A reliable method to study cue-, priming-, and stress-induced reinstatement of cocaine self-administration in mice

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Abstract

Rationale Cocaine addiction is a relapsing psychiatric disorder with a high prevalence in developed countries. To date, the reinstatement model has been difficult to implement in mice. The design of an appropriate reinstatement model in mice is required in order to use genetically modified animals with the aim of clarifying the mechanisms involved in cocaine relapse.

Objectives Our aim was to develop an appropriate model of reinstatement of cocaine-seeking behavior and to investigate the factors that can trigger this reinstatement by using an operant intravenous self-administration procedure in mice. Discrete cues, priming injection of cocaine, and exposure to stress were the stimuli used to reinstate cocaine-seeking behavior.

Material and methods Mice were trained to acquire intravenous self-administration of cocaine (1 mg/kg per infusion) on a fixed ratio 1 (FR1) schedule of reinforcement. After achieving the acquisition criteria, animals were led to extinguish the operant behavior. Subsequently, under extinction conditions, mice were tested after the administration of a cocaine priming injection (10 mg/kg i.p.), the presentation of a light cue associated with cocaine administration, or the exposure to a stressful situation (0.21 mA electric footshock).

Results Under our experimental conditions the three stimuli successfully reinstated an extinguished cocaine-seeking behavior. Reexposure to cocaine effects by a priming injection was revealed as the strongest stimulus, capable of reinstating cocaine-seeking behavior.

Conclusions The effective reinstatement model that we have developed will become a useful tool for future understanding of the neurobiological basis of cocaine addiction and relapse, specifically, with the use of genetically modified mice.

Keywords Cocaine · Mice · Self-administration · Operant behavior · Extinction · Reinstatement · Drug seeking · Priming · Environmental cues · Stress

Abbreviations

i.p. intraperitoneally
i.v. intravenous
FR fixed ratio
CPP conditioned place preference
Introduction

Drug addiction is a chronic psychiatric disease characterized by compulsive drug seeking and consumption followed by periods of abstinence and episodes of intense drug craving which lead to high rates of relapse (DSM-IVTR 2004). The repeated cycles of cessation and relapse remain the major clinical problem in treating drug addiction (Miller and Gold 1994; Weiss et al. 2001). Cocaine is one of the most widely used psychostimulant drugs of abuse. Thus, the prevalence of cocaine use in the European Union is 12 million people, and it is estimated that 4.5 million Europeans currently consume cocaine (EMCDDA 2005; Watson 2007; Wiessing 2005). Cocaine produces euphoric effects, increases motor activity (Woolverton and Johnson 1992), and enhances dopaminergic activity by blocking dopamine reuptake by the binding of the dopamine transporter (Rothman and Baumann 2003). In humans, relapse to cocaine and heroin abuse or the intense desire for these drugs (craving) can be generated by reexposure to the consumed drug, by stimuli usually associated with drug intake, and by stress (see Bossert et al. 2005 for a review).

Whereas acute drug reinforcing properties are essential for the initial phases of the addiction, other complex behavioral processes are crucial for the consolidation of this chronic relapsing disorder (Koob and LeMoal 2001). Operant intravenous drug self-administration is the most complete and reliable approach to investigate the addictive potential of drugs in rodents. Several aspects of the addiction process can be dissected by using this paradigm, including acquisition of an operant behavior to obtain the drug, maintenance, extinction, and reinstatement of such a behavior. Most studies conducted in mice to evaluate extinction and reinstatement of drug-seeking behavior have been performed in the conditioned place preference (CPP) paradigm (Tzschentke 2007) or in operant models using monkeys and rats (Weiss 2005; Shaham et al. 2003; Le Foll and Goldberg 2005). However, only three studies have modeled cocaine reinstatement after extinction in mice (Highfield et al. 2002; Fuchs et al. 2003; Kruzich 2007). Furthermore, most of these studies investigated the potential of cocaine priming administration and environmental cues to produce reinstatement of an extinguished drug-seeking behavior, whereas the exposure to stress was investigated to a lesser extent. However, stressful conditions are the most common situations precipitating reinstatement of an addictive behavior in humans (Bossert et al. 2005).

