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Review

Neurobiology of relapse to heroin and cocaine seeking: An update and clinical implications

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Abstract

The central problem in the treatment of cocaine and heroin addiction is high rates of relapse to drug use after periods of forced or self-imposed abstinence. Relapse can be modeled in laboratory animals a reinstatement procedure in which responding for drug is extinguished and then reinstated by acute exposure to the drug, drug cues, or stress. In this review, we first summarize data from recent (2003–2005) studies on the neural substrates involved in reinstatement of heroin and cocaine seeking. We also discuss the neural mechanisms underlying the progressive increase in cocaine seeking after withdrawal (incubation of cocaine craving). Finally, we provide an update on several novel candidate medications for relapse prevention suggested by recent preclinical studies, and we discuss the translation of findings from nonhuman laboratory studies to the clinical phenomenon of relapse.

Keywords: Cocaine; Conditioned drug cues; Extinction; Heroin; Incubation; Reinstatement; Relapse; Review; Stress

Contents

1. Introduction

The central problem for treatment of heroin and cocaine addiction remains the return to drug use after periods of abstinence (relapse) (Mendelson and Mello, 1996; O'Brien, 1997; Wallace, 1989). Studies in humans provide evidence that relapse to heroin or cocaine use or craving for these drugs can be triggered by exposure to the self-administered drug (de Wit, 1996; Meyer and Mirin, 1979), drug-associated cues (Carter and Tiffany, 1999; Childress et al., 1993) or stress (Sinha, 2001). We and others have argued that this clinical scenario can be modeled in a reinstatement model using laboratory rats and monkeys

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(Epstein and Preston, 2003; Shaham et al., 2003; Spealman et al., 1999; Stewart, 2000), but see Katz and Higgins (2003) for a different view.

In the operant version of the reinstatement model, laboratory animals are trained to self-administer drugs and are then subjected to extinction training during which lever presses are not reinforced with drugs. Reinstatement of extinguished lever responding (the operational measure of drug seeking) is determined after such manipulations as noncontingent priming injections of the drug (de Wit and Stewart, 1981; Stretch et al., 1971), exposure to cues associated with drug intake (Davis and Smith, 1976; Meil and See, 1996) or exposure to stress (Erb et al., 1996; Shaham and Stewart, 1995). During testing for reinstatement, extinction conditions remain in effect (drug is not available).

In the conditioned place preference (CPP) variation of the reinstatement model, laboratory animals are trained to associate a distinct environment with drug injections and are then subjected to extinction training during which they are exposed to the same environment in the absence of drug. Resumption of preference for that environment is then determined after noncontingent priming injections of the drug (Mueller and Stewart, 2000; Parker and McDonald, 2000) or exposure to stress (Sanchez and Sorg, 2001).

The effects of drug-associated cues on relapse to drug seeking can also be examined in extinction tests that are administered at different days after the termination of drug self-administration (Di Ciano and Everitt, 2004a; Lu et al., 2004b; Tran-Nguyen et al., 1998). This permits characterization of the time course of susceptibility to relapse to drug seeking.

In this review we summarize selected results from preclinical studies on reinstatement of cocaine or heroin (or morphine) seeking. In 2000–2003, several comprehensive reviews on this topic were published (Kalivas and McFarland, 2003; See, 2002; Shaham et al., 2003; Shalev et al., 2002; Stewart, 2000, 2003); these reviews covered the literature from 1971 to 2002. Therefore, our review is an update, focusing on studies published from January 2003 to April 2005, and complementing a recent review on the neurobiology of drug craving and relapse by Weiss (2005). Our Medline and ISI searches of drug studies in which investigators used reinstatement and extinction procedures found 129 published papers since 2003, reflecting the increasing popularity of these procedures during the last decade (Fig. 1). To conserve space, the first part of our review (on the neuroanatomy of reinstatement of heroin or cocaine seeking) omits studies in which drugs were injected only systemically. In the second part of the review, we discuss potential relapse-prevention medications that emerge from reinstatement and extinction studies and also address issues related to the translation of findings from non-human laboratory experiments to clinical settings.

2. Brain sites and circuits underlying relapse to heroin and cocaine seeking

In the following sections, we discuss recent developments in the understanding of the brain sites and circuits underlying relapse induced by cocaine or heroin priming, drug cues, and stress. Within each section, we briefly summarize the state of knowledge as of 2002 and then discuss data from neuroanatomical studies that were published in 2003–2005.

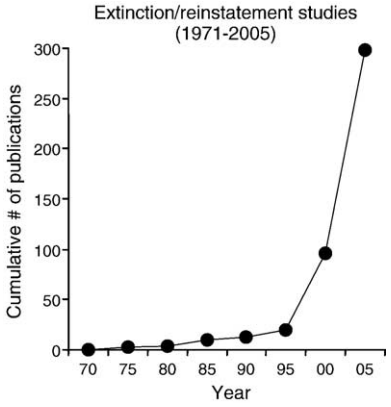

Fig. 1. Cumulative number of published studies that used extinction and reinstatement procedures in laboratory animals to study relapse to opiates, psychostimulants, or alcohol seeking. Source: Pubmed and ISI.

2.1. Cocaine and heroin priming

A large body of evidence indicates that the reinforcing effects of heroin and cocaine are mediated by the drugs' actions in the ventral tegmental area (VTA, the cell body region of the mesolimbic dopamine system) and the nucleus accumbens (a terminal region of this system) (Wise, 1996). Results from studies using systemic drug injections indicate that dopamine neurotransmission mediates cocaine-induced reinstatement (Self and Nestler, 1998; Shalev et al., 2002; Spealman et al., 1999). There is also evidence implicating dopamine neurotransmission in heroin-induced reinstatement (Ettenberg et al., 1996; Norman et al., 1999; Shaham and Stewart, 1996). Reinstatement induced by cocaine priming is mediated by activation of dopamine D2-like, but not dopamine D1-like, receptors (Alleweireldt et al., 2003; De Vries et al., 1999; Khroyan et al., 2000; Marinelli et al., 2003; Self et al., 1996; Wise et al., 1990).

In earlier studies, Stewart (1984) and Stewart and Vezina (1988) found that intra-VTA infusions of morphine, which increases dopamine cell firing and release, reinstate heroin or cocaine seeking, and intra-accumbens infusions of amphetamine, which increases local dopamine release, reinstate heroin seeking. Based on these studies and studies using systemic drug injections, there was—until recently—a consensus that accumbens dopamine activity mediates reinstatement induced by cocaine and heroin priming.

