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ORIGINAL INVESTIGATION

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Cocaine seeking over extended withdrawal periods in rats: different time courses of responding induced by cocaine cues versus cocaine priming over the first 6 months

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Abstract *Rationale and objectives:* We previously found time dependent increases, or incubation, of cocaine seeking induced by re-exposure to cocaine cues over withdrawal periods of up to 3 months. Here, we studied cocaine seeking induced by re-exposure to cocaine cues or cocaine itself over an extended withdrawal period of 6 months. *Methods:* Rats were trained to self-administer intravenous cocaine for 6 h/day for 10 days. Cocaine seeking induced by re-exposure to cocaine cues or cocaine itself, as measured in extinction or drug-induced reinstatement tests, respectively, was then assessed 1 day, or 1, 3 or 6 months after withdrawal. Rats were first given six 1-h extinction sessions wherein lever presses resulted in contingent presentations of cues previously paired with cocaine infusions. Subsequently, reinstatement of drug seeking induced by cocaine injections (expt 1: 0, 5, and 15 mg/kg, IP; expt 2: 0, 2.5, and 5 mg/kg) was assessed during three 1-h sessions. *Results:* Profound time dependent changes in responsiveness to cocaine cues in the extinction tests were observed, with low responding after 1 day, high responding after 1 and 3 months, and intermediate responding after 6 months of withdrawal. In contrast, no significant time dependent changes in cocaine-induced drug seeking were found; acute re-exposure to cocaine effectively reinstated responding at all withdrawal periods. *Conclusions:* Results indicate that the withdrawal period is a critical modulator of drug seeking provoked by re-exposure to cocaine cues, but not cocaine itself. Results also indicate that while the incubation of responsiveness to cocaine cues is a long lasting phenomenon, it is not permanent.

Keywords Craving · Cocaine cues · Extinction · Priming · Reinstatement · Relapse · Self-administration · Withdrawal

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Introduction

Relapse to cocaine use after prolonged abstinence is a major clinical problem (O'Brien 1997). In an attempt to account for this relapse, Gawin and Kleber (1986) postulated that craving induced by exposure to cocaine-associated cues increases over the first several weeks of abstinence, and remains high over extended abstinence periods. In rats, exposure to cocaine cues increases non-reinforced lever pressing (the operational measure of drug seeking in extinction-reinstatement studies) several months after withdrawal from the drug (Meil and See 1996; Ciccocioppo et al. 2001). However, until recently (see Tran-Nguyen et al. 1998), the impact of the drug withdrawal period on vulnerability to drug seeking induced by exposure to drug-associated cues has not been systematically examined. In the study of Tran-Nguyen et al. (1998), the authors reported that extinction responding, a behavior induced by re-exposure to the reward-associated cues (Catania 1992), is somewhat elevated (i.e. results did not reach statistical significance) after 1 month or 1 week of withdrawal from cocaine than after 1 day. In a subsequent study, this group reported that extinction responding was significantly higher after 3 weeks of withdrawal from cocaine than after 1 day (Neisewander et al. 2000). In a study with heroin-trained rats, Shalev et al. (2001) reported that lever pressing during extinction follows an inverted U-shaped curve, with significantly higher responding after 6, 12, and 25 days of withdrawal from heroin than after 1 or 66 days.

Based on these previous findings, we assessed time dependent changes in responsiveness to cocaine cues after withdrawal from cocaine in two ways (Grimm et al. 2001). First, we determined non-reinforced lever pressing in

extinction tests in the presence of a houselight and other contextual cues that had indicated drug availability during training, but in the absence of a discrete tone-light cue that had been paired with the drug injections during training. Second, we determined cue-induced reinstatement of cocaine seeking in a test wherein lever presses led to contingent presentations of the tone-light cue, which serves as a conditioned reinforcer during testing (Robbins 1975).

We found time dependent increases, or incubation, of responsiveness to cocaine cues in the two tests, with significantly higher responding after 1–2 months than after 1–7 days (Grimm et al. 2001). Subsequently, we found that responsiveness to cocaine cues in the extinction and cue-induced reinstatement tests is significantly higher after 1 or 3 months of withdrawal than after 1 day (Grimm et al. 2003). In both studies, responding in the extinction and cue-induced reinstatement tests followed a similar time course and was highly correlated. Thus, while different sets of cocaine cues induce drug seeking in these tests, they probably provoke a similar motivational state that incubates over time. In the present study, we addressed two questions arising from these findings.

First, we determined whether the enhanced responsiveness to cocaine cues is maintained after a longer withdrawal period of 6 months. Second, we studied whether cocaine-induced drug seeking after withdrawal, as measured in a test for drug priming-induced reinstatement (de Wit and Stewart 1981), follows the time course of drug seeking induced by re-exposure to cocaine cues. Re-exposure to cocaine induces craving and relapse in humans (O'Brien 1997) and many studies reported that cocaine priming reinstates drug seeking in laboratory animals (Stewart 2000; Shalev et al. 2002; Shaham et al. 2003). Several studies have shown that cocaine priming reliably reinstates cocaine seeking after withdrawal periods of up to 3–6 weeks (Erb et al. 1996; De Vries et al. 1998, 1999; Tran-Nguyen et al. 1998; Deroche-Gamonet et al. 2003). Some of these studies also assessed time dependent changes in cocaine priming-induced reinstatement of cocaine seeking and reported that this reinstatement either increased (see Tran-Nguyen et al. 1998) or decreased (see Deroche-Gamonet et al. 2003) over the 1st month after withdrawal from the drug. Thus, no clear picture has yet to emerge concerning the impact of the cocaine withdrawal period on the magnitude of cocaine-induced reinstatement of drug seeking.

