacr1*	 t_{19}=3.0 t_{18}=1.6 t_{19}=1.5 t_{18}=1.7 t_{19}=3.1 t_{19}=0.3 t_{19}=0.6 t_{19}=1.6 t_{18}=2.1 t_{19}=2.7	 0.007* 0.125 0.151 0.101 0.006* 0.806 0.576 0.129 0.053 0.015*	
Supplementary figure S1A: Validation of HDAC5 expression after AAV injection—HDAC5 overexpression	Virus condition (within) Protein	 t_{10}=-10.8	 <0.001*	
Supplementary figure S1B: Validation of HDAC5 expression after AAV injection—HDAC5 knockdown	Virus condition (within) mRNA Protein	 t_3=6.8 t_3=4.3	 0.006* 0.023*	
Supplementary figure S2A: Self-administration training—DMS	Infusion: Training day (within) Virus condition (between) Virus condition * Training day interaction Lever Presses: Lever (within) Training day (within) Virus condition (between) Lever * Training day interaction	 $F_{9,198}$=54.4 $F_{1,22}$=1.8 $F_{9,198}$=1.2 $F_{1, 22}$=156.0 $F_{9,198}$=6.9 $F_{1,22}$=1.1 $F_{9,198}$=8.8	 <0.001* 0.191 0.300 <0.001* <0.001* 0.313 <0.001*	 0.71 0.08 0.05 0.88 0.24 0.05 0.29

Figure number	Factor name	F-value	*p*-value	Partial Eta^2
	Lever * Training day*Group interaction	$F_{9,198}$=2.5	0.011*	0.10
Supplementary figure S2B: Self-administration training—DLS	Infusion: Training day (within) Virus condition (between) Virus condition * Training day interaction Lever Presses: Lever (within) Training day (within) Virus condition (between) Lever * Training day interaction Lever * Training day*Group interaction	 $F_{9,189}$=18.3 $F_{1,20}$=0.5 $F_{9,189}$=0.7 $F_{1, 21}$=111.7 $F_{9,189}$=5.7 $F_{1,21}$=0.9 $F_{9,189}$=7.6 $F_{9,189}$=0.2	 <0.001* 0.479 0.724 <0.001* <0.001* 0.763 <0.001* 0.994	 0.47 0.02 0.03 0.84 0.21 0.00 0.27 0.01
Supplementary figure S3C: Sub-region specific HDAC5 knockdown—HDAC5 mRNA expression	**DMS** Virus condition (between) **DLS** Virus condition (between)	 t_{20}=7.8 t_{20}=8.0	 <0.001* <0.001*	
Supplementary figure S3D: Sub-region specific HDAC5 knockdown—Relapse test day 2 and 30 (30 min)	**DMS** Withdrawal day (within) Virus condition (between) Virus condition * Withdrawal day interaction **DLS** Withdrawal day (within) Virus condition (between) Virus condition * Withdrawal day interaction	 $F_{1,22}$=20.2 $F_{1,22}$=0.6 $F_{1,22}$=1.2 $F_{1,21}$=62.4 $F_{1,21}$=3.2 $F_{1,21}$=3.5	 <0.001* 0.811 0.291 <0.001* 0.087 0.074	 0.48 0.00 0.05 0.75 0.13 0.14
Supplementary figure S3E: Sub-region specific HDAC5 knockdown—Relapse test day 30 (3 h)	**DMS** Virus condition (between) **DLS** Virus condition (between)	 t_{22}=0.68 t_{21}=1.4	 0.504 0.174	
Supplementary figure S3E: Sub-region specific HDAC5 knockdown—Relapse test day 30 (3 h) time course	**DMS** Session minute (within) Virus condition (between) Virus condition * Session minute interaction Post hocs [Virus condition (between)]: 30 min 60 min 90 min 120 min 150 min 180 min **DLS** Session minute (within)	 $F_{5,110}$=18.3 $F_{1,22}$=0.46 $F_{5,110}$=0.11 t_{22}=0.55 t_{22}=0.43 t_{22}=0.69 t_{22}=0.70 t_{22}=0.14 t_{22}=0.45 $F_{5,105}$=29.5	 <0.001* 0.504 0.991 0.589 0.675 0.499 0.491 0.888 0.655 <0.001*	 0.45 0.02 0.01 0.58

Figure number	Factor name	F-value	*p*-value	Partial Eta^2
	Virus condition (between)	$F_{1,21}$=2.5	0.132	0.11
	Virus condition * Session minute interaction	$F_{5,105}$=1.8	0.125	0.08
	Post hocs [Virus condition (between)]:			
	30 min	t_{21}=2.0	0.062	
	60 min	t_{21}=1.3	0.196	
	90 min	t_{21}=1.5	0.143	
	120 min	t_{21}=1.4	0.177	
	150 min	t_{21}=1.2	0.235	
	180 min	t_{21}=-0.6	0.561	