This view has been challenged by data from a series of studies by Kalivas and McFarland. Based on their data, these investigators argue that cocaine priming-induced reinstatement is mediated not by accumbens dopamine transmission, but by glutamatergic activation of accumbens α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors (Kalivas and McFarland, 2003). The initial evidence for this hypothesis was derived from three studies. Cornish et al. (1999; Cornish and Kalivas, 2000) reported that while activation of both

accumbens AMPA and dopamine receptors reinstates cocaine seeking, cocaine-induced reinstatement is attenuated by blockade of AMPA, but not dopamine, receptors (with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and fluphenazine, respectively). Subsequently, in a study unprecedented in its scope, McFarland and Kalivas (2001) examined the role of 10 brain sites in cocaine priming-induced reinstatement. They found that cocaine-induced reinstatement is attenuated by bilateral reversible inactivation (by the gamma-aminobutyric acid (GABA) receptor agonists muscimol and baclofen) of the VTA, dorsal prefrontal cortex, accumbens core, or ventral pallidum, but not by inactivation of the substantia nigra, ventral prefrontal cortex, accumbens shell, central and basolateral amygdala, or mediodorsal thalamus. These investigators also showed that cocaine-induced reinstatement is attenuated after "disconnection" of the dorsal prefrontal cortex from the ventral pallidum by unilaterally inactivating each of the sites in the contralateral hemispheres. Finally, they showed that cocaine-induced reinstatement is attenuated by injections of a mixed dopamine receptor antagonist (fluphenazine) into the dorsal prefrontal cortex but not the accumbens core or the ventral pallidum, and that infusing dopamine into the dorsal prefrontal cortex reverses the effect of VTA inactivation on reinstatement. Based on these findings, the authors hypothesized that cocaine-induced reinstatement results from activation of the VTA-dorsal prefrontal cortex dopamine pathway, leading to the activation of the dorsal prefrontal cortex-accumbens core glutamate pathway, and subsequent activation of the accumbens core–ventral pallidum pathway, presumably a GABAergic pathway (Kalivas and McFarland, 2003).

Results from a paper published by Park et al. (2002) provided initial independent support for McFarland and Kalivas' hypothesis. They found that infusions of a mixed dopamine receptor antagonist (flupenthixol) into the prefrontal cortex or an AMPA receptor antagonist (CNQX) into the accumbens attenuate reinstatement induced by intra-prefrontal cortex cocaine injections, while infusions of cocaine into the prefrontal cortex reinstate cocaine seeking. During the last two years, several other studies have further examined the role of glutamate and dopamine within the mesocorticolimbic system in cocaine priming-induced reinstatement.

Results from several of these studies support McFarland and Kalivas' hypothesis. McFarland et al. (2003) reported that repeated cocaine self-administration and subsequent withdrawal decreases basal levels of accumbens glutamate, which is accompanied by sensitization to the effect of cocaine priming on glutamate release. They also found that inactivation of the dorsal prefrontal cortex not only attenuates cocaine-induced reinstatement, but also cocaine-induced increases in the release of glutamate, but not dopamine, in the accumbens. Baker et al. (2003) reported that the decrease in basal glutamate levels in the accumbens after withdrawal is mediated by diminished activity of a non-synaptic cystine–glutamate transporter. Baker et al. also found that stimulating cystine/glutamate exchange with *N*-acetylcysteine, which restores accumbens basal extracellular glutamate levels, blocks cocaine-induced elevations in glutamate release and reinstatement. These findings were corroborated by Bowers et al. (2004) who studied the role of AGS3 (an activator of G protein signaling 3) in reinstatement of cocaine seeking. They found that cocaine self-administration and subsequent withdrawal upregulates AGS3 in the prefrontal cortex; when they upregulated AGS3 in the prefrontal cortex by a virus-vector manipulation, they found decreased basal glutamate levels and increased sensitivity to cocaine-induced glutamate release in the accumbens. Most important, they found that cocaine priming-induced reinstatement is attenuated by prefrontal cortex infusions of antisense oligonucleotides that reverse the cocaine-induced upregulation of AGS3.

Additional support for McFarland and Kalivas' hypothesis derives from data from several other studies. Sun et al. (2005) and Sun and Rebec (2005) found that cocaine-induced reinstatement is attenuated by dorsal prefrontal cortex infusions of either dopamine D1-like or D2-like receptor antagonists or by VTA infusions of the glutamate receptor antagonist kynurenate (a manipulation that results in decreased dopamine cell firing and release), but see Capriles et al. (2003) for a different result after prefrontal cortex infusions of dopamine D1- and D2-like receptor antagonists. Sanchez et al. (2003) found that cocaine-induced reinstatement of CPP was blocked by infusions of a dopamine D1-like receptor antagonist (SCH 23390) into the prefrontal cortex. Suto et al. (2004) reported that cocaine seeking is reinstated by infusions of AMPA into the accumbens, a finding that provides independent replication of the earlier data of Cornish et al. (1999; Cornish and Kalivas, 2000).

However, McFarland and Kalivas' hypothesis cannot account for several recent findings. Cocaine and amphetamine seeking are reinstated by stimulation of the basolateral amygdala and ventral subiculum (Hayes et al., 2003; Taepavarapruk and Phillips, 2003; Vorel et al., 2001), which send glutamatergic projections to the accumbens (Groenewegen et al., 1987; Kelley et al., 1982). More important, cocaine-induced reinstatement is attenuated by basolateral amygdala lesions, antagonism of dopamine D1-like receptors in the basolateral amygdala or central amygdala, or reversible inactivation of the ventral subiculum by lidocaine (Alleweireldt et al., in press; Sun and Rebec, 2003; Yun and Fields, 2003), but see Black et al. (2004) for a different result after ventral subiculum inactivation. Finally, Anderson et al. (2003) reported that cocaine-priming induced reinstatement is attenuated by infusions of a dopamine D1-like receptor antagonist (SCH 23390) into the accumbens shell, but not core.

Taken together, the results from the studies reviewed above and those from other recent (Placenza et al., 2004; Zavala et al., 2003) and earlier (Stewart, 2000) studies indicate that cocaine-induced reinstatement is mediated by both dopaminergic and glutamatergic transmission in the accumbens and other mesocorticolimbic sites. We suspect that the differing conclusions among the studies reviewed above are related to differing methodologies. McFarland and Kalivas (2001) primarily used GABAergic agonists, while investigators who reached different conclusions primarily used dopamine receptor antagonists (Alleweireldt et al., in press; Anderson et al., 2003). The importance of this methodological issue is highlighted in a recent study by Yun et al. (2004); they found that accumbens

infusions of a dopamine D1-like receptor antagonist (SCH 23390) and tetrodotoxin have very different effects on cue-induced reinstatement of food seeking, suggesting that behavioral changes induced by global reversible inactivation methods may not be mimicked by selective antagonism of dopamine and possibly other neurotransmitter receptors.