In the present experiments, we studied cocaine seeking induced by re-exposure to cocaine cues and to cocaine itself, as measured in tests for resistance to extinction and cocaine priming-induced reinstatement, respectively, after 1 day, and 1, 3, and 6 months of withdrawal. In the extinction test, rats were re-exposed to cocaine contextual cues (i.e. the drug environment) and lever pressing resulted in the contingent presentations of the discrete tone-light cue that had been temporally paired with cocaine infusions during training. Based on theoretical reviews (Stewart et al. 1984; Robinson and Berridge 1993), which argue that drug cues activate neuronal circuits involved in motivational states induced by drug exposure, we postulated that cocaine seeking induced by re-exposure to cocaine cues or cocaine itself would follow a similar time course after withdrawal.

Materials and methods

Subjects, surgery and apparatus

The subjects were 118 male Long–Evans rats (Charles River, Raleigh, N.C., USA) weighing 350–400 g (11–13 weeks old) prior to surgery. The rats were group housed (two or three per cage) prior to surgery and individually housed after surgery. The rats were maintained in the animal facility under a reversed 12:12 h light/dark cycle with food and water freely available. Procedures followed the "Principles of Laboratory Animal Care" (NIH publication no. 86-23, 1996). Rats were surgically implanted under anaesthesia (xylazine 10 mg/kg+ketamine 100 mg/kg, IP) with intravenous catheters as previously described (Shalev et al. 2000). Buprenorphine (0.01 mg/kg, SC) was given as an analgesic after surgery. Rats were given 5–7 days to recover from surgery and catheters were flushed with sterile saline (0.05 ml) containing gentamicin (0.8 mg/ml) every 24–48 h during the recovery and training phases. The self-administration boxes were controlled by a Med Associates (Georgia, Vt., USA) system and had two levers located 9 cm above the floor, but only one lever (an active, retractable lever) activated the infusion pump. Presses on the other lever (an inactive, stationary lever) were also recorded. The cannula on the rat's skull was connected to a liquid swivel that was connected to the syringe of the infusion pump.

Procedures

The experiments included three phases (Fig. 1). During training, rats were trained to lever press for cocaine. During the withdrawal phase, rats were housed in the animal facility. During testing, lever presses were not reinforced with cocaine. On the test day, rats were first tested for resistance to extinction for six 1-h sessions in the presence of the cocaine-associated cues. Rats were then tested for reinstatement induced by cocaine injections during three 1-h extinction sessions.

The procedure to assess responsiveness to cocaine cues after withdrawal is different from those we previously used. As mentioned in the Introduction, we previously assessed responsive-

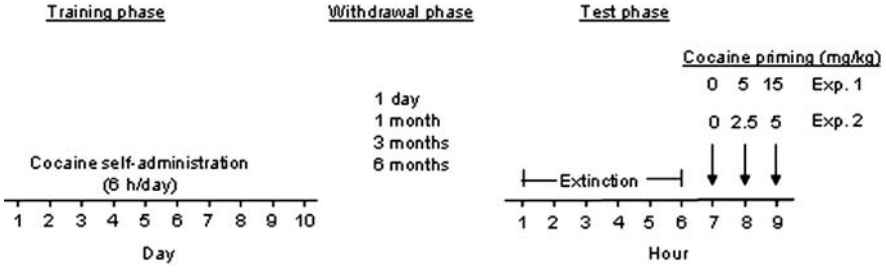

Fig. 1 Timeline of the experimental procedures

ness to cocaine cues after withdrawal in two ways (Grimm et al. 2001, 2003). Initially, we determined resistance to extinction in the presence of the houselight and other contextual cues that had indicated drug availability during training, but in the absence of a discrete tone-light cue that had been temporally paired with the drug injections. Subsequently, we determined cue-induced reinstatement of cocaine seeking in a test wherein responding led to contingent presentations of the tone-light cue, which serves as a conditioned reinforcer during this test. We found that responding in the extinction and the cue-induced reinstatement tests followed a similar time course and was highly correlated (Grimm et al. 2001, 2003), suggesting that the different sets of cocaine cues, which control behavior in these two tests, induce a similar motivational state that underlies the incubation of drug seeking after withdrawal. Therefore, in the present study, we assessed responsiveness to cocaine cues in an extinction test in which rats were re-exposed to the contextual cues previously associated with cocaine availability and lever presses resulted in contingent presentations of a discrete tone-light cue that had been temporally paired with each cocaine infusion during training. Similar extinction procedures have been used to assess behavioral and neuronal mechanisms underlying responsiveness to cues previously paired with drug (e.g. Phillips et al. 1994; Shalev et al. 2001; Lu et al. 2004) or non-drug reinforcers (e.g. Balleine and Dickinson 1998). After extinction testing, rats were tested for cocaine priming-induced reinstatement, allowing us to assess cocaine seeking induced by re-exposure to cocaine cues and cocaine itself in the same subject.