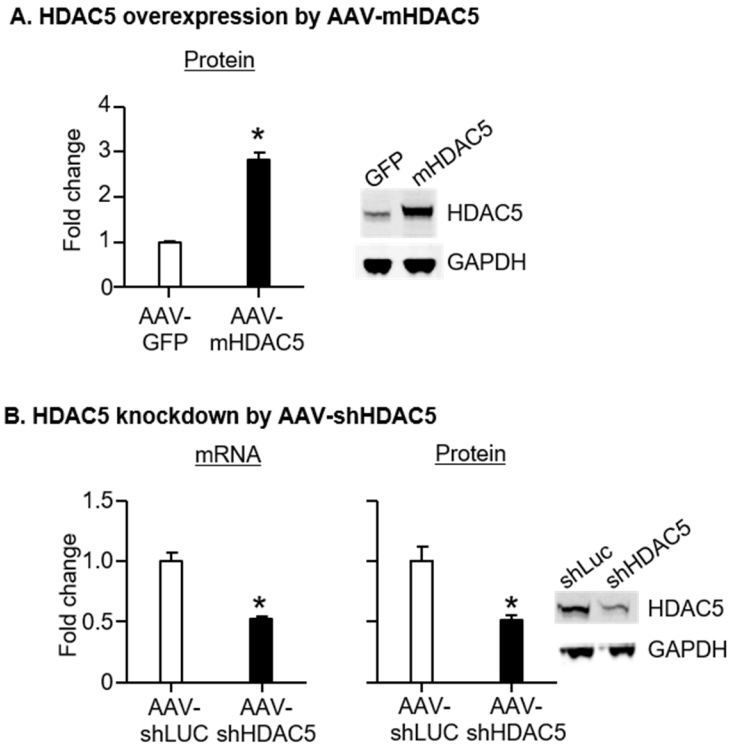

Figure S1. *Validation of HDAC5 expression after HDAC5 expression by AAV infection.* **(A)** HDAC5 protein expression after AAV-mHDAC5 injections into DS. Data are presented as fold change of mean values in the AAV-GFP injected hemisphere. *p<0.05; n=6 per group. **(B)** HDAC5 expression after AAV-shHDAC5 injections into DS. Data are presented as fold change of mean values in the AAV-shLUC injected hemisphere. Left: *Hdac5* mRNA. Right: HDAC5 protein, *p<0.05; n=4 per group.

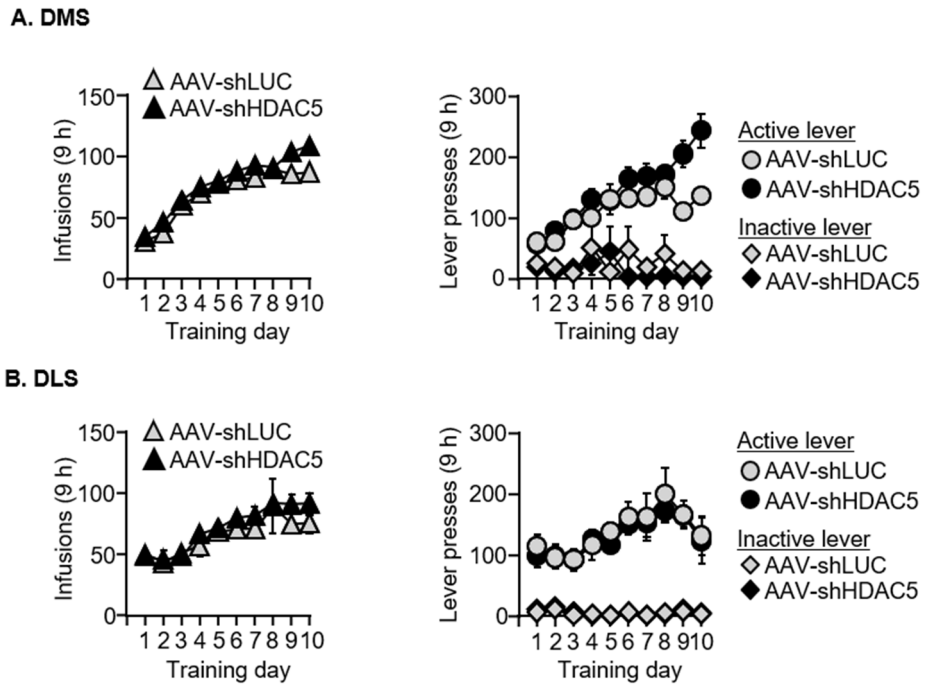

Figure S2. *Meth self-administration training*. Data are mean ± SEM number of Meth (0.1 mg/kg/infusion) infusions, active and inactive lever presses during the ten 9-h daily self-administration sessions in Exp. 3 (**A:** DMS, total n=24; **B:** DLS, total n=23). During training, active lever presses were reinforced on an FR1 20-s timeout reinforcement schedule and Meth infusions were paired with a 5-s tone-light cue.