Finally, over the last two years, three studies examined the neuroanatomy of priming-induced reinstatement of morphine or heroin seeking. Wang et al. (2003) reported that 6-OHDA lesions of the VTA or the accumbens block morphine-induced reinstatement, as measured in the CPP procedure. In an elegant study that combined microdialysis with the CPP reinstatement procedure in mice, Ventura et al. (in press) reported that morphine-induced elevations in prefrontal cortex noradrenaline release mediate morphine-induced dopamine release in the accumbens and morphine-induced reinstatement of drug CPP, suggesting a novel role of prefrontal cortex noradrenaline in relapse to opiate drugs. The data from these two studies confirm and extend earlier findings on the role of mesocorticolimbic dopamine in reinstatement of opiate seeking by drug priming (Stewart, 2000).

In another study, Luo et al. (2004) trained rats to self-administer heroin or saline (a control condition) and after extinction of the drug-reinforced responding they examined in different groups the effect of heroin priming injections on reinstatement or fMRI signal in several brain areas, including components of the mesocorticolimbic system. The main finding was that rats with a history of heroin self-administration showed profound tolerance to the effect of heroin priming on the fMRI signal. The significance of these findings, however, is not clear because previous studies have shown that the effects of heroin and dopaminergic drugs on reinstatement of heroin seeking is highly correlated with their ability to induce locomotor sensitization (De Vries et al., 1998, 1999; De Vries and Shippenberg, 2002).

2.2. Cocaine and heroin cues

In humans, relapse-provoking drug-associated stimuli can be divided into two general categories: discrete drug cues (e.g., drug paraphernalia) that are associated with the acute rewarding effects of the drug, and contextual drug cues (e.g., a specific environment such as a local bar) that predict drug availability. In laboratory animals, drug seeking can be reinstated by three types of conditioned cues: discrete cues, discriminative cues, and contextual cues. Recent findings on the neuroanatomy of reinstatement induced by these different cues are summarized in the first three subsections below. In the final subsection we describe neuroanatomical findings from studies in which an extinction procedure was used to study time-dependent increases in cue-induced cocaine seeking after withdrawal, a phenomenon termed incubation of cocaine craving (Grimm et al., 2001; Lu et al., 2004b).

2.2.1. Discrete cues

In the discrete cue-induced reinstatement procedure, rats are first trained to self-administer a drug. During training, lever responding leads to drug infusions that are temporally paired with a discrete cue (e.g., tone, light). Lever pressing is then extinguished in the absence of the discrete cue. During the reinstatement test, re-exposure to the discrete cue, which is earned contingently by responding on the drug-associated lever, reinstates drug seeking (Davis and Smith, 1976; Meil and See, 1996). In early studies, See (2002) used reversible tetrodotoxin and permanent (excitotoxic) lesions and found that both the basolateral and central amygdala, but not the accumbens, are involved in discrete cue-induced reinstatement of cocaine seeking; BLA inactivation also attenuated discrete cue-induced reinstatement of heroin seeking (Fuchs and See, 2002). These investigators also reported that basolateral amygdala infusions of dopamine, but not glutamate, receptor antagonists attenuate cue-induced reinstatement of cocaine seeking (See et al., 2001). This observation was recently confirmed in another report in which the authors used a second-order schedule to examine the motivational effects of cocaine cues (Di Ciano and Everitt, 2004b).

During the last two years, several studies have further examined the brain sites involved in discrete cue-induced reinstatement of cocaine seeking. These studies confirmed the role of the amygdala and dopamine in this brain area in discrete cue-induced reinstatement. Ledford et al. (2003) reported that basolateral amygdala infusions of amphetamine potentiate the effect of the discrete cues on reinstatement. This effect is not likely to be due to the priming effect of amphetamine because drug infusions had no effect on non-reinforced lever responding in the absence of the discrete cue. Alleweireldt et al. (in press) reported that infusions of a dopamine D1-like receptor antagonist (SCH 23390) into the basolateral or central amygdala attenuate discrete cue-induced reinstatement of cocaine seeking. An important finding in this report was that SCH 23390 infusions into areas dorsal or dorsolateral to the amygdala (basal ganglia and somatosensory/insular cortex) also attenuated reinstatement induced by discrete cues or cocaine priming. This finding highlights the importance of using anatomical control conditions in studies using intracranial injections (Wise and Hoffman, 1992). The relatively nonselective effect of SCH 23390 on the two types of reinstatement also raises questions concerning interpretation of data from previous studies in which SCH 23390 was administered intracranially, especially considering its lipophilicity.

In other studies, McLaughlin and See (2003) reported that discrete cue-induced cocaine seeking is attenuated by tetrodotoxin inactivation of the dorsal, but not ventral, prefrontal cortex. Tetrodotoxin, however, inactivates both cell bodies and fibers of passage, and therefore findings from studies using this method do not conclusively demonstrate a role of a given brain site in behavior. Fuchs et al. (2004b) reported that discrete cue-induced cocaine seeking is attenuated by inactivation of the lateral (but not medial) orbitofrontal cortex by muscimol and baclofen. Interestingly, in this study, permanent excitotoxic (*N*-methyl-D-aspartate or NMDA) lesions did not mimic the effect of reversible inactivation. However, the effect of the two manipulations cannot be readily compared because the NMDA lesions were performed prior to the training phase rather than

after the extinction phase. Finally, Fuchs et al. (2004a) reported that discrete cue-induced cocaine seeking is attenuated by muscimol and baclofen inactivation of the accumbens core, but not the shell. This is a surprising finding because an earlier paper from this group reported no such effect from inactivation of the accumbens with tetrodotoxin (Grimm and See, 2000).

Taken together, results from studies during the last two years confirm the importance of both the basolateral and central amygdala, and of dopamine in these amygdala sites, in discrete cue-induced reinstatement of cocaine seeking. These recent studies also implicate other brain mesocorticolimbic areas, including the dorsal prefrontal cortex, the lateral orbitofrontal cortex, and the accumbens core. The most interesting and unexpected finding is the recent observation of Alleweireldt et al. (in press), potentially implicating dopamine in the basal ganglia and somatosensory/insular cortex in discrete cue-induced reinstatement of cocaine seeking. Finally, no studies published in the last two years addressed the brain sites involved in discrete cue-induced reinstatement of heroin seeking.

2.2.2. Discriminative cues

In the discriminative cue-induced reinstatement procedure, rats are trained to self-administer a drug or saline in the presence of distinct discriminative stimuli; one set of stimuli signals drug availability (S^+) and the other signals saline availability (S^-). Lever pressing is then extinguished in the absence of the discriminative stimuli. During the reinstatement test, re-exposure to the S^+ reinstates drug seeking (Alleweireldt et al., 2001; McFarland and Ettenberg, 1997; Weiss et al., 2000). Early studies showed that exposure to cocaine discriminative cues increases dopamine release in the accumbens and amygdala and induces the expression of the immediate early gene Fos (a marker of neuronal activity) in the basolateral amygdala and dorsal prefrontal cortex; the effect of the discriminative cues on Fos expression was reversed by systemic injections of a dopamine D1-like receptor antagonist (Ciccocioppo et al., 2001; Weiss et al., 2000). These interesting findings, however, were not followed by intracranial injections of the dopamine receptor antagonist in order to confirm the correlational findings from the microdialysis and Fos expression studies. To date, there has been only one pharmacological study on the effect of discriminative cue-induced reinstatement of heroin seeking: McFarland and Ettenberg (1997), using a runway procedure, reported that this form of reinstatement is not affected by the preferential dopamine D2-like receptor antagonist haloperidol.