Training Rats were trained for 10 days to self-administer cocaine HCl (NIDA, USA) during six 1-h daily sessions that were separated by 5 min. Rats were brought to the self-administration chambers daily and training sessions started at the onset of the dark cycle. Cocaine (1.0 mg/kg per infusion) was dissolved in saline and was delivered at a volume of 0.13 ml over 4.5 s. Cocaine infusions were accompanied by a 5-s tone-light compound cue. A fixed-ratio-1 reinforcement schedule was used, with a 40-s timeout period after each infusion. Each session began with the insertion of the active lever and the illumination of a red houselight that remained on for the entire session. At the end of each session, the houselight was turned off and the active lever retracted. For rats that did not initiate cocaine self-administration, food was removed from the chambers during the six 1-h sessions for up to 5 days. The number of cocaine infusions was limited to 15/h to minimize cocaine overdose. Twenty-seven of the 118 rats were excluded due to loss of catheter patency during training (n=9), poor health or death during the training and withdrawal phases (n=15), or failure to acquire cocaine self-administration (<10 infusions/day, n=3).

Withdrawal phase Rats were individually housed in the animal facility and were handled 3 times per week. Different groups were tested for cocaine seeking at the different withdrawal periods.

Testing

Experiment 1 Groups of rats (n=10–14 per group) were tested for resistance to extinction and for cocaine-induced reinstatement after withdrawal periods of 1 day, or 1, 3 or 6 months. Three days prior to testing, rats were given daily saline injections (1 ml/kg, IP). The conditions during testing were identical to those of training, with the exception that lever presses did not lead to cocaine infusions. On the test day, extinction responding was first assessed during six 1-h sessions (that were separated by 5 min) wherein lever presses were not reinforced with cocaine, but resulted in contingent presentations of the discrete tone-light cue. Rats were subsequently tested for reinstatement induced by cocaine injections (0, 5, and 15 mg/kg, IP) during three 1-h sessions that were separated by 5 min. Saline and cocaine injections were given just prior to the test sessions in an ascending order to minimize carry-over effects of residual cocaine. The two cocaine doses were chosen based on evidence that the 5 mg/kg dose does not consistently reinstate cocaine seeking (Schenk and Partridge 1999; Schenk et al. 2000; Park et al. 2002), while 15 mg/kg is within the effective dose range for reinstatement (Shalev et al. 2002). Thus, the potentially low threshold dose of

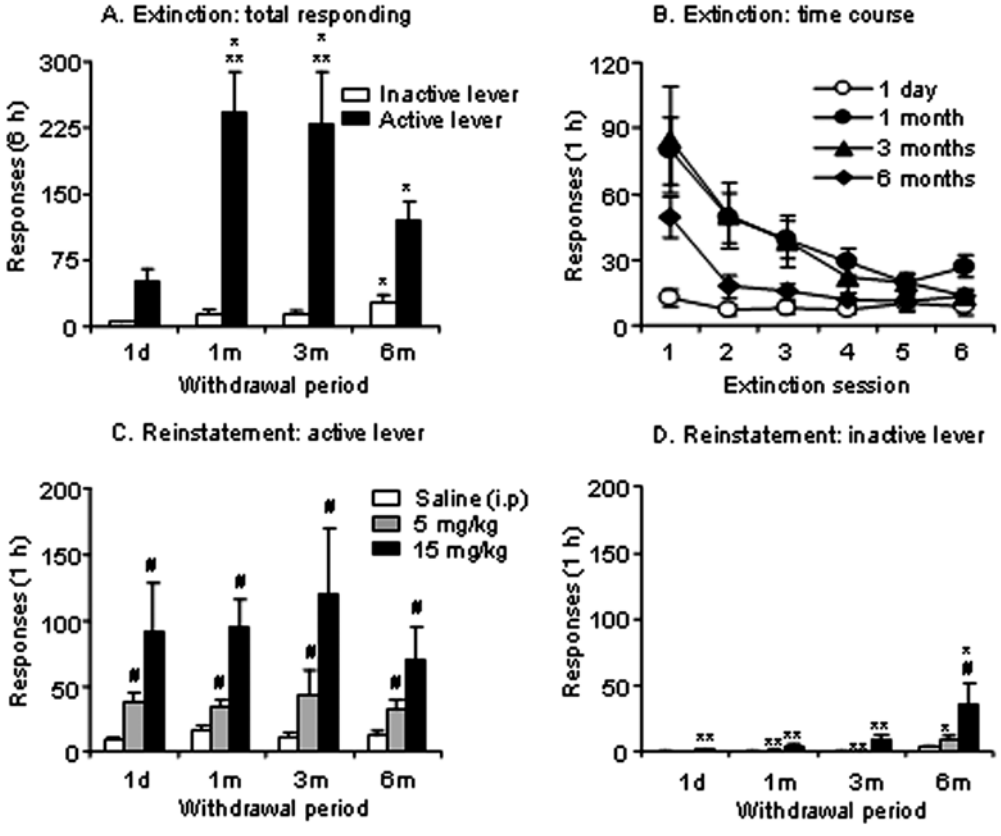

Fig. 2 Experiment 1. **A** Extinction—total responses: mean (±SEM) responses on the previously active lever and inactive lever during the six 1-h sessions of extinction, conducted in the presence of the cocaine-associated cues, but in the absence of cocaine. **B** Extinction—responses per hour: mean active lever responses at each session of extinction. **C** Cocaine-induced reinstatement—active lever: mean active lever responses during the three 1-h sessions after injections of saline and cocaine (5 and 15 mg/kg. IP). **D** Cocaine-induced reinstatement—inactive lever: mean inactive lever responses during these three 1-h sessions. *Different from day 1, P<0.05; **different from 6 months, P<0.05; #different from saline (zero dose) within each withdrawal period, P≤0.05 (n=10–14 per withdrawal period)

5 mg/kg, which also does not induce locomotor activity in naïve male Long–Evans rats (unpublished observations), may provide a sensitive measure of differential responsiveness to cocaine at the different withdrawal periods.