The lack of validated operant models of cocaine reinstatement in mice is mainly due to the complexity of the experimental technique, namely, the surgical procedure in these animal species (Epstein et al. 2006). During the last decade, the use of genetically modified mice has led to important advances in the knowledge of the neurobiological substrates underlying drug addiction. The existence of these mutant animals represents a relevant tool to unveil the neurobiological mechanisms involved in relapse, so the development of a reliable drug reinstatement model in mice becomes imperative.

In the present study, we investigated the reinstatement of a previously extinguished cocaine-seeking behavior by using an operant self-administration procedure in outbred mice. The reinstatement of drug seeking was evaluated under three different experimental conditions: (1) presentation of a visual cue associated with drug delivery, (2) priming injection of cocaine administered at the home cage, and (3) exposure to a stressful situation (an electric footshock). Additionally, we describe and characterize here for the first time the acquisition and extinction of cocaine self-administration in the case of outbred mice.

Materials and methods

Animals

Male CD1 outbred mice (Charles River, France) were used in this study. The animals (weighing 20–25 g upon arrival at the laboratory) were individually housed in clear plastic cages in an animal vivarium maintained on a 12-h light-dark cycle (lights on at 0800 hours), at constant temperature (21±1°C) and humidity (55±10%). Food and water were provided ad libitum, except during experimental sessions. Mice were handled daily in order to habituate them and minimize handling stress during the experiments. Animal care and experimental procedures were in strict accordance with institutional and international standards (the European Communities Council Directive 86/609/EEC, 24 November 1986) and were approved by the local Ethics Committee (CEEA-PRBB).

Drugs

Cocaine hydrochloride was obtained from Ministerio de Sanidad y Consumo (Spain) and prepared in sterile 0.9% physiological saline. We used a dose of 1 mg/kg per infusion during the training phase of the self-administration experiments and 10 mg/kg intraperitoneally (i.p.) for the cocaine priming injection.

Operant cocaine self-administration

Apparatus Self-administration training and testing occurred in operant chambers (Model ENV-307A-CT, MED Associates, Inc., Georgia, VT, USA) equipped with two retractable levers. One of them was selected as the reinforced lever for delivering the drug and the other as the non-reinforced lever. Active pressing on the reinforced...
lever resulted in a cocaine infusion while pressing on the non-reinforced lever had no consequences. Chambers were made of aluminum and clear acrylic, had grid floors connected to an electrical shocker (ENV-414, MED Associates, Inc., St. Albans, VT, USA), and were housed in sound- and light-attenuated boxes equipped with fans to provide ventilation and ambient noise. A stimulus light, located above the reinforced lever, was paired contingently with the delivery of the drug. When mice responded on the reinforced lever, the chamber light went off, the stimulus light went on, and a drug infusion was delivered. Cocaine was infused via a syringe that was mounted on a micro-infusion pump (PHM-100A, MED Associates, Inc., Georgia, VT, USA) and connected via Tygon tubing (0.96 mm o.d., Portex Fine Bore Polythene Tubing, Portex Limited, Hythe, Kent, UK) to a single channel liquid swivel (375/25, Instech Laboratories, Plymouth Meeting, PA, USA) and to the mouse intravenous (i.v.) catheters. The swivel was mounted on a counterbalanced arm above the operant chamber.

Surgery

Mice were anesthetized under isoflurane anesthesia (1.5–2.0%) and then implanted with indwelling intravenous silastic catheters (Soria et al. 2005). Briefly, a 6-cm length of silastic tubing (0.3 mm inner diameter, 0.6 mm outer diameter) (Silastic®, Dow Coming, Houdeng-Goegnies, Belgium) was fitted to a 22-gauge steel cannula (Semat, Herts, UK) that was bent at a right angle and then embedded in a cement disk (Dentalon Plus, Heraeus Kulzer, Wehrheim, Germany) with an underlying nylon mesh. The catheter tubing was inserted 1.3 cm into the right jugular vein and anchored with suture. The remaining tubing ran subcutaneously to the cannula, which exited at the midscapular region. All incisions were sutured and coated with antibiotic ointment (Bactroban, GlaxoSmithKline, Madrid, Spain). After surgery, animals were allowed to recover for 3 days prior to initiation of self-administration sessions. The catheter was flushed daily with a saline solution. The patency of intravenous catheters was evaluated periodically (approximately every 5–6 days) and whenever drug self-administration behavior appeared to deviate dramatically from that observed previously by infusion of 0.1 ml thiobarbital (5 mg/ml) through the catheter. If prominent signs of anesthesia were not apparent within 3 s of the infusion, the mouse was removed from the experiment.

