Epigenetic Mechanisms in Drug Relapse

SUPPLEMENTAL INFORMATION

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Supplemental Table S1. Glossary of epigenetic and physiological terms

Term	Description
BDNF (Brain derived neurotrophic factor)	Neurotrophin that regulates cell growth, survival, and differentiation
BRD4 (Bromodomain-containing protein 4)	Transcriptional regulator that binds to acetylated lysines on the histones and recruits transcription factors
BRG1 (BRM/SWI2-related gene 1)	Chromatin remodeling ATPase
CDK5 (Cyclin-dependent kinase 5)	Member of the cyclin-dependent kinase family involved in neural development and synaptic plasticity
Chromatin remodeling	Process that modifies chromatin structure to allow transcription to occur.
CK2α (Casein kinase II alpha)	Protein kinase that phosphorylates BRD4 and enables the recruitment of transcription factors to BRD4
cFos	Immediate early gene and transcription factor widely used as a cellular marker of neuronal activity
DNA methylation	Process that adds methyl group to DNA
DNMT3A2 (DNA methyltransferase 3A2)	Class of de novo DNA methyltransferases that adds methyl groups to CpG sites on DNA
Epigenetic mechanisms	Regulation of gene expression mediated through non-DNA encoded mechanisms
G9a (Euchromatic histone-lysine N-methyltransferase 2)	Histone methyltransferase, responsible for methylation of lysines 9 and 27 on histone H3
HAT (Histone acetyltransferase)	Enzyme that adds acetyl group to the N-terminal tail of histones
HDAC (Histone deacetylase)	Enzyme that removes the acetyl group from the N-terminal tail of histones
H3Q5dop (Histone 3 glutamine 5 dopaminylation)	Addition of dopamine to glutamine 5 of the histone H3 N-terminal tail (1)
Histone lysine demethylase	Enzyme that removes the methyl group from lysines of the N-terminal tail of histones
HMT (Histone methyltransferase)	Enzyme that adds the methyl group to the N-terminal tail of histones
Histone modifications	Post-translational modifications to histones that increase or decrease gene expression
INO80 (Inositol auxotroph 80)	Chromatin remodeler that regulates transcription through functions such as histone exchange and nucleosome sliding(2, 3)
KDM5C (Lysine demethylase 5C)	Histone lysine demethylase that removes the methyl group from lysine 4 on histone 3 (H3K4)
KDM6B (Lysine demethylase 6B)	Histone lysine demethylase that removes the methyl group from lysine 27 on histone 3 (H3K27)
MLL1 (Mixed-lineage leukemia 1)	Histone lysine methyltransferase that adds the methyl group to H3K4
NPAS4 (Neuronal PAS domain protein 4)	Immediate early gene and transcription factor regulated by HDAC5
PARP-1 (Poly[ADP-ribose] polymerase 1)	Enzyme that plays a role in transcription regulation through activation of transcription factors and regulatory complexes
PRDM2 (PR/SET domain 2)	Histone methyltransferase that methylates lysine 9 on histone H3
Smad3 (SMAD family member 3)	Transcription factor in the TGF-β signaling pathway
TGF-β (Transforming growth factor beta) family	Regulatory proteins that play a role in many cellular processes including plasticity
UPS (Ubiquitin-proteasome system)	Network of proteins involved in protein degradation

Supplemental Table S2. Summary of epigenetic changes beyond 24 h after non-contingent repeated drug administration.

Abbreviations: AD: abstinence day; ChIP: chromatin immunoprecipitation; dmPFC: dorsomedial prefrontal cortex; H3: histone H3; H3K27ac: acetylation at the 27th lysine residue of H3; H3K27me3: trimethylation at the 27th lysine residue of H3; H3K36me3: trimethylation at the 36th lysine residue of H3; H3K4me3: trimethylation at the 4th lysine residue of H3; H3K9me2: dimethylation at the 9th lysine residue of H3; H3K9me3: trimethylation at the 9th lysine residue of H3; Histone H4; H4R3me2a: asymmetrical dimethylation at the 3rd arginine residue of H4; HAT: histone acetylase; HDAC: histone deacetylase; HPC: hippocampus; i.p.: intraperitoneal injection; IHC: immunohistochemistry; IL; infralimbic cortex; KDM: histone lysine demethylase; LC: locus coeruleus; Meth: methamphetamine; mPFC: medial prefrontal cortex; NAc: nucleus accumbens; OFC; orbitofrontal cortex; PFC: prefrontal cortex; PL: prelimbic cortex; qPCR: quantitative polymerase chain reaction; VTA: ventral tegmental area; WB: western blotting. Symbols: ▲: increase; ▼: decrease; ─: no change.

