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Preclinical validation of a novel cocaine exposure therapy for relapse prevention

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Abstract

Background: Cocaine not only induces intense rewarding sensations but also craving for more cocaine particularly during abstinence, an effect that contributes, together with other factors, to relapse. Here we sought to prevent this effect by extinguishing the conditioned interoceptive cues of cocaine which are thought to be acquired during repeated cocaine use.

Methods: Cocaine-induced craving was studied in rats using the well-validated model of drug-primed reinstatement of cocaine seeking. To extinguish the conditioned interoceptive effects of cocaine, rats received daily repeated cocaine priming in the absence of drug reinforcement.

Results: Cocaine-primed reinstatement of cocaine seeking dramatically decreased with repeated cocaine priming regardless of the testing dose and even following a history of extended access to cocaine self-administration. The extinction of cocaine-primed reinstatement of cocaine seeking was enduring, generalized to stress – another major trigger of drug craving and relapse – and was context-dependent.

Conclusions: These findings clearly show that it is feasible to prevent the ability of cocaine and stress to induce cocaine seeking using an approach designed to extinguish the drug's conditioned interoceptive cues. Though this preclinical extinction approach has limitations that will need to be overcome in future research (i.e., its context-dependency), it may nevertheless represent a promising basis for the development of a novel exposure therapy against cocaine relapse.

Keywords

Relapse prevention; interoception; extinction; reinstatement; exposure therapy; insula

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Cocaine not only evokes intense rewarding sensations but also induces craving for more cocaine. This latter effect is especially obvious in individuals with cocaine addiction and is thought to contribute, together with other factors, to relapse after abstinence (1-3). Cocaine-induced craving can be studied in animals in the drug reinstatement model (4). In this model, responding for the drug (e.g., pressing a lever) is first extinguished by discontinuing drug reinforcement and then reinstated by drug priming (i.e., non-contingent re-exposure to the drug), drug-associated cues or stress. Importantly, during reinstatement testing, responses continue to be unrewarded as during extinction and, therefore, reflect genuine drug seeking (5).

Over the past two decades, great strides have been made in understanding the neurobiology of cocaine-primed reinstatement (6-13), but to date this knowledge has not translated into significant treatment advances. In fact, little progress has been made in clarifying the underlying behavioral mechanisms of cocaine-primed reinstatement. According to an interoceptive conditioning hypothesis, with repeated cocaine use, some of the peripheral interoceptive effects of cocaine would acquire strong conditioned properties for signaling drug availability and for eliciting drug seeking (14-22). During reinstatement testing, the acute re-experience of these conditioned interoceptive effects would contribute to the reinstatement of cocaine seeking, a phenomenon that has been demonstrated very recently (19). Interestingly, this hypothesis is consistent with recent research involving the interoceptive inputs to the insular cortex in drug craving and/or relapse in both humans (23) and animals (24, 25).

One unique prediction of the interoceptive conditioning hypothesis – with potential major translational implications – is that repeated cocaine priming should eventually lead to a progressive loss of its conditioned interoceptive effects. Indeed, during extinction, since the interoceptive cues generated by cocaine no longer predict response reinforcement, these cues should progressively lose their conditioned predictive value and thus their ability to reinstate extinguished drug responding. This effect is called extinction of drug priming to distinguish it from extinction of operant behavior produced by discontinuing drug reinforcement. In addition, if stress reinstates cocaine seeking by mimicking some of the drug's interoceptive effects, which has been proposed previously (14), then the extinction of cocaine-primed reinstatement should generalize to stress-primed reinstatement. The generalization to stress is another unique prediction of the interoceptive conditioning hypothesis. The main goal of the following series of experiments was to test these different predictions. Since extinction of conditioned behaviors, including drug self-administration, is generally context-dependent (26, 27), another goal of the present study was to determine whether and to what extent extinction of cocaine-primed reinstatement is also context-dependent.

Methods and Materials

A total of five experiments were conducted: experiments 1 and 2 were conducted at The Scripps Research Institute (La Jolla, CA, USA), and experiments 3-5 were conducted at the CNRS and the Université de Bordeaux (Bordeaux, France).

Animals and housing

Thirty naive, young adult (2.5 months old at the beginning of the experiments), male, Wistar rats (Charles River, France or USA) were used in the present study. The rats were housed in groups of two or three and maintained in a light- and temperature-controlled vivarium. All behavioral testing occurred during the dark phase of the light-dark cycle. Food and water were freely available. Three days after their arrival in the laboratory, rats were prepared with catheters for cocaine self-administration in the jugular vein, as described previously (14, 28).