Procedure

Cocaine self-administration training

Cocaine self-administration sessions were performed as previously described (Soria et al. 2005). Responding was maintained by cocaine (1 mg/kg per injection) delivered in 58.75 μl over 4 s. Daily self-administration started with a priming injection of the drug, lasted for 120 min, and was conducted 7 days per week. The house light was on at the beginning of the session for 3 s and off during the remaining time of the session. Each daily session started with the presentation of the reinforced and non-reinforced levers, a priming injection of the drug. and a 4-s presentation of the light cue (located above the reinforced lever). Presses on the reinforced lever led to cocaine infusions and the presentation of the light cue for 4 s. Mice were trained to lever press for cocaine under a fixed ratio 1 (FR1) schedule of reinforcement. A 30-s time-out period was established after obtaining of each reinforcement. During this 30-s period, the cue light was off and no drug infusions were provided after pressing the reinforced lever. Non-reinforced lever presses and all the responses performed during the 30-s time-out period were also recorded. The session was terminated after 75 reinforcements were delivered or after 2 h, whichever occurred first. The criteria for the acquisition was achieved when mice maintained a stable responding with less than 20% deviation from the mean of the total number of cocaine infusions earned in three consecutive sessions (80% of stability), with at least 75% responding on the reinforced lever, and a minimum of ten reinforcements per session. Considering that relapse was evaluated in a second step, only those animals showing a reliable drug self-administration behavior continued the experiment. The percentage of animals which achieved the acquisition criteria was 78.13%. Once the acquisition criteria were achieved, the extinction period started.

Extinction and reinstatement

The extinction procedure was modified from Highfield et al. (2002). In this phase, pressing the reinforced lever resulted in the activation of the pump to maintain the usual experimental environment but animals did not receive drug infusions. Also, the stimulus light was off during this period. Mice were given two 2-h daily extinction sessions separated by 1 h in which the levers were retracted (Fig. 1). Extinction sessions were conducted 6 days per week until reaching the extinction criteria. These criteria were achieved when, in the first 2 h of the extinction session, mice made a mean number of responses in two consecutive extinction sessions of less than 30% of the responses performed during the last day of the cocaine training phase. A small percentage of animals never achieved the extinction criteria (10%) and were excluded from the following experiments. The following day after achieving the extinction criteria, three different experimental conditions were evaluated to induce reinstatement of the cocaine self-administration behavior: the presentation of a conditioned...
Cocaine-induced reinstatement

Animals received an injection (10 mg/kg i.p.) at their home cage and immediately after were confined to the self-administration boxes to start the reinstatement test. No cue light was associated with the reinforced lever pressing to avoid a possible context bias in the procedure. The remaining conditions during the test day were the same as during the training sessions except that no cocaine was available. A control group of five animals received an i.p. injection of saline on the day before the cocaine-induced reinstatement test and immediately after were confined to the self-administration box following the same procedure as for cocaine-injected animals. This control group makes it possible to ensure that cocaine priming-induced relapse was specific to cocaine administration and not triggered by other factors such as the injection procedure.

Stress-induced reinstatement

Animals received a 2-s footshock (0.21 mA) every min during a 5-min period (total footshocks: 5) and immediately after levers were presented in the self-administration boxes to start the reinstatement test during a 2-h session. As in the case of cocaine-induced reinstatement, no cue light or cocaine were presented after pressing the reinforced lever.

Statistical analysis

The results were analyzed using a multivariate analysis of variance (MANOVA), with time (days) and lever (reinforced vs non-reinforced) as the within-subjects factors, when analyzing acquisition and extinction of the self-administration behavior. In the case of reinstatement experiments, phase of the experiment (last day of cocaine, first day of extinction, mean of extinction and relapse) as well as lever (reinforced vs non-reinforced) were the within-subjects factors. In all of the cases, the dependent variable was the number of lever presses accomplished in a 2-h period. When significant overall interactions were found, further analyses of partial interactions were carried out. Post hoc analyses were performed with Fisher’s LSD test when the initial p value was significant. All data were analyzed with Statistica software (StatSoft Inc., France). A result was considered significant if p<0.05. All the results are expressed as mean ± SEM.