Drug type	Brain regions	Species	Drug administration procedure	Abstinence day	General category of epigenetic marks/enzymes	Specific epigenetic modifications/enzymes (detecting methods)	Major findings	Citations
Cocaine	NAc	Mouse	Repeated (14 days × 20 mg/kg, i.p.)	AD14	Histone acetylation	H3 acetylation on *Bdnf* I and III promoters (ChIP)	─	(4)
Cocaine	NAc	Mouse	Repeated (28 days × 20 mg/kg, i.p.)	AD28	DNA methyltransferase	*Dnmt1*; *Dnmt3a*; *Dnmt3b* (qPCR)	*Dnmt1*: ─ *Dnmt3a*: ▲ *Dnmt3b*: ─	(5)
Cocaine	NAc	Mouse	Repeated (7 days × 20 mg/kg, i.p.)	AD7	Histone methylation	H3K9me3 (WB)	▼	(6)
Cocaine	NAc	Mouse	Repeated (7 days × 20 mg/kg, i.p.)	AD5 AD10	Histone deacetylase	*Sirt1*; *Sirt2* (qPCR)	*Sirt1*: ▲; *Sirt 2*: ─ *Sirt1*: ─; *Sirt 2*: ─	(7)
Cocaine	NAc	Mouse Rat	Repeated (10 days × 20 mg/kg, i.p.) Repeated (10 days × 10 mg/kg, i.p.)	AD7	Subunits of chromatin-remodeling complex	BRD2; BRD3; BRD4 (WB)	─ ─ ─	(8)
Cocaine	NAc	Mouse	Repeated (7 days × 20 mg/kg, i.p.)	AD7 AD14 AD28 AD42	Histone methylation	H4R3me2a; H3K9me2 H3K36me3 (WB)	H4R3me2a: ▲ ─ ─ ─ H3K9me2: ─ (all timepoints) H3K36me3: ─ (all timepoints)	(9)
Cocaine	NAc	Mouse	Repeated (7 days × 20 mg/kg, i.p.)	AD7	Subunits of chromatin-remodeling complex	*Baz1a* (qPCR)	─	(10)
Cocaine	NAc	Mouse	Repeated (7 days × 15 mg/kg, i.p.)	AD28	DNA methyltransferase	*Dnmt1*; *Dnmt3a*; *Dnmt3b* (qPCR); DNMT activity (DNMT activity assay kit)	*Dnmt1*: ─ *Dnmt3a*: ▲ *Dnmt3b*: ▲ DNMT activity: ▲	(11)
Cocaine	NAc	Mouse	Repeated (10 days × 20 mg/kg, i.p.)	AD28	Transcription factor; histone acetylation and histone methylation	*Nr4a1*(qPCR); Nr4a1 binding at *Nr4a1* promoter; H3K27me3 on *Nr4a1* promoter;	─ ▲ ─	(12)

						H3K27ac on *Nr4a1* promoter; H3K4me3 on *Nr4a1* promoter; Nr4a1 binding at *Cartpt* promoter; H3K27me3 on *Cartpt* promoter; H3K27ac on *Cartpt* promoter; H3K4me3 on *Cartpt* promoter (ChIP)	▲ ▲ ▲ ▼ ▲ ▲	
Cocaine	mPFC	Mouse	Repeated ((4 days × 20 mg/kg, i.p.)	AD3 AD7 AD14 AD30	Histone lysine demethylase	KDM6B (WB)	▲ ▲ ▲ –	(13)
Cocaine	PL and IL	Mouse	Repeated ((4 days × 20 mg/kg, i.p.)	AD7	Histone lysine demethylase	KDM6B (IHC)	PL: ▲ IL: –	
Cocaine	VTA	Mouse	Repeated (14 days × 20 mg/kg, i.p.)	AD14	Histone acetylation	H3 acetylation on *Bdnf* I and III promoters (ChIP)	–	(4)
Amphetamine	Striatum	Mouse	Repeated (7 dyas × 4 mg/kg, i.p.)	AD5	Histone acetylation and methylation	HDAC1 and H3K9me2 binding at *cFos* promotor(ChIP)	▲	(14)
Amphetamine	NAc	Rat	Repeated (14 days × 1 mg/kg, i.p.)	AD14	DNA methylation	Global DNA methylation(radio-labelled [^{3}H]-dCTP extension assay)	▲	(15)
	mPFC						▲	
	OFC						▲	
Meth	HPC	Mouse	Repeated (4 days × 2 mg/kg, i.p)	AD2	DNA methyltransferase and transcription factor	DNMT1;DNMT3A; DNMT3B; MECP2 (WB)	DNMT1: ▼ DNMT3A: ▼ DNMT3B: ▼ MECP2: ▼	(16)
	PFC						DNMT1: ▲ DNMT3a: – DNMT3b: ▲ MECP2: ▲	
Morphine	VTA	Rat	Repeated (every other day: 10, 20, 30, 40, 50 mg/kg, i.p.)	AD7	Histone methylation and acetylation	H3K9me3 and H3K9/K14ac on *Bdnf* promoter II and III (ChIP)	H3K9me3: ▼ (II and III) H3K9/K14ac: – (II and III)	(17)
	LC						H3K9me3: ▼ (II and III) H3K9/K14ac: ▲(II); – (III)	
Morphine	VTA	Rat	Repeated (14 days × 5 mg/kg)	AD14	RNA polymerase II (Pol II); various histone modifications and enzymes; transcription factor	Binding at *Bdnf* promoters (ChIP): Pol II; H3 acetylation; H4 acetylation; H3K4me3; H3K9me2; H3K9me3; H3K27me3; H3K36me3; HDAC complex; Mll1; G9a; PRC2, PRC1; CREB; NURR1	Pol II: ▼ (II, IV, VI) H3 acetylation: ▼ (II) H4 acetylation: – (II) H3K4me3: ▲ (II) H3K9me2: – (II) H3K9me3: – (II) H3K27me3: ▲ (II) H3K36me3: – (II) HDAC complex: ▲ (II) Mll1: ▲ (II); G9a: – (II) PRC1: ▲ (II); PCR2: ▼ (II) CREB: ▲ (VI) pCREB: ▼ (I, II and IV) NURR1: ▼ (I, II)	(18)
Morphine	NAc	Mouse	Repeated (7 days × 20 mg/kg, i.p.)	AD5 AD10	Histone deacetylase	*Sirt1*; *Sirt2* (qPCR)	*Sirt1*: ▲; *Sirt 2*: – *Sirt1*: –; *Sirt 2*: –	(7)

