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REVIEW ARTICLE

Relapse to opioid seeking in rat models: behavior, pharmacology and circuits

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Lifetime relapse rates remain a major obstacle in addressing the current opioid crisis. Relapse to opioid use can be modeled in rodent studies where drug self-administration is followed by a period of abstinence and a subsequent test for drug seeking. Abstinence can be achieved through extinction training, forced abstinence, or voluntary abstinence. Voluntary abstinence can be accomplished by introducing adverse consequences of continued drug self-administration (e.g., punishment or electric barrier) or by introducing an alternative nondrug reward in a discrete choice procedure (drug versus palatable food or social interaction). In this review, we first discuss pharmacological and circuit mechanisms of opioid seeking, as assessed in the classical extinction-reinstatement model, where reinstatement is induced by reexposure to the self-administered drug (drug priming), discrete cues, discriminative cues, drug-associated contexts, different forms of stress, or withdrawal states. Next, we discuss pharmacological and circuit mechanisms of relapse after forced or voluntary abstinence, including the phenomenon of "incubation of heroin craving" (the time-dependent increases in heroin seeking during abstinence). We conclude by discussing future directions of preclinical relapse-related studies using opioid drugs.

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INTRODUCTION

The main obstacle in treating addiction across drug classes is high relapse rates during abstinence [1, 2]. High relapse rates are likely a major contributor to the opioid crisis in the United States, a crisis that in 2016 has led to 42,249 deaths attributable to an opioid overdose [3]. Basic research focusing on animal models of opioid relapse allows for the investigation of mechanisms of human-relevant relapse-provoking events, with the goal of identifying novel treatments for relapse prevention [4].

In rodent relapse models, rats learn to self-administer a drug by pressing a lever to earn an intravenous drug infusion, undergo a period of abstinence, and are then tested for relapse to drug seeking. Abstinence can be achieved through extinction, (the extinction-reinstatement model) [4, 5], forced (homecage) abstinence, or voluntary abstinence in the drug environment [6]. Voluntary abstinence can be achieved by either introducing adverse consequences to the drug taking or seeking response (shock punishment or electric barrier) [7, 8] or an alternative nondrug reward in the form of palatable food [9] or social interaction [10].

In the extinction-reinstatement model, drug seeking can be reinstated by reexposure to the drug itself [11], discrete cues previously paired with drug infusions [12], discriminative cues or contexts that previously predicted drug availability [13, 14], stressors [15], or spontaneous opioid withdrawal [16]. These stimuli also induce relapse and craving in humans [17–21]. However, a limitation of the extinction-reinstatement model is that formal operant extinction is not the cause of abstinence in human former drug users. Therefore, abstinence-based relapse models [6, 22] attempt to mimic the human condition where abstinence is either forced (incarceration or inpatient treatment) or voluntary due to the negative consequences of chronic drug use or the availability of nondrug alternative rewards [23–25]. These newer models, however, do not fully capture the complex nature of human abstinence, because in rat models, the adverse consequences of drug taking (punishment) or seeking (electric barrier), or the experience of a nondrug reward, occur in close temporal proximity to the rat's behavior, while in humans, those events are often delayed [26].

In this review, we first discuss pharmacological and circuit mechanisms of relapse to opioid seeking in the extinction-reinstatement model and in abstinence-based models. We conclude by briefly discussing future directions of preclinical relapse-related studies using opioid drugs. Due to space limitations, we do not discuss reinstatement of opioid preference, as assessed in the conditioned place preference model, and only describe selected research findings on underlying mechanisms. In Supplementary Table 1, we provide a complete list of all operant-based reinstatement/relapse studies using opioid drugs that we identified on PubMed, and their major findings (see also Fig. 1 that shows the number of preclinical relapse-related opioid papers over time). In Table 1, we outline the different models used to study relapse to opioid seeking along with historical citations. In Fig. 2, we provide a graphical description of the main brain areas and pathways involved in different forms of relapse to heroin seeking. In Box 1, we discuss methodological and statistical issues related to the study of drug relapse in the different animal models. In Box 2, we discuss issues related to the predictive validity of opioid relapse models.

EXTINCTION-BASED RELAPSE MODELS

In this section, we review results from studies in which reinstatement was induced by drug priming, discrete cues,

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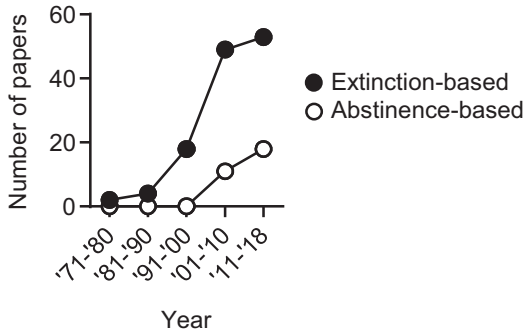

Fig. 1 Number of empirical papers on relapse to opioid seeking in extinction-based and abstinence-based rodent models. This figure illustrates that most studies on relapse to opioid seeking in rat models have used the classical extinction-reinstatement model.

Table 1. Animal models of opioid reinstatement/relapse

Experimental manipulation	Historical citations: opioid drugs	Number of papers
Reinstatement-based relapse models		
Drug priming	[160, 161]	63
Discrete cues	[39, 160]	43
Discriminative cues	[58, 59]	4
Context	[64]	11
Stress	[15, 79]	39
Withdrawal	[16, 103]	3
Reacquisition	[106, 111]	6
Abstinence-based relapse models		
Forced abstinence and incubation	[121, 162]	24
Voluntary abstinence: punishment/electric barrier	[8, 136]	4
Voluntary abstinence: food choice	[143]	1

Note: Several reinstatement-related papers published results with more than one reinstating stimulus (e.g., drug priming and stress, drug priming and drug cue) and appear in more than one category. Historical citations refer to the initial papers published with a given model.

discriminative cues, contextual cues, stressors, or withdrawal states. We also review results from studies on reacquisition of opioid self-administration after extinction.

Drug priming

In the operant version of the drug priming-induced reinstatement model, the effect of noncontingent injections of the self-administered drug or other drugs on reinstatement of drug seeking is determined after extinction of the drug-reinforced response [4].

The receptor types reported to be involved in reinstatement induced by heroin (or opioid agonist) priming are the mu-opioid receptor (MOR), the dopamine D1- and D2-family receptor (Drd1 and Drd2), and the cannabinoid 1 (CB1) receptor. Systemic injections of the preferential MOR antagonist naltrexone, the nonselective dopamine receptor antagonist flupenthixol, the Drd1 (SCH2330) or Drd2 (raclopride) antagonists, and the CB1 receptor antagonist SR141716A decrease heroin priming-induced reinstatement, while systemic injections of morphine (a MOR agonist), bromocriptine or quinpirole (preferential Drd2 agonists), HU210 or other CB1 receptor agonists reinstate heroin seeking [27–32]. The role of Drd2 activation-induced reinstatement, however, is dependent on the duration of abstinence: systemic injections of quinpirole reinstate heroin seeking during early (first week) but not late (3 weeks) abstinence [29]. More recent evidence suggests a role of the dopamine D3 receptor (Drd3) in drug priming-induced reinstatement: You et al. [33] reported that systemic injections of selective Drd3 antagonists (CAB2-015 and BAK4-54) decrease reinstatement induced by priming injections of the prescription opioid oxycodone.

The main brain regions reported to be involved in opioid priming-induced reinstatement are ventral tegmental area (VTA), nucleus accumbens (NAc), and dorsomedial prefrontal cortex (dmPFC). A critical site of action for MOR's role in reinstatement is VTA, where local injections of morphine reinstate heroin seeking [34], presumably through indirect activation of VTA dopamine neurons projecting to NAc [35]. In support of this idea, NAc injections of the indirect dopamine agonist amphetamine (but not morphine) reinstate heroin seeking [36]. Additionally, NAc core injections of either SCH23390, sulpiride (a preferential Drd2 antagonist), or fluphenazine (a mixed Drd1/Drd2 antagonist) decrease heroin priming-induced reinstatement [37]. There is also evidence for a critical role of glutamate transmission in NAc core that originates from dmPFC in heroin priming-induced reinstatement. Reversible inactivation of dmPFC with muscimol + baclofen prevents heroin priming-induced increases in extracellular glutamate in NAc, and local NAc injections of CNQX (an AMPA/kainite receptor antagonist) or ifenprodil (an NMDA NR2B receptor antagonist) decrease heroin priming-induced reinstatement [37, 38].

Studies using reversible inactivation procedures show a role of neuronal activity in multiple brain areas in heroin priming-induced reinstatement. Reversible inactivation of basolateral amygdala (BLA) with the sodium channel blocker tetrodotoxin (TTX), which inhibits both cell bodies and fibers of passage, decreases heroin priming-induced reinstatement [39]. This finding was confirmed in a comprehensive study using muscimol + baclofen inactivation, which only inhibits neuronal activity of cell bodies. Rogers et al. [40] reported that in addition to BLA, reversible inactivation of central amygdala (CeA), dmPFC, ventromedial PFC (vmPFC), bed nucleus of stria terminalis (BNST), NAc core and shell, VTA, substantia nigra (SN), dorsolateral striatum (DLS), and ventral pallidum (VP) decreases heroin priming-induced reinstatement.

Discrete cues

In the discrete cue-induced reinstatement model, rats are trained to self-administer a drug; lever presses lead to drug infusions that are paired with a discrete cue (tone, light). Lever pressing is then extinguished without the drug and the discrete cue. During reinstatement testing, lever pressing results in contingent presentations of the discrete cue (a conditioned reinforcer manipulation) in the absence of the drug [12].

The receptor types reported to be involved in discrete cue-induced reinstatement are Drd1, Drd2, Drd3, 5-HT2A receptor, and the orexin 1 receptor (Ox1R). Systemic injections of risperidone (Drd2 and 5-HT2A receptor antagonist), L-stepholidine (partial Drd1 agonist/Drd2 antagonist), SR 21502 (Drd3 antagonist), and SB335867 (Ox1R antagonist) decrease discrete cue-induced reinstatement of heroin or remifentanil (short-acting synthetic MOR agonist) seeking [41–45].