Experiment 2 As shown in Fig. 2C, under our experimental conditions, 5 mg/kg cocaine effectively reinstated cocaine seeking at all withdrawal periods. Therefore, it is possible that differential responsiveness to cocaine-induced reinstatement after withdrawal would emerge with a lower dose of cocaine. This possibility was studied in expt 2, in which rats were tested with doses of 2.5 and 5 mg/kg. The other purpose of expt 2 was to replicate both the extinction results and those of reinstatement induced by 5 mg/kg cocaine in expt 1. Groups of rats (n=9–13 per group) were tested for resistance to extinction and for cocaine-induced reinstatement after withdrawal periods of 1 day, or 1, 3 or 6 months. The experimental conditions were the same as those of expt 1, with the exception that rats were tested for reinstatement induced by lower doses of cocaine (0, 2.5, and 5 mg/kg, IP, ascending order) during the three 1-h sessions.

Statistical analyses

Data were analyzed separately for responding on the active and inactive levers. Data from the extinction and reinstatement tests were analyzed with a mixed-model ANOVA, using the between-subjects factor of withdrawal period, and the within-subjects factor of extinction session or cocaine dose, respectively. Post-hoc analyses were performed with Fisher PLSD test (two-tailed).

Results

Training No significant differences were found for the number of cocaine infusions among the groups tested at the different withdrawal periods (P-values >0.05 for expts 1 and 2). The mean±SEM (data collapsed from expts 1 and 2, total n=91) number of cocaine infusions during the last 3 training days were 50.6±6.0, 53.5±5.2, and 53.5±5.3, respectively.

Extinction Figures 2A,B, 3A,B present the mean±SEM number of non-reinforced presses on the active and inactive levers during the extinction tests in expts 1 and 2, respectively. In both experiments, active lever responding during the extinction test was significantly higher after one and three months of withdrawal than after 1 day or 6 months, and responding after 6 months was significantly higher than after 1 day. Analyses revealed significant effects of withdrawal period [F(3,44)=6.8 and F(3,33) =13.4, P<0.01], extinction session [F(5,220)=36.4 and F (5,195)=88.8, P<0.01], and withdrawal period by extinction session [F(15,220)=4.3 and F(15,195)=11.2, P<0.01] for expts 1 and 2, respectively. Analyses of inactive lever responding (a potential measure of general activity and/or response generalization, see "Discussion") also revealed a significant effect of withdrawal period [F(3,44)=3.7, and F (3,39)=3.0, P<0.05 for expts 1 and 2, respectively], an effect primarily resulting from increased inactive lever