Oxycodone	HPC	Rat	Repeated (4 days × 3 mg/kg, i.p.)	AD10 (post extinction) AD11 (post stress-induced reinstatement)	DNA methylation DNA methyltransferase and ten-eleven translocations methylcytosine dioxygenase	Global 5-mc (5-mc DNA ELISA kit) *Dnmt1*;*Dnmt3a*;*Dnmt3b* (qPCR) *Tet1*;*Tet2*;*Tet3* (qPCR)	5-mc: ▼ *Dnmt1*: ▼; *Dnmt3a*: ─ *Dnmt3b*: ─ *Tet1*: ▲; *Tet2*: ─; *Tet3*: ─	(19)
Ethanol	PFC	Rat	Repeated (two consecutive days with two days gap for 2 weeks × 3 g/kg, 25% v/v, i.p.)	AD14	Histone acetyl transferase and histone deacetylase	HAT and HDAC activity (ELISA)	─	(20)
Ethanol	PFC	Mouse	Alcohol vapor (72 h continuous exposure)	AD21	Various histone modification enzymes and complexes	Microarrays	─	(21)
			Alcohol vapor (16 h/day × 3 days)	AD3	Histone acetylation	H3K9ac (WB) H3K14ac H3K27ac	─ ─ ─	
	mPFC	Rat	Alcohol vapor 14 h/day × 7 weeks)	AD21	DNA methylation DNMT1 5MeC	Global methylation kit IHC IHC	▲ ▲ in neurons ▲ in neurons	
Ethanol	dmPFC	Rat	Alcohol vapor (14 h/day × 7 weeks)	AD21	Histone demethylase	*Kdm6b* (qPCR)	*Kdm6b*: ▼	(22)
					Histone methyltransferase Histone methylation	*Prdm2* (qPCR) H3K9me1 (WB)	*Prdm2*: ▼ ▼	
	NAc				DNA methylation	Global methylation kit	▲	
					Histone demethylase and histone methylation	KDM6B (qPCR and WB) H3K27me3 (WB) H3K27me3 binding (ChIP-seq)	*Kdm6b* mRNA: ▼ KDM6B protein: ▲ H3K27me3: ▼ H3K27me3 (ChIP-seq): ▼	
Ethanol	NAc	Mouse	Alcohol vapor (16 h/day × 3 days)	AD3	Histone acetylation	H3K9ac (WB) H3K14ac H3K27ac	─ ─ ─	(23)
Ethanol	HPC	Rat	Repeated (3 g/kg, 15% v/v, i.p., twice 9 h apart)	AD2	Histone deacetylase	HDAC2(HDAC activity assay, qPCR, WB and Flow cytometry)	HDAC activity: ▲ *Hdac2* mRNA: ▲ HDAC2 protein: ▲ HDAC2+ Neurons %: ▲	(24)
Ethanol	HPC	Rat	Drinking water (6% v/v x 16 weeks)	AD3	Histone acetylation	H3K9ac at *Grin2b* promoter (ChIP)	H3K9ac: ▼	(25)
Nicotine	NAc	Rat	Repeated (14 days × 0.3 mg/kg, i.p.)	AD14	DNA methylation	Global DNA methylation(radio-labelled [^{3}H]-dCTP extension assay)	▲	(15)
	mPFC						▲	
	OFC						▲	

Supplemental Table S3. Summary of epigenetic changes beyond 24 h after drug self-administration.

Abbreviations: AD: abstinence day; CC: corpus callosum; CeA: central amygdala; ChIP-seq: chromatin immunoprecipitation followed by massively parallel DNA sequencing; ChIP: chromatin immunoprecipitation; Co-IP: co-immunoprecipitation; DEG; differentially expressed genes; DNMT: DNA methyltransferase; DS: dorsal striatum; GO: gene ontology; H3: histone H3; H3K4me3: trimethylation at the 4th lysine residue of H3; H3K4me3Q5dop: H3 lysine 4 trimethylation in combination with H3 glutamine 5 dopaminylation on the same histone tail; H3K9K14Ac2: H3 di-acetyl lysine 9, lysine 14; H3Q5dop: H3 glutamine 5 dopaminylation; HAT: histone acetyltransferase; HDAC: histone deacetylase; HPC: hippocampus; Inf: infusions; Me-DIP: methylated DNA immunoprecipitation; Meth: methamphetamine; mPFC: medial prefrontal cortex; NAc: nucleus accumbens; OFC: orbitofrontal cortex; PFC: prefrontal cortex; qPCR: quantitative polymerase chain reaction; TSS: transcriptional start site; VTA: ventral tegmental area; WB: Western blot. Symbols: ▲: increase; ▼: decrease; ─: no change.