Results

Acquisition and extinction of cocaine self-administration behavior in CD1 outbred mice

Three different groups of mice were trained to self-administer cocaine, one for each experimental condition for reinstatement. An analysis of variance among these groups was performed to evaluate any possible difference during the acquisition phase. The main effect of group was not significant (F(12,28)=0.46, p=0.636), and the results of acquisition were then pooled for the three experimental groups (Fig. 2a). During the training period, mice readily learned to discriminate between reinforced and non-reinforced levers, as revealed by the main effect of lever (F(1,30)=48.91, p<0.001). A significant interaction between time and lever (F(7,210)=3.76, p<0.001) indicated that animals increased the number of responses on the reinforced lever along the time. The mean acquisition time was 8.31±0.52 days, and the mean drug intake during the last 3 days of training was 24.87±1.43 mg/kg of cocaine.

During the extinction phase, mice were given two 2-h daily extinction sessions separated by 1 h in which the levers were...
retracted and the cue light was off (Fig. 1). Only the number of responses performed during the first 2 h were analyzed and compared. An analysis of variance among the three independent groups was also performed to evaluate any possible difference during the extinction of the self-administration behavior. Since the main effect of group was not significant ($F(2,30)=0.15, p=0.865$), data of extinction of the different experimental groups were analyzed together (Fig. 2b). A significant interaction was observed between time (days) and levers ($F(13,117)=2.63, p<0.01$), indicating a decrease in the number of lever presses on the reinforced lever during this phase of the experiment. Post hoc analysis showed no significant difference between the number of responses on the reinforced or on the non-reinforced lever during the last 4 days of extinction, indicating that drug-seeking behavior had decayed during the first extinction sessions and was stable before the beginning of the reinstatement tests. The mean time to fulfill extinction criteria was 14.24±1.68 days. Interestingly, the typical “extinction burst” (Cooper et al. 1987) was observed during the first day of extinction, on which mice showed a “craving-like” state, evidenced by a number of responses on the reinforced lever that was more than threefold the one observed during the last day of acquisition (Fig. 2b). Indeed, mice still showed a preference for the active lever ($p<0.001$) on the first day of extinction. The pattern of lever pressing revealed that the responses mainly occurred at the beginning of the extinction session. Post hoc analysis showed that the responses mainly occurred at the beginning of the extinction session (Fig. 2c). At the end of extinction, the number of responses was significantly lower compared to cocaine self-administration training phase ($p<0.001$) and no lever discrimination was observed ($p=0.989$).

Cue-induced reinstatement

After extinction of cocaine self-administration behavior, the cue light was presented on the test day to evaluate the reinstatement of drug-seeking behavior (Fig. 3a). Two-way MANOVA revealed a main effect of the experimental phase ($F_{3,36}=23.41, p<0.001$), lever ($F_{1,12}=36.79, p<0.001$), and a significant interaction between these two factors ($F_{3,36}=$...
The day prior to the test for cocaine reinstatement, five of the ten animals that had acquired the extinction criteria were randomly selected in order to have a control group which received a saline injection before cocaine priming ("saline-induced relapse" group) (see "Materials and methods" section). No differences were observed in this control group between the number of responses performed on the reinforced lever in comparison to the mean of the last 2 days of the extinction phase (p=0.920), and no lever discrimination or preference was observed (p=0.484), excluding the presence of drug-seeking behavior induced by the injection (Fig. 4a).

