

Recent developments in animal models of drug relapse

Nathan J Marchant, Xuan Li and Yavin Shaham

Drug craving and relapse to drug use during abstinence are defining features of addiction. Evidence indicates that drug craving and relapse in humans are often provoked by acute exposure to the self-administered drug, drug-associated cues, or stress. During the last two decades, this clinical scenario has been primarily studied at the preclinical level using the classical reinstatement model. However, a single preclinical model cannot capture the complicated nature of human drug relapse. Therefore, more recently, we and others have developed several other models to study different facets of human drug relapse. In this review, we introduce and discuss recent findings from these other relapse models, including incubation of drug craving, reacquisition and resurgence models, and punishment-based and conflict-based relapse models.

Address

Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD, USA

Corresponding authors: Marchant, Nathan J (nathan.marchant@nih.gov), Shaham, Yavin (Yavin.shaham@nih.gov)

Current Opinion in Neurobiology 2013, **23**:675–683

This review comes from a themed issue on **Addiction**

Edited by **Barry Everitt** and **Ulrike Heberlein**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 29th January 2013

0959-4388/\$ – see front matter, Published by Elsevier Ltd.

<http://dx.doi.org/10.1016/j.conb.2013.01.003>

Introduction

A main problem for treatment of drug addiction is relapse to drug use after periods of abstinence [1]. In drug addicts, drug craving and relapse during abstinence are often triggered by acute re-exposure to the drug [2], drug-associated cues [1], or stress [3]. This clinical scenario has been primarily studied over the last two decades using the classical reinstatement model [4]. In this model, laboratory animals are tested for reinstatement of drug seeking induced by drug-priming [5], discrete cues [6], discriminative cues [7], contextual cues [8], or stress [9,10], following drug self-administration training and subsequent extinction of the drug-reinforced responding. This phenomenological similarity or face validity has led to a dramatic increase in the use of this model in the addiction field (over 900 papers over the last decade, PubMed search). Face validity is of course not a sufficient condition for a valid animal model of human diseases [11,12]. However, evidence is emerging for predictive validity of the reinstatement model for heroin and alcohol

relapse: naltrexone, buprenorphine, or methadone, which decrease drug relapse in humans, decrease drug-priming or cue-induced reinstatement of drug seeking in rats [13]. Additionally, potential medications that decrease stress-induced craving in humans (clonidine, lofexidine, or prazosin) decrease stress-induced reinstatement of drug seeking in rats [3].

The reinstatement model is currently the most commonly used animal model of drug relapse. However, it is obvious that a single preclinical model, which involves an operant extinction component, cannot capture the complicated nature of human drug relapse [13,14]. Therefore, more recently, we and others have developed and used several other models to study different facets of human drug relapse. These include incubation of drug craving [15,16], reacquisition and resurgence models [17–19], and punishment-based and conflict-based relapse models [20,21,22*]. Here, we introduce and discuss recent findings from these relapse models. **Table 1** provides the experimental phases in these models of relapse and the reinstatement model.

Animal models of drug relapse

Incubation of drug craving

Incubation of drug craving refers to the time-dependent increases in cue-induced drug seeking after withdrawal from cocaine, heroin, alcohol, methamphetamine, and nicotine [23,24]. This phenomenon has been demonstrated using established procedures used to assess cue-induced drug seeking [25–27], including extinction, cue-induced reinstatement, and acquisition of new response procedures [23,24]. To study the neuronal mechanisms of incubation of drug craving, we and others have primarily assessed cue-induced drug seeking in a single extinction test performed at different days after withdrawal from the drug. During testing, rats are brought to the drug self-administration environment/context (operant chambers) and lever-presses (or nose-pokes) lead to contingent presentations of discrete cues previously paired with drug infusions [24]. In previous studies (published before 2009), we and others have demonstrated a causal role of ERK and glutamate in CeA, and calcium permeable GluA2-lacking AMPA receptors in NAc in incubation of cocaine craving; our studies also suggest a role of VTA BDNF, and potentially NAc and amygdala BDNF, in this incubation [24,28**,29]. Below we summarize results from several recent (since 2009) papers on mechanisms of incubation of drug craving. We also discuss results from selected studies in which cue-induced drug seeking in extinction tests was assessed after several weeks of withdrawal, but not early withdrawal.

Table 1

Experimental conditions in the different phases of the operant models of drug relapse

Name	Phase 1 (training)	Phase 2 (abstinence)	Phase 3: Test condition (relapse)	Strengths
				weaknesses
Reinstatement model	Lever presses or nose-poke for drug infusions or delivery	Extinction of operant responding	Reinstatement of non-reinforced responding induced by drug priming, drug cues, or stress	-Reliable and reproducible behavioral effects -Evidence for predictive validity
				-Abstinence (achieved by operant extinction) does not mimic the human condition -Model does not capture negative consequences of drug use
Incubation of drug craving model	Same	Home-cage	Non-reinforced extinction responding in the presence of drug-associated cues at different days after withdrawal	-Model increased craving for the drug after a period of abstinence/withdrawal
				-Model does not capture negative consequences of drug use
Reacquisition model	Same	Extinction of operant responding	Lever presses or nose-poke for drug infusions or delivery	-Models the rapid resumption of drug use after a period of abstinence/extinction
				-Abstinence achieved by operant extinction -The model does not capture negative consequences of drug use -The presence of drug during testing complicates interpretation
Resurgence model	Same	Extinction of the original drug-reinforced responding; responding on a different lever or device is reinforced by a non-drug reward	Removal of the non-drug reward induces reinstatement of non-reinforced responding on the drug-associated lever	-Models resumption of drug seeking after an alternative appetitive behavior is no longer reinforced
				-Abstinence does not mimic the human condition -The model does not capture negative consequences of drug use
Punishment-based relapse model	Same	Lever presses for drug infusions or delivery are suppressed by response-contingent shock	Resumption of non-reinforced responding by drug priming (or other drug or non-drug stimuli)	-Models relapse after drug use is suppressed by a negative consequence of drug intake
				-Individual differences in threshold for suppression of drug-reinforced responding -Potential consequences of repeated shock exposure on behavior outside the context of punishment (e.g., non-specific behavioral suppression) -It is unknown whether the model can detect stress- <i>induce</i> -relapse
Conflict-based relapse model	Same	An electric barrier must be crossed to gain access to the drug-associated lever; shock intensity is gradually increased until cessation of drug self-administration occurs	Resumption of non-reinforced responding by non-contingent presentation of the drug cues. The electric barrier remains ON during the relapse test	-Models relapse after drug use is suppressed by a negative consequence of drug seeking (prior to drug taking)
				-Individual differences in threshold for suppression of drug seeking -Large and variable individual differences in response to cues in the relapse tests -Model has not been shown to detect drug-priming- and stress-induced relapse