Drug type	Brain region	Species	Operant training	Abstinence day	General category of epigenetic mark/enzyme	Specific epigenetic mark/enzyme	Major findings	Citations
Cocaine	NAc	Rat	6 h/d × 10 d (0.5 mg/kg/inf)	AD45	DNMT	*Dnmt3a1* *Dnmt3a2* (qPCR)	─ ─	(26)
Cocaine	NAc	Rat	2 h/d × 10 d (0.7 mg/kg/inf)	AD28	Genome-wide analysis Transcription factors	DEGs *Nr4a1* *Ef21* *Ndn* (RNA-seq)	344 ─ ▼ ▲	(12)
					Transcription factors	*Nr4a1* *Ef21* *Ndn* (qPCR)	─ ▼ ▲	
	PFC				Genome-wide analysis	DEGs (RNA-seq)	45	
	VTA					DEGs (RNA-seq)	1,929	
Cocaine	NAc	Rat	6 h/d × ≥ 5 d, 4 trials/h × 24 h/d × 10 d discrete-trial schedule (1.5 mg/kg/inf)	AD10/AD100	Immediate early genes	*Egr1* *Nr4a1* *Fos* (qPCR)	▼/▼ ─/▼ ▼/▼	(27)
	mPFC					*Egr1* *Nr4a1* *Fos* (qPCR)	─/─ ▼/▼ ▼/▼	
	mPFC			AD10	Histone acetylation	Acetylated histone H3 (K9-14) at promoters of *Egr1* *NPY* (ChIP)	 ▼ ▼	
Cocaine	mPFC	Rat	6 h/d × ≥ 5 d, 4 trials/h × 24 h/d × 10 d discrete-trial schedule (1.5 mg/kg/inf)	AD10	Genome-wide analysis	Changed genes (microarray analysis)	668 (direction not specified)	(28)
	NAc			AD100	Genome-wide analysis	Changed genes (microarray analysis)	346 (direction not specified)	
	mPFC			AD10/AD100	Transcription factor	*Erg1* *Fos* *Nr4a1* (qPCR)	─/─ ▼/▼ ▼/▼	
	NAc			AD10/AD100	Transcription factor	*Egr2* *Fos* *Nr4a1* (qPCR)	─/▼ ▼/▼ ─/▼	
Cocaine	NAc	Rat	2 h/d × 10 d (1 mg/kg/inf)	AD7	Transcription factor	p-SMAD3 SMAD3 (WB)	▲ ─	(29)

						SMAD3 binding at promoters of *Ctnnb1* *Grin2a* *Mef2d* *Cap2* *Dbn1* *Pdyn* *Cfl1*(ChIP)	 ▲ ▲ ▲ ▲ ▲ – –	
Cocaine	NAc	Mouse	3 h/d × 3 weeks (0.75 mg/kg/inf)	AD28	DNA methylation	*Dmnt1* *Dmnt3a* *Dmnt3b* (qPCR)	– ▲ –	(5)
Cocaine	VTA	Rat	6 h/d × 10 d (0.5 mg/kg/inf)	AD30	H3 dopaminylation Histone methylation Histone Transglutaminase	H3Q5dop H3K4me3Q5dop H3K4me3 H3 Tgm2 (WB)	▲ – – – –	(1)
			1 h/d × 10 d (0.5 mg/kg/inf)		H3 dopaminylation Histone methylation Histone Transglutaminase	H3Q5dop H3K4me3Q5dop H3K4me3 H3 Tgm2 (WB)	– – – – –	
			6 h/d × 10 d (0.5 mg/kg/inf)		Genome-wide analysis	DEGs (RNA-seq)	2,590	
Cocaine	NAc	Rat	6 h/d × 10 d (0.75 mg/kg/inf)	AD30	DNA methylation	DNA methylation (Me-DIP)	▲	(30)
Cocaine	CC	Rat	3 h/d × 14 d (0.75 mg/kg/inf)	AD30	DNA methylation	Methylation of *Sox10* (sodium bisulfite conversion and PCR)	▼	(31)
Cocaine	NAc	Rat	2 h/d × 10 d (1 mg/kg/inf)	AD7	Chromatin remodeler Transcription factor	BRG1 p-SMAD3 SMAD3 (WB)	▲ ▲ –	(32)
					Chromatin remodeler Transcription factor	BRG1 interaction with SMAD3 (Co-IP)	▲	
					Chromatin remodeler	BRG1 binding at promoters of *Ctnnb1* *Grin2a* *Mef2d* *Cap2* *Dbn1* *Pdyn* (ChIP)	 ▲ – ▲ ▲ ▲ –	
Cocaine	NAc	Rat	2 h/d × 10 d (1 mg/kg/inf)	AD7	Transcription factors	p-SMAD1/5 SMAD1 SMAD5 RUNX2 (WB)	▲ ▲ ▲ ▲	(33)
					Transcription factor	RUNX2 binding at promoters of *Egr3* *Dnml1* *Tgfbr1* *Smarca4* (ChIP)	 ▲ ▲ – ▲	
Cocaine	NAc	Rat	6 h/d × 10 d (0.5 mg/kg/inf)	AD30	Chromatin remodeler Transcription factor	INO80 EGR1 (WB)	▲ ▼	(34)

					Transcription factor	EGR1 binding at promoter of *Trim3* (ChIP)	▼	
					Chromatin remodeler	INO80 binding near TSS of *Yy1* *Jmjd6* *Ddx39b* (ChIP-seq)	 ▲ ▲ ▲	
					Transcription factor Histone demethylase	*Yy1* *Jmjd6* *Ddx39b* (qPCR)	▲ ▲ ▲	
Cocaine	VTA	Rat	2 h/d × 14 d (0.25 mg/59 µl/inf)	AD7	HAT	CBP binding at promoters containing *Bdnf* exon I *Bdnf* exon IV (ChIP)	 ▲ –	(35)
					Histone acetylation	H3K9K14Ac2 binding at promoters containing *Bdnf* exon I *Bdnf* exon IV (ChIP)	 ▲ –	
Cocaine	HPC	Rat	2 h/d x 12 (0.5 mg/kg/inf)	AD3/AD10	Genome-wide analysis and ten-eleven translocations methylcytosine dioxygenase DNA methylation	Tet3 target genes (microarray analysis) 5-hmC Differential enrichment peaks	9▲/16▲ ▲/▲ 1511/1842	(36)
Meth	Striatum	Rat	15 h/d × 8 d (0.1 mg/kg/inf)	AD30	Genome-wide analysis	DEGs (microarray analysis)	82 ▲ 591 ▼	(37)
Meth	CeA	Rat	3 h/session × 3 sessions/d × 10 sessions in 14 d (0.1 mg/kg/inf)	AD35	Genome-wide analysis	GO analysis (histone methylation) DEGs (RNA-seq)	Histone methylation ▲ 1940 (30) ▲, 277 (22) ▼	(38)
	OFC					DEGs (RNA-seq)	19▲, 36 ▼	
Meth	DS	Rat	15 h/d × 8 d (0.1 mg/kg/inf)	AD30	Immediate early genes	*cFos* *FosB* ∆*fosb* (qPCR)	– – –	(39)
					Immediate early genes Histone methylation Transcription factor	cFos FosB ∆FosB H3K4me3 p-CREB CREB (WB)	▼ – ▼ – ▼ ▼	
Meth	DS	Rat	3 h/session × 3 sessions/d × 10 sessions in 14 d (0.1 mg/kg/inf)	AD2/AD35	HDACs HAT Histone methyltransferases	*Hdac1* *Hdac2* *Hdac3* *Hdac4* *Hdac5* *Sirt1* *Sirt2* *Crebbp* *Suv39h1* *G9a* *GLP* *Mll1*	–/– –/– –/– –/– –/– –/– –/– –/– –/– –/– –/– –/▲	(40)

