Stress and Relapse to Drug Seeking: Studies in Laboratory Animals Shed Light on Mechanisms and Sources of Long-Term Vulnerability

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Relapse is a major characteristic of drug addiction disorders and remains the primary problem for treatment. Recently, there has been hope that these disorders may be amenable to pharmacological treatments that have successfully treated other psychopathological disorders. Pharmacological approaches to drug abuse have tended to be guided by the primary drug used by the individual, though substitution has been the guiding principle in some instances, as in the case of methadone maintenance in opioid addiction. Alternatively, blockade or antagonism of the effects of the primary drug being abused has been tried, as in the case of using naltrexone to treat opioid or alcohol addiction. Though reportedly successful in some populations, it is not clear that these approaches effectively control craving for 'highs' or euphoric experiences or a return to drug use as a response to stressful life experiences. Recent experimental studies of the factors that induce craving and relapse to drug use in both humans and laboratory animals, such as drug-related cues, re-exposure to the drug itself, or exposure to stressful events, have shown that the effects of these different events are mediated by dissociable neurochemical circuitry.
In humans and laboratory animals, relapse to drug seeking can be induced after extended periods of abstinence by re-exposure to the drug itself, exposure to drug-related cues, and by exposure to stress. In recent years, we have been concerned primarily with the study of stress-induced relapse to drug seeking using an animal model of relapse, the reinstatement procedure. A major finding from this work is that the constellation of neurochemical events involved in relapse to drug seeking induced by acute stress is to a large degree distinct from that involved in relapse induced by re-exposure to the drug previously used or to drug-related cues. We have identified two brain systems, the corticotropin-releasing factor (CRF) and noradrenergic (NA) systems, that play major roles in footshock stress-induced relapse but have little effect on drug-induced relapse. An implication of these findings is that no single pharmacological approach to the treatment of addiction is likely to be effective.

A second important finding that is beginning to emerge from these studies is that the motivation underlying drug seeking induced by events that precipitate relapse is intensified by the duration and amount of previous exposure to a drug and by the time since withdrawal of the drug. Exposure to drugs of abuse appears to induce long-lasting increases in the sensitivity of the neural circuitry activated by events that induce relapse to drug seeking, making the individual vulnerable to relapse for extended periods after abstinence. This latter evidence stands in contrast to the view that the period of acute withdrawal from the abused drug has the greatest vulnerability for relapse.

Work in our laboratory has been concerned primarily with factors provoking relapse, that is, the reinitiation of drug craving and seeking after abstinence. We are trying to understand why individuals remain vulnerable to relapse even when drugs are unavailable for long periods of time or users are successful in curbing their own drug use for extended periods. This issue is different from (though related to) that of what leads to excessive drug intake once the individual begins a cycle of drug taking. This latter issue is being explored by Koob and colleagues, who are examining the changes in reward efficacy that occur in drug-free periods immediately following periods of drug exposure. Using electrical brain stimulation to assess reward thresholds, it has been found, for example, that the thresholds for intracranial self-stimulation are raised 22–23 h after an injection of cocaine or amphetamine, but are lowered in response to the drug when injections are given repeatedly but infrequently. Koob and colleagues have argued that the increase in reward threshold that occurs in the period before daily drug injections or after termination of heavy drug use would devalue the rewarding effect of injections taken during that time (tolerance), leading individuals to increase the total amount of drug taken. In a recent study, they found during a period of prolonged daily access to cocaine (6 h)
that the threshold for intracranial self-stimulation was higher than baseline 3–22 h after each session, and that cocaine intake increased over days in the first hour of the daily self-administration sessions. These data may help to account for the escalation of drug intake that occurs during periods of heavy daily use. To compound the situation, however, are the results of the studies mentioned above, showing that when drug administration is infrequent (say, weekly), the increase in reward threshold before injections diminishes, and there is a progressive decrease in threshold induced by injections of the drug themselves. These progressive decreases in drug-induced threshold are not seen when the drugs are administered daily. Such decreases in threshold when they did occur would be expected to increase the rewarding value of the drug and lead animals to work harder to obtain the drug (sensitization). Whatever the neurobiological adaptations that underlie the initial tolerance and subsequent sensitization of reward processes as assessed by brain stimulation reward, it is likely that it is the latter that play a critical role in relapse by increasing the sensitivity of former drug users to events such as drug-related cues, priming injections of drugs, and stressors. Tolerance of the rewarding effects of a drug occurs during periods of intense exposure, whereas sensitization takes time to develop after termination of drug exposure. The challenge for those interested in relapse is to identify the neurobiological adaptations underlying this increased sensitivity that develop over time after drug exposure.