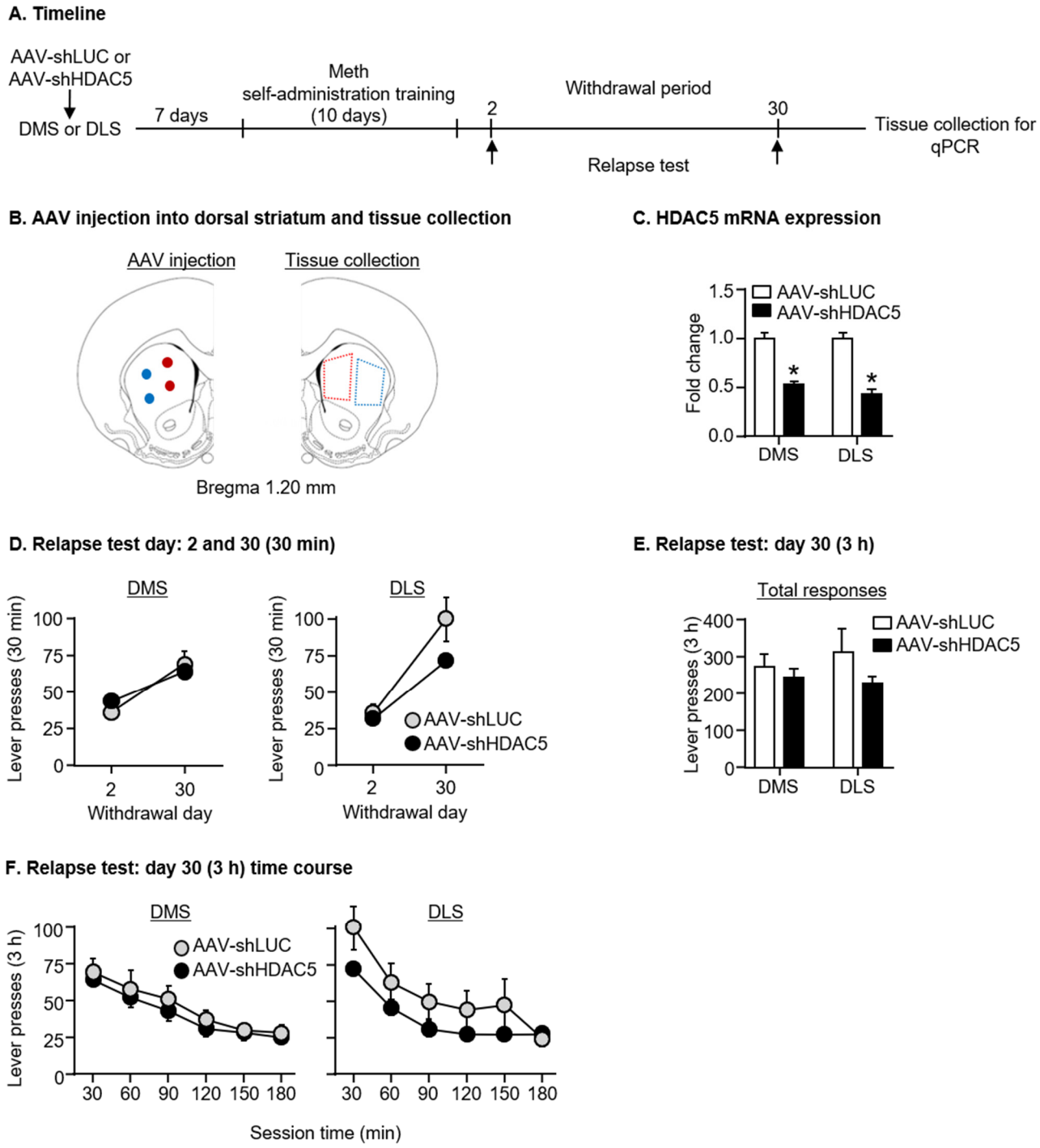

Figure S3. *Knocking down HDAC5 expression in DMS or DLS alone had no effect on incubation of Meth craving.* **(A)** Timeline of the experiment. **(B)** Left: representative anatomical location of AAV injections into DMS (red dots) or DLS (blue dots) [mm from Bregma (15)]; Right: a representative schematic of DMS or DLS tissue collection (DMS: red quadrangle; DLS: blue quadrangle). **(C)** *Hdac5* mRNA expression. Data

are presented as fold change of mean values in the AAV-shLUC group. Error bars indicate SEM. *p<0.05; n=9-13 per group. **(D)** Relapse test on withdrawal days 2 and 30: Data are mean±SEM of responses on the previously active lever during the 30-min relapse test on withdrawal day 2 and the first 30 min of the 3-h relapse test on withdrawal day 30. **(E)** Relapse test on withdrawal day 30: Data are mean±SEM of responses on the previously active lever during the 3-h relapse test. During testing, active lever presses led to contingent presentations of the tone-light cue previously paired with Meth infusions during training, but not Meth. n=10-13 per group.

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