					Histone lysine demethylase DNA methyltransferase	*Kdm1a* *Dnmt3a* (qPCR)	–/– –/–	

Supplemental Table S4. Epigenetic mechanisms of drug relapse assessed by conditioned place preference procedure.

Abbreviations: AAV: adeno-associated virus; AD: abstinence day; BLA: basolateral amygdala; CeA: central amygdala; HDAC: histone deacetylase; KDM: histone lysine demethylase; Meth: methamphetamine; MHb: medial habenula; NAc: nucleus accumbens; shRNA: short hairpin RNA.

Drug type	Brain region	Species	Acquisition	Extinction/ Reconditioning	Drug-primed reinstatement	Abstinence day	Epigenetic manipulation	Behavioral outcomes	Citations
Cocaine	Systemic	Mouse	2 days × 20 mg/kg, i.p.	8 days (non-confined)	10 mg/kg, i.p.	AD10	HDAC inhibitor (NaBut), 8 days × 1.2 g/kg, i.p., immediately after extinction	Facilitated extinction and decreased reinstatement	(41)
				2 days (confined)	N/A	AD5	HDAC inhibitor (NaBut), 2 days × 1.2 g/kg, i.p., immediately after extinction (exposure to drug-paired compartment)	Facilitated extinction	
				8 days (confined)	10 mg/kg, i.p.	AD12	HDAC inhibitor (NaBut), 8 days × 1.2 g/kg, i.p., immediately after extinction	Decreased reinstatement	
				2 days (confined)	10 mg/kg, i.p.	AD5	HDAC inhibitor (NaBut), 2 days × 1.2 g/kg, i.p.,10 h after extinction	No effect on extinction or reinstatement	
				N/A	10 mg/kg, i.p.	AD5	HDAC inhibitor (NaBut), 2 days × 1.2 g/kg, i.p. during abstinence	No effect on extinction or reinstatement	
Cocaine	Systemic	Mouse	4 days × 5 or 15 mg/kg, i.p	7 days (non-confined)	N/A	N/A	HDAC inhibitor (NaBut), 1.2 g/kg, i.p. before 1st conditioning session	Increased resistance to extinction	(42)
				7 days (non-confined)			HDAC inhibitor (NaBut), 1.2 g/kg, i.p. immediately after 1st extinction session	Increased resistance to extinction	
			4 days × escalating dose (3, 6, 12, 24 mg/kg), i.p.	8 days (4 days × non-confined + 4 days × confined	11.25 mg/kg	AD12	HDAC inhibitor (NaBut), 5 days × 1.2 g/kg, i.p. immediately after extinction	Inhibited extinction, no change in reinstatement	
							HDAC inhibitor (NaBut), 4 days × 1.2 g/kg, i.p. immediately after 2nd extinction session	No change in extinction or reinstatement	
Cocaine	Systemic	Mouse	2 days × 20 mg/kg, i.p.	4 days (non-confined)	N/A	N/A	HDAC inhibitor (NaBut), 4 days × 0.3, 0.6 or 1.2 g/kg, i.p. immediately after 2nd extinction session	No change on extinction	(43)
				Post-extinction reconditioning (1 day × 5 mg/kg, i.p.)		AD21	N/A	Increased extinction with 0.3 g/kg NaBut	
Cocaine	Systemic	Mouse	2 days × 20 mg/kg, i.p.	6 days (non-confined)	10 mg/kg, i.p.	AD7	HDAC3 inhibitor (RGFP966), 2 days × 3 or 10 mg/kg, s.c.,	Facilitated extinction and decreased	(44)