THE REINSTATEMENT PROCEDURE

In the reinstatement model of relapse, rats are trained to self-administer a drug by pressing one of two levers, and are then exposed to a period when the drug is no longer available but they are free to try to obtain it (a period of extinction training). Eventually, animals stop responding on the lever and to many of the stimuli previously associated with drug delivery, making it possible to test for the ability of various events to reinitiate drug seeking. During these tests for the reinitiation of drug seeking, animals are given access to the levers, but the drug remains unavailable. It is on this background of renewed drug-seeking or relapse that we are able to begin a search for pharmacological and neurochemical manipulations that block or attenuate such behavior. Most of our and others' early work on drug-induced reinstatement of drug seeking was carried out using a within-session procedure, in which self-administration, extinction, and reinstatement tests are made within the same session. More recently, we have demonstrated that a between-session procedure can be used to study factors involved in relapse to drug seeking after extended periods of abstinence, many days and weeks after the last exposure to drug. Using this procedure, the periods of self-administration training, extinction training, and reinstatement tests can be separated by days and weeks. This procedure has increased the face-validity of the reinstatement model of relapse in laboratory animals by allowing for the study of factors such as the extent and amount of initial exposure to drug-taking and the effect of the passage of time since last exposure on the susceptibility to relapse.

STRESS AND RELAPSE TO HEROIN SEEKING: THE RELATION TO RELAPSE INDUCED BY HEROIN ITSELF

In our initial work on stress-induced reinstatement, rats were trained to
self-administer heroin intravenously for two 3-hour sessions a day for 12 days and were then given extinction training for up to 28 days. Subsequently over a four day period, animals were given four tests for reinstatement in a counterbalanced order, after (1) an injection of saline, (2) an injection of heroin (drug priming), (3) an injection of morphine followed by the opioid receptor antagonist, naltrexone (precipitated withdrawal), and (4) 10 min of intermittent footshock (acute stress). Both heroin and footshock reinstated heroin seeking; precipitated withdrawal did not. After remaining in their home cages for 4 to 6 weeks, tests for reinstatement showed that both heroin and footshock effectively induced relapse.33

The original finding—that exposure to brief stress mimicked the effect of the priming injection of heroin, whereas the induction of an aversive state of withdrawal does not—led us to suggest that stress contributes to relapse by activating the same neural systems as those activated by heroin. The idea was soon dispelled, however, when we found that manipulations that effectively blocked the effects of priming injections of heroin were without effects on stress-induced relapse to heroin seeking. In one study, we compared the effectiveness of heroin priming injections, injections of the opioid receptor antagonist (naloxone), and footshock stress in rats trained to self-administer heroin and then maintained on continuous heroin treatment through the use of osmotic minipumps. During the first seven days of continuous heroin treatment, all animals received extinction training. In subsequent tests for reinstatement given under continuous heroin treatment, naloxone did not induce drug seeking and the effect of the heroin priming injections was minimal, but footshock stress strongly reinstated heroin-seeking. The critical finding from this experiment was that when animals were under opioid (heroin) maintenance sufficient to reduce the effectiveness of heroin injections, footshock stress retained its ability to instigate reinstatement of heroin seeking.34

In another set of experiments, we compared the effects of a number of receptor antagonists on heroin seeking induced by heroin and by footshock stress. Following a period of heroin self-administration, extinction sessions were given during which saline was substituted for heroin. In nine groups of animals, the effects on relapse induced by footshock or priming injections of heroin or saline were studied after pretreatment with either naltrexone, a D1-like dopamine receptor antagonist (SCH 23390), a D2-like dopamine receptor antagonist (raclopride), a mixed dopamine antagonist (flupenthixol decanoate), or saline. Naltrexone, flupenthixol, raclopride, and the highest dose of SCH 23390 attenuated heroin-induced relapse; only the mixed dopamine receptor antagonist, flupenthixol, attenuated footshock-induced relapse.35 These results provided strong evidence for the idea that the neurochemical events underlying stress- and heroin-induced relapse are not identical. Thus, although complete blockade of dopaminergic function was able to suppress the effects of footshock on heroin seeking, the greater sensitivity of the heroin priming injections to these antagonists led us to search for other systems that might be mediating the effect of stress on relapse to heroin seeking.