NAc and mPFC are critical for discrete cue-induced reinstatement of heroin seeking, and this reinstatement is associated with immediate early gene (Fos, Zif268, and others) induction in both brain areas [46, 47]. Inactivation of NAc core with muscimol + baclofen or the entire NAc with TTX decreases discrete cue-induced reinstatement [40, 48]. Injections of fluphenazine into NAc core or SCH23390 into NAc core (but not shell) also decrease this reinstatement [37, 49]. Extracellular glutamate in NAc core is increased during discrete cue-induced reinstatement testing and

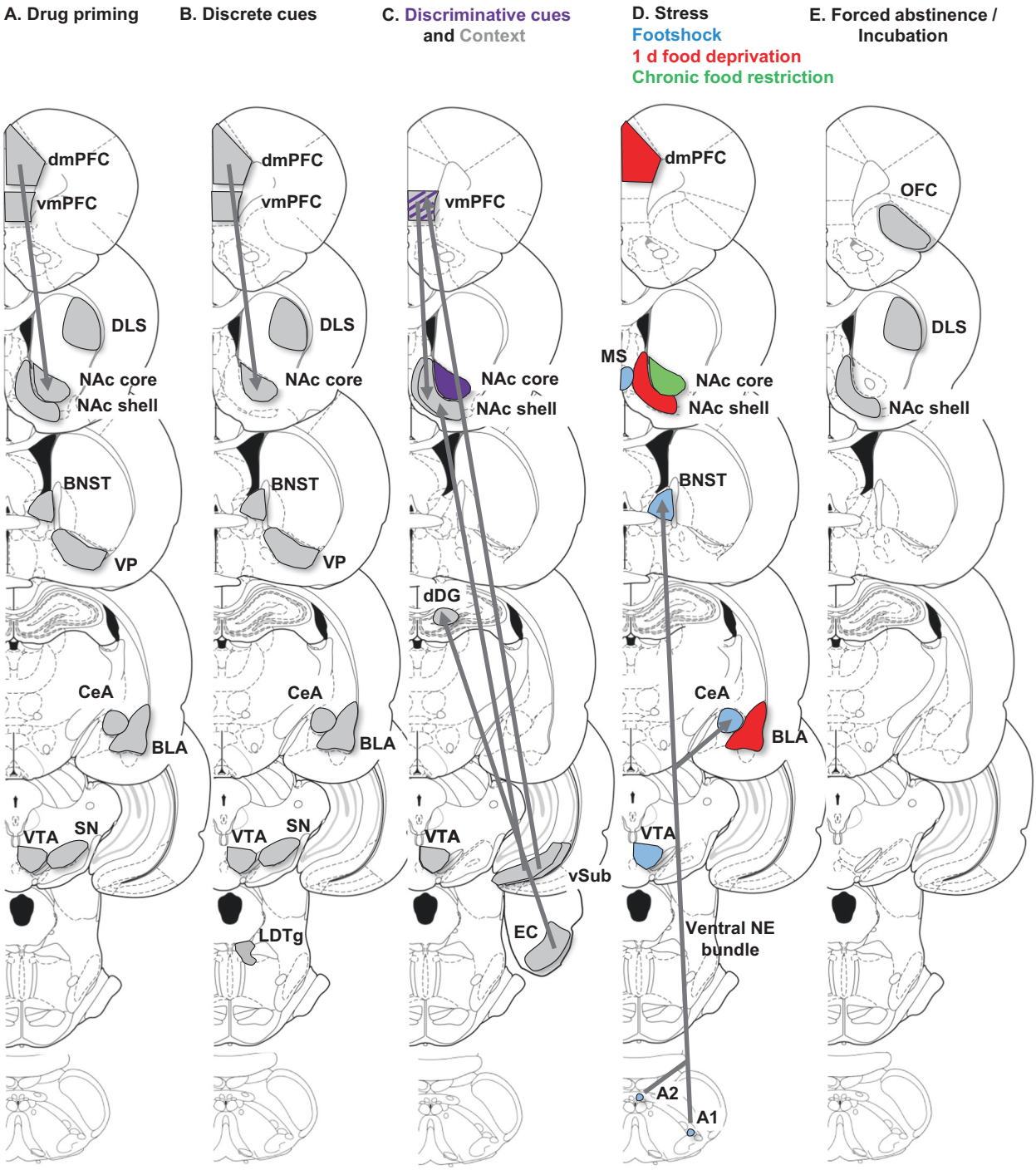

Fig. 2 Brain areas and pathways involved in relapse to opioid seeking. Coronal sections of the rat brain showing brain areas and pathways implicated in reinstatement/relapse of opioid seeking induced by **a** drug priming, **b** discrete cues, **c** discriminative cues and context, and **d** different stressors. **e** Brain areas implicated in relapse to opioid seeking and incubation of opioid craving after forced abstinence. *Abbreviations*: A1 and A2 noradrenergic cell body nuclei; BLA basolateral amygdala; BNST bed nucleus of stria terminalis; CeA central nucleus of amygdala; dmPFC dorsal medial prefrontal cortex; dDG dorsal dentate gyrus; DLS dorsolateral striatum; EC entorhinal cortex; LDTg lateral dorsal tegmental nucleus; MS medial septum; NE norepinephrine; NAc nucleus accumbens; SN substantia nigra; VP ventral pallidum; vSub ventral subiculum; VTA ventral tegmental area

local CNQX injections decrease this reinstatement [37]. Shen et al. [50] reported that heroin self-administration decreases glutamate uptake and surface expression of glutamate transporter-1 (GLT-1) in NAc core. This decrease causes spillover of synaptic glutamate to extrasynaptic NMDA receptors. Systemic injections of ceftriaxone (a GLT-1 activator) restore NAc core glutamate uptake and decrease discrete cue-induced reinstatement of heroin seeking, an effect reversed by GLT-1 knockdown [50]. There is also evidence for a role of NAc acetylcholine in discrete cue-induced reinstatement; NAc injections of physostigmine (acetylcholinesterase inhibitor) decrease this reinstatement [48].

Regarding mPFC, reversible inactivation (muscimol + baclofen) of dmPFC or vmPFC decreases discrete cue-induced reinstatement of heroin seeking [40], but see Schmidt et al.

Box 1 Methodological and statistical issues in reinstatement/relapse studies

Due to space limitations, we do not discuss specific confounds in data interpretation in the studies we reviewed. Below, we discuss two general issues. The first issue is the appropriate experimental design and statistical analyses in reinstatement studies. In many studies, authors have used an "incomplete" experimental design where the experimental manipulations (e.g., antagonist pretreatment and reversible inactivation) were only tested for their effect on reinstatement induced by the reinstating stimulus (e.g., drug priming, intermittent footshock) without determining the manipulations' effect on baseline responding after extinction to determine their unique effect on reinstatement. This is problematic because the effect of different drug priming doses or different intermittent footshock intensities on reinstatement follows an inverted U-shaped dose–response curve [77, 163]. Thus, a given neuropharmacological manipulation can decrease responding during reinstatement testing due to either potentiation (shift-to-the-left) or inhibition (shift-to-the-right) of the effect of drug priming or footshock on drug seeking. This interpretation issue can be partially avoided if authors use at a minimum a standard 2×2 factorial design where the effect of the experimental manipulation (e.g., vehicle/muscimol + baclofen) on operant responding during testing is assessed with and without the reinstating stimulus (saline priming versus drug priming, no cues versus cues, context B versus context A, no stress versus stress). From a statistical perspective, if the experimental manipulation selectively decreases reinstatement induced by a given reinstating stimulus without changing baseline extinction responding, this would be reflected in the factorial analysis of variance as a significant interaction between the two factors (experimental manipulation × reinstatement condition).

The second issue is whether decreases (or increases) in responding on the lever previously paired with drug self-administration (active lever) during reinstatement testing reflect the effect of the experimental manipulations on drug seeking versus nonspecific performance-impairing effects. One common practice is to determine the effect of the experimental manipulations on the lever that was not paired with drug self-administration (inactive lever) [164]. However, because responding on this lever is low, nonspecific performance deficits cannot be adequately assessed. Additionally, if the experimental manipulations increase responding on the inactive lever, it may reflect response generalization that commonly occurs under extinction conditions [165]. Another way to assess nonspecific effects of pharmacological/brain manipulations is to determine their impact on lever pressing after extinction in the absence of the reinstating stimulus (see above). However, as in the case of inactive lever responses, because responding on the active lever is low after extinction, nonspecific sedative effects are difficult to assess. Another common method is to assess the effects of the experimental manipulations on unconditioned locomotor activity. This is a problematic practice, because a given experimental manipulation can have opposite effects on learned operant response in a familiar environment versus unconditioned locomotion in a novel environment. For example, psychostimulants increase locomotor activity but typically decrease high-rate operant responding [166], while benzodiazepines decrease locomotor activity but under certain conditions can increase low-rate operant responding [167].

We advocate three methods to rule out nonspecific effects of different experimental manipulations on reinstatement of drug seeking [164]: determine the effect of a given experimental manipulation on (1) ongoing high-rate operant responding for a nondrug reward (e.g., palatable food), (2) reinstatement of nondrug reward seeking, and (3) reinstatement induced by more than one reinstating stimulus. However, while data interpretation is straightforward if the experimental manipulations have a "selective" effect using one of these methods, a "nonselective" decrease in operant responding in these control conditions does not always imply performance deficits. To the extent that addictive drugs act on brain systems of nondrug rewards [168], experimental manipulations that decrease drug seeking may also decrease food taking and seeking. Additionally, a given experimental manipulation (reversible inactivation of dorsomedial prefrontal cortex (dmPFC)) can decrease reinstatement induced by multiple stimuli [169, 170].

In conclusion, the use of fully factorial experimental design and multiple measures to assess potential nonselective effects of different experimental manipulations on drug seeking is critical for accurate interpretation of data from studies using animal models of drug relapse.

Box 2 The predictive validity of opioid reinstatement/relapse models

The basic premise of the studies described in our review is that a better understanding of neuropharmacological mechanisms of opioid reinstatement/relapse in rat models will lead to novel treatments to prevent opioid relapse in humans. To date, however, this premise has yet to materialize. Studies using relapse models (and other preclinical addiction models) with opioid and other addictive drugs have yet to impact clinical practice or lead to FDA-approved medications [171, 172]. This state-of-affairs has led some authors to question the predictive validity (in the narrow sense, the ability of the animal model to predict medication efficacy in the modeled human condition) of reinstatement/relapse models [24] and preclinical addiction models and brain-based addiction research in general [173]. Regarding predictive validity of animal relapse models, it is useful to consider two sub-categories: (1) postdictive validity or whether medications known to decrease human relapse also decrease relapse/reinstatement in the animal model, and (2) prospective predictive validity or whether medications identified in the relapse models decrease human relapse [174].

For opioid drugs, the available evidence suggests reasonable postdictive validity. Opioid agonist maintenance therapy that decreases drug use and relapse in humans [102, 175] also decreases some forms of relapse in the animal model. Thus, chronic delivery of heroin, buprenorphine, or methadone via osmotic minipumps during the extinction and reinstatement phases decreases heroin priming-induced reinstatement of heroin seeking [163, 176, 177]. Chronic delivery of buprenorphine also decreases extinction responding in a nondrug-associated context to discrete cues previously paired with oxycodone and decreases reacquisition of oxycodone self-administration after extinction (Bossert et al. [178] Society for Neuroscience Abstract and poster presentation, 2018).

However, the prospective predictive validity of opioid relapse models has yet to be established. The alpha-2 adrenoceptor agonist clonidine decreases footshock stress-induced reinstatement of heroin seeking in rats [82]. These observations led to human studies showing that clonidine modestly decreases heroin craving and heroin relapse in a double-blind human clinical study [179]. However, another potentially promising receptor target, the CRF1 receptor, whose blockade decreases stress-induced reinstatement of heroin seeking and stress-induced reinstatement of other addictive drugs [76], was ineffective in human laboratory studies [179–182].

The mixed evidence regarding the prospective predictive validity of the extinction-reinstatement model across drug classes and other mixed evidence from other animal models (see Box 1 [171]) has led us to recently introduce alternative voluntary abstinence-based animal models; these models more closely mimic the human condition [9, 10, 183], and hopefully will show good predictive validity. Improved predictive validity may also be achieved by testing medications on not only the effect of nonreinforced drug seeking in the different models but also on reinforced drug seeking (the reacquisition model), which more closely parallels relapse in humans.

[46] for different results for dmPFC. Injections of SCH23390 or PEPA (an allosteric AMPA receptor potentiator) in vmPFC decrease this reinstatement [51, 52]. Van den Oever et al. [53] reported that discrete cue-induced reinstatement of heroin seeking is associated with decreased synaptic AMPA receptor subunit GluA2 in mPFC, resulting in decreased AMPA/NMDA current ratio in mPFC pyramidal neurons. Systemic or vmPFC, but not dmPFC, injections of TAT-$GluR_{23Y}$ (a peptide that inhibits GluA2 endocytosis) decrease discrete cue-induced reinstatement. These results show that GluA2 subunit endocytosis and resulting synaptic depression in vmPFC play a key role in discrete cue-induced reinstatement [53].