All procedures were conducted in conformity with the National Institutes of Health Guide for the Care and Use of Laboratory Animals or in accordance with institutional and international standards of care and use of laboratory animals [UK Animals (Scientific Procedures) Act, 1986; and associated guidelines; European Communities Council Directive 86/609/EEC, 24 November 1986; French Directives concerning the use of laboratory animals (décret 87-848, 19 October 1987)].

Surgery

Anesthetized rats [experiments 1 and 2: halothane-oxygen mixture, 1.0-1.5% halothane; experiments 3-5: mixture of xylazine (15 mg/kg, i.p.) and ketamine (110 mg/kg, i.p.)] were surgically prepared with silastic catheters in the right jugular vein that exited the skin in the middle of the back about 2 cm below the scapulae. After surgery, catheters were flushed daily with 0.2 ml of a sterile antibiotic solution containing heparinized saline (280 IU/ml) and Timentin® (100 mg/ml; SmithKline Beecham Pharmaceuticals, Philadelphia, PA, USA) or ampicillin (Panpharma, France). When a catheter leak was suspected, the patency of the catheter was checked by intravenous administration of methohexital sodium (1%, Eli Lilly, Indianapolis, IN, USA) or etomidate (1 mg/kg, Braun Medical, France), a short-acting anesthetic. Behavioral testing began 7-10 days after surgery.

Drug

Cocaine HCl was obtained from the National Institute on Drug Abuse (experiments performed in the USA) or from the Coopération Pharmaceutique Française (France) and was dissolved in sterile physiological saline (0.9%).

Experiments 1 and 2

Acquisition and extinction of cocaine self-administration—Cocaine self-administration was conducted in operant chambers that were previously described (14). One week after surgery, operant-naïve rats were trained to self-administer intravenous cocaine (0.25 mg per injection) for 21 (experiment 2) to 28 sessions (experiment 1) on a fixed-ratio 1 time-out 20 s schedule of reinforcement, as described elsewhere (14). Each daily self-administration session lasted 2 h. Following acquisition and stabilization of cocaine self-administration (variation between the last three self-administration sessions less than 15%), rats were tested under extinction for several days. Briefly, during extinction, responding on the active lever turned on the time-out cue above the lever but was no longer rewarded by cocaine. Each daily extinction session lasted 2 h. Daily extinction testing continued until average responding on the active lever decreased below 20 responses per hour (i.e., within a maximum of 18 extinction days).

Footshock stress

Footshock (alternating current; 0.86 mA; 0.5 s trains) were delivered through a scrambler (Coulbourn Instruments) to the grid floor of the operant chamber (14). They were administered intermittently for 15-min according to a variable interval schedule (mean interval: 40 s; range: 10-70 s).

Experiment 1: Extinction of cocaine-primed reinstatement

Seven rats ($n = 7$) were tested in this experiment. The effects of repeated cocaine on cocaine-induced reinstatement took place after extinction of cocaine self-administration. On the first day, rats received a vehicle injection (1 ml/kg, i.p.) immediately before the regular daily 2 h extinction session. On the following 6 days, they received one priming dose of cocaine (15 mg/kg, i.p.) immediately before extinction testing.

Experiment 2: Effects of extinction of cocaine priming on stress-primed reinstatement

Eleven rats were tested in this experiment. After extinction of cocaine self-administration, one group of rats ($n = 6$) was tested for stress-induced reinstatement of cocaine seeking both before and after repeated cocaine priming (as described above). Stress-primed reinstatement was precipitated by exposing rats to intermittent electric footshock for 15 min immediately before the regular daily 2 h extinction session. Each stress testing day was preceded by a baseline day during which the 15 min period of intermittent footshock was replaced by a 15 min period without footshock. Another group of rats ($n = 5$) was tested as the first group, except that they received vehicle instead of cocaine between the two tests of stress-induced reinstatement.

Experiment 3: Extinction of cocaine-primed reinstatement following extended access to cocaine self-administration

Cocaine self-administration was conducted in operant chambers that were previously described (28). One week after intravenous surgery, operant-naïve rats were trained to self-administer intravenous cocaine (0.25 mg per injection) during 32 daily sessions on a fixed-ratio 1 schedule of reinforcement. Each daily self-administration session lasted 6 h (instead of 2 h as in experiments 1 and 2). All self-administration sessions began with extension of the lever. No noncontingent injections of cocaine were given, except on rare occasions when a subject failed to respond within the first 30 min in which case it received two passive injections approximately 20 s apart. To favor incubation of cocaine craving, rats were tested for cocaine-induced reinstatement following 10 days of withdrawal from extended access to cocaine self-administration (5). To extinguish the priming effect of cocaine, a total of 10 daily tests of cocaine-primed reinstatement were performed. Each priming test day consisted of an initial 90-min period of extinction during which lever pressing was no longer rewarded by cocaine delivery. This extinction period was followed by 3 priming doses of cocaine (0.25, 0.5 and 1 mg) administered intravenously every 45 min by increasing the duration of the injection (4, 8 and 16 s), as described previously (28). Lever pressing remained unrewarded following each priming dose of cocaine. Cocaine-induced drug-seeking behavior (i.e., non-reinforced lever pressing) was counted for 45 min after each dose and compared to the 45-min period preceding the first priming dose (considered as the dose 0). To assess the persistence of extinction of cocaine priming, reinstatement testing was interrupted for 10 days (during which time rats were left undisturbed in their home cage) and was then renewed once. Note that the present study was not designed to measure the exact magnitude of cocaine intake escalation due to extended drug access. To do that would have required to first baseline rats under limited access conditions.