Koya *et al.* [30] provided evidence for a role of ventral (infralimbic/ventral prelimbic), but not dorsal (dorsal prelimbic/anterior cingulate), mPFC in incubation of cocaine craving. Time-dependent increases in extinction responding were associated with large (ventral mPFC) or

modest (dorsal mPFC) increases in ERK phosphorylation (used as an index of neuronal activation). After 30 withdrawal days, reversible inactivation (using agonists of GABA_A and GABA_B receptors) of ventral, but not dorsal, mPFC decreased extinction responding. After 1

withdrawal day, reversible *activation* (using GABA_A + GABA_B receptor antagonists) of ventral, but not dorsal, mPFC injections increased extinction responding. Additional evidence for a role of mPFC in incubation of drug craving is the finding that weaker incubation of heroin craving in adolescent rats is associated with blunted cue-induced Fos expression in this brain region; this effect was observed in both the infralimbic and prelimbic regions [31[•]].

Another PFC region implicated in incubation of drug (heroin) craving is the lateral OFC. Fanous *et al.* [32^{••}] found that reversible inactivation of this brain area decreased extinction responding on withdrawal day 14 but not day 1. These authors also used the Daun02 inactivation procedure [33] to demonstrate a causal role of selectively activated lateral OFC neurons (neuronal ensembles) in incubation of heroin craving.

Pacchioni [34] reported that reversible inactivation of dorsolateral striatum decreases 'incubated' (high) extinction responding after 60 days of withdrawal from cocaine. However, dorsolateral striatum inactivation also decreased 'non-incubated' (low) extinction responding on withdrawal day 1, suggesting a general role of this brain area in cue-induced cocaine seeking in extinction tests [35^{••}] rather than a unique role in incubation of cocaine craving. Xi *et al.* [36[•]] reported that blockade of D3 receptors in NAc core or shell or CeA, but not dorsal striatum or BLA, decrease 'incubated' cue-induced cocaine seeking after 21–28 withdrawal days. Whether this site-specific effect of the D3-receptor antagonist is selective for incubated (late withdrawal) versus non-incubated (early withdrawal) cocaine seeking is unknown, because systemic injections of the antagonist decreased extinction responding in tests performed during both early (day 2) or late (day 30) withdrawal [36[•]].

Gao *et al.* [37] reported that lesions of dorsolateral striatum and NAc shell, as well as blockade of D1-family, but not D2-family, receptors decreases extinction responding after 3 weeks of withdrawal from morphine. On the basis of the results of Pacchioni *et al.* [34], and previous studies on the role of dorsolateral striatum and NAc shell dopamine in cue-induced drug seeking [38^{••},39,40], it is likely that the results of Gao *et al.* [37] reflect a general role of D1-family receptors in cue-induced heroin seeking, independent of the withdrawal period. In a Fos-mapping study in mice, Madsen *et al.* [41] found that exposure to morphine cues during late withdrawal (21 days) extinction tests increased Fos expression in NAc shell and core. These authors also found increased Fos expression in other brain areas, including anterior cingulate cortex, OFC, BNST, VTA, BLA. However, because Fos expression data are correlative, the role of these brain areas in incubation of morphine craving is a subject for future research.

Lu *et al.* [42] and Airavaara *et al.* [43] provided evidence for a role of VTA GDNF in incubation of cocaine, but not heroin, craving. Lu *et al.* [42] reported that viral over-expression of GDNF or exogenous GDNF injections in VTA potentiate incubation of cocaine craving. More important, chronic delivery of anti-GDNF antibodies during withdrawal days 1–14 prevented incubation of cocaine craving, implicating endogenous GDNF in this phenomenon. In contrast, Airavaara *et al.* [43] reported no effect on incubation of heroin craving of either acute exogenous VTA GDNF injections or chronic delivery of anti-GDNF antibodies in VTA during the withdrawal period.

Li *et al.* [44[•]] recently further studied the role of BDNF in NAc core and shell in incubation of cocaine craving. They confirmed an earlier report [45] that after prolonged withdrawal from cocaine NAc BDNF levels are increased, and further showed that after 90 withdrawal days, but not 25 or 48 days, surface expression of phosphorylated TrkB (the preferential BDNF receptor) are increased. Analysis of BDNF protein levels in NAc sub-regions demonstrated increased BDNF levels after 45 (core but not shell) or 90 (both core and shell) withdrawal days. Finally, Li *et al.* [44[•]] used a viral-vector approach to knockdown TrkB in NAc core and shell. Surprisingly, they found that TrkB knockdown in NAc core, *increased* extinction responding on withdrawal day 1, but not days 30 or 90. In contrast, TrkB knockdown in NAc shell decreased incubated extinction responding on withdrawal day 90, but not days 1 or 45. A potential interpretation of this complicated data set is that during early withdrawal basal BDNF transmission in NAc core suppresses cue-induced cocaine seeking, while after very prolonged withdrawal periods elevated BDNF in NAc shell maintains high levels of 'incubated' cue-induced cocaine seeking. The latter idea is in agreement with the idea that while mesolimbic BDNF plays a role in maintaining incubated cue responding after prolonged withdrawal periods, BDNF does not mediate the development of incubation of cocaine craving [46].

