

# Recent developments in animal models of drug relapse

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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.

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## 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-induce-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<sub>A</sub> and GABA<sub>B</sub> receptors) of ventral, but not dorsal, mPFC decreased extinction responding. After 1

withdrawal day, reversible *activation* (using GABAa + GABA<sub>b</sub> 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<sup>••</sup>]. Thiel *et al.* [57<sup>••</sup>] 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<sup>••</sup>,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<sup>••</sup>] 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<sup>••</sup>].

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 remifentanil (a short-acting opiate agonist) after punishment-induced suppression of the drug-reinforced responding cause faster reacquisition of remifentanil 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 remifentanil after punishment. In contrast, using a reinstatement model, they reported that heroin, but not lorazepam, priming injections reinstate remifentanil 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  $\mu$ -opioid receptor, was given chronically (via osmotic mini-pumps) during the withdrawal period. In heroin-trained but not methamphetamine-trained rats (+)-naltrexone decreased incubated cue-induced drug seeking seeking in an extinction test performed on withdrawal day 13.

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