STRESS AND RELAPSE TO COCAINE AND OTHER DRUGS

Soon after finding that brief exposure to stress reinstated drug seeking in heroin-trained rats, we went on to determine whether similar effects would be seen in rats trained to self-administer other drugs. In cocaine-trained rats, footshock stress was as effective in inducing relapse as
it was in rats trained to self-administer heroin. Furthermore, the effects seem to be enhanced 4–6 weeks after the last exposure to cocaine. Similar effects were found in other laboratories in rats trained to self-administer nicotine and ethanol, though interestingly not in rats trained to lever press for food or sucrose solutions. These findings show that exposure to stress effectively reinstates drug seeking in animals experienced in the self-administration of several drugs of abuse of different pharmacological classes and demonstrate that the neurochemical systems involved in stress-induced relapse are different from those mediating the maintenance of the self-administration of particular drugs and relapse induced by reexposure to these drugs.

STRESS-INDUCED RELAPSE AND HORMONES OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

Acute exposure to many drugs of abuse activates the hypothalamic-pituitary-adrenal axis in a manner similar to acute exposure to stress. There is evidence that prior exposure to stress augments the responses to drugs of abuse and that exposure to stimulant drugs augments the acute responses to stress. There is also considerable evidence suggesting that the adrenal hormone, corticosterone, facilitates the initiation of the self-administration of psychostimulant drugs and ethanol, and that manipulations that decrease circulating levels of corticosterone also decrease instances of self-administration in rats trained to self-administer cocaine and ethanol. We examined, therefore, the role of the adrenal hormone, corticosterone, and corticotropin-releasing factor (CRF) in both stress- and heroin-induced relapse in animals trained to self-administer heroin after several days of extinction training. Tests for reinstatement were given after priming injections of saline and heroin and after intermittent footshock. To study the role of corticosterone, we tested animals after adrenalectomy, after chronic exposure to the corticosterone synthesis inhibitor, metyrapone (100 mg/kg, SC, twice daily), or after acute exposure to metyrapone. Neither complete absence of corticosterone induced by adrenalectomy (carried out after training) nor chronic suppression induced by daily exposure to metyrapone (from the beginning of extinction) nor acute suppression induced by a single injection of metyrapone (3 hours before testing) altered the reinstatement of heroin-seeking induced by footshock or heroin. In fact, these treatments appeared to potentiate the reinstatement by footshock. The suppression or loss of corticosterone would be expected to increase levels of CRF, suggesting that CRF systems might be important in the effects of stress. We therefore tested the effects of acute intracerebroventricular (ICV) injections of CRF (0.3 and 1.0 µg) and the CRF antagonist, alpha-helical CRF (3 and 10 µg) in animals trained under the same conditions and found that acute exposure to CRF reinstated heroin-seeking, whereas the CRF receptor antagonist, alpha-helical CRF, attenuated stress-induced relapse. Although occasional suppression of reinstatement by priming injections of heroin was observed, the effect of the CRF antagonist on reinstatement by heroin was not consistent. Together, these results suggested that stress-induced corticosterone is not required for either heroin- or stress-induced reinstatement, whereas CRF, the major brain peptide involved in stress, appears to play a major role in relapse to drug-seeking induced by stressors but not heroin.