							after 1st and 2nd extinction sessions	reinstatement (10mg/kg)	
Cocaine	Systemic	Mouse	4 days × 20 mg/kg, i.p.	N/A	N/A N/A	AD5 AD7	KDM6B inhibitor (GSK-J4), 5 days (AD3 to 7) × 0.5 mg/kg, i.p.)	No change in CPP Decreased CPP	(13)
					N/A N/A 10 mg/kg, i.p.	AD5 AD7 AD14	KDM6B inhibitor (GSK-J4), 3 days (AD5 to 7) × 0.5 mg/kg, i.p.)	No change in CPP Decreased CPP Decreased reinstatement	
Cocaine	Systemic	Mouse	3 days × 20 mg/kg, i.p.	6 days (non-confined)	5 mg/kg, i.p.	AD9	BET inhibitor [(+)JQ1, 25 mg/kg, prior to daily extinction sessions)	Decreased reinstatement	(45)
	NAc	Mouse					CK2α inhibitor (CX4945, 100 μM, prior to daily extinction	Decreased extinction and reinstatement	
Cocaine	Systemic	Rat	5 days × 15 mg/kg, i.p	N/A	N/A	AD1 AD7 AD14	PARP-1 inhibitor (ABT-888), 7.5 or 15 mg/kg, i.p., injected 50 min before 1st CPP tests	Decreased CPP Decreased CPP Decreased CPP	(46)
	CeA/BLA	Rat	5 days × 15 mg/kg, i.p.			AD1 AD7 AD14	PARP-1 inhibitor (PJ-34, 50 μM/0.5 μl, i.c., injected 30 min before 1st CPP tests	CeA: Decreased CPP Decreased CPP Decreased CPP No change with BLA	
	CeA	Rat	5 days × 15 mg/kg, i.p.			AD1 AD7 AD14	Lentivirus-PARP-1-shRNA, injected days before training	Decreased CPP No change in CPP No change in CPP	
	CeA	Rat	5 days × 15 mg/kg, i.p.			AD1 AD7 AD14	Lentivirus-D3ZLJ1(downstream target of PARP-1), injected days before training	Decreased CPP Decreased CPP Decreased CPP	
Cocaine	MHb	Mouse	4 days × 10 mg/kg, i.p.	6 days (non-confined)	5 mg/kg, i.p.	AD8	AAV-DIO-HDAC3 (expressed in cholinergic neurons), injected days before training	No change in reinstatement	(47)
Meth	Systemic	Mouse	2 days × 1 mg/kg, i.p.	12 days (confined)	1 mg/kg, i.p.	AD13	HDAC inhibitor (NaBut), 6 days × 0.4 g/kg, i.p., immediately after extinction (exposure to drug-paired compartment)	Facilitated extinction and decreased reinstatement	(48)
							HDAC inhibitor (NaBut), 1 day × 0.4 g/kg, i.p., 30 min before Meth injection for the reinstatement test	No change in reinstatement	
Meth	NAc	Mouse	3 days × 2 mg/kg, i.p.	N/A	N/A	AD2	Mll1 siRNA, 12 h before pre-test	Decreased CPP	(49)
				N/A	N/A	AD2	KDM5C siRNA, 12 h before pre-test	No change in CPP	
				CPP test on AD2	N/A	AD6	KDM5C siRNA, 24 h after the 1st CPP test	Decreased CPP after consolidation	
Morphine	Systemic	Rat	4 days × 5 mg/kg, i.p.	8 days (non-confined)	3 mg/kg, s.c.	AD11	HDAC inhibitor (NaBut), 8 days × 1.2 g/kg, i.p., immediately after extinction	Facilitated extinction and decreased reinstatement	(50)

				2 days (confined)		AD4	HDAC inhibitor (NaBut), 2 days × 1.2 g/kg, i.p., immediately after extinction (exposure to drug-paired compartment)	Facilitated extinction and decreased reinstatement	
Morphine	BLA	Rat	4 days × 10 mg/kg, i.p.	16 days (confined)	10 mg/kg, i.p.	AD17	HDAC inhibitor (TSA), 12 days × 165 nm/1 µl, before extinction training	Facilitated extinction and decreased reinstatement	(51)

Supplemental Table S5. Epigenetic mechanisms of operant drug relapse.

Abbreviations: 5-AZA: 5-azacytidine; AAV: adeno-associated virus; AD: abstinence day; Amph: amphetamine; BLA: basolateral amygdala; dCas9: CRISPR; dmPFC: dorsomedial prefrontal cortex; dn: dominant negative; DNMT: DNA methyltransferase; DS: dorsal striatum; H3: Histone H3; HAT: histone acetyltransferase; HDAC: histone deacetylase; HSV: herpes simplex virus; hSyn: synapsin promotor; Inf: infusion; LA: lateral nucleus of the amygdala; mPFC: medial prefrontal cortex; NAc: nucleus accumbens; PhB: phenylbutyrate; RNAi: RNA interference; SAM: S-adenosylmethionine; sgRNA: single-guide RNA; shRNA: short hairpin RNA; VP64 transcriptional activation domain; VTA: ventral tegmental area. *Other drug-associated behaviors were tested. #Observed incubation of drug seeking.