Wolf and colleagues continued their mechanistic studies on the role of calcium-permeable GluA2-lacking AMPA receptors in NAc in incubation of cocaine craving [47^{••}]. This group and others replicated the initial findings of accumulation of these receptors in NAc after prolonged but not early withdrawal from cocaine [48–50]. They also recently identified a role of mGluR1 in accumulation of GluA2-lacking AMPA receptors in NAc. In electrophysiology studies using cocaine-experienced rats after prolonged withdrawal (>45 days), agonist activation of mGluR1 eliminated the facilitation of glutamate transmission by GluA2-lacking AMPA receptors [51[•]], indicating the removal of GluA2-lacking AMPA receptors from synapses. In follow-up studies, this group demonstrated the functional significance of this molecular

mechanism in incubation of cocaine craving. They reported that NAc mGluR1 surface expression progressively decreases after withdrawal from cocaine [52]. Additionally, preventing the decrease of cocaine-withdrawal-induced mGluR1 surface expression and increasing mGluR1 function by systemic injections of a positive allosteric modulator of this receptor decreased 'incubated' cue-induced cocaine seeking after prolonged withdrawal [53].

Edwards *et al.* [54] reported that NAc core (but not NAc shell or dorsal striatum) GluA1-S845 (an index of PKA activity) and ERK phosphorylation increase after extinction tests performed after 21 withdrawal days, but not 1 day. The relevance of these data to incubation of cocaine craving is unknown in the absence of follow-up studies in which local ERK or PKA activity is manipulated; additionally, under the authors' training conditions, extinction responding after 1 day was higher than after 21 days, but this effect was not statistically significant.

Finally, several studies have demonstrated that enriched environment during the withdrawal period decreases incubation of cocaine craving [55,56,57**]. Thiel *et al.* [57**] found that short (1 day), but not long (28 days), environmental enrichment increases BDNF levels in hippocampus (dorsal and ventral). Interestingly long, but not short, enrichment prevents cue-induced CeA ERK activation during the late withdrawal extinction tests. Time-dependent cue-induced glutamate-mediated ERK activation in CeA plays a critical role in incubation of cocaine craving [58,59]. Thus, Thiel *et al.* [57] data suggest that environmental enrichment reduces incubation of cocaine craving by reversing the time-dependent increased sensitivity of CeA neurons to cocaine cues. Data from an earlier study of Thiel *et al.* [55] provide support for this hypothesis by demonstrating that enriched environment inhibits cue-induced cocaine seeking and Fos expression in CeA after 30 withdrawal days. The authors also reported enrichment-induced decreases in cue-induced Fos expression in other mesocorticolimbic and nigrostriatal brain areas, including infralimbic mPFC and OFC, recently implicated in incubation of cocaine craving [32**,33]. Thus, it is likely that other brain areas in addition to the CeA play a role in the inhibitory effect of enrichment on incubation of cocaine craving.

In summary, incubation of drug craving is used to model increased cue-induced drug craving after an extended period of withdrawal from the drug. To study the neural mechanisms of incubation of drug craving, it is critical to identify manipulations that selectively affect late withdrawal 'incubated' cue-induced drug seeking but not 'non-incubated' early withdrawal drug seeking. We and others have recently reported several brain regions (e.g. ventral mPFC, OFC, NAc, VTA), molecular mechanisms (e.g. GDNF, GluA2-lacking AMPA receptors, and ERK)

that are critical for incubation of cocaine craving. The mechanisms underlying incubation of craving for other drugs of abuse are currently unknown.

Reacquisition after extinction

In the operant conditioning paradigm, reacquisition refers to the resumption of the reward-reinforced operant response after extinction. Reacquisition is typically more rapid than the original training, demonstrating that extinction training does not erase the originally learned associations [60]. Recently, Willcocks and McNally [18] used a variation of the ABA renewal procedure [61] to demonstrate that reacquisition is context independent. They found similar rates of reacquisition of alcoholic beer self-administration in the AAA (training, extinction, reacquisition in the same context), AAB (training and extinction in the same context, and reacquisition in a novel context), and ABA (training and reacquisition in the same context, and extinction in a novel context). Perry and McNally [62] reported that systemic naloxone (a preferential mu opioid receptor antagonist) injections attenuate reacquisition of alcoholic beer self-administration, suggesting a role of mu opioid receptors in this reacquisition. However, a role of delta and kappa opioid receptors cannot be ruled out, because at the doses used (1.25 and 2.5 mg/kg) naloxone likely also blocks these receptors [63].

Three studies have explored central mechanisms of reacquisition of operant alcohol seeking. Canicella *et al.* [19] found that VTA injections of exogenous GDNF attenuate this reacquisition. However, it has not been demonstrated that local inhibition of endogenous GDNF activity affects re-acquisition of alcohol self-administration. Willcocks and McNally [64**] recently used a reversible inactivation approach [65] to determine the role of mPFC subregions (prelimbic, infralimbic, dorsal peduncular) in reacquisition of alcoholic beer self-administration; they also assessed the effect of inactivation of these regions on context-induced reinstatement. Infralimbic or dorsal peduncular inactivation had a minimal effect on reacquisition or context-induced reinstatement. In contrast, inactivation of the prelimbic area had opposite effects on reacquisition (potentiation) versus context-induced reinstatement (inhibition). This surprising finding suggests that the role of the prelimbic area in drug relapse depends on drug availability during the relapse test, and challenges the notion that prelimbic mPFC activation invariably promotes drug seeking [66**].

Logrip and Zorrilla [67] found that prior stress exposure (conditioned fear training) selectively increased reacquisition (but not initial acquisition) of alcohol self-administration. This effect was also selective to rats that consumed lower amounts of alcohol before extinction training. These authors also reported that increased reacquisition in the low drinking rats was correlated with an

increase in *Pde10a* mRNA (a dual-specificity cyclic adenosine monophosphate and cyclic guanosine monophosphate hydrolyzing enzyme) in prelimbic mPFC. The functional significance of this molecular finding is unknown.