We next examined the role of corticosterone and intracerebroventricular (ICV) injections of CRF on stress- and
c cocaine-induced reinstatement of cocaine seeking.\(^5\) In this study, we tested the effects of ICV injections of the potent CRF receptor antagonist, D-Phe CRF\(_{12-41}\), in animals that were either adrenalectomized, adrenalectomized with corticosterone replacement, or intact. Rats were allowed to self-administer cocaine for 10–14 days and were then placed on an extinction schedule during which saline was substituted for cocaine. Tests for reinstatement were given after presentation of intermittent footshock and after priming injections of saline and cocaine. Two main findings were obtained from these experiments. First, the CRF receptor antagonist, D-Phe CRF\(_{12-41}\), blocked footshock-induced reinstatement at all doses tested, whereas reinstatement by priming injections of cocaine was not consistently attenuated by pretreatment with the CRF receptor antagonist. Thus, CRF appears to play a critical role in stress-induced reinstatement but not in reinstatement induced by priming injections of cocaine. Second, footshock did not reinstate cocaine seeking in adrenalectomized animals, though it was effective in both intact animals and animals with corticosterone replacement. Furthermore, D-Phe CRF\(_{12-41}\) blocked footshock-induced reinstatement in adrenalectomized animals with corticosterone replacement. Thus, although reinstatement of cocaine seeking by footshock stress requires minimal, basal levels of corticosterone, stress-induced increases in corticosterone do not play a role in the effect. These data show that the CRF receptor antagonist must be acting at extra hypothalamic sites and not via the hypothalamic-pituitary-adrenal axis to mediate these effects on stress-induced reinstatement. We have also tested a non-peptidergic antagonist, CP-154,526 (butyl-[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-yl]-ethylamine), that can be administered systemically. This compound effectively attenuated stress-induced relapse in both heroin- and cocaine-trained animals.\(^5\)

Studies done in rats trained to self-administer ethanol have provided a remarkably similar set of results; adrenalectomy had no effect on stress-induced reinstatement, whereas both ICV injections of D-Phe CRF\(_{12-41}\) and systemic injections of CP-154,526 attenuated footshock-induced reinstatement of ethanol seeking.\(^5\)

CRF SYSTEMS OF THE BRAIN AND STRESS-INDUCED REINSTATEMENT

The data from these experiments using ICV injections of the CRF-receptor antagonists demonstrate that CRF plays a role in stress-induced reinstatement that is independent of the hypothalamic-pituitary-axis, leading us to next determine which extra-hypothalamic sites might be critical. Two brain sites that contain CRF receptors\(^5\) and that appeared likely to be involved in these effects are the amygdala and the bed nucleus of the stria terminalis (BNST). Both sites contain CRF-immunoreactive cells, and both have been implicated in central nervous systems responses to stressful events.\(^5\) For these reasons, we studied the effects of local injections of the CRF receptor antagonist, D-Phe CRF\(_{12-41}\), in the BNST and amygdala on footshock-induced reinstatement of cocaine seeking.

Rats were trained to self-administer cocaine and, after 5 drug-free days, were returned to the self-administration chambers and given daily extinction and reinstatement test sessions. To test the effects of D-Phe CRF\(_{12-41}\) on stress-induced reinstatement, rats were pretreated with vehicle or D-Phe in either the BNST or amygdala before being exposed to intermittent footshock stress. To test whether injections of CRF itself could induce reinstatement, rats were given a
vehicle or CRF in either the BNST or amygdala 15 min before the session. Injections of D-Phe into the BNST completely blocked footshock-induced reinstatement of cocaine seeking; injections of CRF into the BNST were sufficient to induce reinstatement. Injections of these compounds into the amygdala were without effect. These findings suggest that activation of CRF receptors in the BNST, but not in the amygdala, is critical for footshock-induced reinstatement of cocaine seeking. The findings are consistent with those of Davis and colleagues, suggesting that the BNST, and not the amygdala, is responsible for the unconditioned-emotional changes induced by CRF. Lesions of the BNST but not the amygdala interfered with the effects of CRF administered ICV, and intra-BNST injections of CRF enhanced startle responses.

Injections of CRF and its receptor antagonist into the BNST would mimic and block, respectively, the effects of CRF released locally from cells intrinsic to this structure. CRF could be released in the BNST by cells intrinsic to that structure and known to contain CRF or via a projection from the amygdala. In addition to CRF-containing cells intrinsic to the BNST, there is a CRF-containing projection from the amygdala to the BNST, and stress-induced activation of this pathway could be partly responsible for changes in CRF activity in the BNST following exposure to stress. To determine the involvement of the amygdala projection in footshock stress-induced reinstatement of cocaine seeking, we tested rats after the blockade of neurotransmission by infusion of the sodium channel blocker, tetrodotoxin (TTX), in amygdala into one hemisphere, and after the blockade of CRF receptors in the BNST in the other. The logic was that if CRF pathways from amygdala to BNST are involved, the intra-amygdala TTX infusion would interrupt transmission from amygdala to BNST in one hemisphere, while the intra-BNST D-Phe infusion would block the effect of CRF released in the BNST in the other hemisphere. Together, the two infusions would lead to a functional inactivation or ‘lesion’ of the pathway. Animals were also tested after unilateral injections of either TTX or D-Phe, alone, into the amygdala or BNST, respectively. Footshock reinstated cocaine seeking following the unilateral injections, but following the injection of both compounds into their respective sites in each hemisphere, the effect of footshock was attenuated. This simultaneous injection of TTX in the CeA and D-Phe in the BNST did not, however, block footshock stress-induced reinstatement completely, suggesting that CRF-containing neurons intrinsic to the BNST are also involved.