Experiment 4: Context-specificity of extinction of cocaine-primed reinstatement

Ample evidence demonstrates that extinction of conditioned behaviors is context-dependent and therefore does not necessarily generalize across different contexts (26, 27). To test the context-specificity of extinction of cocaine-primed reinstatement, a separate group of rats ($n = 10$) with a long history of cocaine self-administration in one context (context A; number of FR training sessions: 82-98; session length: 2-3h) received in a novel context (context B) repeated cocaine priming injections for 8 consecutive days, as described in experiment 3. Specifically, each priming test day consisted of an initial 90-min period of extinction followed by 3 priming doses of cocaine (0.25, 0.5 and 1 mg) administered intravenously every 45 min by increasing the duration of the injection (4, 8 and 16 s). Lever pressing remained unrewarded following each priming dose of cocaine. Context B differed from context A along several dimensions: identity of the experimenter (context A: Claudia Mihindou; context B: Serge Ahmed); type of experimental room (context A: room 21; context B: room 22); ambient room illumination (context A: red light; context B: white dim light); ambient chamber noise (context A: none; context B: background white noise) and

smell (context A: none programmed; context B: anis). After significant evidence for extinction of cocaine-primed reinstatement in context B, rats were returned to context A and tested for cocaine-primed reinstatement in this context.

Experiment 5: Role of non-reinforced responding in extinction of cocaine-primed reinstatement

This experiment addresses the role of non-reinforced lever responding during drug priming in extinction of cocaine-primed reinstatement. To examine this possibility, we measured the effects of repeated drug priming in the absence of actual responding (i.e., lever retracted). One week after intravenous surgery, rats ($n = 8$) were trained to self-administer cocaine during 30 sessions as described in experiment 3 except that session length was 3h. After acquisition and stabilization of cocaine self-administration, rats were tested for cocaine-primed reinstatement during 7 consecutive days, as described above except that only one priming dose of cocaine was tested. Specifically, each priming test day consisted of an initial 90-min period of extinction followed by 1 priming dose of intravenous cocaine (1 mg). Lever responding following drug priming (i.e., the 45-min period following the priming dose of cocaine) was allowed only on the first and last priming days (i.e., Pr1 and Pr7). During the other priming days, lever responding following drug priming was prevented by lever retraction.

Data analysis

Drug self-administration data and drug-primed reinstatement data were subjected to one-way or two-way analysis of variance (ANOVA), followed by appropriate *post hoc* comparisons using the Newman-Keuls's test. Due to large interindividual variability (coefficient of variation > 100%), stress-primed reinstatement data were subjected to a square root transformation to meet the requirements of homogeneity of error variance. Square-root transformed data were then subjected to one-way or two-way ANOVA, followed by appropriate *post hoc* comparisons using the Newman-Keuls's test. Statistical analyses were performed using Statistica, version 7.1 (Statsoft, Inc France).

Results

Following extinction of stable cocaine self-administration (0.25 mg/injection, 2 h per day for 4-5 weeks), rats ($n = 7$) were tested with repeated daily, passive re-exposure to cocaine or drug priming (15 mg/kg, i.p.; for other details, see Methods). Cocaine strongly reinstated cocaine seeking during the first priming, as compared with vehicle [$F(1, 6) = 8.22$, $P < 0.05$; Fig.1A]. As predicted from the interoceptive conditioning hypothesis of drug reinstatement, this reinstating effect decreased with repeated cocaine priming [$F(5, 30) = 6.87$, $P < 0.01$; Fig.1A]. To assess whether this loss of cocaine effects on reinstatement generalizes to stress, two separate groups of rats were trained and then tested as in the first experiment, except that they were tested for stress-induced reinstatement both before and after repeated daily re-exposure to cocaine (group COC, $n = 5$) or vehicle (group VEH, $n = 6$) (Fig.1B). As shown in Fig.1C, stress dramatically reinstated cocaine seeking above the extinction baseline level in a similar manner in both groups of rats [$F(1, 9) = 5.79$, $P < 0.05$]. However, after repeated daily re-exposure to cocaine and extinction of its priming effect [Drug priming x Group interaction: $F(4, 36) = 6.78$, $P < 0.01$; Fig.1D], stress totally lost its ability to reinstate cocaine seeking [Stress x Group interaction: $F(5, 30) = 19.35$, $P < 0.01$; Fig.1E].