Brain region	Drug type	Species	Operant training	Extinction	Relapse paradigm(s)	Abstinence day	Epigenetic manipulation	Behavioral outcomes	Citations
Systemic	Cocaine	Rat	4 discrete trials/h × 24 h access × 10 d (1.5 mg/kg/inf)	Within-session extinction/cue-induced reinstatement schedule		AD14	HDAC inhibitor (NaBut, 200 mg/kg, i.p.), injected daily during abstinence	No effect on extinction, decreased cue-induced reinstatement	(52)
Systemic	Cocaine	Rat	1 h/d × 10 d (0.33 mg/kg/inf)	N/A	Drug-primed drug seeking	AD21	HDAC inhibitor (TSA, 0.3 mg/kg, i.v.), injected daily for 4 d prior to testing	Decreased drug-primed drug seeking	(53)
							HDAC inhibitor (PhB, 100 mg/kg, i.v.), injected daily for 4 d prior to testing	Decreased drug-primed drug seeking	
							HDAC inhibitor (PhB, 20 mg/kg, i.v.), injected daily for 4 d prior to testing)	No effect on drug-primed drug seeking	
Systemic	Cocaine	Rat	1 h/d × 12 d or 24 d (1 mg/kg/inf)	1 h/d × 8 d, cue presentation session in novel context (24 h after last extinction session)	Cue-induced reinstatement	AD10	HAT (garcinol, 10 mg/kg, i.p.), injected 30 after cue presentation session	Decreased cue-induced reinstatement (specific to light but not tone cue)	(54, 55)
Systemic	Cocaine	Rat	1 h/d × 12 d (1 mg/kg/inf)	1 h/d × 8 d, cue presentation session in novel context (24 h after last extinction session)	Cue-induced reinstatement	AD10	HDAC inhibitor (TsA, 2.5 mg/kg, i.p.), injected 45 min after cue presentation session	Increased cue-induced reinstatement	(54)
							HAT (garcinol, 10 mg/kg, i.p.), injected 30 min after cue presentation session; HDAC inhibitor (TsA, 2.5 mg/kg, i.p.), injected 45 min after cue presentation session	No effect on cue-induced reinstatement (garcinol occluded TsA-mediated increase in cue-induced reinstatement)	
Systemic	Cocaine	Rat	1 h/d × 12 d (1 mg/kg/inf)	1 h/d × 8 d, cue presentation	Cue-induced reinstatement	AD10	HAT (garcinol, 10 mg/kg, i.p.), injected 6 h after cue presentation session	No effect on cue-induced reinstatement	(55)

				session in novel context (24 h after last extinction session)	Drug-primed reinstatement		HAT (garcinol, 10 mg/kg, i.p.), injected 30 after drug presentation session	No effect on drug-primed reinstatement	
Systemic	Heroin	Rat	4 h/d × 14 d (0.1-0.025 mg/kg/inf)	2 h/d × 14 d	Drug-primed reinstatement	AD15	HDAC inhibitor (NaBut, 400 mg/kg, i.p.), injected 6 h prior to testing	Increased drug-primed reinstatement	(56)
							HDAC inhibitor (NaBut, 400 mg/kg, i.p.), injected 12 h prior to testing	No effect on drug-primed reinstatement	
Systemic	Nicotine	Rat	1 h/d × 12 d (30 µg/kg/inf)	1 h/d × ≥ 6 d, 1 h/d × ≥ 2 d (between reinstatement tests)	Cue-induced reinstatement, drug-primed reinstatement, cue+drug-primed reinstatement	~AD7, AD10, AD13	HDAC inhibitor (NaBut, 100 mg/kg, i.p.), injected immediately after extinction sessions	No effect on extinction (potentially due to low levels of responding), no effect on cue-induced reinstatement, decreased drug- and drug+cue-induced reinstatement	(57)
							HDAC inhibitor (NaBut, 100 mg/kg, i.p.), injected 6 h after extinction sessions	No effect on drug- or drug+cue-induced reinstatement	
Systemic	Amph	Rat	1 h/d × 8 d (0.1 mg/kg/inf)	1 h/d × 10 d, 1 h/d × 4 d (between reinstatement tests)	Cue-induced reinstatement, drug-primed reinstatement	AD11, AD13	HDAC inhibitor (TsA, 0.3 mg/kg, i.v.), injected 30 min prior to extinction and reinstatement sessions)	No effect on extinction, decreased cue-induced reinstatement, no effect on drug-primed reinstatement	(58)
Systemic	Cocaine	Rat	2 h/d × ≥ 10 d (0.75 mg/kg/inf)	2 h/d × ≥ 10 d, 2 h/d × 2 d between reinstatement tests	Cue-induced reinstatement, drug-primed reinstatement	~AD11, AD14	MET (L-methionine) supplementation (10.4 mmol/ml, 1 ml/kg, s.c.), injected daily 1-2 h prior to every behavior session	No effect on self-administration, extinction or cue-induced reinstatement, but decreased drug-primed reinstatement	(59)
NAc	Cocaine	Rat	3 h/d × 5 d/week × 3-4 weeks (0.5 mg/kg/inf)	3 h/d × 5 d	Cue-induced reinstatement, drug-primed reinstatement, stress-induced reinstatement*	AD22	HSV-G9a, injected prior to cocaine behaviors or self-administration training (virus was not expressed during reinstatement tests)	Increased stress-induced reinstatement	(60)
NAc	Cocaine	Rat	3 h/d × 5 d/week × 3-4	3 h/d × 5 d	Cue-induced reinstatement, drug-primed reinstatement,	AD20	AAV-RNAi-G9a, 4-7 d prior to self-administration training	Decreased stress-induced reinstatement	(61)