NORADRENERGIC SYSTEMS OF THE BRAIN AND STRESS-INDUCED REINSTATEMENT

The finding that CRF receptors in the BNST are critically involved in stress-induced reinstatement of drug seeking led to two obvious questions: how does exposure to stress activate CRF systems of the brain, and what pathways are critical to the effect? One neurotransmitter that had a high probability of being involved was noradrenaline (NA). NA projections to the forebrain arise from two groups of cells, the locus coeruleus (LC) and the lateral tegmental nuclei. The LC neurons send heavy projections via the dorsal NA bundle to the cortex, hippocampus, and hypothalamus, and are thought to partly mediate responses to stress. The lateral tegmental neurons innervate, via the ventral NA bundle, the amygdala, septum, and nucleus accumbens and send a dense projection to the BNST, but their role in stress has only recently begun to be
The CRF system is known to interact with the LC-NE system in the modulation of stress responses; ICV or intra-LC injections of CRF activate the LC neurons and elicit stress-like responses, whereas injections of CRF receptor antagonists attenuate responses to certain stressors. In addition, there is evidence for CRF-NA interactions in terminal regions of NA neurons in the BNST. These findings, and those of attenuation of footshock-induced reinstatement by CRF receptor antagonists, point to a role for the LC-NE system in relapse induced by stressors.

In our initial attempt to explore the role of NA in stress-induced relapse, we administered the alpha-2 adrenoceptor agonists, clonidine, lofexidine, and guanabenz, systemically. Low doses of these compounds preferentially activate alpha-2 autoreceptors, resulting in decreased NA cell firing and release. Subsequently, we tried to identify which NA systems were critical for the effect of footshock.

In cocaine-trained rats, we found that clonidine attenuates footshock- but not cocaine priming-induced reinstatement. Similar findings were obtained with lofexidine and guanabenz. In addition, we showed using in vivo microdialysis that the three alpha-2 adrenoceptor agonists decreased NA release in the prefrontal cortex and amygdala under basal conditions and blocked footshock stress-induced NA release in these areas. In heroin-trained rats, we found that systemic and ICV injections of clonidine effectively block reinstatement induced by intermittent footshock, pointing to a central site of action. Furthermore, LC infusions of clonidine or its charged analogue, ST-91, had minimal effect on footshock stress-induced reinstatement. Therefore, we next studied the contribution of the lateral tegmental NA neurons by using selective 6-OHDA lesions to the ventral NA bundle after heroin self-administration training. The lesions, which reduced NA levels in the hypothalamus and the BNST by 60–70%, had no effect on responding during the extinction of heroin-taking behavior, but significantly attenuated footshock stress-induced reinstatement. Therefore, because the lateral tegmental NA neurons have major projections to the BNST and CeA, we proposed that footshock stress increases NA release in the BNST and CeA.

In order to test the hypothesis that stress induces reinstatement of drug-seeking by increasing NA release in BNST and CeA, we investigated the effects of bilateral infusions of a cocktail of beta-1 and beta-2 NA receptor antagonists into either the BNST or the region of the CeA on footshock-induced reinstatement of response in cocaine-trained rats. We then compared the effects of such infusions on reinstatement induced by priming injections of cocaine.