The generality of the major outcome of the above experiments is possibly limited by several factors. First, rats had relatively limited access to cocaine self-administration before reinstatement testing. Previous research has amply demonstrated that following more extended access to cocaine self-administration, rats are more likely to escalate drug

consumption (29), to work harder (30) and to take more risk to seek or obtain cocaine (31). There is now also strong evidence that the ability of cocaine to reinstate cocaine seeking after extinction is increased following extensive cocaine self-administration (28, 32-34). It is thus important to determine whether and to what extent the rapid extinction of cocaine priming seen in rats with limited exposure to cocaine can generalize to rats with more extended drug exposure. Second, only one priming dose of cocaine was tested. It is possible that the extinction of cocaine craving is a dose-dependent phenomenon that does not necessarily occur at higher doses. Third, as in the majority of previous research in the field, the route of administration during reinstatement testing (i.e., i.p.) differed from the route of drug self-administration (i.e., i.v.). In contrast, in human cocaine users, relapse does not generally involve a change in route of administration. Finally, the above experiments did not document the long-term persistence of the extinction of cocaine craving. To address all of these potential limitations, rats ($n = 12$) were given extended access to cocaine self-administration (i.e., 6 h per day, 5-6 days per week for 6-7 weeks compared with 2 h per day, 5-6 days per week for 4-5 weeks). Rats were then tested for cocaine-induced reinstatement with different intravenous doses of cocaine (for additional information, see Experiment 3 in Methods and Materials). The persistence of extinction of cocaine craving was measured following a 10-day period of interruption from repeated drug priming.

As expected, extended access to cocaine was associated with a dramatic increase in drug consumption [$F(31, 341) = 33.69$, $P < 0.01$; Fig.2A]. This dramatic increase probably reflects both initial acquisition of operant behavior (i.e., probably first week) and escalation of cocaine intake per se. As a result, the total amount of self-administered cocaine was approximately four-fold greater than in the first two experiments (1832.6 ± 65.9 versus 432.2 ± 51.0 mg/kg). During the first reinstatement day (Priming 1 or Pr1) following extended cocaine use, cocaine induced a robust, dose-dependent reinstatement of cocaine seeking [$F(3, 33) = 12.25$, $P < 0.01$; Fig.2B], comparable to that reported in a previous study using a similar methodology (28) (for the within-session pattern of cocaine-primed reinstatement, see Figure S1 in Supplement). However, following repeated drug priming (Pr10), there was a downward shift in the dose-effect curve for cocaine reinstatement in all rats [Repeated priming: $F(1, 10) = 22.48$, $P < 0.01$; Fig.3A], with the exception of one statistical outlier not included here that responded progressively more to cocaine (see Figure S2 in the Supplement). This outcome shows that the extinction of cocaine craving was independent of the priming dose. To better follow the temporal course of the extinction of cocaine craving, responses to the highest priming dose of cocaine were compared across repeated testing days. Cocaine-induced reinstatement decreased gradually with repeated daily testing [$F(9, 90) = 8.03$, $P < 0.01$; Fig.3B]. Importantly, this extinction was persistent as there was no significant evidence for spontaneous recovery following a 10-day period of interruption from repeated drug priming (Post) as compared with the last 3 priming days (Pre) [$F(1, 10) = 1.08$; Fig.3B, inset].

To test for the context-specificity of the priming extinction effect, rats that were trained for cocaine self-administration in context A were tested for repeated cocaine priming in a novel context B (see Methods and Materials, experiment 4). As expected, regardless of the dose tested, cocaine-primed reinstatement of cocaine seeking decreased with repeated drug priming in context B [Repeated priming: $F(1, 9) = 8.04$, $P < 0.02$; Fig.4A]. However, when returned to the initial training context A, cocaine recovered most of its priming efficacy and this priming response was present regardless of the dose tested [Context: $F(1, 9) = 34.42$, $P < 0.01$; Fig.4B]. Finally, lever responding during repeated drug priming was apparently not a critical factor for the priming extinction effect, since this effect occurred in most rats (7 out of 8) even when responding was prevented by lever retraction (see Methods and Materials, experiment 5; see Figure S3 in the Supplement).