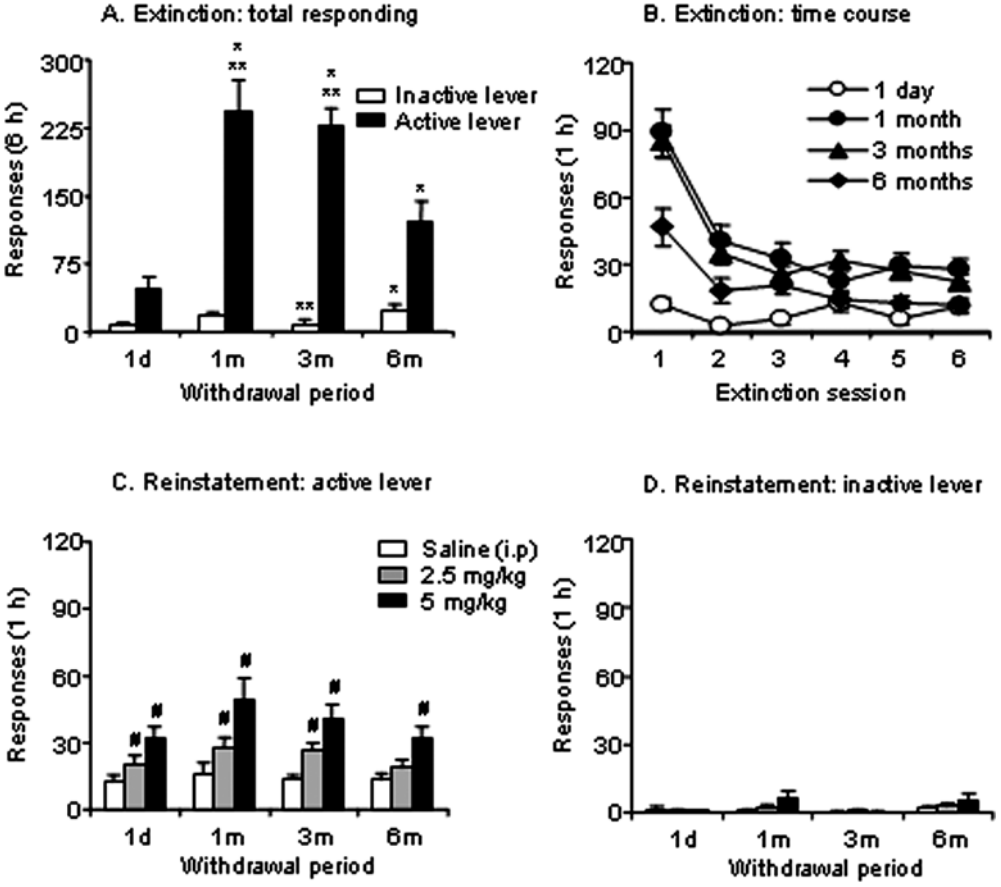

Fig. 3 Experiment 2 **A** Extinction—total responses: mean (±SEM) responses on the previously active lever and inactive lever during the six 1-h sessions of extinction. **B** Extinction—responses per h: mean active lever responses at each session of extinction. **C** Cocaine-induced reinstatement—active lever: mean active lever responses during the three 1-h sessions after injections of saline or cocaine (2.5 and 5 mg/kg. IP). **D** Cocaine-induced reinstatement—inactive lever: mean inactive lever responses during these three 1-h sessions. *Different from day 1, P<0.05; **different from 6 months, P<0.05; $^{\#}$different from saline (zero dose) within each withdrawal period, P<0.05 (n=9–13 per withdrawal period)

responding in the 6-month withdrawal groups (Fig. 2A, 3A). Therefore, a change-score analysis (active lever minus inactive lever presses) was performed. This change-score analysis replicated that of active lever responding (data not shown), suggesting that time dependent changes in inactive lever responding cannot account for the results obtained for active lever responding.

Reinstatement Figures 2C,D, 3C,D present the mean±SEM number of non-reinforced presses on the active and inactive levers during the tests for cocaine-induced reinstatement in expts 1 and 2, respectively. In both experiments, cocaine reinstated extinguished active lever responding, but no significant time dependent changes were found. Analyses revealed a significant effect of cocaine dose [$F(2,88)=27.7$ and $F(2,78)=43.0$; $P<0.01$ for expts 1 and 2, respectively]. No significant effects were found for withdrawal period ($P>0.2$) or cocaine dose by withdrawal period ($P>0.4$) in either experiment. Analysis of inactive lever responding for expt 1 revealed a significant effect of cocaine dose [$F(3,44)=3.9$; $P<0.05$], an effect resulting from increased responding in the 6-month withdrawal group (Fig. 2D). A subsequent change-score analysis, however, replicated the statistical analysis of active lever responding (data not shown). No significant effects were found in the analysis of inactive lever responding in expt 2 (Fig. 3D).

Discussion

We studied cocaine seeking induced by re-exposure to cocaine cues or cocaine itself over a withdrawal period of 6 months. We found profound time dependent changes in responsiveness to cocaine cues as measured in the extinction tests, with low responding after 1 day, high responding after 1 and 3 months, and intermediate responding after 6 months. In contrast, no significant time dependent changes in reinstatement of drug seeking induced by cocaine priming were found. Cocaine priming reinstated extinguished lever pressing at all withdrawal periods. These data suggest that the cocaine withdrawal period is a critical modulator of drug seeking induced by re-exposure to cocaine cues, but not cocaine itself.

Drug seeking induced by re-exposure to cocaine cues after withdrawal

The present data extend our previous findings of time dependent increases in responsiveness to cocaine cues (as measured in tests for resistance to extinction and cue-induced restatement) over withdrawal periods of up to 3 months (Grimm et al. 2001, 2003). The new finding here is that the enhanced responsiveness to cocaine cues is no longer observed after 6 months of withdrawal; cocaine seeking was significantly lower at this time point than after 1 or 3 months. However, even after 6 months of withdrawal, extinction responding was significantly higher than after 1 day, suggesting that the cocaine cues maintain their ability to control behavior even after very prolonged withdrawal periods.

An important issue to consider is the degree to which the extinction data of the 6-month groups reflect a reduction in the motivational effects of cocaine cues after extended withdrawal. Due to methodological limitations, it is not possible in our studies to control for the potential effect of age on responding induced by cocaine cues. Also, the rats in the 6-month group had the highest responding on the inactive lever. Inactive lever responding is often used to measure general (non-specific) activity in reinstatement studies. However, an alternative account for increased responding on this lever during extinction and reinstatement is response generalization (Shalev et al. 2002). Previous research demonstrates time dependent flattening of the generalization gradient of conditioned cues after reward removal (Riccio et al. 1992). Thus, increased responding on the inactive lever in the 6-month groups during both extinction (Fig. 2A, 3A) and reinstatement (Fig. 2D) may be due to response generalization, potentially due to the forgetting of some of the stimulus attributes of the levers. Based on these considerations, the decrease in active lever responding in the 6-month groups may be related to some loss of stimulus control rather than to decreases in the motivational effects of the cocaine cues.

Several recent studies assessed responsiveness to cocaine cues over the first several weeks of withdrawal from cocaine and found either modest time dependent changes (Tran-Nguyen et al. 1998; Neisewander et al. 2000; Semenova and Markou 2003) or no changes (Di Ciano and Everitt 2002; Deroche-Gamonet et al. 2003; Marinelli et al. 2003). Modest time dependent increases in cue-induced reinstatement were found over longer withdrawal periods in the studies of Ciccocioppo et al. (2001) and Meil and See (1997). The investigators in these two studies, however, only measured two relatively late withdrawal time points (21 days and 4 months or 21 days and 40 days, respectively) and in one of the two studies (Meil and See 1997) the test for cue-induced reinstatement at the second time point was performed several weeks after extinction. Thus, spontaneous recovery, the resumption of the extinguished conditioned response that occurs after time has passed following the conclusion of extinction (Bouton and Swartzentruber 1991), may account for the data of Meil and See (1997).