Rats were trained to self-administer cocaine and, after a 5–7 day drug-free period, were given extinction sessions followed by a test for footshock stress-induced (15 min of intermittent footshock, 0.8 mA) or cocaine-induced (20 mg/kg IP) reinstatement. Before the test, different groups of rats were given bilateral infusions of one of four doses of a cocktail of the beta-1 and beta-2 receptor antagonists, betaxolol and ICI 181,555 (vehicle, 0.25, 0.5, and 1 nmol of each compound in 0.5 µl) either into the BNST or CeA. We observed a dose-dependent reduction of stress-induced reinstatement after infusions into the BNST and a complete blockade of stress-induced reinstatement after infusions into the CeA at all doses tested. The same treatments did not block cocaine-induced reinstatement when given at either site. These data suggest that stress-induced NA activation in the BNST and the region of the CeA is critical to relapse to drug seeking induced by stress,
but not to relapse induced by priming injections of cocaine. Furthermore, we hypothesize that noradrenergic activity leads to activation of CRF neurons in these regions.

The finding that the behavioral effect of beta-NA antagonists was dose-dependent in the BNST but not the CeA region suggests that the CeA modulates the activity of BNST in response to stressful stimulation. Although it is known that there are NA projections to both amygdala and BNST and that there are CRF containing cells in both regions, as discussed above, the amygdala sends a dense CRF projection to the BNST. It is possible, therefore, that even low doses of NA antagonists infused in the CeA region could have had profound effects on reinstatement because they could have prevented the release of CRF in the BNST.

THE ROLE OF THE PREFRONTAL CORTEX IN STRESS-INDUCED REINSTATEMENT

As mentioned previously, a major finding from recent studies on reinstatement is that different constellations of neurochemical events and anatomical pathways mediate relapse induced by stress, drugs, and drug-related cues. It has been found, for example, that cocaine cue-induced reinstatement depends on the integrity of the basolateral amygdala (BLA) and mesocorticolimbic dopaminergic projections to this region, whereas reinstatement induced by cocaine does not; rather, the latter is mediated by mesocorticolimbic dopamine system and dopaminergic/glutamatergic interactions in the nucleus accumbens and ventral pallidum. As discussed in the present review, we have found brain CRF and NA systems of the amygdala and BNST to be critically involved in stress-induced reinstatement. In spite of these clear dissociations, the systems that preferentially mediate reinstatement of drug seeking by cues, drugs, and stressors are intimately connected. It is likely, therefore, that activation within one subsystem can affect other subsystems and may lead finally to the engagement of a common pathway that controls the reinitiation of response in all these circumstances. Because the medial prefrontal (mPFC) and orbitofrontal cortex (OFC) have been implicated in both cue- and drug-induced reinstatement in laboratory animals and craving human addicts, we explored the role of these regions in stress-induced reinstatement.

Groups of rats were trained to self-administer cocaine and after 10 drug-free days were exposed to extinction and reinstatement test sessions. In one set of experiments, the effects of inactivation of the prelimbic (PL), infralimbic (IL), or orbitofrontal cortex (OFC) by tetrodotoxin (TTX, 5 ng/0.5 μl/side) on reinstatement induced by footshock or priming injections of cocaine were determined. In a second set of experiments, the effects of infusions of the D1-like and D2-like dopamine receptor antagonists (SCH 23390 and raclopride) were studied using the same methods. TTX infusions into the PL cortex blocked both footshock and cocaine-induced reinstatement. TTX into OFC attenuated footshock-induced reinstatement but had no effect on cocaine-induced reinstatement. Infusions into IL were ineffective. Infusions of SCH 22390 (0.25 μg/0.5 μl/side) into either PL or OFC blocked footshock-induced reinstatement, whereas raclopride (5 μg/0.5 μl/side) had no effect on footshock-induced reinstatement in either area. These results suggest that the PL and OFC regions form part of the circuitry mediating the effects of footshock stress on reinstatement of drug seeking and, combined with those of others, that the PL region may be a common pathway for cue, drug, and footshock stress-induced reinstatement of drug seeking. Figure 1
provides a diagrammatical summary of the pathways implicated in stress-induced reinstatement.

**FIGURE 1.** Diagram representing present knowledge of the systems of the brain critical for the induction of reinstatement by footshock stress: CeA (central amygdala) and BNST (bed nucleus of the stria terminalis) — CRF (corticotropin-releasing hormone rich regions); LTg (noradrenergic [NA] cell groups of the lateral tegmental nuclei); A8 and A10 (dopaminergic [DA] cell groups), projecting to mPFC (medial prefrontal cortex). BLA (basolateral amygdala) and mPFC and the DA projections are critical for cue-induced reinstatement; NAc (nucleus accumbens), VP (ventral pallidum) and mPFC are critical for drug-induced reinstatement.