Nic Dhonnchadha and colleagues determined the effect of pharmacological manipulations aimed at enhancing consolidation of extinction learning by potentiating NMDA-mediated glutamatergic transmission [68] on reacquisition of cocaine self-administration [69–71]. They found that systemic injections of the glycine partial agonist D-cycloserine, or the glycine transporter-1 inhibitors RO4543338 or Org24598 before daily extinction training decrease subsequent reacquisition of cocaine self-administration in rats and monkeys. In contrast, repeated injections of these drugs had no effect on resumption of cocaine self-administration during abstinence in the absence of extinction training. The central mechanisms involved in the inhibitory effect of increasing glycine transmission during extinction on reacquisition are unknown.

In summary, reacquisition is potentially an important and underutilized relapse model that can be used to model the clinical scenarios of a ‘lapse leading to relapse’ and rapid resumption of drug use after cue exposure therapy [72]. The neuronal mechanisms of reacquisition are largely unknown but based on the provocative findings of Willcocks and McNally [64••] on the opposite role of prelimbic mPFC in reacquisition versus context-induced reinstatement, it is likely that the neuronal circuits of reacquisition are different from the circuits identified in studies on reinstatement of non-reinforced responding.

Resurgence

In the operant conditioning paradigm, resurgence refers to recovery of an extinguished operant response after discontinuation of reinforcement of an alternative response [73•]. A typical resurgence experiment includes 3 phases. In the first, rats are trained to press on Lever 1 for food reward. In the second, lever presses on Lever 1 is not reinforced (extinction) while responding on Lever 2 is reinforced by the food reward [74], or Lever 1 is extinguished before Lever 2 training [75]. In the third phase (test), lever responding is not reinforced on either lever, and resumption of responding on Lever 1 serves as the operational measure of ‘resurgence’ or resumption of extinguished reward seeking [74].

Timothy Shaham and colleagues have used a variation of this ‘resurgence’ procedure to demonstrate this phenomenon in rats trained to lever-press for alcohol [17] or cocaine [76••] in phase 1 who underwent extinction of drug-reinforced responding while a different operant response (chain pull or nose-poke) led to food delivery during phase 2. Of note, the resurgence effect with cocaine-trained rats

was significantly more robust than that observed with alcohol-trained rats. Finally, resurgence of cocaine seeking was blocked by systemic injection of the D1-family receptor antagonist SCH 23390 [76••].

In summary, resurgence is a promising animal model of relapse after completion of contingency management treatment when the alternative reward (money or vouchers contingent on clean urine samples) is no longer available [77]. Resurgence of cocaine seeking is dependent on D1-family receptor activation, extending previous results on the critical role of this receptor in relapse to drug seeking [26].

Punishment-based and conflict-based relapse models

The experimental phases in punishment-based and conflict-based relapse models are similar to the reinstatement model, with the exception that drug-taking behavior is suppressed by an aversive shock before the relapse tests. In punishment-based relapse models, this is achieved by administering the shock after the rat performs the operant response [20]. In the conflict-based relapse model, drug taking and seeking is suppressed by introducing an electric barrier in front of the drug-associated lever [22•]. Punishment or conflict models can be used to study mechanisms of drug relapse during abstinence after suppression of drug-reinforced responding by negative consequences [78].

Punishment-based relapse models

In an initial study, Panlilio *et al.* [20] found that priming injections of remifentanyl (a short-acting opiate agonist) after punishment-induced suppression of the drug-reinforced responding cause faster reacquisition of remifentanyl self-administration. These authors [79•] also reported that priming injections of heroin or the benzodiazepine lorazepam cause resumption of non-reinforced lever responding (relapse), as well as reacquisition of remifentanyl after punishment. In contrast, using a reinstatement model, they reported that heroin, but not lorazepam, priming injections reinstate remifentanyl seeking.

Economidou *et al.* [80] used a punishment model to study the role of individual differences in impulsivity (as assessed in the 5-choice serial reaction-time task) and cocaine exposure history (extended versus limited access) in the propensity for cue-induced relapse; this relapse was assessed in a single extinction test one week after punishment-induced suppression of cocaine-reinforced responding. They found that after an extended access drug self-administration, relapse to cocaine seeking after punishment had occurred in the high but not low impulsive rats, an effect that was reversed by the Attention Deficit Hyperactivity disorder medication atomoxetine.

We [21] recently modified the ABA context-induced reinstatement procedure [8] to demonstrate context-induced

relapse to alcohol seeking alcohol-preferring P rats after punishment in a non-drug context. A main finding in our study was that the magnitude of non-reinforced lever-presses in the relapse tests in the original alcohol self-administration context (A) after punishment of alcohol-reinforced responding in a different context (B) was substantially higher than that observed after extinction training in the B context.

In summary, several recent studies have successfully incorporated punishment and shock-conflict models to study drug relapse during abstinence periods induced by adverse consequences. While the central mechanisms of relapse after punishment or conflict are unknown, there is evidence that at least for drug priming, these mechanisms are likely to be to some degree different from the mechanisms of drug-priming-induced reinstatement after extinction [79^{*}].

A conflict-based relapse model

There is a long history of the use of conflict procedures to assess motivation to seek rewards [81,82]. Cooper *et al.* [22^{*}] recently adapted a conflict-based procedure as an animal model of the human condition of self-imposed abstinence and relapse episodes that involve making a choice between the desire for the drug and its adverse consequence. They reported that about half of the rats whose cocaine-reinforced responding was suppressed by increasing shock intensities of the 'electric barrier' near the drug-paired lever, resumed drug seeking (in the presence of the electric barrier) during tests for discrete-cue-induced relapse. Recently, Barnea-Ygael *et al.* [83] replicated this finding and also demonstrated that imposing 14 abstinence days in the home-cage causes a significant decrease in the proportion of rats demonstrating cue-induced relapse. This surprising finding was not predicted by results from incubation of drug craving studies on time-dependent increases in cue-induced drug seeking after withdrawal (see above). The authors speculated that these discrepant results might be due to the development of incubation of fear [84] to the shock-associated context that override the putative incubation of craving for cocaine cues.