On the following day, the total group of animals (n=10) received a noncontingent cocaine injection (10 mg/kg i.p.) and

Cocaine-induced reinstatement

The conditions during this experiment were the same as in the training sessions except that no cocaine and no light cue were available when the mouse pressed the reinforced lever. 19.64, p<0.001), indicating that mice behaved in a different way regarding reinforced and non-reinforced levers among the different phases of the experiment. Post hoc analysis of the interaction showed that, after light cue presentation, animals increased their responses on the reinforced lever when compared with the extinction phase (p<0.05), revealing cue-induced reinstatement of the drug-seeking behavior (Fig. 3). However, no lever discrimination or preference was observed in this phase of testing (p=0.120, n.s.).
immediately after were confined to the self-administration boxes for the reinstatement test, under the same conditions as previously described for the “saline-induced relapse” group (Fig. 4a). As in the case of cue-induced reinstatement, two-way MANOVA revealed a main effect of the phase of the experiment (F_{3,24}=8.06, p<0.001) and lever (F_{1,8}=14.77, p<0.01) as well as a significant interaction between these two factors (F_{3,24}=4.15, p<0.05), indicating that mice behaved differentially with regard to the reinforced and the non-reinforced levers among the different phases of the experiment. Post hoc analysis showed that cocaine administration induced an increase in the responses on the reinforced lever when compared with responses during the last day of extinction. Besides, a preference for the reinforced lever was observed (p<0.01) during cocaine testing, revealing altogether that cocaine priming induced a reliable reinstatement of drug-seeking behavior.

Stress-induced reinstatement

The exposure of mice that have previously extinguished a cocaine self-administration behavior to electric footshocks produced reinstatement of operant responding on the reinforced lever, according to the relapse criterion established (at least ten responses on the reinforced lever as well as double the number of responses elicited during extinction on the reinforced lever). Two-way MANOVA did not show time (F_{2,18}=0.25, p=0.78, n.s.) or lever effect (F_{1,9}=2.83, p=0.13, n.s.), but a significant interaction between these two factors (F_{2,18}=4.96, p<0.05), indicating that mice behaved in a different way regarding the reinforced and non-reinforced levers among the phases of the experiment (Fig. 5a). Fisher’s LSD test performed on the interaction showed that, after electric footshock administration, animals increased their responses on the reinforced lever when compared with the extinction phase. Mice performance on the reinforced lever during relapse evaluation did not significantly differ from the one observed during the acquisition of cocaine self-administration behavior. However, as in the case of cue-induced reinstatement, no lever discrimination or preference was observed in this phase of testing (p=0.143, n.s.).

No correlation was found between the total amount of cocaine consumed during acquisition and maintenance periods and the rate of reinstatement observed after cue-, priming-, or stress- induced relapse (data not shown).

Discussion

In the present study we have established and validated a reliable method for evaluating acquisition, extinction, and reinstatement of cocaine-seeking behavior in CD1 outbred mice by using an operant self-administration paradigm. Here, we show for the first time reinstatement of cocaine-seeking behavior in mice induced by the three classic stimuli: (1) a non contingent priming injection of cocaine (10 mg/kg i.p.), (2) exposure to a discrete cue (light) associated with the drug delivery and intake, and (3) exposure to a stressor (electric footshock).

Acquisition of cocaine self-administration was achieved in about 8 days, as similarly reported in previous studies (Soria et al. 2005, 2006). Subsequently, animals were subjected to an extinction period in which lever pressing was not reinforced with cocaine. The extinction of cocaine-seeking behavior lasted for 14.24±1.68 days. Under our experimental conditions, the i.p. priming injection of cocaine produced the highest effect on reinstatement of drug-seeking behavior. Remarkably, this is the first time
that a cocaine i.p. priming injection triggers the reinstatement of an operant responding for cocaine in mice. Moreover, an important discriminative effect between the reinforced and the non-reinforced lever was observed for cocaine-induced reinstatement. The dose of cocaine priming (10 mg/kg i.p.) was chosen based on previous studies evaluating cocaine rewarding effects (Martin et al. 2000). In addition, this dose is known to increase the extracellular levels of dopamine in the mice nucleus accumbens (Soria et al. 2005). These data support the hypothesis that our observations involving cocaine priming were due to the psychotropic effects of the drug. A possible effect of stress induced by the injection procedure was ruled out by the fact that animals receiving a saline injection did not reinitiate cocaine-seeking behavior. A limitation of our experimental procedure is that mice also were primed with an infusion of cocaine (1 mg/kg) during training, in order to facilitate the acquisition of the self-administration behavior. Recently, it has been shown that cocaine may act both as a discriminative stimulus as well as a reinforcer in the reinstatement paradigm currently used (Shalev et al. 2002; Keiflin et al. 2008). Under our experimental conditions, we were not able to dissociate the portion of reinstatement induced by the discriminative properties of cocaine from the one induced by its incentive motivational properties. Nevertheless, now that we have validated this model of reinstatement in mice, further investigation aimed at dissociating the contribution of the discriminative and motivational properties of cocaine in reinstatement is warranted.