During the last two years, three papers were published on the neuroanatomy of discriminative cue-induced reinstatement of cocaine seeking. Black et al. (2004) reported that this form of reinstatement was not affected by lidocaine inactivation of the ventral or dorsal subiculum in rats trained to earn cocaine infusions under a second-order reinforcement schedule. The interpretation of this result, however, is confounded by the fact that the reinstatement tests started 7 days after the completion of the extinction phase. This time-off period can lead to spontaneous recovery of the drug-taking behavior (Di Ciano and Everitt, 2002; Shaham et al., 1997a), a phenomenon that may not be dependent on hippocampal function. In another study, Yun and Fields (2003) reported that discriminative cue-induced reinstatement of cocaine seeking is attenuated by excitotoxic lesions of the basolateral amygdala. In a third study, Ghitza et al. (2003) found that accumbens shell neurons are selectively activated by exposure to discriminative cocaine cues during the tests for reinstatement. These correlational data suggest a role for the accumbens shell in discriminative cue-induced reinstatement of cocaine seeking, but this hypothesis should be experimentally confirmed by intracranial injection or lesion studies.Taken together, data from recent studies confirm the earlier finding of Weiss and colleagues on the role of the basolateral amygdala and accumbens in discriminative cue-induced reinstatement of cocaine seeking. An issue for future research is whether discriminative cue-induced reinstatement involves the dorsal prefrontal cortex, an area that is involved in reinstatement of cocaine seeking induced by drug priming, stress, and discrete and contextual cues. Finally, more studies are needed on the neuroanatomy of discriminative cue-induced reinstatement of heroin seeking.

2.2.3. Contextual cues

In the context-induced reinstatement procedure, which is based on the "renewal" procedure of Bouton and Bolles (1979), rats are trained to self-administer a drug in one context; drug infusions are paired with a discrete tone-light cue. Lever pressing is then extinguished in the presence of the discrete cue in a different, non-drug context. The contexts differ in terms of visual, auditory, tactile, olfactory, and circadian cues. During the reinstatement test, re-exposure to the original drug self-administration context reinstates drug seeking (Crombag et al., 2002; Crombag and Shaham, 2002). Studies on the brain sites involved in context-induced reinstatement of heroin or cocaine seeking were not performed prior to 2004. During the last year, two studies were published on this topic.

In a comprehensive anatomical study, Fuchs et al. (2005) reported that context-induced reinstatement of cocaine seeking is attenuated by inactivation of the dorsomedial prefrontal cortex, BLA, or dorsal hippocampus. These authors also found that tetrodotoxin inactivation of the dorsal hippocampus does not alter cocaine- or discrete cue-induced reinstatement. These findings are in agreement with previous work in fear conditioning on the partial neuroanatomical dissociation between the behavioral effects of discrete and contextual cues (LeDoux, 2000).

We used a negative modulator of glutamate transmission, the group II metabotropic glutamate ($mGluR_{2/3}$) receptor agonist LY379268 (Schoepp, 2001), to examine the role of glutamate in context-induced reinstatement of heroin seeking. We found that this form of reinstatement is attenuated by systemic and VTA (but not substantia nigra) infusions of LY379268, suggesting a role for VTA glutamate (Bossert et al., 2004).

Taken together, results from recent studies suggest that the hippocampus, VTA, prefrontal cortex, and basolateral amygdala are involved in context-induced reinstatement of drug seeking. However, the findings concerning the role of the hippocampus,

prefrontal cortex, and basolateral amygdala were derived from studies in which tetrodotoxin was used, and should be confirmed in future studies in which investigators use selective receptor ligands or fiber-sparing lesion methods.

2.2.4. Incubation of cocaine craving

Several studies prior to 2003 examined the time course of susceptibility to drug seeking after different periods of withdrawal. Neisewander and co-workers reported that extinction responding, a behavior induced by re-exposure to reward-associated cues (Catania, 1992), is greater after 3–4 weeks than after 1 day of withdrawal from cocaine (Neisewander et al., 2000; Tran-Nguyen et al., 1998). Shalev et al. (2001a) found that extinction responding follows an inverted U-shaped curve, with higher responding after 6, 12 or 25 days than after 1 or 66 days of withdrawal from heroin. Based on these findings, Grimm et al. (2001, 2003) assessed cocaine seeking after withdrawal in extinction and discrete cue-induced reinstatement tests and found that the responsiveness to cocaine cues in both tests progressively increases over the first 60 days of withdrawal and persist for up to 90 days. Grimm et al. concluded that craving (a motivational state elicited by exposure to drug-associated cues, which often precedes and accompanies drug seeking) incubates after withdrawal from cocaine self-administration. Findings from recent studies on incubation with drug and non-drug reinforcers are described in a recent review (Lu et al., 2004b). Below, we summarize recent studies on neurobiological mechanisms potentially mediating the incubation of cocaine craving.

The incubation of cocaine craving may be related to long-lasting molecular neuroadaptations induced by cocaine self-administration and subsequent withdrawal (Lu et al., 2004b). In this regard, many studies have shown that cocaine exposure leads to upregulation of AMPA and NMDA glutamate receptors in the VTA and accumbens (Carlezon and Nestler, 2002; Kalivas et al., 2003). Extending these previous results, we found that cocaine self-administration induced long-lasting (up to 90 days after drug withdrawal) upregulation in the expression of glutamate receptor subunits, including NMDA1 receptor in the VTA and accumbens, and GluR1 and GluR2 receptor in the accumbens (Lu et al., 2003a). More recently, we found that cocaine self-administration and subsequent withdrawal (1 or 30 days) also regulates glutamate receptor expression in the amygdala. In the basolateral amygdala, GluR1 receptor, but not GluR2 receptor levels were increased on days 1 and 30, NMDA2A receptor levels were increased on day 1, and NMDA2B receptor levels were decreased on day 30 of withdrawal from cocaine. In the central amygdala, GluR1 receptor but not GluR2 receptor levels were increased on days 1 and 30 and NMDA1 receptor levels were increased on day 30 (Lu et al., 2005b). The relevance of these alterations to incubation is not known because unlike extinction responding, which increases over time, glutamate receptor upregulation is observed during both early and late withdrawal.