**WHAT IS THE BASIS OF THE REINITIATION OF DRUG SEEKING INDUCED BY ACUTE EXPOSURE TO STRESS?**

The observation that brief exposure to stress reinstates drug-seeking behavior implies a change in the motivational state of the animal that alters responses to stimuli in its environment. Traditionally, the term motivation is invoked by the observation that a particular goal-directed behavior, such as food seeking, occurs at some times and not others, with more or less vigor and persistence. The ease with which a behavior is engaged by environmental stimuli, its persistence, and the energy expended to obtain the goal all appear to depend on internal changes that alter stimulus effectiveness and readiness to act.

On the basis of our studies showing that a priming injection of previously self-administered drug can reinstate drug seeking, we have argued that the priming injection acts to renew the significance or salience of the drug-related environmental stimuli drawing the animal to approach the lever and engage in lever pressing. Thus, after extinction, a priming injection of the previously self-administered drug (and presumably exposure to stress) can be said to renew the salience of the lever and surrounding stimuli. We have used the conditioned place preference (CPP) procedure to explore this hypothesis directly.

In this procedure, a particular stimulus complex, or environment, is paired with the effects of the drug, without the animal having to learn to make a response to obtain the drug, and a second environment is explicitly paired with the absence of the drug. On the test trial, the animal is allowed, while in a drug-free state, to move freely between the area previously paired with drug and the non-drug environment. If the animal stays longer in the presence of stimuli previously paired with the drug (the conditioned cues), these stimuli can be said to have acquired secondary or conditioned incentive properties through pairings with the rewarding effects of the drug.

This procedure can test the idea that a priming injection of the drug used to develop the CPP, given after extinction conditions, acts to restore the salience or attractiveness of the environment previously paired with drug. We have argued that if, after extinction of the CPP, the rat
is given a test trial after a priming injection of the drug and if the animal stays longer in the presence of the cues previously associated with the drug, the priming injection can be said to increase the salience, attractiveness, or positive valence of those cues. In fact, we have found just that; following the extinction of the CPP by repeatedly pairing both compartments with saline or by giving repeated tests in the absence of drug, the former preference for the "drug-paired" compartment can be completely reinstated by giving a single injection of the drug before the test.\textsuperscript{91-93}

Recently, this same idea has been explored with respect to the effects of stress. Rats were trained to lever press to obtain access to a 10% ethanol-solution; ethanol-reinforced responses were accompanied by the onset of a light stimulus that served as a conditioned stimulus paired with ethanol. After extinction training given in the absence of both ethanol and the light, lever pressing was reinstated by response-contingent presentations of the conditioned light cue or by exposure to brief intermittent footshock. Animals tested after footshock with the light cue present showed greatly enhanced response compared to animals tested with either footshock or the light cues only.\textsuperscript{94} Interestingly, it was also found that although the effects were additive, each was dependent on different neurochemical systems. The effects of footshock stress were blocked by ICV infusions of a CRF receptor antagonist, whereas the effect of conditioned ethanol cues was blocked by injections of the opioid receptor antagonist naltrexone. It required injections of both antagonists to block the enhanced reinstatement effect induced by the footshock/conditioned cue combination. Again, these data suggest that the state of stress induced by prior exposure to footshock enhanced the salience and effectiveness of the drug-related cues to engage the animal in drug seeking behavior.

\section*{THE DURATION AND AMOUNT OF DRUG EXPOSURE ENHANCES STRESS-INDUCED REINSTATEMENT OF DRUG SEEKING}

Evidence has accumulated that shows that the motivation underlying drug seeking induced by events that precipitate relapse is intensified by the duration and amount of previous exposure to a drug. Two studies bear on this issue. It has been shown, for example, that compared to rats given 1 h of heroin self-administration training daily, rats given 11 hours show enhanced response to footshock stress in tests for reinstatement.\textsuperscript{95} Similarly, it has been found that in rats trained to lever-press for ethanol, an extended period of exposure to ethanol vapor led to enhanced sensitivity to footshock-induced reinstatement.\textsuperscript{94} These results point to long-lasting effects of previous drug exposure on subsequent sensitivity to stress-induced drug seeking. There is increasing evidence\textsuperscript{14,96} that such long-lasting experience-dependent changes involve modifications in connectivity and effectiveness of the circuitry that take time to develop following the termination of drug exposure, but this extensive literature cannot be covered here.