Discussion

This study clearly demonstrates that the ability of cocaine to trigger drug seeking can be extinguished rather rapidly, regardless of the priming dose and the route of drug administration. In addition, the extinction of cocaine-primed reinstatement was observed even following a long history of cocaine self-administration that was shown in previous research to increase the reinforcing and incentive effects of cocaine (35). Importantly, the extinction of cocaine-primed reinstatement persisted for at least 10 days, generalized to stress – another well-known powerful precipitant of drug craving and relapse in humans with cocaine addiction (36) – and was context-dependent. The present study is consistent with a previous, though largely overlooked, study in rats showing that the priming effects of caffeine on cocaine seeking also decreased with repeated priming (37). Caffeine can generate cocaine-like interoceptive stimuli in rats (20). It is also partially consistent with a study in monkeys showing that the priming effect of low and moderate, but not high, doses of amphetamine can be extinguished with repeated testing in 2 of 3 individuals (38). Overall these results support the interoceptive conditioning hypothesis of drug reinstatement (14–16, 18–20) and suggest a novel approach to therapy against relapse to cocaine addiction after abstinence. This novel approach should not be confused with agonist-based substitution therapy (39–41) whose primary goal is to replace illegal cocaine use with a safe, long-acting drug substitute (e.g., continuous amphetamine administration) and not to prevent relapse by extinguishing the conditioned interoceptive effects of cocaine, as advocated here.

Traditional cue exposure therapies attempted to extinguish the conditioned effects of exteroceptive cues predictive of drug reinforcement (e.g., pictures of drug paraphernalia) with mixed clinical efficacy (42, 43). However, in real life, the interoceptive cues generated by the drug itself are more contiguous to and more predictive of drug use than exteroceptive predictive stimuli (19). In the laboratory, these differences would explain why drug- or stress-induced reinstatement is generally more intense than reinstatement induced by re-exposure to conditioned exteroceptive cues. Thus, the extinction of the conditioned interoceptive effects of cocaine in abstinent individuals may considerably increase the efficacy of traditional cue exposure therapies. In humans with cocaine addiction, such extinction could be achieved under medical supervision either by passive re-exposure to priming doses of cocaine without response reinforcement or to a cocaine analog that mimics the peripheral interoceptive effects of cocaine without crossing the blood-brain barrier [e.g., cocaine-methiodide (19)]. If this “behavioral vaccination” approach proved effective, it would be consistent with the treatment of other medical conditions by passive exposure to the pathogenic agent (here the abused drug).

This novel drug exposure approach has, however, several limitations that must be overcome before considering its potential clinical utility. First, though cocaine-primed reinstatement dramatically decreased with repeated drug priming, it was not completely abolished, leaving a small residual effect (see Figures 1A, 1D, 3A and 4A). Further research is required to determine what the potential clinical significance of this residual effect is and whether it can eventually be overcome with more extended extinction of drug priming. Second, though most rats were sensitive to the priming extinction effect, a few resisted to the priming extinction effect (see also (38)). For instance, one rat of a total of 12 rats that had extended access to cocaine self-administration remained responsive to the priming effect of cocaine and even tended to be increasingly responsive with repeated priming. This potential undesirable complication in few individuals should be considered in eventual future clinical applications of the drug exposure therapy. Finally, like other extinction phenomena (26), the extinction of cocaine-primed reinstatement was context-dependent. This context-dependency could limit the potential clinical utility of the proposed drug exposure therapy, since the medical context in which this type of therapy might take place would be different

from the habitual drug use context of individuals with cocaine addiction. One possible way to overcome or minimize this important limitation would be to approximate the drug use context in the hospital or the clinic, a strategy that has been already employed for other research purposes (44). Alternatively, a cocaine analog that does not cross the blood brain barrier might be formulated for use in the “real world”. Another possible strategy for preventing the context-dependency of the cocaine priming extinction effect could also involve extinction in multiple distinct contexts and not in one single context, as tested here. Such approach was recently shown to abolish almost completely the context-dependency of extinction of ethanol-conditioned exteroceptive cues in rats (45).