There is also evidence for a role of extracellular matrix (ECM) in both NAc core and vmPFC in discrete cue-induced reinstatement. After extinction, ECM proteins are downregulated in NAc and mPFC [54]. After this reinstatement, ECM proteins in mPFC are condensed in the perineuronal nets that surround GABAergic interneurons, resulting in increased inhibitory tone of synaptic inputs onto mPFC pyramidal neurons [54]. Matrix metalloproteinases, which remodel the ECM, are upregulated in NAc core after discrete cue-induced reinstatement [55] and ventricular injections of the matrix metalloproteinase inhibitor FN-439 decrease this reinstatement [54]. Finally, as with heroin priming, the projection from dmPFC to NAc core is critical for discrete cue-induced reinstatement [37].

Studies using reversible inactivation procedures show a role of neuronal activity in additional brain areas in discrete cue-induced reinstatement. Reversible inactivation of VTA with TTX decreases this reinstatement [48], while increasing VTA acetylcholine levels with physostigmine injections increases discrete cue-induced reinstatement. However, fiber-sparing inactivation of VTA with muscimol + baclofen has no effect on this reinstatement [40]. Reversible inactivation of CeA, BLA, BNST, DLS, SN, and VP also decreases discrete cue-induced reinstatement to heroin seeking [39, 40].

Finally, lateral dorsal tegmental nucleus (LDTg) injections of the acetylcholinesterase inhibitor galantine decrease discrete cue-induced reinstatement, an effect reversed by local injections of

the muscarinic receptor antagonist scopolamine [56]. From a circuit perspective, the mechanisms underlying the effect of increasing acetylcholine tone in LDTg on this reinstatement are unknown.

Discriminative cues

The experimental procedure of discriminative cue-induced reinstatement includes three phases. During discrimination training, rats are trained to self-administer a drug in the presence of a discriminative cue (DS+) and to self-administer saline in the presence of a different discriminative cue (DS−). During extinction, lever pressing is extinguished in the absence of the discriminative cues and the drug. During reinstatement testing, the rats are exposed to the DS+ or DS− and lever responding is assessed under extinction conditions [57]. The role of discriminative cues in reinstatement of heroin seeking after extinction has also been determined in a runway model in which rats receive a drug infusion in the goal box in the presence of the DS+ and saline infusion in the presence of the DS− during the training phase [58]. In both models, reexposure to DS+, but not DS− reliably reinstates heroin seeking after extinction [58–60].

Two studies provide evidence for the role of Drd2 and CB1 receptors in discriminative cue-induced reinstatement of heroin seeking. In the runway model, McFarland and Ettenberg [58] reported that systemic injections of haloperidol (a preferential Drd2 antagonist) decrease this reinstatement. In the self-administration model, Alvarez-Jaimes et al. [61] reported that vmPFC or NAc core injections of SR141716A decrease discriminative cue-induced reinstatement of heroin seeking.

Context

In the context-induced reinstatement model used in the studies described below, rats are first trained to self-administer a drug in one context (context A) in the presence of the discrete cue. Lever pressing and the response to the discrete cue is then extinguished in a different nondrug context (context B). The contexts typically differ from each other in tactile, visual, auditory, and circadian features. During reinstatement testing, the rats are exposed to context A, which previously predicted drug availability, and lever presses result in the delivery of the discrete cues [13]. The operational definition of context-induced reinstatement is significantly greater nonreinforced responding in context A than in context B [62].

There is evidence for a role of dopamine, opioid, and glutamate systems, as well as VTA, NAc shell, vmPFC, and ventral subiculum (vSub) in context-induced reinstatement of opioid seeking. Systemic injections of the preferential MOR antagonist naltrexone, but not the delta opioid receptor (DOR) or kappa opioid receptor (KOR) antagonists naltrindole or LY2456302, respectively, decrease context-induced reinstatement of oxycodone seeking [63]. Systemic injections of LY379268, a group II metabotropic glutamate receptor (mGluR2/3) agonist that inhibits evoked glutamate release, decrease context-induced reinstatement of heroin seeking [64]. The systemic effect of LY379268 is mimicked by site-specific injections of the drug into the VTA and NAc shell but not SN or dorsal striatum [64, 65]. LY379268 injections into NAc core also decrease context-induced reinstatement but the effective doses are 3–10 times higher than the dose required in NAc shell, suggesting that the higher effective dose in the core could be due to diffusion to the shell. Additional evidence for a role of NAc shell but not core is that injections of SCH23390 into the medial or lateral NAc shell but not core decrease context-induced reinstatement of heroin seeking [49]. We also found that injections of SCH23390 into DLS, but not dorsomedial striatum, decrease context-induced reinstatement of heroin seeking [66]. Thus, context-induced reinstatement involves glutamate transmission in VTA, dopamine transmission in DLS, and both dopamine and glutamate transmission in NAc.

vmPFC sends glutamatergic projections to NAc shell [67] and we found that reversible inactivation (muscimol + baclofen) of vmPFC, but not dmPFC, decreases context-induced reinstatement of heroin seeking [68]. This effect is mimicked by selectively inactivating vmPFC context-activated Fos-positive neurons using the Daun02 inactivation procedure [68]. Context-induced reinstatement is associated with increased Fos expression in vmPFC neurons that project to NAc shell, and using an asymmetrical disconnection procedure [69], we found that pharmacological disconnection of vmPFC from NAc shell with muscimol + baclofen and SCH23390, respectively, decreases context-induced reinstatement. These data suggest that an interaction between glutamatergic projections from vmPFC to NAc shell and Drd1-mediated dopamine transmission in NAc shell is critical for context-induced reinstatement of heroin seeking [70]. More recently, we showed that muscimol + baclofen into ventral subiculum (vSub) and pharmacological disconnection of vSub (muscimol + baclofen) from NAc shell (SCH23390) decreases this reinstatement [71, 72].

vSub also sends glutamatergic projections to vmPFC [73] and context-induced reinstatement of heroin seeking is associated with activation (Fos induction) of the vSub-vmPFC pathway [72, 74]. Wang et al. [74] reported that functional disconnection of vSub (muscimol + baclofen or inhibitory DREADD) and vmPFC (blockade of internalization of GluA2 or clozapine-N-oxide) decreases context-induced reinstatement of heroin seeking. However, we found that disconnection of this projection with contralateral muscimol + baclofen inactivation of both areas has no effect on this reinstatement [72]. Thus, the role of the vSub-vmPFC projection in context-induced reinstatement has not been independently confirmed. Finally, using muscimol + baclofen or inhibitory DREADD, Ge et al. [75] reported that functional disconnection of the entorhinal cortex projection to dorsal dentate gyrus decreases context-induced reinstatement of heroin seeking.

Stress

In the operant variation of the stress-induced reinstatement model, rats are first trained to self-administer a drug in the presence of a discrete cue. Lever pressing is then extinguished in the presence of this cue. During reinstatement testing under extinction conditions (in the presence of the discrete cue), rats are exposed to different stressors [76, 77]. Notably, footshock-induced reinstatement is more readily observed in rats with a history of extended access heroin self-administration (compared to short access) [78] and is critically dependent on the context of stress exposure [79]. Below we discuss neuropharmacological findings on the mechanisms of intermittent footshock, acute food deprivation, and chronic food restriction. In Supplementary Table 1, we also summarize results from studies using the alpha-2 adrenoceptor antagonist yohimbine as a putative pharmacological stressor that potently reinstates drug seeking [76]. However, we do not describe these studies in the main text because results from a study of Chen et al. [80] challenge this notion. These authors showed that at a dose used in many reinstatement studies, yohimbine causes a modest conditioned place preference and increases operant lever pressing that was not previously reinforced with either food or drug. These observations indicate that in rat relapse models yohimbine does not appear to induce a stress-like state that motivates drug seeking.

Intermittent footshock. There is evidence for a role of dopamine, norepinephrine (NE), and corticotropin-releasing factor (CRF) in intermittent footshock-induced reinstatement of heroin seeking. Regarding dopamine, intermittent footshock-induced reinstatement of heroin seeking is associated with increased extracellular dopamine levels in NAc, and systemic injections of the nonselective Drd1/Drd2 antagonist flupenthixol decrease this reinstatement [27]. More recently, Wang et al. [81] reported that

footshock-induced reinstatement of heroin seeking is associated with increased glutamate and dopamine levels in VTA, and that local blockade of ionotropic glutamate receptors prevents this reinstatement. Together, these data suggest a role of dopamine and VTA in footshock-induced reinstatement of heroin seeking.

Regarding NE, systemic or ventricular injections of the alpha-2 adrenoceptor agonist clonidine decrease footshock-induced reinstatement of heroin seeking [82]. Repeated injections of the alpha-2 adrenoceptor agonist lofexidine during the extinction and reinstatement phases decrease footshock-induced reinstatement of speedball (heroin–cocaine mixture) seeking [83]. The critical NE projection involved in footshock-induced reinstatement is the ventral NE bundle that originates in the lateral tegmental nuclei (A1, A2, and A4) and innervates the BNST, CeA, NAc, and other subcortical areas; in contrast, the dorsal NE bundle that originates from the locus coeruleus (LC, A6 area) and innervates mPFC and other cortical areas [84, 85] does not play a role in footshock-induced reinstatement. Shaham et al. [82] found that 6-hydroxydopamine (6-OHDA) lesions of the ventral NE bundle decrease footshock-induced reinstatement; in contrast, inhibition of NE cell firing and release by injections of clonidine (or its charged analogue ST-91) into the LC has no effect on this reinstatement. The likely lateral tegmental nuclei projection areas that play a role in footshock stress-induced reinstatement are the BNST and CeA: reversible inactivation of these brain areas with TTX decreases this reinstatement (see Fig. 7 in [77]).

Regarding CRF, there is evidence that activation of extrahypothalamic CRF systems but not hypothalamic CRF, which leads to activation of the hypothalamic–pituitary–adrenal (HPA) axis, is critical for footshock stress-induced reinstatement of heroin seeking [77]. Systemic injections of the CRF1 receptor antagonist CP154,526 or ventricular injections of the nonselective CRF receptor antagonist alpha-helical CRF [9–41] decrease footshock-induced reinstatement, while ventricular injections of CRF reinstate heroin seeking [86, 87]. In contrast, adrenalectomy potentiates this reinstatement and systemic injections of the corticosterone synthesis inhibitor metyrapone mimic the effect of footshock stress on reinstatement [86]. The reasons for the unexpected stress-like effects of metyrapone and adrenalectomy-induced potentiation of footshock stress-induced reinstatement are unknown, but these results indicate that stress-induced activation of the HPA axis, which leads to increased plasma corticosterone levels, does not play a role in footshock stress-induced reinstatement of heroin seeking.

Finally, electrical stimulation of medial septum (MS) decreases footshock-induced reinstatement of heroin seeking while MS inactivation with TTX reinstates heroin seeking [79, 88]. These findings suggest a role of the MS in footshock-induced reinstatement, but they should be interpreted with caution regarding the role of this brain region in this reinstatement, because both manipulations act on both fibers-of-passage and cell bodies in MS.

Food deprivation and restriction. A second stressor that reliably reinstates heroin seeking after extinction is acute 1-day food deprivation [79]. There is evidence that hormones involved in energy balance that influence hunger and satiety states like leptin and neuropeptide-Y (NPY) contribute to food deprivation-induced reinstatement of heroin seeking. Ventricular injections of leptin decrease this reinstatement [89], while ventricular injections of the orexigenic peptide NPY induce reinstatement [90]. Additionally, systemic injections of the NPY Y5 receptor antagonist Lu AA33810, but not the NPY Y1-receptor antagonist BIBO 3304, decrease acute food deprivation-induced reinstatement of heroin seeking [91]. As with footshock, there is evidence that extrahypothalamic but not hypothalamic CRF is critical to food deprivation-induced reinstatement of heroin seeking. Ventricular injections the CRF antagonist alpha-helical CRF decrease this reinstatement while adrenalectomy has no effect [92].