			weeks, (0.5 mg/kg/inf)		stress-induced reinstatement*				
NAc	Cocaine	Rat	6 h/d × 10 d (0.5 mg/kg/inf)	Within-session extinction/cue-induced reinstatement schedule[#]		AD1, AD45	AAV-dnmt3a2-shRNA, injected on AD2 or prior to self-administration training	Decreased cue-induced reinstatement	(26)
NAc	Cocaine	Rat	2 h/d × 10 d (1 mg/kg/inf)	Within-session extinction (1 h/session × 8-10 sessions)	Drug-primed reinstatement	AD7	HSV-dnSmad3, injected 3 d prior to testing	Decreased drug-primed reinstatement	(29)
							HSV-Smad3, injected 3 d prior to testing	Increased drug-primed reinstatement	
NAc	Cocaine	Rat	2 h/d × ≥ 3 weeks (~0.89 mg/kg/inf)	2 h/d × 8 d, 2 h/d × 2 d (between reinstatement tests)	Context-induced reinstatement, cue-induced reinstatement, drug-primed reinstatement	AD9, AD13, AD16	HDAC3 inhibitor (RGFP966), injected 20 min prior to first extinction session)	Decreased context-induced and cue-induced reinstatement, no effect on drug-primed reinstatement	(62)
NAc	Cocaine	Rat	3 h/d × 15-20 d (0.5 mg/kg/inf)	3 h/d × 6 d	Cue-induced reinstatement, drug-primed reinstatement, stress-induced reinstatement	AD13	AAV-HDAC5, 21 d prior to self-administration	No effect on cue-induced reinstatement, drug-primed reinstatement or stress-induced reinstatement	(63)
							AAV-HDAC5 3SA, 21 d prior to self-administration	Decreased cue-induced reinstatement, decreased drug-primed reinstatement, no effect on stress-induced reinstatement	
NAc	Cocaine	Mouse (*Npas4* cKO^{NAc})	2 h/d × 10-14 d (0.5 mg/kg/inf)	3 h/d × ≥ 12 d	Cue-induced reinstatement	AD19-27	AAV-Cre-eGFP	No effect on cue-induced reinstatement	
NAc	Cocaine	Rat	2 h/d × 10 d (1 mg/kg/inf)	Within-session extinction (1 h/session × 8-10 sessions)	Cue-induced reinstatement	AD7	BRG1 inhibitor (PF13, 30 mmol/L), injected 1/d × 4 d with last injection 24 h before test	Decreased cue-induced reinstatement	(32)
							HSV-Brg1, injected 3 d prior to testing	Increased cue-induced reinstatement	
							HSV-dnSmad3, injected 3 d prior to testing	Decreased cue-induced reinstatement	
NAc	Cocaine	Rat	6 h/d × 10 d (0.75 mg/kg/inf)	N/A	Drug seeking[#]	AD30	DNMT inhibitor (RG108, 100 μM), AD29 and immediately prior to test on AD30	Decreased drug seeking	(30)

							Methyl donor (SAM, 500 μM), AD29 and immediately prior to test on AD30	Increased drug seeking	
NAc	Cocaine	Rat	6 h/d × 10 d (0.5 mg/kg/inf)	N/A	Drug seeking[#]	AD30	HSV-INO80 ΔNC-EQ, injected 3 d prior to testing	Decreased drug seeking	(34)
							HSV-INO80, injected 3 d prior to testing	Increased drug seeking	
NAc	Cocaine	Rat	2 h/d × 10 d (0.7 mg/kg/inf)	N/A	Drug seeking	AD28	hSynGW-mCherry-IRES-dCas9-VP64 sgRNA-*Nr4a1*, injected on AD21	Decreased drug seeking	(12)
NAc	Heroin	Rat	3 h/d × 10 d (0.02 mg/kg/inf)	Within-session extinction (1 h/session × 8-10 sessions)	Drug-primed reinstatement	AD5	HDAC2/3 inhibitor (MI-192, 1 μM/mL), injected 1/d × 3 d with last injection 24 h before test	Decreased drug-primed reinstatement	(64)
DS	Meth	Rat	3 h/session × 3 sessions/d × 10 sessions in 14 d (0.1 mg/kg/inf)	N/A	Drug seeking[#]	AD30	AAV-shHDAC5, injected 1 week prior to self-administration training	Decreased drug seeking	(65)
						AD2	AAV-shHDAC5, injected 1 week prior to self-administration training	No effect on drug seeking	
						AD30	AAV-mHDAC5, injected 1 week prior to self-administration training	Increased drug seeking	
						AD2	AAV-mHDAC5, injected 1 week prior to self-administration training	No effect on drug seeking	
VTA	Cocaine	Rat	6 h/d × 10 d (0.5 mg/kg/inf)	N/A	Drug seeking	AD30	Lentivirus expressing H3.3Q5A, injected on AD1	Decreased drug seeking	(1)
							Lentivirus expressing H3.3, injected on AD1	No effect on drug seeking	
LA	Cocaine	Rat	1 h/d × 12 d (1 mg/kg/inf)	1 h/d × 8 d, cue presentation session in novel context (24 h after last extinction session)	Cue-induced reinstatement	AD10	HAT (garcinol, 500 ng), injected 1 h after cue presentation session	Decreased cue-induced reinstatement (via reconsolidation disruption)	(54)
BLA	Cocaine	Rat	1 h/session × 3 sessions/d × 10 d (0.75 mg/kg/inf)	3 h/d × 10 d, 15 min cue presentation session (24 h after last extinction session), 3 h/d × 2 d extinction sessions between reinstatement tests	Cue-induced reinstatement, cue+drug reinstatement	AD12, AD15	DNMT inhibitor (5-AZA, 1 μg/side), injected immediately after cue presentation session	Decreased cue-induced reinstatement and cue+drug reinstatement	(66)
							DNMT inhibitor (5-AZA, 1 μg/side), injected 6 h after cue presentation session	No effect on cue-induced reinstatement and cue+drug reinstatement	

BLA	Cocaine	Rat	1 h/session × 3 sessions/d × 10 d (0.75 mg/kg/inf)	Cue presentation session (24 h after last training session), exctinction sessions (3 h/d × 5 d) between drug seeking and reinstatement tests	Drug seeking, cue+drug reinstatement	AD2, AD8	DNMT inhibitor (5-AZA, 1 µg/side), injected immediately after cue presentation session	Decreased drug seeking and cue+drug reinstatement	
dmPFC	Alcohol	Rat	30 min/d × 15 d (0.1 mL, 10% alcohol)	30 min/d × 16 d	Stress-induced reinstatement	AD17	Lentivirus expressing shRNA targeting *Prdm2*, injected 1 week prior to self-administration	Increased stress-induced reinstatement*	(67)

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