\section*{TIME SINCE LAST EXPOSURE TO DRUG TAKING ENHANCES STRESS-INDUCED REINSTATEMENT OF DRUG SEEKING}

A second important finding from the research is that animals become more susceptible to events that induce drug-seeking with the passage of time after withdrawal of the drug. It has been shown, for example, that following withdrawal from cocaine self-administration, reinstatement by cocaine-related cues is enhanced by the passage of time. In recent studies, different groups of cocaine-trained rats were tested for extinction and cue-induced relapse at a number of time points, 1–60 days,
following the termination of drug self-administration sessions. A progressive and dramatic increase was observed in the number of responses made during extinction and on the test for reinstatement as a function of time since drug termination, suggesting an enhanced motivational effect of cues as a function of time.97

A similar type of study has been done on relapse induced by footshock stress. Rats were trained to self-administer heroin. After termination of drug-taking, different groups were given extinction sessions and tests for footshock-induced reinstatement of response at different time points after training.98 It was found that response during extinction and in tests for footshock-induced reinstatement peaked around 12 days after termination of drug treatment but remained elevated in tests carried out at 25 days. As in the study of cocaine-trained rats, extinction response and reinstatement were low one day after drug-taking but increased over days. The fact that extinction response did not increase after 25 days, as it had in cocaine-trained rats, is likely due to differences in the long-lasting effects of opioids and stimulants on specific systems. The findings that the effects of both stress and cues on reinstatement of drug seeking increase with the passage of time after withdrawal and that stress augments the effectiveness of cue-induced reinstatement94 point to the potential for even greater effects of stress in the presence of salient-conditioned drug cues with time after withdrawal. Such findings lend support to the idea that individuals remain vulnerable to stress-induced relapse for extended periods after abstinence. The implications of such findings for treatment of addiction are that whatever approach is taken, treatment will have to be multifaceted and maintained over an extended period of time after the initial termination of drug use.

Studies in laboratory animals have shown that acute exposure to footshock stress reinstates drug-seeking behavior in animals trained to self-administer a number of drugs of abuse from different classes, including heroin, cocaine, ethanol, and nicotine. These studies are providing insight into the systems of the brain involved in the mediation of stress-induced relapse. In particular it has been found that the neurochemical systems mediating stress-induced relapse are different from those mediating relapse induced by drugs and drug-related cues.

Extrahypothalamic corticotropin-releasing factor (CRF)-rich regions of the brain in the amygdala and bed nucleus of the stria terminalis (BNST) are critically involved in stress-induced relapse in animals trained to self-administer heroin, cocaine, and ethanol. ICV infusions of CRF receptor antagonists block stress-induced reinstatement of heroin, cocaine, and ethanol seeking without affecting relapse induced by priming injections of the drugs themselves. Studies in cocaine-trained animals show that blockade of CRF receptors in the BNST prevents stress-induced relapse without affecting reinstatement induced by priming injections of cocaine.

Studies in heroin- and cocaine-trained rats have shown that systemic injections of low doses of alpha-adrenoceptor agonists, sufficient to reduce firing in noradrenergic (NA) neurons, and the NA neurons projecting from the lateral tegmental nuclei of the lower brain stem are critically involved in stress-induced reinstatement. Furthermore, it has been found in cocaine-trained rats that the blockade of beta-NA receptors in the amygdala and BNST prevents stress-induced reinstatement without affecting cocaine-induced reinstatement. These data and previous anatomical findings support the idea that
stress-induced NA release in these regions activates CRF neurons leading to reinstatement of drug seeking.

Taken together these findings support the view that no single pharmacological or behavior approach to treatment of addiction is likely to be sufficient.

Finally, there is evidence that stress-induced drug seeking is enhanced by the amount of previous exposure to drugs and that it increases with the passage of time after withdrawal, as is drug seeking induced by drug related cues. In addition, drug seeking induced by drug-related cues is enhanced by a brief exposure to stress before the session, suggesting that the state induced by exposure to stress augments the motivational significance of these cues.

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