Opioid and dopamine systems also contribute to acute food deprivation-induced reinstatement of heroin seeking. Systemic injections of the KOR antagonist nor-BNI, but not the MOR antagonist naltrexone, decrease acute food deprivation-induced reinstatement of heroin seeking [93]. Systemic injections of SCH23390 but not raclopride or NGB2904 (a Drd3 antagonist), also decrease this reinstatement [94]. Tobin et al. [95] also found that injections of SCH23390 into NAc shell, dmPFC, and BLA, but not NAc core or vmPFC, decrease acute food deprivation-induced reinstatement.

More recently, Shalev [96] reported that in rats that underwent heroin self-administration and extinction of the drug-reinforced responding, chronic food *restriction* for 10 days (~80–90% of free-feeding body weight) potentiates spontaneous recovery of heroin seeking. Spontaneous recovery refers to the resumption of the extinguished conditioned response that occurs after time has passed following the conclusion of extinction [97]. In subsequent studies, Shalev and colleagues reported that chronic food restriction during 14 days of forced abstinence also potentiates heroin seeking as assessed in a single extinction session [98–100].

Neuropharmacological studies have shown a role of dopamine, but not CRF or corticosterone in chronic food restriction-induced potentiation of heroin seeking during forced abstinence. Food restriction-induced potentiation of heroin seeking is associated with increases in extracellular dopamine in NAc core and shell. Additionally, NAc shell injections of the selective Drd1 antagonist SCH39166 decrease heroin seeking in both the food-restricted and food-sated conditions, while NAc core injections selectively decrease food restriction-induced potentiation of heroin seeking [100], suggesting a critical role of NAc core in this effect. In contrast, systemic injections of the CRF1 receptor antagonist R121919, ventricular injections alpha-helical CRF [9–41], or systemic injections of the glucocorticoid receptor antagonist RU486 have no effect on food restriction-induced potentiation of heroin seeking [101]. The negative data with the CRF receptor antagonists indicate that dissociable mechanisms play a role in *acute food deprivation*-induced reinstatement after extinction versus *chronic food restriction*-induced increases in heroin seeking, as assessed in a single extinction session.

Withdrawal

In opioid-dependent drug users, abstinence from the drug induces aversive withdrawal symptoms that promote relapse to opioid use [21, 102]. However, this important relapse-related human phenomenon has been rarely studied in rat models because it has been a challenge to empirically demonstrate that opioid withdrawal can induce reinstatement after extinction. In an early study, Stewart and Wise [103] used the within-session variation of the reinstatement model in which rats self-administer heroin for 2–3 h/day (limited access), then undergo extinction training for several hours, and are then tested for reinstatement [11]. They reported that morphine priming injections reinstate heroin seeking while naltrexone priming injections, which presumably precipitate opioid withdrawal, do not. Subsequently, Shaham and Stewart [15] reported that naltrexone-precipitated withdrawal (induced by injecting morphine 45 min before the test session and naltrexone 40 min later) has no effect on reinstatement. Next, they showed that in rats implanted with heroin-containing minipumps during the extinction and reinstatement phases, acute injections of naloxone, which induce withdrawal symptoms, do not reinstate heroin seeking. In contrast, spontaneous withdrawal 24 h after removal of the heroin-containing minipump induces reinstatement of heroin seeking [16].

More recently, Zhou et al. [104] reported that systemic naltrexone injections 1 day after heroin self-administration (a precipitated withdrawal manipulation) potentiate heroin seeking, as assessed in a single extinction session. At present, the

mechanisms of spontaneous withdrawal-induced reinstatement or precipitated withdrawal-induced potentiation of heroin seeking during early abstinence are unknown.

Reacquisition

In the reacquisition procedure, rats are first trained to self-administer a drug and then undergo extinction training. Next, the rats are tested for reacquisition of drug self-administration under conditions identical to those of training [105].

Studies on the neuropharmacological mechanisms of reacquisition of opioid seeking have focused on the role of NE. In an early study, Davis et al. [106] reported that pretreatment with NE-depleting agents (diethyldithiocarbamate or U-14,624; dopamine β-hydroxylase inhibitors) decreases reacquisition of morphine self-administration after extinction, suggesting an important role of NE in this reacquisition. However, potential evidence against this conclusion is that increasing brain NE by repeated pretreatment with a high but not a low dose of the monoamine oxidase inhibitor selegiline decreases reacquisition of morphine self-administration [107, 108]. From a mechanistic perspective, however, the selegiline data are difficult to interpret because this drug also increases brain levels of dopamine and serotonin. Indeed, systemic injections of yohimbine, which increases brain NE levels [109], have no effect on reacquisition of heroin self-administration [110].

Together, the role of NE in reacquisition of opioid self-administration has not been clearly established and the brain areas involved in reacquisition of opioid self-administration are largely unknown. To our knowledge, this question was investigated in one study by Olmstead et al. [111]; they reported that lesions of pedunculopontine tegmental nucleus have no effect on reacquisition of heroin self-administration.

ABSTINENCE-BASED RELAPSE MODELS

In this section, we review findings on relapse and incubation of opioid seeking after forced or voluntary abstinence.

Forced abstinence and incubation of drug craving

Forced abstinence studies include three phases: training, abstinence (withdrawal), and relapse testing. During training, rats are trained to self-administer a drug in the presence of drug-associated discrete cues. During abstinence, rats are housed in their homecage for different abstinence periods. During relapse testing under extinction conditions, lever pressing leads to contingent presentations of the discrete cues [112]. We and others have been using a variation of this forced abstinence procedure to study "incubation of drug craving" where rats or mice are tested for relapse at different abstinence days [113, 114]. In rodent studies, incubation of drug craving refers to the time-dependent increase in drug seeking during abstinence [115]. Recent studies on incubation of craving in humans have shown time-dependent increases in cue-induced subjective craving during abstinence for nicotine [116], methamphetamine [117], and alcohol [118]. To date, incubation of drug craving has not been reported in opioid drug users.

Forced abstinence. There is evidence for the role of striatal dopamine in relapse to opioid seeking after forced abstinence. Gao et al. [119] reported that 6-OHDA lesions of DLS and NAc shell, blockade of Drd1 but not Drd2 in NAc shell and blockade of either receptor in DLS decreases relapse to morphine seeking after 3 weeks of forced abstinence in rats. It is likely that other brain areas play a role in opioid relapse after forced abstinence. Madsen et al. [120] reported that in mice, this relapse is associated with Fos induction in NAc core, CeA, and SN. However, the causal role of these brain areas in relapse to morphine seeking after forced abstinence is unknown.

Incubation of craving. Incubation of heroin craving, as assessed in extinction sessions at different forced abstinence periods (1–66 days), was first demonstrated in a study on the time-course of extinction responding and subsequent footshock stress-induced reinstatement of heroin seeking [121]. The data from this study were the inspiration for the subsequent study in cocaine-trained rats where the term incubation of drug craving was first used [115]. Incubation of heroin craving was also demonstrated in an acquisition of a new conditioned response procedure where after initial pairing of a discrete cue with drug infusions, rats perform a new operant response that is only reinforced by the discrete cue [122]. Subsequent studies determined the pharmacological and circuit mechanisms of incubation of heroin craving.

There is evidence for a role of MOR and the toll-like receptor 4 (TLR4), which is primarily expressed in microglia, in incubation of heroin craving. Theberge et al. [123] reported that NAc MOR mRNA levels decrease after 1 day of abstinence and return to baseline levels after 11 and 30 days. They also reported that systemic injections of naloxone decrease incubated heroin seeking after 15 days of forced abstinence but have no effect on lower, nonincubated heroin seeking on day 1. In a subsequent study, Theberge et al. [124] reported that chronic minipump delivery of the selective TLR4 antagonist (+)-naltrexone during the first two weeks of forced abstinence decreases the development of incubation of heroin craving. In contrast, acute systemic injections of (+)-naltrexone during the late abstinence-relapse test have no effect on incubated heroin craving.

The main brain regions reported to be involved in incubation of heroin craving are mPFC and orbitofrontal cortex (OFC). A role of mPFC in incubation of heroin craving was examined in three correlational immediate early gene studies. Doherty et al. [125] reported that compared to adult male rats, adolescent male rats show weaker incubation of heroin craving; this weaker incubation is associated with lower Fos induction in dmPFC and vmPFC. Kuntz et al. [126] reported that incubation of heroin craving is associated with elevated *egr1* (early growth response 1) and *egr2* mRNA expression in mPFC. Finally, in a subsequent study, Kuntz-Melcavage et al. [127] reported that incubation of heroin craving is associated with time-dependent increases in *Bdnf* (brain-derived neurotrophic factor), *Dusp5* (dual specificity phosphatase 5), and *Calb 1* (calbindin 1) mRNA levels in mPFC. Fanous et al. [128] demonstrated a key role of the OFC in incubation of heroin craving. Incubation of heroin craving was associated with Fos induction in OFC. Reversible inactivation (muscimol + baclofen) of lateral OFC decreases heroin seeking after 14 days of forced abstinence but not 1 day. Additionally, selective ablation of incubation-associated Fos-positive neurons using the Daun02 procedure [129] decreases "incubated" heroin seeking on forced abstinence day 14.

Airavaara et al. [130] reported no effect on incubation of heroin seeking after either acute VTA or NAc GDNF injections or chronic delivery of anti-GDNF antibodies into VTA or NAc during the forced abstinence period. As acute VTA GDNF injections potentiate incubation of cocaine craving and chronic delivery of anti-GDNF antibodies into VTA prevents the emergence of incubation of cocaine craving [131], the data of Airavaara et al. and Lu et al. studies indicate dissociable mechanisms for incubation of heroin versus cocaine craving.

Finally, Blackwood et al. [132] studied molecular adaptations in striatal and hippocampal opioid receptors during incubation of oxycodone craving after forced abstinence. They reported that this incubation was associated with increased MOR mRNA expression but decreased MOR protein expression in dorsal

striatum. They also reported decreased MOR and KOR mRNA expression but increased MOR and KOR protein levels in hippocampus. A question for future research is whether these alterations in opioid receptor, and in particular the unexpected opposite mRNA and protein expression changes, play a role in incubation of opioid craving.

Voluntary abstinence induced by adverse consequences of drug intake

Voluntary abstinence from drug self-administration in rats can be achieved using two classical learning procedures: response-contingent operant punishment [133] and electric barrier-induced suppression of operant responding [134]. In the punishment-based relapse model, this is achieved by administering a shock after the rat lever presses for the drug [8, 22]. In the electric barrier-based relapse model (also termed conflict-based relapse model), drug self-administration is suppressed by introducing an electric barrier in front of the drug-paired lever [7]. During subsequent relapse testing, drug seeking is precipitated by exposure to drug-priming injections or drug-associated cues or contexts, or by allowing the rats to self-administer the drug (reacquisition) [6]. To date, very few studies assessed relapse to opioid seeking after punishment or electric barrier exposure and the mechanisms underlying these forms of relapse are unknown.

Panlilio et al. [8] reported that priming injections of remifentanil after punishment-induced suppression of the drug-reinforced responding cause faster reacquisition of remifentanil self-administration. In a subsequent study, these authors reported that priming injections of heroin or the benzodiazepine lorazepam induce relapse to remifentanil seeking and facilitate reacquisition of remifentanil self-administration after punishment [135].