Additional research will be necessary to determine the identity of the conditioned peripheral interoceptive cues of cocaine, the mechanisms by which they lose their ability to induce craving, and how the extinction of drug-induced craving can be accelerated and maintained over time and across different contexts to reliably and durably protect abstinent individuals against relapse. Nevertheless, based on recent research, we hypothesize that the extinction of drug-primed craving and its generalization to stress will involve, at least partially, long-lasting changes in neural processing within the interoceptive insular cortex (23-25). Of particular interest, it will also be important to determine whether stress-primed reinstatement can also be extinguished with repeated stress priming. Consistent with this possibility, a recent study using a pharmacological stressor in rats (i.e., yohimbine, a sympathomimetic drug with strong anxiogenic properties) showed that repeated exposure to yohimbine during extinction can subsequently attenuate footshock stress-induced reinstatement of cocaine seeking (46). Finally, regardless of the behavioral and neural mechanisms involved in the extinction of cocaine craving, it is hypothesized that repeated, medically assisted priming of cocaine craving without relapse opportunity may also help individuals with cocaine addiction to rebuild a sense of control over drug taking and seeking. For instance, during repeated priming of cocaine craving without relapse, cocaine-abstinent individuals may eventually learn that craving is a transient experience that they can cope with merely by anticipating and waiting for its dissipation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Jaffe JH, Cascella NG, Kumor KM, Sherer MA. Cocaine-induced cocaine craving. *Psychopharmacology (Berl)*. 1989; 97:59–64. [PubMed: 2496428]
2. Mahoney JJ 3rd, Kalechstein AD, De La Garza R 2nd, Newton TF. A qualitative and quantitative review of cocaine-induced craving: the phenomenon of priming. *Prog Neuropsychopharmacol Biol Psychiatry*. 2007; 31:593–599. [PubMed: 17270333]
3. Volkow ND, Wang GJ, Ma Y, Fowler JS, Wong C, Ding YS, et al. Activation of orbital and medial prefrontal cortex by methylphenidate in cocaine-addicted subjects but not in controls: relevance to addiction. *J Neurosci*. 2005; 25:3932–3939. [PubMed: 15829645]

4. Epstein DH, Preston KL, Stewart J, Shaham Y. Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. *Psychopharmacology (Berl)*. 2006; 189:1–16. [PubMed: 17019567]
5. Grimm JW, Hope BT, Wise RA, Shaham Y. Neuroadaptation. Incubation of cocaine craving after withdrawal. *Nature*. 2001; 412:141–142. [PubMed: 11449260]
6. Anderson SM, Famous KR, Sadri-Vakili G, Kumaresan V, Schmidt HD, Bass CE, et al. CaMKII: a biochemical bridge linking accumbens dopamine and glutamate systems in cocaine seeking. *Nat Neurosci*. 2008; 11:344–353. [PubMed: 18278040]
7. Baker DA, McFarland K, Lake RW, Shen H, Tang XC, Toda S, et al. Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. *Nat Neurosci*. 2003; 6:743–749. [PubMed: 12778052]
8. Ciccocioppo R, Sanna PP, Weiss F. Cocaine-predictive stimulus induces drug-seeking behavior and neural activation in limbic brain regions after multiple months of abstinence: reversal by D(1) antagonists. *Proc Natl Acad Sci U S A*. 2001; 98:1976–1981. [PubMed: 11172061]
9. Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, et al. Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature*. 2008; 454:118–121. [PubMed: 18500330]
10. Graham DL, Edwards S, Bachtell RK, DiLeone RJ, Rios M, Self DW. Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. *Nat Neurosci*. 2007; 10:1029–1037. [PubMed: 17618281]
11. Lu L, Hope BT, Dempsey J, Liu SY, Bossert JM, Shaham Y. Central amygdala ERK signaling pathway is critical to incubation of cocaine craving. *Nat Neurosci*. 2005; 8:212–219. [PubMed: 15657599]
12. Moussawi K, Pacchioni A, Moran M, Olive MF, Gass JT, Lavin A, et al. N-Acetylcysteine reverses cocaine-induced metaplasticity. *Nat Neurosci*. 2009; 12:182–189. [PubMed: 19136971]
13. Vorel SR, Liu X, Hayes RJ, Spector JA, Gardner EL. Relapse to cocaine-seeking after hippocampal theta burst stimulation. *Science*. 2001; 292:1175–1178. [PubMed: 11349151]
14. Ahmed SH, Koob GF. Cocaine- but not food-seeking behavior is reinstated by stress after extinction. *Psychopharmacology (Berl)*. 1997; 132:289–295. [PubMed: 9292629]
15. Banks ML, Czoty PW, Nader MA. The influence of reinforcing effects of cocaine on cocaine-induced increases in extinguished responding in cynomolgus monkeys. *Psychopharmacology (Berl)*. 2007; 192:449–456. [PubMed: 17318506]
16. Bickel WK, Kelly TH. The relationship of stimulus control to the treatment of substance abuse. *NIDA Res Monogr*. 1988; 84:122–140. [PubMed: 3147379]
17. Brown PL, Kiyatkin EA. Sensory effects of intravenous cocaine on dopamine and non-dopamine ventral tegmental area neurons. *Brain Res*. 2008; 1218:230–249. [PubMed: 18514638]
18. Gerber GJ, Stretch R. Drug-induced reinstatement of extinguished self-administration behavior in monkeys. *Pharmacol Biochem Behav*. 1975; 3:1055–1061. [PubMed: 817297]
19. Wise RA, Wang B, You ZB. Cocaine serves as a peripheral interoceptive conditioned stimulus for central glutamate and dopamine release. *PLoS One*. 2008; 3:e2846. [PubMed: 18682722]
20. Schenk S, Partridge B. Cocaine-seeking produced by experimenter-administered drug injections: dose-effect relationships in rats. *Psychopharmacology (Berl)*. 1999; 147:285–290. [PubMed: 10639687]
21. Redish AD, Jensen S, Johnson A. A unified framework for addiction: vulnerabilities in the decision process. *Behav Brain Sci*. 2008; 31:415–437. discussion 437–487. [PubMed: 18662461]
22. Bergquist KL, Fox HC, Sinha R. Self-reports of interoceptive responses during stress and drug cue-related experiences in cocaine- and alcohol-dependent individuals. *Exp Clin Psychopharmacol*. 18:229–237. [PubMed: 20545387]
23. Naqvi NH, Rudrauf D, Damasio H, Bechara A. Damage to the insula disrupts addiction to cigarette smoking. *Science*. 2007; 315:531–534. [PubMed: 17255515]
24. Contreras M, Ceric F, Torrealba F. Inactivation of the interoceptive insula disrupts drug craving and malaise induced by lithium. *Science*. 2007; 318:655–658. [PubMed: 17962567]
25. Hollander JA, Lu Q, Cameron MD, Kamenecka TM, Kenny PJ. Insular hypocretin transmission regulates nicotine reward. *Proc Natl Acad Sci U S A*. 2008; 105:19480–19485. [PubMed: 19033203]