The differences between the present data and some of the previous data may be related to the higher daily intake of cocaine in our studies (in which rats were trained for 6 h/day under a fixed-ratio-1 schedule) than in studies in which rats were trained for either 1–3 h/day (Tran-Nguyen et al. 1998; Deroche-Gamonet et al. 2003; Marinelli et al. 2003) or under a second-order schedule (Di Ciano and Everitt 2002; Semenova and Markou 2003), which results in low daily drug intake. High daily cocaine intake during training induces long lasting neuroadaptations (Grimm et al. 2003; Lu et al. 2003), which may not be induced by low daily cocaine intake; these neuroadaptations may

potentially mediate the time dependent changes in responsiveness to cocaine cues in our studies. Other methodological factors, including the rat's strain, the schedule of reinforcement, and the use of within-subjects design (see Deroche-Gamonet et al. 2003; Semenova and Markou 2003) may also contribute to the different results.

Reinstatement of drug seeking induced by cocaine priming after withdrawal

Another novel finding in this report is the persistent susceptibility of rats to the priming effect of cocaine on reinstatement after extended withdrawal periods. A low dose of cocaine (5 mg/kg, IP) effectively reinstated lever pressing after extinction at all withdrawal periods, and an even lower dose of 2.5 mg/kg modestly reinstated cocaine seeking for up to 3 months. As mentioned, previous studies, in which total daily cocaine intake during training was much lower than in the present report, found that 5 mg/kg cocaine does not induce a significant increase in lever responding (Schenk and Partridge 1999; Park et al. 2002; Schenk and Gittings 2003), but see Schenk et al. (2000). The robust effect on reinstatement of the low doses of cocaine priming at the different withdrawal periods may be due to the development of persistent psychomotor sensitization induced by exposure to high cocaine doses during the training phase. Previous studies have shown that psychomotor sensitization is associated with reinstatement of cocaine (De Vries et al. 1998, 2002) or amphetamine (Vezina et al. 2002) seeking induced by drug priming.

Two recent studies assessed time dependent changes in cocaine-induced reinstatement over the 1st month of withdrawal. Using one cocaine dose (15 mg/kg, IP) in a between-subjects design, Tran-Nguyen et al. (1998) reported that lever pressing induced by cocaine priming was higher after 1 month than after 1 or 7 days. In contrast, using a range of doses (0.2–1.6 mg/kg,IV) in a within-subjects design, Deroche-Gamonet et al. (2003) reported that responding to cocaine priming was lower after 30 days than after 5 days. Here, we did not find time dependent changes in cocaine-induced reinstatement after withdrawal. Our data may be different from those of Deroche-Gamonet et al. (2003) because we used a between-subjects design, while these authors used a within-subjects design. There are also many procedural differences between our study and that of Tran-Nguyen et al. (1998) that may account for the different results. In the study of Tran-Nguyen et al. (1998) rats were trained for 3 h/day on a variable-ratio-5 schedule, drug priming injections were given during training, rats were tested for cocaine priming-induced reinstatement after 2 h of non-contingent exposure to cues (which had a modest, non-significant, effect on reinstatement), and priming tests were conducted in the absence of the discrete cues previously paired with cocaine infusions. In contrast, in the present study, daily sessions were for 6 h and lever pressing was reinforced under a fixed-ratio-1 schedule (resulting in much higher daily intake than in Tran-Nguyen et al.'s study), priming injections were not given during training, and tests were conducted in the presence of the discrete tone-light cue.

Different time course of cocaine seeking induced by cocaine cues or cocaine itself after withdrawal

Based on theoretical reviews (Stewart 1984; Robinson and Berridge 1993), we postulated that cocaine seeking induced by re-exposure to cocaine cues and cocaine itself would follow a similar time course after withdrawal. The present data do not confirm this hypothesis. One possibility is that the different time courses are due to the use of different procedures to measure cocaine seeking induced by re-exposure to cocaine cues and cocaine itself: resistance to extinction in which lever presses result in response-contingent cue presentations versus reinstatement of extinguished lever pressing by non-contingent cocaine injections. Contingent and non-contingent cocaine injections have different effects on cue-controlled responding under a second-order schedule (Markou et al. 1999). In addition, two studies using progressive (Morgan et al. 2002) and second-order (Di Ciano and Everitt 2002) schedules demonstrated modest time dependent increases in lever pressing over the first several weeks after withdrawal from cocaine self-administration (but see Semenova and Markou 2003). In addition, Lynch and Taylor (2003) recently found that after 10 days of withdrawal, female, but not male, rats increase cocaine self-administration under a progressive ratio schedule. However, the magnitude of the time dependent changes in drug-taking behavior in these studies was much lower than the magnitude of the time dependent changes in responsiveness to cocaine cues in our studies (~500% increase from 1 day to 1 month). Thus, it appears unlikely that the contingency of drug exposure would have significantly altered the time course of cocaine-induced reinstatement of drug seeking that was observed here. It is also unlikely that we would have obtained a similar time course for cocaine seeking induced by re-exposure to cocaine cues and cocaine itself if we had used the cue-induced reinstatement test, which is operationally more similar to the cocaine-induced reinstatement test because it is conducted after extinction of the operant responding. The time course of extinction responding (in the presence of the tone-light cue) over the first 3 months of withdrawal in the present experiment is very similar to that observed for cue-induced reinstatement in our previous work (Grimm et al. 2003). Under both conditions, responding was substantially higher after 1 or 3 months of withdrawal than after 1 day.