Two studies demonstrated relapse to heroin seeking after electric barrier suppression of heroin seeking. Peck et al. [136] reported that a higher proportion of heroin-trained rats than cocaine-trained rats resume drug seeking after electric barrier suppression of drug self-administration during a relapse test in which the electric barrier was maintained. For both drugs, the authors observed large individual differences during the relapse tests, most likely because the tests were performed in the presence of the electric barrier. These authors also found that heroin-trained rats housed in an enriched environment achieve abstinence at lower electric barrier shock intensities than rats housed in standard cages [137]. However, because the authors did not test for relapse after electric barrier suppression of drug self-administration in this study, a question for future research is whether environmental enrichment also protects against relapse in the electric barrier conflict model.

Voluntary abstinence induced by a nondrug reward in a choice procedure

Previous studies by Ahmed and colleagues have shown that most rats given a mutually exclusive choice between a drug (cocaine or heroin) versus saccharin or sucrose solution strongly prefer the nondrug reward [138–140]. Based on these findings, we recently introduced two rat models in which drug relapse and incubation of drug craving is assessed after choice-induced voluntary abstinence. In the first model, we use palatable high-carbohydrate food as the alternative nondrug reward [9, 141, 142]. In the second model, we use operant access to social interaction with a peer as the alternative nondrug reward [10].

The experimental procedure for both models include four phases: (1) palatable food or "social interaction" self-administration training in the presence of distinct discriminative and discrete nondrug reward-associated cues, (2) drug self-administration training in the presence of distinct discriminative and discrete drug-associated cues, (3) voluntary abstinence during which the rats are given mutually exclusive choice sessions between the palatable food or social interaction and the drug, and (4) tests for relapse to drug seeking in extinction tests in the presence of the discriminative and discrete drug-associated cues during early or late abstinence. The operational definition of voluntary abstinence is complete (zero choices of drug infusions) or almost complete (a small number of choices of drug infusions) suppression of drug taking during the choice sessions [143].

In our initial study using the voluntary abstinence food-choice model, we found reliable incubation of methamphetamine craving after voluntary abstinence [9]. In a subsequent study, we tested the generality of the food-choice voluntary abstinence model to heroin and reported that this manipulation prevents the emergence of incubation of heroin craving in male and female rats [143]. These data support the notion that environmental contexts and events play different roles in opioid versus psychostimulant reward and relapse [144–146]. The mechanisms underlying the inhibitory effect of food-choice voluntary abstinence on incubation of heroin craving are unknown.

CONCLUSIONS AND FUTURE DIRECTIONS

We reviewed studies on pharmacological and circuit mechanisms of relapse to opioid seeking as assessed in the classical operant extinction-reinstatement model, the established forced abstinence and incubation of craving model, and the newer voluntary abstinence-relapse models where abstinence is achieved by introducing adverse consequences to the drug taking (punishment) or seeking (electric barrier) behavior, or by introducing operant nondrug rewards (palatable food or social interaction) in a mutually exclusive discrete choice procedure.

As seen in Fig. 2, several interconnected brain areas and projections play a role in drug priming-, discrete cue-, context-, and stress-induced reinstatement of opioid seeking, with an important role of dopamine and glutamate transmission in mesolimbic (VTA-to-NAc), mesocortical (VTA-to-mPFC), and mPFC-to-NAc projections. In contrast, much less is known about the brain areas and circuits controlling discriminative cue-induced reinstatement and relapse to opioid seeking after forced abstinence. To date, no published data are available on brain mechanisms of reacquisition after extinction or relapse to opioid seeking in the newer voluntary abstinence models.

In our view, the most important question for future research is whether for a given relapse-provoking stimulus the neuropharmacological mechanisms that control drug seeking are dependent on the method used to achieve abstinence—extinction, homecage forced abstinence, voluntary abstinence induced by adverse consequences, and voluntary abstinence induced by providing rats an alternative reward in a discrete choice procedure. Based on the limited available data we suspect that investigators will identify substantial differences [147]. For example, the benzodiazepine lorazepam reinstates remifentanil seeking after punishment but not extinction [135]. Additionally, reversible inactivation of the BLA increases context-induced reinstatement of cocaine seeking after punishment but decreases context-induced reinstatement after extinction [148]. Furthermore, the method used to achieve abstinence can further modulate the rat's response to the relapse-provoking stimulus. For example, incubation of heroin craving is observed after forced abstinence but not food choice-induced voluntary abstinence [142]. And incubation of methamphetamine craving is observed after forced abstinence or food choice-induced voluntary abstinence but not after social choice-induced voluntary abstinence [10].

Another question for future research is whether the mechanisms that control relapse to drug seeking induced by cues and contexts previously associated with opioid self-administration are different from those that control relapse in the presence of the drug itself (reacquisition). Based on recent studies of McNally and colleagues we suspect that future mechanistic studies will identify

more differences than similarities. In this regard, Willcocks and McNally [149] showed that reversible inactivation of dmPFC decreases context-induced reinstatement of alcohol seeking but accelerates acquisition of alcohol self-administration after extinction. Other evidence that suggests that reinstatement and reacquisition of opioid-taking behavior involve dissociable mechanisms is that yohimbine reliably reinstates opioid seeking after extinction [150, 151], but has no effect on reacquisition of heroin self-administration [110].

Another issue for future research is the mechanisms of relapse to opioid seeking in rat models of polydrug self-administration. With a few exceptions [83, 152], mechanisms of relapse in the studies reviewed here were performed in rats that self-administered a single opioid drug. However, many human addicts are polydrug users [153, 154] and this human condition can be assessed in relapse models. For example, Leri and Stewart [155] reported that in rats trained to self-administer heroin and cocaine, heroin priming reinstates heroin but not cocaine seeking, while cocaine priming reinstates cocaine but not heroin seeking. More recently, Rubio et al. [152] reported that under similar experimental conditions, discrete cues associated with heroin self-administration reinstate heroin but not cocaine seeking, while discrete cues associated with cocaine self-administration reinstate cocaine but not heroin seeking. These polydrug relapse models can be used to study mechanisms underlying relapse to polydrug use, a potentially important research area from a human translation perspective [153, 155].

Two other issues for future research are sex as a biological variable and the use of transgenic mice in mechanistic studies of opioid relapse. Regarding the first issue, it is unknown whether there are sex differences in opioid relapse models, because with a few exceptions (see [15, 143] in the studies we reviewed), investigators have almost exclusively used male rats. This state-of-affair in opioid research is in sharp contrast with psychostimulant research where the topic of sex differences in relapse/reinstatement has been extensively studied and documented [156–158]. Regarding the second issue, the studies we reviewed exclusively used rats and in general investigators have not incorporated transgenic mice to study cell-types and circuit mechanisms of opioid relapse using optogenetic and chemogenetic tools. The main reason for this state-of-affairs is the technical difficulties of using mice in long-term intravenous self-administration studies and the fact that oral opioid self-administration procedures with potent opioid synthetic agonists, which can serve as reliable reinforcers in rodents [159], are yet to be used in operant relapse studies using mice.

Finally, in the context of the current US opioid crisis, it will be important to determine if there are mechanistic differences in reinstatement/relapse between heroin and prescription opioids within a given reinstatement/relapse model. Also important from this perspective is to examine the effect of pain on relapse in preclinical models of opioid reinstatement/relapse.

In closing, we hope that our review will serve as a useful resource to the increasing number of addiction researchers who have begun to use animal models of opioid relapse due to the shift in funding to opioid research to address the US opioid crisis. We also hope that our review will encourage investigators to use the potentially more human-relevant models of relapse after voluntary abstinence in mechanistic studies, which may lead to the development of new medications to prevent relapse to opioid use.

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ADDITIONAL INFORMATION

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REFERENCES

1. Hunt WA, Barnett LW, Branch LG. Relapse rates in addiction programs. J Clin Psychol. 1971;27:455–6.
2. Sinha R. New findings on biological factors predicting addiction relapse vulnerability. Curr Psychiatry Rep. 2011;13:398–405.
3. Seth P, Scholl L, Rudd RA, Bacon S. Overdose deaths involving opioids, cocaine, and psychostimulants—United States, 2015–2016. MMWR Morb Mortal Wkly Rep. 2018;67:349–358.
4. Shaham Y, Shalev U, Lu L, de Wit H, Stewart J. The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology. 2003;168:3–20.
5. Stewart J, de Wit H. Reinstatement of drug-taking behavior as a method of assessing incentive motivational properties of drugs. In: Bozarth MA, ed. Methods of assessing the reinforcing properties of abused drugs.. New York, NY: Springer-Verlag; 1987. p. 211–27.
6. Venniro M, Caprioli D, Shaham Y. Animal models of drug relapse and craving: From drug priming-induced reinstatement to incubation of craving after voluntary abstinence. Prog Brain Res. 2016;224:25–52.
7. Cooper A, Barnea-Ygael N, Levy D, Shaham Y, Zangen A. A Conflict Rat Model of Cue-Induced Relapse to Cocaine Seeking. Psychopharmacology. 2007;194:117–25.
8. Panlilio LV, Thorndike EB, Schindler CW. Reinstatement of punishment-suppressed opioid self-administration in rats: an alternative model of relapse to drug abuse. Psychopharmacology. 2003;168:229–35.
9. Caprioli D, Venniro M, Zeric T, Li X, Adhikary S, Madangopal R, et al. Effect of the novel positive allosteric modulator of metabotropic glutamate receptor 2 AZD8529 on incubation of methamphetamine craving after prolonged voluntary abstinence in a rat model. Biol Psychiatry. 2015;78:463–73.
10. Venniro M, Zhang M, Caprioli D, Golden SA, Heins C, Hoots JK, et al. (2018): Volitional social interaction prevents drug addiction. *Nat Neurosci*. (in press).
11. de Wit H, Stewart J. Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology. 1981;75:134–43.
12. Meil WM, See RE. Conditioned cued recovery of responding following prolonged withdrawal from self-administered cocaine in rats: an animal model of relapse. Behav Pharmacol. 1996;7:754–63.
13. Crombag HS, Shaham Y. Renewal of drug seeking by contextual cues after prolonged extinction in rats. Behav Neurosci. 2002;116:169–73.
14. Katner SN, Magalong JG, Weiss F. Reinstatement of alcohol-seeking behavior by drug-associated discriminative stimuli after prolonged extinction in the rat. Neuropsychopharmacology. 1999;20:471–9.
15. Shaham Y, Stewart J. Stress reinstates heroin self-administration behavior in drug-free animals: an effect mimicking heroin, not withdrawal. Psychopharmacology. 1995;119:334–41.
16. Shaham Y, Rajabi H, Stewart J. Relapse to heroin-seeking in rats under opioid maintenance: the effects of stress, heroin priming, and withdrawal. J Neurosci. 1996;16:1957–63.
17. de Wit H. Priming effects with drugs and other reinforcers. Exp Clin Psychopharmacol. 1996;4:5–10.
18. Sinha R, Shaham Y, Heilig M. Translational and reverse translational research on the role of stress in drug craving and relapse. Psychopharmacology. 2011;218:69–82.
19. Jaffe JH, Cascella NG, Kumor KM, Sherer MA. Cocaine-induced cocaine craving. Psychopharmacology. 1989;97:59–64.
20. O'Brien CP, Childress AR, Mclellan TA, Ehrman R. Classical conditioning in drug dependent humans. Ann NY Acad Sci. 1992;654:400–15.
21. Wikler A. Dynamics of drug dependence. Implications of a conditioning theory for research and treatment. Arch Gen Psychiatry. 1973;28:611–6.