26. Bouton ME. Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. *Biol Psychiatry*. 2002; 52:976–986. [PubMed: 12437938]
27. Crombag HS, Bossert JM, Koya E, Shaham Y. Review. Context-induced relapse to drug seeking: a review. *Philos Trans R Soc Lond B Biol Sci*. 2008; 363:3233–3243. [PubMed: 18640922]
28. Ahmed SH, Cador M. Dissociation of psychomotor sensitization from compulsive cocaine consumption. *Neuropsychopharmacology*. 2006; 31:563–571. [PubMed: 16034440]
29. Ahmed SH, Koob GF. Transition from moderate to excessive drug intake: change in hedonic set point. *Science*. 1998; 282:298–300. [PubMed: 9765157]
30. Paterson NE, Markou A. Increased motivation for self-administered cocaine after escalated cocaine intake. *Neuroreport*. 2003; 14:2229–2232. [PubMed: 14625453]
31. Vanderschuren LJ, Everitt BJ. Drug seeking becomes compulsive after prolonged cocaine self-administration. *Science*. 2004; 305:1017–1019. [PubMed: 15310907]
32. Kippin TE, Fuchs RA, See RE. Contributions of prolonged contingent and noncontingent cocaine exposure to enhanced reinstatement of cocaine seeking in rats. *Psychopharmacology (Berl)*. 2006; 187:60–67. [PubMed: 16598453]
33. Knackstedt LA, Kalivas PW. Extended access to cocaine self-administration enhances drug-primed reinstatement but not behavioral sensitization. *J Pharmacol Exp Ther*. 2007; 322:1103–1109. [PubMed: 17601982]
34. Mantsch JR, Yuferov V, Mathieu-Kia AM, Ho A, Kreek MJ. Effects of extended access to high versus low cocaine doses on self-administration, cocaine-induced reinstatement and brain mRNA levels in rats. *Psychopharmacology (Berl)*. 2004; 175:26–36. [PubMed: 15042275]
35. Ahmed, SH. Escalation of drug use. In: Olmstead, MC., editor. *Neuromethods: Animal Models of Drug Addiction*. Humana Press; 2009. in press
36. Sinha R. The role of stress in addiction relapse. *Curr Psychiatry Rep*. 2007; 9:388–395. [PubMed: 17915078]
37. Schenk S, Worley CM, McNamara C, Valadez A. Acute and repeated exposure to caffeine: effects on reinstatement of extinguished cocaine-taking behavior in rats. *Psychopharmacology (Berl)*. 1996; 126:17–23. [PubMed: 8853212]
38. Stretch R, Gerber GJ. Drug-induced reinstatement of amphetamine self-administration behaviour in monkeys. *Can J Psychol*. 1973; 27:168–177. [PubMed: 4197125]
39. Grabowski J, Shearer J, Merrill J, Negus SS. Agonist-like, replacement pharmacotherapy for stimulant abuse and dependence. *Addict Behav*. 2004; 29:1439–1464. [PubMed: 15345275]
40. Mattick RP, Darke S. Drug replacement treatments: is amphetamine substitution a horse of a different colour? *Drug Alcohol Rev*. 1995; 14:389–394. [PubMed: 16203339]
41. Moeller FG, Schmitz JM, Herin D, Kjome KL. Use of stimulants to treat cocaine and methamphetamine abuse. *Curr Psychiatry Rep*. 2008; 10:385–391. [PubMed: 18803911]
42. Conklin CA, Tiffany ST. Applying extinction research and theory to cue-exposure addiction treatments. *Addiction*. 2002; 97:155–167. [PubMed: 11860387]
43. Myers KM, Carlezon WA Jr. Extinction of drug- and withdrawal-paired cues in animal models: Relevance to the treatment of addiction. *Neurosci Biobehav Rev*. 2010 in press.
44. Larsen H, Engels RC, Granic I, Overbeek G. An experimental study on imitation of alcohol consumption in same-sex dyads. *Alcohol Alcohol*. 2009; 44:250–255. [PubMed: 19240054]
45. Chaudhri N, Sahuque LL, Janak PH. Context-induced relapse of conditioned behavioral responding to ethanol cues in rats. *Biol Psychiatry*. 2008; 64:203–210. [PubMed: 18423574]
46. Kupferschmidt DA, Tribe E, Erb S. Effects of repeated yohimbine on the extinction and reinstatement of cocaine seeking. *Pharmacol Biochem Behav*. 2009; 91:473–480. [PubMed: 18824018]