In summary, the conflict-based relapse model has substantial intuitive appeal, because of all models described in our review, it most closely to models the typical human condition of a conflict situation that eventually leads to either continued abstinence or relapse to drug seeking. The limitation of the model, however, is its low reliability: many rats do not respond to the cue-induced relapse manipulation, and those who do respond, demonstrate high variability in responding [22^{*}]. Additionally, relapse induced by drug priming or stress has not been demonstrated in the conflict model. Thus, the conflict model is currently limited to the study of individual differences in cue-induced relapse.

Conclusions and future directions

The reinstatement model has been used for many years to identify neuropharmacological mechanisms of drug relapse [85]. However, like most if not all animal models, the reinstatement model has limitations, and no single animal model can capture the complicated nature of human drug relapse [13,14]. As a natural progression in the addiction field, several alternative relapse models have emerged, each aims to simulate different aspects of human drug relapse. The incubation of drug craving model is used to study mechanisms of time-dependent increases in cue-induced drug seeking after withdrawal. The reacquisition model is used to study the rapid resumption of drug self-administration after extinction. The resurgence model is used to study reinstatement of drug seeking induced by removal of an alternative non-drug reward. Finally, punishment-based and conflict-based models are used to study the mechanisms of drug relapse after drug self-administration has been suppressed by negative consequences.

With the exception of incubation of drug craving, the brain sites and circuits controlling relapse in the above models are unknown. An important question for future research is whether similar or different neuronal mechanisms control relapse behavior in the different models. We suspect that future mechanistic studies will identify more differences than similarities among the relapse models, because relapse to drug seeking in each model is likely mediated by unique psychological mechanisms with unique neural substrates. For example, punished, but not extinguished, opiate-taking behavior is reinstated by systemic injections of the benzodiazepine lorazepam [79^{*}]. In addition, the role of mPFC in relapse to drug seeking is, surprisingly, model-dependent. Many studies have demonstrated that reversible inactivation of dorsal mPFC attenuates reinstatement of drug seeking [85,86]. In contrast, Willcocks and McNally [64^{**}] recently showed that, in the reacquisition model, reversible inactivation of dorsal mPFC *potentiates* alcohol-taking behavior.

Notes added in the proofs

Recently, Theberge *et al.* [87] demonstrated an important role of the toll-like receptor 4 (TLR4, innate immune system pattern recognition receptor) in incubation of heroin but not methamphetamine craving. The TLR4 antagonist (+)-naltrexone, which is inactive at the μ -opioid receptor, was given chronically (via osmotic minipumps) during the withdrawal period. In heroin-trained but not methamphetamine-trained rats (+)-naltrexone decreased incubated cue-induced drug seeking in an extinction test performed on withdrawal day 13.