22. Marchant NJ, Li X, Shaham Y. Recent developments in animal models of drug relapse. Curr Opin Neurobiol. 2013;23:675–83.
23. Epstein DH, Preston KL. The reinstatement model and relapse prevention: a clinical perspective. Psychopharmacology. 2003;168:31–41.
24. Katz JL, Higgins ST. The validity of the reinstatement model of craving and relapse to drug use. Psychopharmacology. 2003;168:21–30.
25. Marlatt AG. Models of relapse and relapse prevention: a commentary. Exp Clin Psychopharmacol. 1996;4:55–60.
26. de Wit H, Epstein DH, Preston KL. Does human language limit translatability of clinical and preclinical addiction research? Neuropsychopharmacology. 2018;43:1985–8.
27. Shaham Y, Stewart J. Effects of opioid and dopamine receptor antagonists on relapse induced by stress and reexposure to heroin in rats. Psychopharmacology. 1996;125:385–91.
28. De Vries TJ, Homberg JR, Binnekade R, Raaso H, Schoffelmeer AN. Cannabinoid modulation of the reinforcing and motivational properties of heroin and heroin-associated cues in rats. Psychopharmacology. 2003;168:164–9.
29. De Vries TJ, Schoffelmeer AN, Binnekade R, Raaso H, Vanderschuren LJ. Relapse to cocaine- and heroin-seeking behavior mediated by dopamine D2 receptors is time-dependent and associated with behavioral sensitization. Neuropsychopharmacology. 2002;26:18–26.
30. Wise RA, Murray A, Bozarth MA. Bromocriptine self-administration and bromocriptine- reinstatement of cocaine-trained and heroin-trained lever pressing in rats. Psychopharmacology. 1990;100:355–60.
31. Fattore L, Spano MS, Cossu G, Deiana S, Fratta W. Cannabinoid mechanism in reinstatement of heroin-seeking after a long period of abstinence in rats. Eur J Neurosci. 2003;17:1723–6.
32. Fattore L, Spano S, Cossu G, Deiana S, Fadda P, Fratta W. Cannabinoid CB(1) antagonist SR 141716A attenuates reinstatement of heroin self-administration in heroin-abstinent rats. Neuropharmacology. 2005;48:1097–104.
33. You ZB, Gao JT, Bi GH, He Y, Boateng C, Cao J, et al. The novel dopamine D3 receptor antagonists/partial agonists CAB2-015 and BAK4-54 inhibit oxycodone-taking and oxycodone-seeking behavior in rats. Neuropharmacology. 2017;126:190–9.
34. Stewart J. Reinstatement of heroin and cocaine self-administration behavior in the rat by intracerebral application of morphine in the ventral tegmental area. Pharmacol Biochem Behav. 1984;20:917–23.
35. Wise RA. Opiate reward: sites and substrates. Neurosci Biobehav Rev. 1989;13:129–33.
36. Stewart J, Vezina P. A comparison of the effects of intra-accumbens injections of amphetamine and morphine on reinstatement of heroin intravenous self-administration behavior. Brain Res. 1988;457:287–94.
37. LaLumiere RT, Kalivas PW. Glutamate release in the nucleus accumbens core is necessary for heroin seeking. J Neurosci. 2008;28:3170–7.
38. Shen H, Moussawi K, Zhou W, Toda S, Kalivas PW. Heroin relapse requires long-term potentiation-like plasticity mediated by NMDA2b-containing receptors. Proc Natl Acad Sci USA. 2011;108:19407–412.
39. Fuchs RA, See RE. Basolateral amygdala inactivation abolishes conditioned stimulus- and heroin-induced reinstatement of extinguished heroin-seeking behavior in rats. Psychopharmacology. 2002;160:425–33.
40. Rogers JL, Ghee S, See RE. The neural circuitry underlying reinstatement of heroin-seeking behavior in an animal model of relapse. Neuroscience. 2008;151:579–88.
41. Lai M, Chen W, Zhu H, Zhou X, Liu H, Zhang F, et al. Low dose risperidone attenuates cue-induced but not heroin-induced reinstatement of heroin seeking in an animal model of relapse. Int J Neuropsychopharmacol. 2013; 16:1569–75.
42. Yue K, Ma B, Chen L, Tian X, Ru Q, Gan Y, et al. L-Stepholidine, a naturally occurring dopamine D1 receptor agonist and D2 receptor antagonist, attenuates heroin self-administration and cue-induced reinstatement in rats. Neuroreport 2014;25:7–11.
43. Galaj E, Manuszak M, Babic S, Ananthan S, Ranaldi R. The selective dopamine D3 receptor antagonist, SR 21502, reduces cue-induced reinstatement of heroin seeking and heroin conditioned place preference in rats. Drug Alcohol Depend. 2015;156:228–33.
44. Porter-Stransky KA, Bentzley BS, Aston-Jones G. Individual differences in orexin-I receptor modulation of motivation for the opioid remifentanil. Addict Biol. 2017;22:303–17.
45. Smith RJ, Aston-Jones G. Orexin/hypocretin 1 receptor antagonist reduces heroin self-administration and cue-induced heroin seeking. Eur J Neurosci. 2012;35:798–804.
46. Schmidt ED, Voorn P, Binnekade R, Schoffelmeer AN, De Vries TJ. Differential involvement of the prelimbic cortex and striatum in conditioned heroin and sucrose seeking following long-term extinction. Eur J Neurosci. 2005;22:2347–56.
47. Koya E, Spijker S, Voorn P, Binnekade R, Schmidt ED, Schoffelmeer AN, et al. Enhanced cortical and accumbal molecular reactivity associated with conditioned heroin, but not sucrose-seeking behaviour. J Neurochem. 2006;98:905–15.
48. Zhou W, Liu H, Zhang F, Tang S, Zhu H, Lai M, et al. Role of acetylcholine transmission in nucleus accumbens and ventral tegmental area in heroin-seeking induced by conditioned cues. Neuroscience. 2007;144:1209–18.
49. Bossert JM, Poles GC, Wihbey KA, Koya E, Shaham Y. Differential effects of blockade of dopamine D1-family receptors in nucleus accumbens core or shell on reinstatement of heroin seeking induced by contextual and discrete cues. J Neurosci. 2007;27:12655–63.
50. Shen HW, Scofield MD, Boger H, Hensley M, Kalivas PW. Synaptic glutamate spillover due to impaired glutamate uptake mediates heroin relapse. J Neurosci. 2014;34:5649–57.
51. See RE. Dopamine D1 receptor antagonism in the prelimbic cortex blocks the reinstatement of heroin-seeking in an animal model of relapse. Int J Neuropsychopharmacol. 2009;12:431–46.
52. Chen W, Wang Y, Sun A, Zhou L, Xu W, Zhu H, et al. Activation of AMPA receptor in the infralimbic cortex facilitates extinction and attenuates the heroin-seeking behavior in rats. Neurosci Lett. 2016;612:126–31.
53. Van den Oever MC, Goriounova NA, Li KW, Van der Schors RC, Binnekade R, Schoffelmeer AN, et al. Prefrontal cortex AMPA receptor plasticity is crucial for cue-induced relapse to heroin-seeking. Nat Neurosci. 2008;11:1053–8.
54. Van den Oever MC, Lubbers BR, Goriounova NA, Li KW, Van der Schors RC, Loos M, et al. Extracellular matrix plasticity and GABAergic inhibition of prefrontal cortex pyramidal cells facilitates relapse to heroin seeking. Neuropsychopharmacology. 2010;35:2120–33.
55. Smith AC, Kupchik YM, Scofield MD, Gipson CD, Wiggins A, Thomas CA, et al. Synaptic plasticity mediating cocaine relapse requires matrix metalloproteinases. Nat Neurosci. 2014;17:1655–7.
56. Liu H, Lai M, Zhou X, Zhu H, Liu Y, Sun A, et al. Galantamine attenuates the heroin seeking behaviors induced by cues after prolonged withdrawal in rats. Neuropharmacology . 2012;62:2515–21.
57. Weiss F, Maldonado-Vlaar CS, Parsons LH, Kerr TM, Smith DL, Ben-Shahar O. Control of cocaine-seeking behavior by drug-associated stimuli in rats: effects on recovery of extinguished operant-responding and extracellular dopamine levels in amygdala and nucleus accumbens. Proc Natl Acad Sci USA. 2000;97:4321–6.
58. McFarland K, Ettenberg A. Reinstatement of drug-seeking behavior produced by heroin-predictive environmental stimuli. Psychopharmacology. 1997;131:86–92.
59. Gracy KN, Dankiewicz LA, Weiss F, Koob GF. Heroin-specific stimuli reinstate operant heroin-seeking behavior in rats after prolonged extinction. Pharmacol Biochem Behav. 2000;65:489–94.
60. Zhou W, Zhang F, Tang S, Liu H, Gu J, Yang G. The dissociation of heroin-seeking patterns induced by contextual, discriminative, or discrete conditioned cues in a model of relapse to heroin in rats. Psychopharmacology. 2005;181:197–206.
61. Alvarez-Jaimes L, Polis I, Parsons LH. Attenuation of cue-induced heroin-seeking behavior by cannabinoid CB1 antagonist infusions into the nucleus accumbens core and prefrontal cortex, but not basolateral amygdala. Neuropsychopharmacology. 2008;33:2483–93.
62. Crombag HS, Bossert JM, Koya E, Shaham Y. Review. Context-induced relapse to drug seeking: a review. Philos Trans R Soc Lond B Biol Sci. 2008;363:3233–43.
63. Bossert JM, Hoots JK, Fredriksson I, Adhikary, Zhang M, Venniro M, et al. (2018). Role of mu, but not delta or kappa, opioid receptors in context-induced reinstatement of oxycodone seeking. *Eur J Neurosci.* (in press).
64. Bossert JM, Liu SY, Lu L, Shaham Y. A role of ventral tegmental area glutamate in contextual cue-induced relapse to heroin seeking. J Neurosci. 2004;24:10726–30.
65. Bossert JM, Gray SM, Lu L, Shaham Y. Activation of group II metabotropic glutamate receptors in the nucleus accumbens shell attenuates context-induced relapse to heroin seeking. Neuropsychopharmacology. 2006;31:2197–209.
66. Bossert JM, Wihbey K, Pickens CL, Nair SG, Shaham Y. Role of dopamine D1-family receptors in dorsolateral striatum in context-induced reinstatement of heroin seeking in rats. Psychopharmacology. 2009;206:51–60.
67. Groenewegen HJ, Wright CI, Beijer AV, Voorn P. Convergence and segregation of ventral striatal inputs and outputs. Ann NY Acad Sci. 1999;877:49–63.
68. Bossert JM, Stern AL, Theberge FR, Cifani C, Koya E, Hope BT, et al. Ventral medial prefrontal cortex neuronal ensembles mediate context-induced relapse to heroin. Nat Neurosci. 2011;14:420–2.
69. Gold RM. Aphagia and adipsia produced by unilateral hypothalamic lesions in rats. Am J Physiol. 1966;211:1274–6.
70. Bossert JM, Stern AL, Theberge FR, Marchant NJ, Wang HL, Morales M, et al. Role of projections from ventral medial prefrontal cortex to nucleus accumbens shell in context-induced reinstatement of heroin seeking. J Neurosci. 2012;32:4982–91.