Figure 1.

Extinction of cocaine-primed reinstatement. **(A)** Decrease in cocaine-primed reinstatement with repeated daily re-exposure to the drug. Vehicle or cocaine (15 mg/kg, i.p.) was administered immediately before the onset of each extinction session. Vehicle was administered the day before the first cocaine priming. #, different from vehicle ($p < 0.01$, one-way ANOVA); *, different from the first priming day ($p < 0.05$, Newman-Keuls's test). **(B)** Treatment timeline for group COC and group VEH in the stress-primed reinstatement experiment. During baseline days (BL), the extinction session was preceded by a 15 min period without electric footshock. During stress days (STR), the extinction session was preceded by a 15 min period of intermittent electric footshock. Following the first exposure to stress, animals were rebaselined during three consecutive regular extinction sessions (E) before receiving repeated administration of cocaine (COC) or vehicle (VEH). **(C)** Stress-primed reinstatement before repeated, daily cocaine priming. *, different from baseline ($p < 0.05$). **(D)** Decrease in cocaine-induced reinstatement with repeated daily re-exposure to the

drug. #, different from group VEH ($p < 0.05$, Newman-Keuls's test). (E) Stress-induced reinstatement after repeated, daily cocaine priming. #, different from group VEH ($p < 0.05$, Newman-Keuls's test); *, different from baseline ($p < 0.01$, Newman-Keuls's test).

Figure 2.

Cocaine-primed reinstatement following extended access to cocaine self-administration and as a function of the priming dose. **(A)** Escalation of cocaine self-administration across sessions. Each point represents the number of injections per 6 h session (mean $\pm$ SEM). **(B)** Dose-effect function for cocaine-primed reinstatement. Rats passively received increasing i.v. doses of cocaine (0, 0.25, 0.5, and 1 mg per injection), one dose every 45 min with the first 45 min interval corresponding to behavioral extinction. During reinstatement testing, the lever was extended but lever pressing was no longer rewarded by cocaine. Each point represents the number of unrewarded responses per 45 min period. *, different from dose 0 ($p < 0.05$, Newman-Keuls's test).

Figure 3.

Extinction of cocaine-primed reinstatement following extended access to cocaine self-administration. **(A)** Downward shift in the dose-effect function for cocaine-primed reinstatement between the first (Pr1) and last priming days (Pr10). *, different from Pr1 ($p < 0.05$, Newman-Keuls's test). Each point represents the number of unrewarded responses per 45 min period (mean $\pm$ SEM). For other information, see legend of Fig.2. **(B)** Time-course of the extinction of cocaine-primed reinstatement at the highest priming dose tested. Each point represents the number of unrewarded responses per 45 min period (mean $\pm$ SEM). *, different from testing day 1 ($p < 0.05$, Newman-Keuls's test). **Inset:** Persistence of extinction of cocaine-primed reinstatement. Testing ceased during a period of 10 days after which rats were re-tested for cocaine-induced reinstatement. Following this interruption period, there was no evidence for recovery of the priming effects of cocaine (Post) compared with the last three pre-interruption sessions (Pre).

Figure 4.

Effects of context on extinction of cocaine-primed reinstatement. **(A)** Downward shift in the dose-effect function for cocaine-primed reinstatement between the first (Pr1) and last priming days (Pr9) in a novel context B. Each point represents the number of unrewarded responses per 45 min period (mean $\pm$ SEM). There was a significant main effect of dose and priming session (see text) but no dose $\times$ priming session interaction. **(B)** Upward shift in the dose-effect function for cocaine-primed reinstatement following return to the original training context A. Each point represents the number of unrewarded responses per 45 min period (mean $\pm$ SEM). There was a significant effect of dose and context (see text) but no dose $\times$ context interaction.