Another important finding was that both the light cue and, for the first time, a footshock stressor reinstated cocaine-seeking behavior in CD1 outbred mice. In both cases the number of lever pressings during the relapse testing day was significantly increased specifically on the reinforced lever. However, no discrimination was found between the reinforced and non-reinforced levers when stress or discrete cues were tested to induce reinstatement.

Intravenous self-administration is probably the most reliable approach to investigate the abuse potential of drugs in animals (Collins et al. 1984). The technical complexity of this method is the reason why the majority of these studies have been performed in rats (Shaham et al. 2000; Weiss et al. 2000; De Vries et al. 2001). As a result, mice studies testing reinstatement of drug seeking have currently employed the CPP paradigm, which is based on classic conditioning (Daza-Losada et al. 2007; Tzschentke 2007). Several authors have demonstrated cocaine relapse after a withdrawal period using the CPP paradigm (Itzhak and Martin 2002; Graham et al. 2007; Ribeiro do Couto et al. 2005). Nonetheless, only three studies have tried to characterize the reinstatement of cocaine-seeking behavior in mice after a period of extinction by using the i.v. self-administration paradigm. First, Highfield et al. (2002) showed reinstatement of cocaine seeking in 129X1/SvJ mice. Several discrepancies exist between our study and that conducted by Highfield et al. (2002); whereas 129/SvJ mice exhibited a modest effect of cocaine priming on reinstatement of cocaine-seeking behavior, in our study cocaine priming was the most significant condition to reinitiate drug seeking. Under their experimental conditions, the cocaine dose and the route of administration were different in comparison to our experiment (6 mg/kg i.v. versus 10 mg/kg i.p.). It should be noted that the 129/SvJ strain is known to be less sensitive to cocaine-induced behavioral responses than CD1 outbred or C57BL/6J strains (Schlussman et al. 1998; Miner 1997). All the above-mentioned factors could account for the modest effect of cocaine-induced relapse observed in 129/SvJ animals. It is important to note that the 129/SvJ mouse is not the most suitable strain to investigate a complex phenomenon such as drug addiction since several cognitive deficits have been described in this particular strain (Phillips et al. 1999; Crawley 2003; Bailey et al. 2006). Fuchs et al. (2003) also investigated cocaine reinstatement in mice after extinction and showed that conditioned stimuli associated with cocaine administration reinstated drug-seeking behavior in C57BL/6J mice. The mean number of responses during training and testing was much higher than the one observed under our experimental conditions. However, Fuchs and colleagues (2003) trained their animals during the dark period of the circadian cycle, the active period for most rodents. Also, animals were trained to self-administer sucrose during an overnight session of 16 h before the intravenous surgery. Therefore, both the circadian moment and the previous training history may account for the differences observed in the performance of mice in both studies. On the other hand, the previous work failed to demonstrate cocaine-induced reinstatement of responding when cocaine (doses ranging from 1 to 40 mg/kg) was delivered i.p. This disparity with our results could be explained by the fact that Fuchs et al. (2003) exposed the animals to changes in the food regime during the different phases of the study. In our case, this factor is not present since animals were trained directly to self-administer cocaine in a satiated state. Moreover, the mice strain used in the above-cited study was C57BL/6J, whereas here, for the first time, we have used a CD1 outbred background mouse to study cocaine reinstatement behavior. CD1 mice are a common outbred albino stock, and the outbred mating strategy makes it likely that these mice will have more variable genes (alleles), phenotype, and, possibly, more variable behavior than the inbred strains (Festing 1999). Thus, the use of CD1 mice may be advantageous, since outbred mice are considered to reflect more natural heterogeneous populations (Adams et al. 2002). In addition, considering the present results, CD1
seems to be the most suitable strain for relapse studies in mice, since all three experimental conditions evaluated produced a reliable reinstatement of the operant behavior.