71. Bossert JM, Stern AL. Role of ventral subiculum in context-induced reinstatement of heroin seeking in rats. Addict Biol. 2014;19:338–42.
72. Bossert JM, Adhikary S, St Laurent R, Marchant NJ, Wang HL, Morales M, et al. Role of projections from ventral subiculum to nucleus accumbens shell in context-induced reinstatement of heroin seeking in rats. Psychopharmacology. 2016;233:1991–2004.
73. Jay TM, Witter MP. Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin. J Comp Neurol. 1991;313:574–86.
74. Wang N, Ge F, Cui C, Li Y, Sun X, Sun L, et al. Role of glutamatergic projections from the ventral CA1 to infralimbic cortex in context-induced reinstatement of heroin seeking. Neuropsychopharmacology. 2018;43:1373–84.
75. Ge F, Wang N, Cui C, Li Y, Liu Y, Ma Y, et al. Glutamatergic projections from the entorhinal cortex to dorsal dentate gyrus mediate context-induced reinstatement of heroin seeking. Neuropsychopharmacology. 2017;42:1860–70.
76. Mantsch JR, Baker DA, Funk D, Le AD, Shaham Y. Stress-induced reinstatement of drug seeking: 20 years of progress. Neuropsychopharmacology. 2016;41:335–56.
77. Shaham Y, Erb S, Stewart J. Stress-induced relapse to heroin and cocaine seeking in rats: a review. Brain Res Brain Res Rev. 2000;33:13–33.
78. Ahmed SH, Walker JR, Koob GF. Persistent increase in the motivation to take heroin in rats with a history of drug escalation. Neuropsychopharmacology. 2000;22:413–21.
79. Shalev U, Highfield D, Yap J, Shaham Y. Stress and relapse to drug seeking in rats: studies on the generality of the effect. Psychopharmacology. 2000;150:337–46.
80. Chen YW, Fiscella KA, Bacharach SZ, Tanda G, Shaham Y, Calu DJ. Effect of yohimbine on reinstatement of operant responding in rats is dependent on cue contingency but not food reward history. Addict Biol. 2015;20:690–700.
81. Wang B, You ZB, Wise RA. Heroin self-administration experience establishes control of ventral tegmental glutamate release by stress and environmental stimuli. Neuropsychopharmacology. 2012;37:2863–9.
82. Shaham Y, Highfield D, Delfs J, Leung S, Stewart J. Clonidine blocks stress-induced reinstatement of heroin seeking in rats: an effect independent of locus coeruleus noradrenergic neurons. Eur J Neurosci. 2000;12:292–302.
83. Highfield D, Yap J, Grimm JW, Shalev U, Shaham Y. Repeated lofexidine treatment attenuates stress-induced, but not drug cues-induced reinstatement of a heroin–cocaine mixture (speedball) seeking in rats. Neuropsychopharmacology. 2001;25:320–31.
84. Kvetnansky R, Sabban EL, Palkovits M. Catecholaminergic systems in stress: structural and molecular genetic approaches. Physiol Rev. 2009;89:535–606.
85. Aston-Jones G, Delfs JM, Druhan J, Zhu Y. The bed nucleus of the stria terminalis. A target site for noradrenergic actions in opiate withdrawal. Ann NY Acad Sci. 1999;877:486–98.
86. Shaham Y, Funk D, Erb S, Brown TJ, Walker CD, Stewart J. Corticotropin-releasing factor, but not corticosterone, is involved in stress-induced relapse to heroin-seeking in rats. J Neurosci. 1997;17:2605–14.
87. Shaham Y, Erb S, Leung S, Buczek Y, Stewart J. CP-154,526, a selective, non peptide antagonist of the corticotropin-releasing factor type 1 receptor attenuates stress-induced relapse to drug seeking in cocaine-and heroin-trained rats. Psychopharmacology. 1998;137:184–90.
88. Highfield D, Clements A, Shalev U, McDonald R, Featherstone R, Stewart J, et al. Involvement of the medial septum in stress-induced relapse to heroin seeking in rats. Eur J Neurosci. 2000;12:1705–13.
89. Shalev U, Yap J, Shaham Y. Leptin attenuates acute food deprivation-induced relapse to heroin seeking. J Neurosci. 2001;21:RC129.
90. Maric T, Tobin S, Quinn T, Shalev U. Food deprivation-like effects of neuropeptide Y on heroin self-administration and reinstatement of heroin seeking in rats. Behav Brain Res. 2008;194:39–43.
91. Maric T, Sedki F, Chafetz D, Schoela N, Shalev U. A role for neuropeptide Y Y5 but not the Y1-receptor subtype in food deprivation-induced reinstatement of heroin seeking in the rat. Psychopharmacology. 2011;218:693–701.
92. Shalev U, Finnie PS, Quinn T, Tobin S, Wahi P. A role for corticotropin-releasing factor, but not corticosterone, in acute food-deprivation-induced reinstatement of heroin seeking in rats. Psychopharmacology. 2006;187:376–84.
93. Sedki F, Eigenmann K, Gelinas J, Schouela N, Courchesne S, Shalev U. A role for kappa-, but not mu-opioid, receptor activation in acute food deprivation-induced reinstatement of heroin seeking in rats. Addict Biol. 2015; 20:423–32.
94. Tobin S, Newman AH, Quinn T, Shalev U. A role for dopamine D1-like receptors in acute food deprivation-induced reinstatement of heroin seeking in rats. Int J Neuropsychopharmacol. 2009;12:217–26.
95. Tobin S, Sedki F, Abbas Z, Shalev U. Antagonism of the dopamine D1-like receptor in mesocorticolimbic nuclei attenuates acute food deprivation-induced reinstatement of heroin seeking in rats. Eur J Neurosci. 2013;37:972–81.
96. Shalev U. Chronic food restriction augments the reinstatement of extinguished heroin-seeking behavior in rats. Addict Biol. 2012;17:691–3.
97. Bouton ME, Swartzentruber D. Sources of relapse after extinction in Pavlovian and instrumental learning. Clin Psychol Rev. 1991;11:123–40.
98. D'Cunha TM, Sedki F, Macri J, Casola C, Shalev U. The effects of chronic food restriction on cue-induced heroin seeking in abstinent male rats. Psychopharmacology. 2013;225:241–50.
99. Sedki F, D'Cunha T, Shalev U. A procedure to study the effect of prolonged food restriction on heroin seeking in abstinent rats. *J Vis Exp*. 2013;81:e50751.
100. D'Cunha TM, Daoud E, Rizzo D, Bishop AB, Russo M, Mourra G, et al. Augmentation of heroin seeking following chronic food restriction in the rat: differential role for dopamine transmission in the nucleus accumbens shell and core. Neuropsychopharmacology. 2017;42:1136–45.
101. Sedki F, Abbas Z, Angelis S, Martin J, D'Cunha T, Shalev U. Is it stress? The role of stress related systems in chronic food restriction-induced augmentation of heroin seeking in the rat. Front Neurosci. 2013;7:1–10.
102. Dole VP, Nyswander M. A medical treatment for diacetylmorphine (heroin) addiction. a clinical trial with methadone hydrochloride. J Am Med Assoc. 1965;193:646–50.
103. Stewart J, Wise RA. Reinstatement of heroin self-administration habits: morphine prompts and naltrexone discourages renewed responding after extinction. Psychopharmacology. 1992;108:79–84.
104. Zhou W, Zhang F, Liu H, Tang S, Lai M, Zhu H, et al. Effects of training and withdrawal periods on heroin seeking induced by conditioned cue in an animal of model of relapse. Psychopharmacology. 2009;203:677–84.
105. Li N, He S, Parrish C, Delich J, Grasing K. Differences in morphine and cocaine reinforcement under fixed and progressive ratio schedules; effects of extinction, reacquisition and schedule design. Behav Pharmacol. 2003;14:619–30.
106. Davis WM, Smith SG, Khalsa JH. Noradrenergic role in the self-administration of morphine or amphetamine. Pharmacol Biochem Behav. 1975;3:477–84.
107. Grasing K, He S. Effects of high-dose selegiline on morphine reinforcement and precipitated withdrawal in dependent rats. Behav Pharmacol. 2005;16:1–13.
108. Grasing K, He S, Li N. Selegiline modifies the extinction of responding following morphine self-administration, but does not alter cue-induced reinstatement, reacquisition of morphine reinforcement, or precipitated withdrawal. Pharmacol Res. 2005;51:69–78.
109. Abercrombie EA, Keefe KA, Difrischia DS, Zigmond MJ. Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. J Neurochem. 1989;52:1655–8.
110. Minhas M, Leri F. The effect of heroin dependence on resumption of heroin self-administration in rats. Drug Alcohol Depend. 2014;138:24–31.
111. Olmstead MC, Munn EM, Franklin KB, Wise RA. Effects of pedunculopontine tegmental nucleus lesions on responding for intravenous heroin under different schedules of reinforcement. J Neurosci. 1998;18:5035–44.
112. Fuchs RA, Branham RK, See RE. Different neural substrates mediate cocaine seeking after abstinence versus extinction training: a critical role for the dorsolateral caudate-putamen. J Neurosci. 2006;26:3584–8.
113. Dong Y, Taylor JR, Wolf ME, Shaham Y. Circuit and synaptic plasticity mechanisms of drug relapse. J Neurosci. 2017;37:10867–76.
114. Pickens CL, Airavaara M, Theberge F, Fanous S, Hope BT, Shaham Y. Neurobiology of the incubation of drug craving. Trends Neurosci. 2011;34:411–20.
115. Grimm JW, Hope BT, Wise RA, Shaham Y. Neuroadaptation. Incubation of cocaine craving after withdrawal. Nature. 2001;412:141–2.
116. Bedi G, Preston KL, Epstein DH, Heishman SJ, Marrone GF, Shaham Y, et al. Incubation of cue-induced cigarette craving during abstinence in human smokers. Biol Psychiatry. 2011;69:708–11.
117. Wang G, Shi J, Chen N, Xu L, Li J, Li P, et al. Effects of length of abstinence on decision-making and craving in methamphetamine abusers. PLoS One. 2013;8: e68791.
118. Li P, Wu P, Xin X, Fan YL, Wang GB, Wang F, et al. Incubation of alcohol craving during abstinence in patients with alcohol dependence. Addict Biol. 2015;20:513–22.
119. Gao J, Li Y, Zhu N, Brimijoin S, Sui N. Roles of dopaminergic innervation of nucleus accumbens shell and dorsolateral caudate-putamen in cue-induced morphine seeking after prolonged abstinence and the underlying D1- and D2-like receptor mechanisms in rats. J Psychopharmacol. 2013;27:181–91.
120. Madsen HB, Brown RM, Short JL, Lawrence AJ. Investigation of the neuroanatomical substrates of reward seeking following protracted abstinence in mice. J Physiol. 2012;590:2427–42.
121. Shalev U, Morales M, Hope B, Yap J, Shaham Y. Time-dependent changes in extinction behavior and stress-induced reinstatement of drug seeking following withdrawal from heroin in rats. Psychopharmacology. 2001;156:98–107.
122. Di Ciano P, Everitt BJ. Conditioned reinforcing properties of stimuli paired with self-administered cocaine, heroin or sucrose: implications for the persistence of addictive behaviour. Neuropharmacology. 2004;47:202–13.