In two studies, we examined incubation-associated changes in the levels of brain-derived nerve growth factor (BDNF) (Grimm et al., 2003; Lu et al., 2004a). BDNF is a growth factor involved in synaptic plasticity (Thoenen, 1995) and it plays an important role in the survival and function of midbrain dopamine neurons (Hyman et al., 1991). BDNF infusions into the substantia nigra, VTA, and accumbens increase dopamine utilization and enhance locomotor activity induced by psychostimulant drugs (see Pierce and Bari, 2001), and accumbens infusions of BDNF enhance responding for conditioned stimuli paired with water reward (Horger et al., 1999). BDNF expression in mesocorticolimbic areas is also increased after amphetamine exposure (Meredith et al., 2002). We found time-dependent increases in BDNF protein levels in the VTA, accumbens, and amygdala after withdrawal from cocaine but not sucrose self-administration (Grimm et al., 2003). The accumbens and amygdala showed the greatest increases, but the temporal pattern of increases in the VTA more closely paralleled the time course of incubation. Therefore, we subsequently studied whether intra-VTA BDNF infusions would enhance cocaine seeking after withdrawal, as measured in extinction tests. We hypothesized that BDNF infusions, given at the end of the training phase, would enhance cocaine seeking during the first several days of withdrawal, when lever responding is substantially lower than it is after 1–3 months. We also studied the role of the extracellular signal-regulated kinase (ERK) pathway, which mediates BDNF effects (Poo, 2001).

We found that a single intra-VTA infusion of BDNF induces long-lasting enhancement of cocaine seeking for up to 30 days, an effect reversed by an ERK inhibitor (U0126). However, interpretation of these data in the context of BDNF's role in incubation of cocaine craving is not straightforward, primarily because incubation of reward craving also occurs over the first few weeks after withdrawal from oral sucrose self-administration, and this short-lasting incubation is not associated with increases in mesolimbic BDNF levels. Based on the above results and other considerations (see Lu et al., 2004a), we suggested that mesolimbic BDNF does not mediate the basic process of incubation of reward craving but is likely to be involved in the persistence of responsiveness to cocaine cues after prolonged withdrawal periods.

In a recent study, we describe data indicating a critical role of central amygdala ERK signaling pathway in incubation of cocaine craving (Lu et al., 2005a). Rats were trained to self-administer cocaine for 10 days and then tested for cue-induced drug-seeking after 1 or 30 days of withdrawal. We found that cocaine seeking was substantially greater after 30 withdrawal days than after 1 day and that this increase was associated with increased ERK phosphorylation in the central but not the basolateral amygdala. Furthermore, after 30 days of withdrawal from cocaine, inhibition of central but not basolateral amygdala ERK phosphorylation decreased cocaine seeking. After 1 day of withdrawal, stimulation of central amygdala ERK phosphorylation by NMDA increased cocaine seeking, while after 30 days of withdrawal, inhibition of NMDA receptors by 2-amino-5-phosphopenoic acid (AP-5) decreased both ERK phosphorylation and cocaine seeking. These results suggest that incubation of cocaine craving is mediated by glutamatergic activation of central amygdala ERK signaling pathway.

Finally, Hollander and Carelli (2005) examined the impact of the cocaine withdrawal period (1 or 30 days) on extinction

responding (in the absence of a discrete tone-light cue) and subsequent reinstatement test in which cocaine was given non-contingently at the onset of the session and lever pressing resulted in contingent presentations of the discrete cue. The authors found that lever responding in the extinction and reinstatement tests was higher after 30 days than after 1 day. In two other groups, the authors assessed phasic neuronal activity in the accumbens core and shell during ongoing cocaine self-administration or in a self-administration session conducted after 30 days of withdrawal. Phasic neuronal activity refers to the changes in firing rates in the seconds surrounding the cocaine-reinforced responding or in the minutes spanning the inter-infusion interval. The authors found that after 30 days of withdrawal, the percentage of phasically active neurons was increased in the accumbens core but not the shell. However, unlike the behavioral results in the extinction and reinstatement tests, the duration of the withdrawal period had no effect on cocaine self-administration. The authors' neurophysiological data potentially suggest a role for the accumbens core in the incubation of cocaine craving. This possibility, however, should be confirmed by experiments in which phasic neuronal activity is measured during tests for cocaine seeking in extinction or reinstatement tests in which time-dependent increases in cocaine seeking are observed. Another issue for future research is whether the increased accumbens core neuronal activity during late withdrawal reflects increased sensitivity (sensitization) of the core neurons during late withdrawal or decreased sensitivity (tolerance) of these neurons during ongoing cocaine self-administration.

Taken together, results from recent studies suggest that increases in BDNF expression in mesolimbic dopamine areas after withdrawal from cocaine are likely to be involved in the persistence of responsiveness to cocaine cues, though perhaps not in the initial stages of incubation. The most significant recent finding is that the manifestation of incubation of cocaine craving requires glutamate-dependent activation of the central amygdala ERK pathway. An important question for future research is whether this signaling pathway is also involved in the incubation observed with other drugs such as heroin (Shalev et al., 2001a) and methamphetamine (Shepard et al., 2004), or with non-drug reinforcers such as sucrose (Grimm et al., 2002). Finally, unresolved questions for future research are the source of the glutamate projections to the central amygdala that become hypersensitive to cocaine cues after withdrawal and the central amygdala projection areas that are recruited after ERK activation.

2.3. Stress

A decade ago, Shaham and Stewart (1995) reported that an intermittent-footshock stressor reinstates heroin seeking and suggested that the reinstatement model could be used to identify neuronal mechanisms underlying stress-induced relapse to drug seeking. These findings were extended to rats with histories of self-administering cocaine, nicotine, alcohol, or methamphetamine (Le and Shaham, 2002; Lu et al., 2003b). The effect of intermittent-footshock stress on reinstatement generalized to several other stressors, including acute food deprivation (Highfield et al., 2002; Shalev et al., 2001b) and exposure to the stress hormone corticotropin-releasing factor (CRF) (Shaham et al., 1997b) in the self-administration reinstatement procedure. It also generalized to conditioned fear and restraint in the CPP reinstatement procedure (Sanchez et al., 2003; Sanchez and Sorg, 2001), although these two stressors did not reinstate heroin or cocaine seeking in the self-administration reinstatement procedure (Shaham et al., 2000a). More recently, we and others reported that the α-2 adrenoceptor antagonist yohimbine, which induces stress-like responses in humans and nonhumans, reinstates cocaine seeking in monkeys and methamphetamine and alcohol seeking in rats (Le et al., 2005, 2004; Shepard et al., 2004). Results from neuroanatomical studies conducted prior to 2003 indicate that footshock-induced reinstatement involves extrahypothalamic CRF and noradrenaline in the central amygdala and the bed nucleus of stria terminalis (Erb et al., 2001; Erb and Stewart, 1999; Leri et al., 2002); the noradrenaline appears to originate from the lateral tegmental nuclei but not the locus coeruleus (Shaham et al., 2000b; Wang et al., 2001). Several recent studies have further explored the neural substrates of stress-induced reinstatement of cocaine seeking; the stressor used was intermittent footshock.