The different time courses of drug seeking induced by re-exposure to cocaine cues and cocaine itself may be, in part, related to the experience of aversive withdrawal symptoms during the first few days after cocaine self-administration (Sarnyai et al. 1995, 2001; Mutschler and Miczek 1998; Covington and Miczek 2003), which can be

reversed by cocaine re-exposure. Thus, during early withdrawal from psychostimulants, rats experience reward deficits (Leith and Barrett 1976) and consequently may be less responsive to cocaine-associated cues (Arroyo et al. 1998). Following cocaine priming injections, however, the putative reward deficit state may be ameliorated, leading to the resumption of the motivational effects of the cocaine cues (that are earned contingently during the reinstatement test) in the presence of the drug in the body (Stewart et al. 1984).

Finally, the different time courses of cocaine seeking induced by re-exposure to cocaine cues versus cocaine priming may be a manifestation of different neuroadaptations that occur in the partially overlapping neuronal systems involved in cue-induced and drug-induced cocaine seeking (Shalev et al. 2002; Kalivas and McFarland 2003). One potential brain site in this regard is the basolateral amygdala, which is involved in cue-induced, but not cocaine-induced, drug seeking (Grimm and See 2000; Everitt and Wolf 2002).

Concluding remarks

We studied relapse susceptibility over extended withdrawal periods that had not been previously assessed in animal models. We found that the cocaine withdrawal period is a critical modulator of drug seeking induced by re-exposure to cocaine cues, but not cocaine itself. Another finding is that the long lasting and time dependent enhancement of responsiveness to cocaine cues after withdrawal, which persists for at least 3 months, is not permanent. Finally, together with previous findings on time dependent changes in stress-induced reinstatement of heroin seeking (Shalev et al. 2001), a potential clinical implication of the present data is that the drug withdrawal period should be taken into account in the treatment of drug addiction.

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References

Arroyo M, Markou A, Robbins TW, Everitt BJ (1998) Acquisition, maintenance and reinstatement of intravenous cocaine self-administration under a second-order schedule of reinforcement in rats: effects of conditioned cues and continuous access to cocaine. Psychopharmacology 140:331–344

Balleine BW, Dickinson A (1998) Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. Neuropharmacology 37:407–419

Bouton ME, Swartzentruber D (1991) Sources of relapse after extinction in pavlovian and instrumental learning. Clin Psychol Rev 11:123–140

Catania CA (1992) Learning, 3rd edn. Prentice-Hall, Englewood Cliffs, N.J.

Ciccocioppo R, Sanna PP, Weiss F (2001) 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 USA 98:1976–1981

Covington HE, Miczek KA (2003) Vocalizations during withdrawal from opiates and cocaine: possible expressions of affective distress. Eur J Pharmacol 467:1–13

Deroche-Gamonet V, Martinez A, Le Moal M, Piazza PV (2003) Relationships between individual sensitivity to CS- and cocaine-induced reinstatement in the rat. Psychopharmacology 168:201–207

De Vries TJ, Schoffelmeer AN, Binnekade R, Mulder AH, Vanderschuren LJ (1998) Drug-induced reinstatement of heroin- and cocaine-seeking behaviour following long-term extinction is associated with expression of behavioural sensitization. Eur J Neurosci 10:3565–3571

De Vries TJ, Schoffelmeer AN, Binnekade R, Vanderschuren LJ (1999) Dopaminergic mechanisms mediating the incentive to seek cocaine and heroin following long-term withdrawal of IV drug self-administration. Psychopharmacology 143:254–260

De Vries TJ, Schoffelmeer ANM, Binnekade R, Raasø H, Vanderschuren LJMJ (2002) Relapse to cocaine- and heroin-seeking behavior mediated by dopamine D_2 receptors is time-dependent and associated with behavioral sensitization. Neuropsychopharmacology 26:18–26

de Wit H, Stewart J (1981) Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology 75:134–143

Di Ciano P, Everitt BJ (2002) Reinstatement and spontaneous recovery of cocaine-seeking following extinction and different durations of withdrawal. Behav Pharmacol 13:397–405

Erb S, Shaham Y, Stewart J (1996) Stress reinstates cocaine-seeking behavior after prolonged extinction and drug-free periods. Psychopharmacology 128:408–412

Everitt BJ, Wolf ME (2002) Psychomotor stimulant addiction: a neural systems perspective. J Neurosci 22:3312–3320

Gawin FH, Kleber HD (1986) Abstinence symptomatology and psychiatric diagnosis in cocaine abusers. Clinical observations. Arch Gen Psychiatry 43:107–113

Grimm JW, See RE (2000) Dissociation of primary and secondary reward-relevant limbic nuclei in an animal model of relapse. Neuropsychopharmacology 22:473–479

Grimm JW, Hope BT, Wise RA, Shaham Y (2001) Incubation of cocaine craving after withdrawal. Nature 412:141–142

Grimm JW, Lu L, Hayashi T, Hope BT, Su TP, Shaham Y (2003) Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving. J Neurosci 23:742–747

Kalivas PW, McFarland K (2003) Brain circuitry and the reinstatement of cocaine-seeking behavior. Psychopharmacology 168:44–56

Leith NJ, Barrett RJ (1976) Amphetamine and the reward system: evidence for tolerance and post-drug depression. Psychopharmacology 46:19–25

Lu L, Grimm JW, Shaham Y, Hope BT (2003) Molecular neuroadaptations in the accumbens and ventral tegmental area during the first 90 days of forced abstinence from cocaine self-administration in rats. J Neurochem 85:1604–1613