Recently, Kruzich (2007) reported an operant method for cocaine craving and relapse in mice. The author performed conditioned- and cocaine-primed reinstatement in the same C57BL/6J mice subjected to a food restriction regime. This author used a complex experimental design where mice received cocaine in a contingent way inside the operant chamber, whereas we administered a non contingent i.p. injection of cocaine outside the operant chamber. Under the experimental conditions reported by Kruzich (2007), it was not possible to ensure that the observed reinstatement of cocaine-seeking behavior was only due to the effects of cocaine per se or both to its effects and the presence of environmental visual cues associated with drug delivery.

In contrast to our data, the three studies discussed above (Highfield et al. 2002; Fuchs et al. 2003; Kruzich 2007) demonstrated a significant discrimination between active and inactive lever responses on the cue-induced reinstatement test. The discrepancy could be due to the fact that they used compound conditioned stimuli consisting in a light placed above the reinforced lever plus a tone generator for 2 s, whereas we only used the cue light situated above the reinforced lever. Their conditions would result in a stronger incentive value attributed to the conditioned stimuli and, as a consequence, to a stronger effect on the reinstatement of cocaine seeking when presenting the compound stimulus.

In the case of reinstatement induced by electric stimulation, the use of a cocaine-paired cue during extinction and reinstatement phases is known to be critical for shock-induced reinstatement in rats (Shelton and Beardsley 2005). Therefore, the absence of such a cue during extinction and reinstatement phases could account for the relatively weak effect of shock observed in stress-induced reinstatement in our study.

Human and animal studies have suggested that stress is one of the most important factors to trigger relapse of addictive behaviors after a period of abstinence (Kita et al. 1999; Todd 2004). Indeed, stress was recently revealed as a crucial factor in producing relapse and is strongly dependent on the individual genetically determined emotionality (Bilkei-Gorzo et al. 2008; LeDoux 2000). Footshock stress is a general method to induce reinstatement of drug-seeking behavior in experimental animals (Weiss 2005). Moreover, stress facilitates the acquisition of psychostimulant self-administration in operant paradigms (Goeders and Guerin 1994; Piazza et al. 1990). In a very recent study, nicotine-seeking behavior was reinstated after a brief exposure to footshock in mice presenting high responsivity to stress, but not in those presenting low responsivity (Bilkei-Gorzo et al. 2008). Several studies have also used food deprivation as a stressor in mice (Highfield et al. 2002) and rats (Shalev et al. 2001; De Vry et al. 1989) to reinstate cocaine- and heroin-seeking behavior. However, inconsistent results have been found when evaluating the reinstatement of operant responses using food deprivation, probably due to the participation of additional metabolic factors. As already stated, under our experimental conditions the lack of metabolic state manipulations of the organism allows us to attribute the results specifically to stress.

A body of evidence shows that the neuronal mechanisms underlying stress-induced drug seeking, cues, or priming are not identical. Cocaine-induced reinstatement results from activation of the ventral tegmental area-dorsal prefrontal cortex dopamine pathway, which is responsible for the activation of the dorsal prefrontal cortex-nucleus accumbens core glutamatergic pathway, with a subsequent activation of the accumbens core-ventral pallidum pathway, presumably via a γ-aminobutyric acid (GABA) pathway (Kalivas and McFarland 2003; Maier and Watkins 2005). Several studies have proposed the role of both basolateral and central amygdala in discrete cue-induced reinstatement where the reexposure to the cue is earned contingently by responding on the drug-associated lever (Di Ciano and Everitt 2004; Bossert et al. 2005; Davis et al. 2006). Concerning stress, recent neuroanatomical studies indicate that stress-induced activation of the mesocorticolimbic dopamine and glutamatergic system is critically involved in the reinstatement of drug-seeking responses (Piazza and Le Moal 1996; McFarland et al. 2004).

In conclusion, we have characterized and validated for the first time a model in CD1 outbred mice to evaluate acquisition, extinction, and reinstatement of cocaine-seeking behavior by using the self-administration paradigm. Taking into account the elevated relapse rates observed in cocaine addiction, this method is of high interest to investigate the neurobiological basis involved in this phenomenon, by using genetically modified mice. A better understanding of the neural and molecular events underlying relapse will be crucial to design more effective therapeutic actions and to establish convenient preventive policies.

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