Xi et al. (2004) found that footshock-induced reinstatement of cocaine seeking is attenuated by systemic and intra-accumbens, but not intra-dorsal striatum, infusions of a selective dopamine D_3 antagonist (SB-277011A). Capriles et al. (2003) reported that footshock-induced reinstatement of cocaine seeking is attenuated by reversible tetrodotoxin inactivation of the prelimbic cortex or the orbitofrontal cortex; these effects were mimicked by injections of the dopamine D1-like receptor antagonist SCH 23390 but not the dopamine D2-like receptor antagonist raclopride. Sutton et al. (2003) reported that footshock-induced reinstatement was blocked by viral-mediated over-expression of both GluR1 and GluR2 (AMPA receptor subunits) in the accumbens shell during extinction training; the test was conducted 2 weeks after the viral manipulation, which alters receptor subunit expression for less than 6 days. The mechanisms underlying this unexpected finding are not known.

McFarland et al. (2004) reported that footshock-induced reinstatement of cocaine seeking is attenuated by reversible inactivation (with muscimol and baclofen) of the central amygdala, the bed nucleus of the stria terminalis, the dorsal prefrontal cortex, or the accumbens core and shell; these findings are consistent with those of Xi et al. (2004), Capriles et al. (2003) and earlier results (see above). These investigators also found that footshock-induced reinstatement is attenuated by reversible inactivation of the VTA and ventral pallidum, but not by inactivation of the ventral prefrontal cortex, basolateral amygdala, or mediodorsal thalamus. An additional finding was that footshock-induced reinstatement was attenuated by infusions of the mixed dopamine receptor antagonist fluphenazine into the dorsal prefrontal cortex. Two other interesting findings in this report are that inactivation of the accumbens shell or dorsal prefrontal cortex not only blocked footshock-induced reinstatement of cocaine seeking, but also blocked, respectively,

footshock-induced increases in prefrontal cortex dopamine and accumbens core glutamate release. Based on these data, the authors suggested that footshock stress first activates extended amygdala limbic structures (central amygdala, bed nucleus of the stria terminalis, and accumbens shell) that in turn activate a "motor" circuit including the dorsal prefrontal cortex, accumbens core, and ventral pallidum, which they previously found to be critical for cocaine-priming induced reinstatement.

Recent data from Wang et al. (2005) provide additional evidence for the role of the VTA in stress-induced reinstatement of cocaine seeking and further show that this reinstatement requires a unique cocaine-induced neuroadaptation in the response of VTA glutamatergic neurons to CRF. Wang et al. found that footshock stress induces reinstatement and increases VTA levels of CRF, glutamate, and dopamine, as measured by in vivo microdialysis. The effects of footshock on reinstatement and on VTA glutamate and dopamine release are blocked by a CRF receptor antagonist (α-helical CRF); footshock-induced reinstatement and dopamine release are also blocked by an ionotropic glutamate receptor antagonist (kynurenic acid). Furthermore, the effect of footshock on reinstatement and local release of glutamate and dopamine is mimicked by CRF perfusions into the VTA. Another novel and potentially important finding is that footshock-induced elevations of VTA glutamate and dopamine and CRF-induced elevations of VTA glutamate are observed only in rats with a history of cocaine self-administration, not in drug-naïve rats. Together, these data indicate that an increase in the VTA glutamate response to local CRF in cocaine-experienced animals is critically involved in stress-induced reinstatement of cocaine seeking.

Finally, Kreibich and Blendy (2004) reported that exposure to a forced-swim stress reinstates extinguished cocaine CPP in mice. This effect is associated with increases in phosphorylated cAMP response element-binding protein (phosphorylated CREB) in the amygdala and accumbens, but not the bed nucleus of the stria terminalis and VTA, and is eliminated in knockout mice deficient in alpha and delta isoforms of CREB. The lack of forced-swim stress-induced reinstatement in the knockout mice cannot be attributed to learning deficits because these mice demonstrate normal cocaine CPP and cocaine priming-induced reinstatement. The authors' conclusion was that their data indicate a specific requirement for CREB in stress-induced behavioral responses to drugs of abuse. Despite the high quality of the study and the interesting nature of the results, it is not entirely clear that the data support such a broad conclusion. CREB is a transcription factor that controls the expression of other genes, and acute stress-induced phosphorylation of CREB is not likely to directly mediate behavior within the time frame of the study (less than 60 min). Thus, the induction of phosphorylated CREB by swim stress is likely an index of other neuronal processes in the amygdala and accumbens that are involved in stress-induced reinstatement (e.g., glutamate release that leads to ERK activation and subsequent CREB activation) in wild-type mice. Furthermore, the different behavioral effects in the knockout and wild-type mice may be related to differences in expression of many other genes due to the deletion the alpha and delta isoforms of CREB, resulting in a different behavioral phenotype in the knockout mice.

Taken together, the recent neuroanatomical studies reviewed above indicate that stress-induced activation of mesocorticolimbic dopamine and glutamate is critically involved in reinstatement of cocaine seeking. Vulnerability to stress-induced relapse in cocaine-experienced rats may result from stress-induced increases in the sensitivity of VTA glutamatergic neurons to CRF, in turn resulting in activation of mesolimbic and mesocortical dopaminergic neurons in the accumbens and dorsal prefrontal cortex, respectively—two brain sites in which blockade of dopamine receptors was found to attenuate footshock-induced reinstatement. If such neuroadaptations in the VTA mediate footshock-induced reinstatement with drugs other than cocaine, this could account for the observations that intermittent footshock is not an effective stimulus for reinstatement of food pellet (Ahmed and Koob, 1997; Mantsch and Goeders, 1999) or sucrose (Buczek et al., 1999) seeking.

Finally, during the last 2 years, only one study examined neuronal mechanisms underlying stress-induced reinstatement of heroin seeking. Shalev et al. (2003) reported that 1 day of food deprivation selectively increases Fos induction in the prelimbic cortex of heroin-trained but not saline-trained rats, providing further support for a potential role of the prefrontal cortex in stress-induced reinstatement.

2.4. Summary and conclusions

We have reviewed recent data on the neural substrates of reinstatement of heroin and cocaine seeking induced by exposure to drug priming, different types of cues (discrete, discriminative, and contextual) and stress (almost exclusively in the form of intermittent footshock). We have also reviewed recent data on the neural substrates of incubation of cocaine craving, as measured in extinction tests in the presence of discrete and contextual cues.