Lu L, Dempsey J, Liu S, Bossert J, Shaham Y (2004) A single infusion of BDNF into the ventral tegmental area induces long-lasting potentiation of cocaine-seeking after withdrawal. J Neurosci 24:1604–1611

Lynch WJ, Taylor JR (2003) Sex differences in the behavioral effects of 24-h/day access to cocaine under a discrete trial pocedure. Neuropsychopharmacology (in press)

Marinelli M, Cooper DC, Baker LK, White FJ (2003) Impulse activity of midbrain dopamine neurons modulates drug-seeking behavior. Psychopharmacology 168:84–98

Markou A, Arroyo M, Everitt BJ (1999) Effects of contingent and non-contingent cocaine on drug-seeking behavior measured using a second-order schedule of cocaine reinforcement in rats. Neuropsychopharmacology 20:542–555

Meil WM, See RE (1996) Conditioned cued recovery of responding following prolonged withdrawal from self-administered cocaine in rats: an animal model of relapse. Behav Pharmacol 7:754–763

Meil WM, See RE (1997) Lesions of the basolateral amygdala abolish the ability of drug associated cues to reinstate responding during withdrawal from self-administered cocaine. Behav Brain Res 87:139–148

Morgan D, Brebner K, Lynch WJ, Roberts DC (2002) Increases in the reinforcing efficacy of cocaine after particular histories of reinforcement. Behav Pharmacol 13:389–396

Mutschler NH, Miczek KA (1998) Withdrawal from i.v. cocaine "binges" in rats: ultrasonic distress calls and startle. Psychopharmacology 135:161–168

Neisewander JL, Baker DA, Fuchs RA, Tran-Nguyen LT, Palmer A, Marshall JF (2000) Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. J Neurosci 20:798–805

O'Brien CP (1997) A range of research-based pharmacotherapies for addiction. Science 278:66–70

Park WK, Bari AA, Jey AR, Anderson SM, Spealman RD, Rowlett JK, Pierce RC (2002) Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens. J Neurosci 22:2916–2925

Phillips GD, Howes SR, Whitelaw RB, Wilkinson LS, Robbins TW, Everitt BJ (1994) Isolation rearing enhances the locomotor response to cocaine and a novel environment, but impairs the intravenous self-administration of cocaine. Psychopharmacology 115:407–418

Riccio DC, Ackil J, Burch-Vernon A (1992) Forgetting of stimulus attributes: methodological implications for assessing associative phenomena. Psychol Bull 112:433–445

Robbins TW (1975) The potentiation of conditioned reinforcement by psychomotor stimulant drugs: a test of Hill's hypothesis. Psychopharmacologia 45:103–114

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 18:247–291

Sarnyai Z, Biro E, Gardi J, Vecsernyes M, Julesz J, Telegdy G (1995) Brain corticotropin-releasing factor mediates "anxiety-like" behavior induced by cocaine withdrawal in rats. Brain Res 657:89–97

Sarnyai Z, Shaham Y, Heinrichs SC (2001) The role of corticotropin-releasing factor in drug addiction. Pharmacol Rev 53:209–244

Schenk S, Gittings D (2003) Effects of SCH 23390 and eticlopride on cocaine-seeking produced by cocaine and WIN 35,428 in rats. Psychopharmacology 168:118–123

Schenk S, Partridge B (1999) Cocaine-seeking produced by experimenter-administered drug injections: dose-effect relationships in rats. Psychopharmacology 147:285–290

Schenk S, Partridge B, Shippenberg TS (2000) Reinstatement of extinguished drug-taking behavior in rats: effect of the kappa-opioid receptor agonist, U69593. Psychopharmacology 151:85–90

Semenova S, Markou A (2003) Cocaine-seeking behavior after extended cocaine-free periods in rats: role of conditioned stimuli. Psychopharmacology 168:192–200

Shaham Y, Shalev U, Lu L, De Wit H, Stewart J (2003) The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology 168:3–20

Shalev U, Highfield D, Yap J, Shaham Y (2000) Stress and relapse to drug seeking in rats: studies on the generality of the effect. Psychopharmacology 150:337–346

Shalev U, Morales M, Hope B, Yap J, Shaham Y (2001) Time-dependent changes in extinction behavior and stress-induced reinstatement of drug seeking following withdrawal from heroin in rats. Psychopharmacology 156:98–107

Shalev U, Grimm JW, Shaham Y (2002) Neurobiology of relapse to heroin and cocaine seeking: a review. Pharmacol Rev 54:1–42

Stewart J (1984) Reinstatement of heroin and cocaine self-administration behavior in the rat by intracerebral application of morphine in the ventral tegmental area. Pharmacol Biochem Behav 20:917–923

Stewart J (2000) Pathways to relapse: the neurobiology of drug- and stress-induced relapse to drug-taking. J Psychiatr Neurosci 25:125–136

Stewart J, de Wit H, Eikelboom R (1984) Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. Psychol Rev 91:251–268

Tran-Nguyen TL, Fuchs RA, Coffey GP, O'Dell LE, Baker DA, Neisewander JL (1998) Time-dependent changes in cocaine-seeking behavior and dopamine overflow in the amygdala during cocaine withdrawal. Neuropsychopharmacology 19:48–59

Vezina P, Lorrain DS, Arnold GM, Austin JD, Suto N (2002) Sensitization of midbrain dopamine neuron reactivity promotes the pursuit of amphetamine. J Neurosci 22:4654–4662