Acknowledgments

Research was supported by the National Institute on Drug Abuse, Intramural Research Program. The authors declare that they do not have any conflicts of interest (financial or otherwise) related to the data presented in this manuscript.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. O'Brien CP: **Anticraving medications for relapse prevention: a possible new class of psychoactive medications.** *Am J Psychiatry* 2005, **162**:1423-1431.
 2. de Wit H: **Priming effects with drugs and other reinforcers.** *Exp Clin Psychopharmacol* 1996, **4**:5-10.
 3. Sinha R, Shaham Y, Heilig M: **Translational and reverse translational research on the role of stress in drug craving and relapse.** *Psychopharmacology (Berl)* 2011, **218**:69-82.
 4. Shaham Y, Shalev U, Lu L, de Wit H, Stewart J: **The reinstatement model of drug relapse: history, methodology and major findings.** *Psychopharmacology (Berl)* 2003, **168**:3-20.
 5. de Wit H, Stewart J: **Reinstatement of cocaine-reinforced responding in the rat.** *Psychopharmacology (Berl)* 1981, **75**:134-143.
 6. 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**:754-763.
 7. Weiss F, Maldonado-Vlaar CS, Parsons LH, Kerr TM, Smit 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 U S A* 2000, **97**:4321-4326.
 8. Crombag HS, Shaham Y: **Renewal of drug seeking by contextual cues after prolonged extinction in rats.** *Behav Neurosci* 2002, **116**:169-173.
 9. Shaham Y, Stewart J: **Stress reinstates heroin-seeking in drug-free animals: an effect mimicking heroin, not withdrawal.** *Psychopharmacology (Berl)* 1995, **119**:334-341.
 10. Shepard JD, Bossert JM, Liu SY, Shaham Y: **The anxiogenic drug yohimbine reinstates methamphetamine seeking in a rat model of drug relapse.** *Biol Psychiatry* 2004, **55**:1082-1089.
 11. Sarter M, Bruno JP: **Animal models in biological psychiatry.** In *Biological Psychiatry*. Edited by D'haenen H, den Boer JA, Willner P. Johns Wiley & Sons Ltd.; 2002:1-8.
 12. Geyer MA, Markou A: **Animal models of psychiatric disorders.** In *Psychopharmacology*. Edited by Bloom FE, Kupfer DJ. Raven Press; 1995:787-798.
 13. Epstein D, Preston K, Stewart J, Shaham Y: **Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure.** *Psychopharmacology (Berl)* 2006, **189**:1-16.
 14. Katz J, Higgins S: **The validity of the reinstatement model of craving and relapse to drug use.** *Psychopharmacology (Berl)* 2003, **168**:21-30.
 15. Neisewander JL, Baker DA, Fuchs RA, Tran-Nguyen LT, Palmer A, Marshall JF: **Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment.** *J Neurosci* 2000, **20**:798-805.
 16. Grimm JW, Hope BT, Wise RA, Shaham Y: **Incubation of cocaine craving after withdrawal.** *Nature* 2001, **412**:141-142.
 17. Podlesnik CA, Jimenez-Gomez C, Shahan TA: **Resurgence of alcohol seeking produced by discontinuing non-drug reinforcement as an animal model of drug relapse.** *Behav Pharmacol* 2006, **17**:369-374.
 18. Willcocks AL, McNally GP: **The role of context in re-acquisition of extinguished alcoholic beer-seeking.** *Behav Neurosci* 2011, **125**:541-550.
 19. Carnicella S, Kharazija V, Jeanblanc J, Janak PH, Ron D: **GDNF is a fast-acting potent inhibitor of alcohol consumption relapse.** *Proc Natl Acad Sci U S A* 2008, **105**:8114-8119.
 20. Panlilio L, Thorndike E, Schindler C: **Reinstatement of punishment-suppressed opioid self-administration in rats: an alternative model of relapse to drug abuse.** *Psychopharmacology (Berl)* 2003, **168**:229-235.
 21. 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**(3):256-262.
 22. Cooper A, Barnea-Ygael N, Levy D, Shaham Y, Zangen A: **A conflict rat model of cue-induced relapse to cocaine seeking.** *Psychopharmacology (Berl)* 2007, **194**:117-125.
 - The authors describe an adaptation of a conflict procedure to study cue-induced relapse after drug seeking is suppressed by an aversive consequence.
 23. Lu L, Grimm JW, Hope BT, Shaham Y: **Incubation of cocaine craving after withdrawal: a review of preclinical data.** *Neuropharmacology* 2004, **47**(Suppl 1):214-226.
 24. Pickens CL, Airavaara M, Theberge F, Fanous S, Hope BT, Shaham Y: **Neurobiology of incubation of drug craving.** *Trends Neurosci* 2011, **34**:411-420.
 25. Everitt BJ, Robbins TW: **Neural systems of reinforcement for drug addiction: from actions to habits to compulsion.** *Nat Neurosci* 2005, **8**:1481-1489.
 26. Shalev U, Grimm JW, Shaham Y: **Neurobiology of relapse to heroin and cocaine seeking: a review.** *Pharmacol Rev* 2002, **54**:1-42.
 27. See RE: **Neural substrates of cocaine-cue associations that trigger relapse.** *Eur J Pharmacol* 2005, **526**:140-146.
 28. Wolf ME, Ferrario CR: **AMPA receptor plasticity in the nucleus accumbens after repeated exposure to cocaine.** *Neurosci Biobehav Rev* 2010, **35**:185-211.
 - An excellent review on the role of AMPA receptor plasticity in the behavioral effects of cocaine, including incubation of cocaine craving.
 29. Ghitza UE, Zhai H, Wu P, Airavaara M, Shaham Y, Lu L: **Role of BDNF and GDNF in drug reward and relapse: a review.** *Neurosci Biobehav Rev* 2010, **35**:157-171.
 30. Koya E, Uejima JL, Wihbey KA, Bossert JM, Hope BT, Shaham Y: **Role of ventral medial prefrontal cortex in incubation of cocaine craving.** *Neuropharmacology* 2009, **56**(Suppl 1):177-185.
 31. 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**(3):446-454.
 - An interesting study demonstrating that, contrary to expectations, adult rats are more likely to demonstrate incubation of cocaine craving than adolescent rats. The authors also demonstrated that these age differences are associated with differences in cue-induced mPFC activation.
 32. 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**:11600-11609.
 - The authors used the Daun02 selective inactivation procedure to demonstrate a critical role of a small subset of Fos-activated OFC neurons in incubation of heroin craving.
 33. Koya E, Golden SA, Harvey BK, Guez-Barber DH, Berkow A, Simmons DE, Bossert JM, Nair SG, Uejima JL, Marin MT *et al.*: **Targeted disruption of cocaine-activated nucleus accumbens neurons prevents context-specific sensitization.** *Nat Neurosci* 2009, **12**:1069-1073.
 34. Pacchioni AM, Gabriele A, See RE: **Dorsal striatum mediation of cocaine-seeking after withdrawal from short or long daily access cocaine self-administration in rats.** *Behav Brain Res* 2011, **218**:296-300.
 35. 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**:3584-3588.
 - An excellent study providing the first demonstration that the neuronal mechanisms mediating cue-induced cocaine seeking in extinction tests and cue-induced reinstatement of cocaine seeking after extinction are to some degree dissociable.