123. Theberge FR, Pickens CL, Goldart E, Fanous S, Hope BT, Liu QR, et al. Association of time-dependent changes in mu opioid receptor mRNA, but not BDNF, TrkB, or MeCP2 mRNA and protein expression in the rat nucleus accumbens with incubation of heroin craving. Psychopharmacology. 2012;224:559–71.
124. Theberge FR, Li X, Kambhampati S, Pickens CL, St Laurent R, Bossert JM, et al. Effect of chronic delivery of the Toll-like receptor 4 antagonist (+)-naltrexone on incubation of heroin craving. Biol Psychiatry. 2013;73:729–37.
125. Doherty JM, Cooke BM, Frantz KJ. A role for the prefrontal cortex in heroin-seeking after forced abstinence by adult male rats but not adolescents. Neuropsychopharmacology. 2013;38:446–54.
126. Kuntz KL, Patel KM, Grigson PS, Freeman WM, Vrana KE. Heroin self-administration: II. CNS gene expression following withdrawal and cue-induced drug-seeking behavior. Pharmacol Biochem Behav. 2008;90:349–56.
127. Kuntz-Melcavage KL, Brucklacher RM, Grigson PS, Freeman WM, Vrana KE. Gene expression changes following extinction testing in a heroin behavioral incubation model. BMC Neurosci. 2009;10:95.
128. Fanous S, Goldart EM, Theberge FR, Bossert JM, Shaham Y, Hope BT. Role of orbitofrontal cortex neuronal ensembles in the expression of incubation of heroin craving. J Neurosci. 2012;32:11600–9.
129. Cruz FC, Koya E, Guez-Barber DH, Bossert JM, Lupica CR, Shaham Y, et al. New technologies for examining the role of neuronal ensembles in drug addiction and fear. Nat Rev Neurosci. 2013;14:743–54.
130. Airavaara M, Pickens CL, Stern AL, Wihbey KA, Harvey BK, Bossert JM, et al. Endogenous GDNF in ventral tegmental area and nucleus accumbens does not play a role in the incubation of heroin craving. Addict Biol. 2011;16:261–72.
131. Lu L, Wang X, Wu P, Xu C, Zhao M, Morales M, et al. Role of ventral tegmental area glial cell line-derived neurotrophic factor in incubation of cocaine craving. Biol Psychiatry. 2009;66:137–45.
132. Blackwood CA, Hoerle R, Leary M, Schroeder J, Job MO, McCoy MT, et al. (2018). Molecular adaptations in the rat dorsal striatum and hippocampus following abstinence-induced incubation of drug seeking after escalated oxycodone self-administration. *Mol Neurobiol*. (in press).
133. Azrin NH, Holz WC. Punishment. In: Honig WK, editor.. Operant behavior: areas of research and application.. Englewood Cliffs, N.J: Prentice-Hall; 1966. p. 380–447.
134. Jenkins TN, Warner LH, Warden CJ. Standard apparatus for the study of animal motivation. J Comp Psychol. 1926;6:361–82.
135. Panlilio LV, Thorndike EB, Schindler CW. Lorazepam reinstates punishment-suppressed remifentanil self-administration in rats. Psychopharmacology. 2005;179:374–82.
136. Peck JA, Wercberger R, Kariyeva E, Ranaldi R. Cue-induced resumption of heroin and cocaine seeking in rats using a conflict model of abstinence and relapse. Psychopharmacology. 2013;228:651–8.
137. Peck JA, Galaj E, Eshak S, Newman KL, Ranaldi R. Environmental enrichment induces early heroin abstinence in an animal conflict model. Pharmacol Biochem Behav. 2015;138:20–25.
138. Lenoir M, Ahmed SH. Heroin-induced reinstatement is specific to compulsive heroin use and dissociable from heroin reward and sensitization. Neuropsychopharmacology. 2007;32:616–24.
139. Lenoir M, Cantin L, Vanhille N, Serre F, Ahmed SH. Extended heroin access increases heroin choices over a potent nondrug alternative. Neuropsychopharmacology. 2013;38:1209–20.
140. Ahmed SH, Lenoir M, Guillem K. Neurobiology of addiction versus drug use driven by lack of choice. Curr Opin Neurobiol. 2013;23:581–7.
141. Caprioli D, Venniro M, Zhang M, Bossert JM, Warren BL, Hope BT, et al. Role of dorsomedial striatum neuronal ensembles in incubation of methamphetamine craving after voluntary abstinence. J Neurosci. 2017;37:1014–27.
142. Venniro M, Caprioli D, Zhang M, Whitaker LR, Zhang S, Warren BL, et al. The anterior insular cortex→central amygdala glutamatergic pathway is critical to relapse after contingency management. Neuron. 2017;96:414–27.e418.
143. Venniro M, Zhang M, Shaham Y, Caprioli D. Incubation of methamphetamine but not heroin craving after voluntary abstinence in male and female rats. Neuropsychopharmacology. 2017;42:1126–35.
144. Badiani A. Substance-specific environmental influences on drug use and drug preference in animals and humans. Curr Opin Neurobiol. 2013;23:588–96.
145. Badiani A, Belin D, Epstein D, Calu D, Shaham Y. Opiate versus psychostimulant addiction: the differences do matter. Nat Rev Neurosci. 2011;12:685–700.
146. Montanari C, Stendardo E, De Luca MT, Meringolo M, Contu L, Badiani A. Differential vulnerability to relapse into heroin versus cocaine-seeking as a function of setting. Psychopharmacology. 2015;232:2415–24.
147. Marchant NJ, Campbell EJ, Pelloux Y, Bossert JM, Shaham Y (2018): Context-induced relapse after extinction versus punishment: similarities and differences. *Psychopharmacology* (in press).
148. Pelloux Y, Minier-Toribio A, Hoots JK, Bossert JM, Shaham Y. Opposite effects of basolateral amygdala inactivation on context-induced relapse to cocaine seeking after extinction versus punishment. J Neurosci. 2018;38:51–59.
149. Willcocks AL, McNally GP. The role of medial prefrontal cortex in extinction and reinstatement of alcohol-seeking in rats. Eur J Neurosci. 2013;37:259–68.
150. de Guglielmo G, Kallupi M, Scuppa G, Demopulos G, Gaitanaris G, Ciccocioppo R. Pioglitazone attenuates the opioid withdrawal and vulnerability to relapse to heroin seeking in rodents. Psychopharmacology. 2017;234:223–34.
151. Ghitza UE, Nair SG, Golden SA, Gray SM, Uejima JL, Bossert JM, et al. Peptide YY3-36 decreases reinstatement of high-fat food seeking during dieting in a rat relapse model. J Neurosci. 2007;27:11522–32.
152. Rubio FJ, Quintana-Feliciano R, Warren B, Li X, Witonsky K, Soto del Valle F, et al. (2018). Prelimbic cortex is a common brain area activated during cue-induced reinstatement of cocaine and heroin seeking in a polydrug self-administration rat model. *Eur J Neurosci*. (accepted pending minor revisions).
153. Leri F, Bruneau J, Stewart J. Understanding polydrug use: review of heroin and cocaine co-use. Addiction. 2003;98:7–22.
154. Magura S, Kang SY, Nwakeze PC, Demsky S. Temporal patterns of heroin and cocaine use among methadone patients. Subst Use Misuse. 1998;33:2441–67.
155. Leri F, Stewart J. Drug-induced reinstatement to heroin and cocaine seeking: a rodent model of relapse in poly-drug use. Exp Clin Psychopharmacol. 2001;3:297–306.
156. Becker JB. Sex differences in addiction. Dialog Clin Neurosci. 2016;18:395–402.
157. Becker JB, Chartoff E (2018). Sex differences in neural mechanisms mediating reward and addiction. *Neuropsychopharmacology*. (in press).
158. Carroll ME, Anker JJ. Sex differences and ovarian hormones in animal models of drug dependence. Horm Behav. 2010;58:44–56.
159. Meisch RA, Carroll ME. Oral drug self-administration: Drugs as reinforcers. In: Bozarth MA, editor. Methods of assessing the reinforcing properties of abused drugs.. New York, NY: Springer Verlag; 1987. p. 143–61.
160. Davis WM, Smith SG. Role of conditioned reinforcers in the initiation, maintenance and extinction of drug-seeking behavior. Pavlov J Biol Sci. 1976;11:222–36.
161. de Wit H, Stewart J. Drug reinstatement of heroin-reinforced responding in the rat. Psychopharmacology. 1983;79:29–31.
162. Buccafusco JJ, Bain JN. A 24-h access I.V. self-administration schedule of morphine reinforcement and the estimation of recidivism: pharmacological modification by arecoline. Neuroscience. 2007;149:487–98.
163. Shaham Y. Effect of stress on opioid-seeking behavior: evidence from studies with rats. Ann Behav Med. 1996;18:255–63.
164. Shalev U, Grimm JW, Shaham Y. Neurobiology of relapse to heroin and cocaine seeking: a review. Pharmacol Rev. 2002;54:1–42.
165. Catania CA. Learning. 3rd ed. Englewood Cliffs, N.J: Prentice-Hall; 1992.
166. Sanger DJ, Blackman DE. Rate-dependent effects of drugs: a review of the literature. Pharmacol Biochem Behav. 1976;4:73–83.
167. Geller I, Kulak JT Jr, Seifter J. The effects of chlordiazepoxide and chlorpromazine on a punishment discrimination. Psychopharmacologia. 1962;3:374–85.
168. Wise RA, Rompre PP. Brain dopamine and reward. Annu Rev Psychol. 1989;40:191–225.
169. Kalivas PW, McFarland K. Brain circuitry and the reinstatement of cocaine-seeking behavior. Psychopharmacology. 2003;168:44–56.
170. Bossert JM, Marchant NJ, Calu DJ, Shaham Y. The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. Psychopharmacology. 2013;229:453–76.
171. Heilig M, Epstein DH, Nader MA, Shaham Y. Time to connect: bringing social context into addiction neuroscience. Nat Rev Neurosci. 2016;17:592–9.
172. Epstein DH, Heilig M, Shaham Y (2018). Science-based actions can help address the opioid crisis. *Trends Pharmacol Sci*. (in press).
173. Satel S, Lilienfeld SO. Addiction and the brain-disease fallacy. Front Psychiatry. 2013;4:141.
174. Epstein DH, Preston KL, Stewart J, Shaham Y. Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. Psychopharmacology. 2006;189:1–16.
175. Jasinski DR, Pevnick JS, Griffith JD. Human pharmacology and abuse potential of the analgesic buprenorphine: a potential agent for treating narcotic addiction. Arch Gen Psychiatry. 1978;35:501–16.
176. Leri F, Tremblay A, Sorge RE, Stewart J. Methadone maintenance reduces heroin- and cocaine-induced relapse without affecting stress-induced relapse in a rodent model of poly-drug use. Neuropsychopharmacology. 2004;29:1312–20.
177. Sorge RE, Rajabi H, Stewart J. Rats maintained chronically on buprenorphine show reduced heroin and cocaine seeking in tests of extinction and drug-induced reinstatement. Neuropsychopharmacology. 2005;30:1681–92.
178. Bossert JM, Hoots JK, Negus SS, Blough BE, Shaham Y. Modeling opioid maintenance therapy in rats: Effects of chronic buprenorphine and the biased mu

opioid receptor agonist TRV130 on relapse to oxycodone seeking. Poster abstract at Society for Neuroscience 2018 annual meeting.
179. Kowalczyk WJ, Phillips KA, Jobes ML, Kennedy AP, Ghitza UE, Agage DA, et al. Clonidine maintenance prolongs opioid abstinence and decouples stress from craving in daily life: a randomized controlled trial with ecological momentary assessment. Am J Psychiatry. 2015;172:760–7.
180. Kwako LE, Spagnolo PA, Schwandt ML, Thorsell A, George DT, Momenan R, et al. The corticotropin releasing hormone-1 (CRH1) receptor antagonist pexacerfont in alcohol dependence: a randomized controlled experimental medicine study. Neuropsychopharmacology. 2015;40:1053–63.
181. Schwandt ML, Cortes CR, Kwako LE, George DT, Momenan R, Sinha R, et al. The CRF1 antagonist verucerfont in anxious alcohol-dependent women: translation of neuroendocrine, but not of anti-craving effects. Neuropsychopharmacology. 2016;41:2818–29.
182. Shaham Y, de Wit H. Lost in translation: CRF1 receptor antagonists and addiction treatment. Neuropsychopharmacology. 2016;41:2795–7.
183. Marchant NJ, Khuc TN, Pickens CL, Bonci A, Shaham Y. Context-induced relapse to alcohol seeking after punishment in a rat model. Biol Psychiatry. 2013;73:256–62.