In early studies, footshock- and priming-induced reinstatement (to heroin or cocaine seeking) were shown to respond differentially to ligands acting on opiate, dopamine, CRF, and noradrenaline receptors (Erb et al., 2000, 1998; Leri et al., 2002; Shaham et al., 1997b; Shaham and Stewart, 1996). In addition, Grimm and See (2000) found that reversible inactivation of the basolateral amygdala attenuated discrete cue-induced cocaine seeking but not cocaine taking, while reversible inactivation of the accumbens had opposite effects. Using a second-order reinforcement schedule, Everitt and colleagues have also shown selective effects of pharmacological and neuroanatomical manipulations on cue-induced cocaine seeking prior to first cocaine infusion versus cocaine seeking maintained by subsequent cocaine infusions (Everitt and Robbins, 2000). Based on these findings, a primary conclusion of previous reviews was that the neuronal mechanisms underlying reinstatement induced by drug priming, drug cues, and stress are to a large degree dissociable (Shaham et al., 2000a; Shalev et al., 2002).

The recent neuroanatomical findings reviewed above indicate that this conclusion should be substantially modified. A

common theme in recent studies is a large degree of overlap among the brain sites mediating reinstatement induced by drug priming, drug cues, and stress. For example, decreasing glutamate transmission in the VTA attenuates reinstatement induced by drug priming, contextual cues, or stress (Bossert et al., 2004; Sun et al., 2005; Wang et al., 2005). In addition, blockade of dorsal PFC dopamine receptors attenuates both footshock- and drug-induced reinstatement (Capriles et al., 2003; McFarland et al., 2004; McFarland and Kalivas, 2001), while blockade of these receptors in the central and basolateral amygdala attenuates both drug- and discrete cue-induced reinstatement (Alleweireldt et al., in press).

A possible explanation for the discrepancy between earlier and more recent findings is that drug priming, cues, and stress access a final common reinstatement/relapse circuit via different projections (Kalivas and McFarland, 2003; McFarland et al., 2004; Shaham et al., 2003). McFarland and Kalivas hypothesized that this final common pathway includes a glutamate projection from the dorsal prefrontal cortex to the accumbens core and further argue that activation of glutamate, but not dopamine, transmission in the accumbens core is critical for relapse to drug use. To date, the role of this glutamatergic pathway in relapse behavior has been conclusively demonstrated only for cocaine priming-induced reinstatement (Kalivas, 2004). However, there are data that are incompatible with this hypothesis, indicating that accumbens dopamine is important not only for cocaine priming-induced reinstatement (Anderson et al., 2003), but also for footshock stress-induced reinstatement of cocaine seeking (Xi et al., 2004).

Finally, from a methodological perspective, the most important issue that has emerged during the past 2 years is that infusions of dopamine receptor antagonists into discrete brain areas do not have the same effects on reinstatement as reversible inactivation of the same areas. For example, while muscimol–baclofen inactivation of the accumbens core but not the shell attenuate cocaine-induced reinstatement (McFarland and Kalivas, 2001), the dopamine D1-like receptor antagonist SCH 23390 has opposite effects (Anderson et al., 2003). Thus, while reversible inactivation methods may be useful for initial identification of the role of discrete brain sites in behavior, the results from studies using these methods must be interpreted with great caution. Based on the above considerations, we suggest that future reinstatement studies should primarily use selective receptor antagonists and agonists rather than non-selective reversible-inactivation procedures.

3. Implications for relapse prevention in humans

In the studies reviewed in the previous section, drugs were primarily injected into discrete brain areas. While such studies are important for the understanding of the neurobiology of drug relapse/reinstatement in laboratory animals, the results do not readily translate to the human condition because medications used in the treatment of drug addiction are given systemically. In this regard, many of the reinstatement studies published over the past 2 years were concerned with identifying potential pharmacological treatments for relapse prevention. As with the neuroanatomical studies, the majority of these "medication discovery" studies were conducted with cocaine-trained rats.

A major issue to consider regarding "medication discovery" is that the predictive validity of reinstatement and extinction models has not been established for relapse to cocaine use: there are many pharmacological agents that decrease cocaine seeking in these models, but there are no clinically established pharmaceutical treatments for cocaine addiction that can be used to validate the models. The issue of predictive validity is not addressed here in detail because it was extensively discussed in several recent reviews (Epstein and Preston, 2003; Katz and Higgins, 2003; Shaham et al., 2003). It should be noted that in the case of cocaine, the problem of predictive validity is not unique to extinction and reinstatement models. There are many pharmacological agents that attenuate lever pressing in the more established self-administration and drug discrimination models, yet results from numerous studies that used these models for "medication discovery" are yet to be translated to promising clinical outcomes with cocaine users. However, in the case of heroin and alcohol addiction, medications that are effective to some degree do exist, and results from reinstatement studies demonstrate that they can attenuate heroin or alcohol seeking in reinstatement and extinction tests. For example, acamprosate and naltrexone, which are used clinically in the treatment of alcoholism, attenuate reinstatement induced by alcohol priming (Le et al., 1999) or alcohol discriminative cues (Bachteler et al., 2005; Liu and Weiss, 2002). In addition, Stewart and colleagues recently showed that methadone (Leri et al., 2004) and buprenorphine (Sorge et al., 2005), which are used clinically in the treatment of opiate addiction, attenuate extinction responding and drug priming-induced reinstatement in rats with a history of concurrent self-administration of heroin and cocaine.

The findings of Stewart and co-workers are particularly relevant to the question of predictive validity because the "medications" were given chronically (via osmotic minipumps), rather than acutely as in the vast majority of previous studies, and the rats self-administered both heroin and cocaine in order to model polydrug use, a condition that is very common in humans. Importantly, however, in some of the studies mentioned above, medications found effective in attenuating priming- or cue-induced reinstatement had no effect on footshock-stress-induced reinstatement (Leri et al., 2004; Sorge et al., 2005); see also an earlier finding on the effect of heroin "maintenance" treatment on heroin- versus stress-induced reinstatement (Shaham et al., 1996). The clinical implications of these findings are discussed in more detail below.

From a medication-development perspective, several types of compounds found effective in reinstatement studies may be particularly promising. Results from early reinstatement studies suggesting a role for dopamine D1-like receptor agonists, which attenuate cocaine- and discrete cue-induced reinstatement (Alleweireldt et al., 2002; Self et al., 1996; Spealman et al., 1999), have led to phase II clinical trials with one of these agonists (DAS-431, also called adrogolide) in cocaine-dependent subjects (Heidbreder and Hagan, 2005). Results from other early studies suggesting a role for the α-2 adrenoceptor

agonist lofexidine, which attenuates stress-induced reinstatement of cocaine and speedball (a heroin-cocaine combination) seeking (Erb et al., 2000; Highfield et al., 2001), have led to a study of lofexidine (in combination with naltrexone) for relapse prevention in opiate-dependent patients, with promising preliminary results (Sinha et al., 2003). In addition, results from studies on the effect of CRF1 receptor antagonists on stress-induced reinstatement of drug seeking (Le et al., 2000; Shaham et al., 1998) are likely to lead to clinical trials with human drug addicts when such compounds become available for clinical trials. Recent results from reinstatement studies suggest that other candidates for medication development include compounds that target dopamine D3 receptors, cannabinoid CB1 receptors, and group II metabotropic glutamate ($mGluR_2$ and $mGluR_3$) receptors.