36. Xi ZX, Li X, Li J, Peng XQ, Song R, Gaal J, Gardner EL: **Blockade of dopamine D(3) receptors in the nucleus accumbens and central amygdala inhibits incubation of cocaine craving in rats.** *Addict Biol* 2012, in press
This study demonstrates a potential role of dopamine D3 receptors in incubation of cocaine craving in rats. D3 receptor antagonists are currently in development as a potential medication for cocaine addiction.
37. 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* 2012, in press.
38. Belin D, Jonkman S, Dickinson A, Robbins TW, Everitt BJ: **Parallel and interactive learning processes within the basal ganglia: relevance for the understanding of addiction.** *Behav Brain Res* 2009, **199**:89-102.
An excellent comprehensive review on the psychological processes and neural circuits involved in the motivational effects of drug cues.
39. Crombag H, Bossert JM, Koya E, Shaham Y: **Context-induced relapse to drug seeking: a review.** *Trans R Soc Lond B Biol Sci* 2008, **363**:3233-3243.
40. Feltenstein MW, See RE: **The neurocircuitry of addiction: an overview.** *Br J Pharmacol* 2008, **154**:261-274.
41. 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**:2427-2442.
42. Lu L, Wang X, Wu P, Xu C, Zhao M, Morales M, Harvey BK, Hoffer BJ, Shaham Y: **Role of ventral tegmental area glial cell line-derived neurotrophic factor in incubation of cocaine craving.** *Biol Psychiatry* 2009, **66**:137-145.
43. Airavaara M, Pickens CL, Stern AL, Wihbey KA, Harvey BK, Bossert JM, Liu QR, Hoffer BJ, Shaham Y: **Endogenous GDNF in ventral tegmental area and nucleus accumbens does not play a role in the incubation of heroin craving.** *Addict Biol* 2011, **16**:261-272.
44. Li X, DeJoseph MR, Urban JH, Bahi A, Dreyer JL, Ford MGEKA, Ferrario CR, Loweth JA, Wolf ME: **Different roles of BDNF in nucleus accumbens core versus shell during the incubation of cue-induced cocaine craving and its long-term maintenance.** *J Neurosci* 2013, **33**:1130-1142.
A comprehensive new study demonstrating a complicated time-dependent role of BDNF in NAc core and shell in cue-induced cocaine seeking.
45. Grimm JW, Lu L, Hayashi T, Hope BT, Su TP, Shaham Y: **Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving.** *J Neurosci* 2003, **23**:742-747.
46. Lu L, Dempsey J, Liu SY, Bossert JM, Shaham Y: **A single infusion of brain-derived neurotrophic factor into the ventral tegmental area induces long-lasting potentiation of cocaine seeking after withdrawal.** *J Neurosci* 2004, **24**:1604-1611.
47. Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, Marinelli M, Wolf ME: **Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving.** *Nature* 2008, **454**:118-121.
An important study demonstrating a role of a unique form of cocaine-induced synaptic plasticity in NAc in incubation of cocaine craving.
48. McCutcheon JE, Wang X, Tseng KY, Wolf ME, Marinelli M: **Calcium-permeable AMPA receptors are present in nucleus accumbens synapses after prolonged withdrawal from cocaine self-administration but not experimenter-administered cocaine.** *J Neurosci* 2011, **31**:5737-5743.
49. Ferrario CR, Loweth JA, Milovanovic M, Ford KA, Galinanes GL, Heng LJ, Tseng KY, Wolf ME: **Alterations in AMPA receptor subunits and TARPs in the rat nucleus accumbens related to the formation of Ca(2+)-permeable AMPA receptors during the incubation of cocaine craving.** *Neuropharmacology* 2011, **61**:1141-1151.
50. Mamedi M, Halbout B, Creton C, Engblom D, Parkitna JR, Spanagel R, Luscher C: **Cocaine-evoked synaptic plasticity: persistence in the VTA triggers adaptations in the NAc.** *Nat Neurosci* 2009, **12**:1036-1041.
51. McCutcheon JE, Loweth JA, Ford KA, Marinelli M, Wolf ME, Tseng KY: **Group I mGluR activation reverses cocaine-induced accumulation of calcium-permeable AMPA receptors in nucleus accumbens synapses via a protein kinase C-dependent mechanism.** *J Neurosci* 2011, **31**:14536-14541.
An interesting study suggesting a role of NAc mGluR1 in the accumulation of GluA-lacking AMPA receptors after prolonged withdrawal from cocaine self-administration.
52. Loweth JA, McCutcheon JE, Milovanovic M, Ford KA, Scheyer AF, Marinelli M, Tseng KY, Wolf ME: **Cocaine-induced AMPA receptor plasticity: modulation by metabotropic glutamate receptors.** *Soc Neurosci Abst* 2012:67.08.
53. Loweth JA, Li X, Milovanovic M, Scheyer AF, Olive MF, Tseng KY, Wolf ME: **Metabotropic glutamate receptor 1 (mGluR1) negatively regulates cocaine-induced AMPA receptor plasticity in the nucleus accumbens and attenuates incubation of cocaine craving.** *Soc Neurosci Abst* 2012:669.14.
54. Edwards S, Bachtell RK, Guzman D, Whisler KN, Self DW: **Emergence of context-associated GluR(1) and ERK phosphorylation in the nucleus accumbens core during withdrawal from cocaine self-administration.** *Addict Biol* 2011, **16**:450-457.
55. Thiel KJ, Engelhardt B, Hood LE, Peartree NA, Neisewander JL: **The interactive effects of environmental enrichment and extinction interventions in attenuating cue-elicited cocaine-seeking behavior in rats.** *Pharmacol Biochem Behav* 2010, **97**:595-602.
56. Chauvet C, Goldberg SR, Jaber M, Solinas M: **Effects of environmental enrichment on the incubation of cocaine craving.** *Neuropharmacology* 2012, **63**:635-641.
57. Thiel KJ, Painter MR, Pentkowski NS, Mitroi D, Crawford CA, Neisewander JL: **Environmental enrichment counters cocaine abstinence-induced stress and brain reactivity to cocaine cues but fails to prevent the incubation effect.** *Addict Biol* 2012, **17**:365-377.
An excellent study in which the authors demonstrate that environmental enrichment exposure during the abstinence period attenuates incubation of cocaine craving and prevents cue-induced CeA ERK activation, providing a potential molecular mechanism for the effect of environmental enrichment on incubation of cocaine craving.
58. Lu L, Hope BT, Dempsey J, Liu SY, Bossert JM, Shaham Y: **Central amygdala ERK signaling pathway is critical to incubation of cocaine craving.** *Nat Neurosci* 2005, **8**:212-219.
59. Lu L, Uejima JL, Gray SM, Bossert JM, Shaham Y: **Systemic and central amygdala injections of the mGluR(2/3) agonist LY379268 attenuate the expression of incubation of cocaine craving.** *Biol Psychiatry* 2007, **61**:591-598.
60. Bouton ME: **Context, ambiguity, and unlearning: sources of relapse after behavioral extinction.** *Biol Psychiatry* 2002, **52**:976-986.
61. Bouton ME, Bolles RC: **Role of conditioned contextual stimuli in reinstatement of extinguished fear.** *J Exp Psychol Anim Behav Process* 1979, **5**:368-378.
62. Perry CJ, McNally GP: **Naloxone prevents the rapid reacquisition but not acquisition of alcohol seeking.** *Behav Neurosci* 2012, **126**:599-604.
63. Goldstein A, Naidu A: **Multiple opioid receptors: ligand selectivity profiles and binding sites signatures.** *Mol Pharmacol* 1989, **36**:265-272.
64. Willcocks AL, McNally GP: **The role of medial prefrontal cortex in extinction and reinstatement of alcohol-seeking in rats.** *Eur J Neurosci* 2013, **37**(2):259-268.
An excellent and provocative study demonstrating a dissociable role of dorsal mPFC in context-induced reinstatement of alcoholic beer seeking (inhibition) versus reacquisition of alcoholic beer self-administration (potentiation).
65. McFarland K, Kalivas PW: **The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior.** *J Neurosci* 2001, **21**:8655-8663.
66. Peters J, Kalivas PW, Quirk GJ: **Extinction circuits for fear and addiction overlap in prefrontal cortex.** *Learn Mem* 2009, **16**:279-288.

An excellent review in which the authors present a new conceptualization of the role of different mPFC subregions in promoting or inhibiting drug seeking after extinction.

67. Logrip ML, Zorrilla EP: **Stress history increases alcohol intake in relapse: relation to phosphodiesterase 10A.** *Addict Biol* 2012, **17**:920-933.
68. Davis M, Ressler K, Rothbaum BO, Richardson R: **Effects of D-cycloserine on extinction: translation from preclinical to clinical work.** *Biol Psychiatry* 2006, **60**:369-375.
69. Achat-Mendes C, Nic Dhonnchadha BA, Platt DM, Kantak KM, Spealman RD: **Glycine transporter-1 inhibition preceding extinction training inhibits reacquisition of cocaine seeking.** *Neuropsychopharmacology* 2012, **37**(13):2837-2845.
70. Nic Dhonnchadha BA, Pinard E, Alberati D, Wettstein JG, Spealman RD, Kantak KM: **Inhibiting glycine transporter-1 facilitates cocaine-cue extinction and attenuates reacquisition of cocaine-seeking behavior.** *Drug Alcohol Depend* 2012, **122**:119-126.
71. Nic Dhonnchadha BA, Szalay JJ, Achat-Mendes C, Platt DM, Otto MW, Spealman RD, Kantak KM: **D-Cycloserine deters reacquisition of cocaine self-administration by augmenting extinction learning.** *Neuropsychopharmacology* 2010, **35**:357-367.
72. Carter BL, Tiffany ST: **Meta-analysis of cue-reactivity in addiction research.** *Addiction* 1999, **94**:327-340.
73. Bouton ME, Winterbauer NE, Todd TP: **Relapse processes after the extinction of instrumental learning: renewal, resurgence, and reacquisition.** *Behav Processes* 2012, **90**:130-141.
- An excellent scholarly review on the behavioral processes involved in extinction and reinstatement of reward seeking.
74. Winterbauer NE, Bouton ME: **Mechanisms of resurgence of an extinguished instrumental behavior.** *J Exp Psychol Anim B* 2010, **36**:343-353.
75. Winterbauer NE, Bouton ME: **Mechanisms of resurgence II: response-contingent reinforcers can reinstate a second extinguished behavior.** *Learn Motiv* 2011, **42**:154-164.
76. Quick SL, Pyszczynski AD, Colston KA, Shaham TA: **Loss of alternative non-drug reinforcement induces relapse of cocaine-seeking in rats: role of dopamine D1 receptors.** *Neuropsychopharmacology* 2011, **36**:1015-1020.
- This study provides an elegant demonstration of robust resurgence of cocaine seeking. The authors also reported that resurgence of cocaine seeking is mediated by activation of dopamine D1 receptors.
77. Preston KL, Umbricht A, Epstein DH: **Abstinence reinforcement maintenance contingency and one-year follow-up.** *Drug Alcohol Depend* 2002, **67**:125-137.
78. Klingemann HKH: **The motivation for change from problem alcohol and heroin use.** *Br J Addict* 1991, **86**:727-744.
79. Panlilio LV, Thorndike EB, Schindler CW: **Lorazepam reinstates punishment-suppressed remifentanyl self-administration in rats.** *Psychopharmacology (Berl)* 2005, **179**:374-382.
- The first demonstration of drug-priming induced resumption of drug seeking after suppression of drug seeking by punishment. This study also shows a pharmacological dissociation (with lorazepam) between drug priming-induced reinstatement after extinction versus after resumption of drug seeking after punishment-induced suppression of drug taking.
80. Economidou D, Pelloux Y, Robbins TW, Dalley JW, Everitt BJ: **High impulsivity predicts relapse to cocaine-seeking after punishment-induced abstinence.** *Biol Psychiatry* 2009, **65**:851-856.
81. Olds J, Olds ME: **Positive reinforcement produced by stimulating hypothalamus with iproniazid and other compounds.** *Science* 1958, **127**:1175-1176.
82. Warden CJ: *Animal Motivation: experimental Studies on the Albino Rat.* New York: Columbia University Press; 1931.
83. Barnea-Ygaël N, Yadid G, Yaka R, Ben-Shahar O, Zangen A: **Cue-induced reinstatement of cocaine seeking in the rat "conflict model": effect of prolonged home-cage confinement.** *Psychopharmacology (Berl)* 2012, **219**:875-883.
84. Pickens CL, Golden SA, Adams-Deutsch T, Nair SG, Shaham Y: **Long-lasting incubation of conditioned fear in rats.** *Biol Psychiatry* 2009, **65**:881-886.
85. Bossert JM, Ghitza UE, Lu L, Epstein DH, Shaham Y: **Neurobiology of relapse to heroin and cocaine seeking: An update and clinical implications.** *Eur J Pharmacol* 2005, **526**:36-50.
86. McFarland K, Lapish CC, Kalivas PW: **Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior.** *J Neurosci* 2003, **23**:3531-3537.
87. Theberge FR, Li A, Kambhampati S, Pickens CL, Bossert JM, Baumann MH, Hutchinson MR, Rice RC, Watkins LR, Shaham Y: **Effect of chronic delivery of the Toll-like receptor 4 antagonist (+)-Naltrexone on incubation of heroin craving.** *Biol Psychiatry* 2013 (in press).