Gardner and colleagues found that the dopamine D3 antagonist SB-277011A attenuates reinstatement induced by cocaine priming (Vorel et al., 2002), discrete cues (Gilbert et al., 2005), or footshock stress (Xi et al., 2004). Di Ciano et al. (2003) reported that SB-277011A also attenuates cue-controlled cocaine seeking in a second-order reinforcement schedule. To our knowledge, SB-277011A is the only compound identified in reinstatement studies that attenuates drug-, cue- and stress-induced reinstatement of drug seeking. Another promising candidate is the cannabinoid CB1 receptor antagonist SR141716A. This drug attenuates reinstatement induced by drug priming or discrete cues in rats with a history of cocaine, heroin, or methamphetamine self-administration (Anggadiredja et al., 2004; de Vries et al., 2003, 2001), though it has no effect on reinstatement of cocaine seeking induced by stress (de Vries et al., 2001). Cannabinoid CB1 receptor antagonists are currently in clinical trials with nicotine- and alcohol-dependent subjects (Heidbreder and Hagan, 2005), and we hope that such compounds will also be tested in the near future with opiate- and psychostimulant-dependent subjects. As for group II metabotropic glutamate receptors, Baptista et al. (2004) reported that systemic injections of an $mGluR_{2/3}$ receptor agonist (LY379268) attenuate discriminative cue-induced reinstatement of cocaine seeking, and we found that this agonist attenuates context- and discrete-cue-induced reinstatement of heroin seeking (Bossert et al., 2005). $mGluR_{2/3}$ receptor agonists are in clinical development for the treatment of nicotine dependence (Heidbreder and Hagan, 2005), and based on the above recent studies, it appears that they should also be considered in the treatment of relapse to heroin and cocaine dependence.

Assuming that relapse in humans is shown to fall into subtypes of stimuli comparable to those of reinstatement (drug priming, drug cues, and stress), a unique set of challenges lies ahead. Reinstatement experiments suggest many potential relapse-prevention medications, each of which may be effective regardless of whether the patient's drug of abuse is heroin, cocaine, or both. However, many of those medications are specific to just one or two subtypes of reinstatement, and thus presumably to one or two subtypes of relapse. For example, as mentioned above, the cannabinoid CB1 receptor antagonist SR141716A blocks drug priming- or cue-induced reinstatement of heroin or cocaine seeking, but not stress-induced reinstatement (de Vries et al., 2003, 2001). In contrast, CRF receptor antagonists attenuate stress-induced, but not drug-induced, reinstatement (Shaham et al., 2000a). Such findings pose two types of challenges for clinical studies.

First, clinical trials must be designed to detect the medication's differential effects on subtypes of relapse. Otherwise, a seemingly well-powered trial might produce a false-negative result when many participants succumb to relapse precipitants not blocked by the medication being tested. To distinguish cue-induced from stress-induced relapse without the biases introduced by retrospective assessment, participants will need to be intensively monitored, either through diary methods or frequent contact. We know of no precedent for this approach to clinical trials for substance-abuse treatments, and even if it is methodologically feasible, it cannot permit certainty about the causation of a specific episode of relapse. Nonetheless, it needs to be pursued so that relapse-prevention medications identified in the reinstatement model can be tested appropriately.

Second, if a medication proves effective against one or two subtypes of relapse, it will need to be incorporated into treatment regimens, presumably via polypharmacy with other medications that have proven effective against other subtypes of relapse. This "cocktail" approach—preventing subtypes of relapse-inducing stimuli with a combination of medications—has been shown effective in an elegant preclinical study by Liu and Weiss (2002). They found that the modest reinstatement induced in rats by discriminative alcohol cues is blocked by naltrexone but not by the CRF antagonist d-Phe-CRF, that the modest reinstatement induced by footshock stress is blocked by d-Phe-CRF but not by naltrexone, and that the strong reinstatement induced by a cues+stress combination is blocked by d-Phe-CRF+naltrexone. The need for a "cocktail" approach underscores the importance of focusing on medications that are well-tolerated when co-administered. We suggest that this approach should be considered in future clinical trials with cannabinoid CB1 receptor antagonists combined with CRF1 receptor antagonists or α-2 adrenoceptor agonists. However, it will ultimately be desirable to obviate the need for polypharmacy. In this regard, dopamine D_3 ligands appear especially promising: a dopamine D_3 receptor antagonist was found to block reinstatement of cocaine seeking regardless of whether the precipitant is cocaine priming, cocaine discrete cue, or a stressor (see above).

Finally, an earlier commentary coauthored by one of us (Epstein and Preston, 2003) discussed the applicability of the reinstatement model to the human condition. We concluded that the model appears to have face validity, especially with regard to the likely precipitants of relapse in humans: a "taste" of the drug (i.e., priming), an exposure to drug-associated cues, or a stressful experience. We cautioned that this frequently cited trio of relapse precipitants has not yet been validated by rigorous prospective assessment, but is based instead on retrospective interview data and extrapolations from human laboratory models. Relapse in cocaine and heroin addicts needs to be prospectively differentiated into such categories as "stress-induced" or "cue-induced," perhaps by means of the electronic-diary methods used in cigarette smokers by Shiffman et al. (1997). In the same commentary, we acknowledged that the clearest challenge to the face validity of the reinstatement model is the fact that abstinence occurs through the passive process of

extinction, while in humans, abstinence is typically chosen as the negative consequences of use become more salient than the positive ones. In terms of the model's predictive or construct validity, we argued that this difference "should matter only if the degree of choice involved in initial abstinence alters the precipitants or process of subsequent relapse." There is now evidence that under certain circumstances this can be the case. Panlilio et al. (2003, 2005) have developed a relapse model in which drug-taking behavior is suppressed through punishment with electric shock. They found that in rats that have undergone the punishment procedure, opiate (remifentanil) seeking is reinstated not only by a priming injection of heroin, as expected, but also by the anxiolytic drug lorazepam, a drug that does not reinstate opiate seeking in the original extinction-based reinstatement procedure. The clinical significance of this finding is unknown; it bears an obvious homology to the finding that use of sedative/hypnotic drugs such as ethanol is associated with relapse to other drugs (McKay et al., 1999), and perhaps it is also homologous to the finding that good moods can precipitate relapses (Heather et al., 1991). Based on the above considerations, the punishment-based reinstatement procedure of Panlilio and Schindler appears to be an important methodological development, and this procedure should be used in future studies to identify neurobiological mechanisms of clinically observed relapse precipitants that are not detected in the traditional extinction-based reinstatement procedure.

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