

# Environmental Enrichment Reduces Cocaine Seeking and Reinstatement Induced by Cues and Stress but Not by Cocaine

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Whereas earlier studies have focused on the preventive effects of enriched environments (EE) in drug addiction, in a recent study we suggested that EE can also have 'curative' effects. In fact, we found that cocaine addiction-related behaviors can be eliminated by housing cocaine-treated mice in EE during periods of forced abstinence. However, those results were obtained with two simple models of addiction, conditioned place preference (CPP), and behavioral sensitization. In this study, we used intravenous drug self-administration procedures in rats to further investigate the beneficial effects of EE on cocaine addiction in a reinstatement model of relapse. Singly housed rats learned to self-administer cocaine during 10 consecutive daily sessions (0.6 mg/injection, 6 h/day). They were then housed three per cage in either standard environments (SE) or EE and were kept abstinent in the animal facility until testing for extinction and reinstatement. We found that 30 days of EE significantly and consistently reduced cocaine seeking during a 6-h extinction session. In addition, EE significantly reduced cue- and stress-induced reinstatement. Surprisingly, given our earlier results in mice with CPP, EE did not reduce cocaine-induced reinstatement regardless of the level of exposure to cocaine and the duration of the period of abstinence and exposure to EE. Altogether, these results support the hypothesis that EE can reduce cocaine-induced craving and highlight the importance of positive life conditions in facilitating abstinence and preventing relapse to cocaine addiction.

*Neuropsychopharmacology* (2009) **34**, 2767–2778; doi:10.1038/npp.2009.127; published online 9 September 2009

**Keywords:** environment; addiction; stress; relapse; protracted abstinence; behavioral therapy

## INTRODUCTION

Cocaine addiction is a chronic brain disorder characterized by compulsive drug use that escapes control, by difficulties in remaining abstinent, and by a high risk of relapse. The mechanisms of relapse, and the factors that modulate it, can be studied in animals with reinstatement models (de Wit and Stewart, 1981; Epstein *et al*, 2006; Shalev *et al*, 2002). Environmental conditions have an important function in addiction, with negative environmental conditions increasing (Goeders, 2003; Marinelli and Piazza, 2002; Sinha, 2001) and positive environmental conditions decreasing the risks of addiction (Bardo *et al*, 2001; El Rawas *et al*, 2009; Jessor and Jessor, 1980; Solinas *et al*, 2009). Whereas all earlier studies have focused on the preventive effects of exposure to enriched environments (EE) on drug addiction, in a recent study we showed that EE can also have 'curative' effects and eliminate preexisting addiction-related behaviors (Solinas *et al*, 2008). In fact, exposing mice to a positive EE during a

30-day period of abstinence can reduce cocaine behavioral sensitization, drug seeking, and cocaine-induced reinstatement in conditioned place preference (CPP) procedures in mice (Solinas *et al*, 2008). In addition, the effects of EE on cocaine-induced reinstatement are paralleled by reductions in cocaine-induced activation of the brain circuitry involved in relapse (Solinas *et al*, 2008). Although these findings provide new evidence for the positive effects of EE on cocaine addiction, the models that were used mimic only some of the features of drug addiction in humans, namely the Pavlovian learning mechanisms involved in drug addiction (Bardo and Bevins 2000; Sanchis-Segura and Spanagel, 2006). On the other hand, drug self-administration procedures and reinstatement of extinguished drug-seeking procedures have a higher level of validity (Epstein *et al*, 2006; Shalev *et al*, 2002) because with these procedures it is possible to appropriately mimic more complex aspects of human addiction, including voluntary drug taking and seeking behaviors, in experimental animals (Panlilio and Goldberg, 2007). In reinstatement of self-administration procedures, animals first learn to respond for drug injections and, then, this responding is extinguished by withdrawal of the drug reinforcer. Drug-seeking behavior can then be reinstated by drug priming injections, discrete drug-associated cues, or stressors (de Wit and Stewart,

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Received 3 April 2009; revised 24 July 2009; accepted 25 July 2009

1981; Epstein *et al*, 2006; Shalev *et al*, 2002). Thus, drug self-administration procedures allow for the study of each of these factors that can trigger relapse to drug use in humans (Epstein *et al*, 2006).

In this study, singly housed rats first learned to intravenously self-administer cocaine (0.6 mg/injection) during 6 h daily sessions for 10 days and were then housed three per cage in standard environments (SE) or EE (Laviola *et al*, 2008; Nithianantharajah and Hannan, 2006; van Praag *et al*, 2000). Rats were housed for a 30-day abstinence period in these environmental conditions and then underwent one 6-h extinction session followed by one or two 1-h reinstatement sessions according to a 'between-within' design (Shalev *et al*, 2002; Lu *et al*, 2004b). In these protocols, rats are allowed to self-administer a drug for several sessions and, subsequently, extinction training and relapse tests are performed on the same day after different periods of withdrawal. Reinstatement was triggered by cocaine-associated conditioned cues, by priming injections of cocaine or by the pharmacological stressor yohimbine, a drug that causes anxiety-like symptoms in humans and animals (Bremner *et al*, 1996a; Bremner *et al*, 1996b). As cocaine-induced reinstatement was not reduced by EE under these conditions, in contrast to our earlier findings in mice with CPP procedures (Solinas *et al*, 2008), we conducted three additional experiments in which (1) the level of cocaine exposure was reduced (10 × 3 h daily sessions), (2) the duration of exposure to EE was increased (90 days of abstinence in an EE), and (3) a 'between-day' reinstatement procedure was used (Shalev *et al*, 2002).

## MATERIALS AND METHODS

### Subjects

Adult (11–12 weeks of age) male Sprague–Dawley rats (Janvier, France) experimentally naive at the start of the study were housed in a temperature- and humidity-controlled room and maintained on a 12-h light/dark cycle

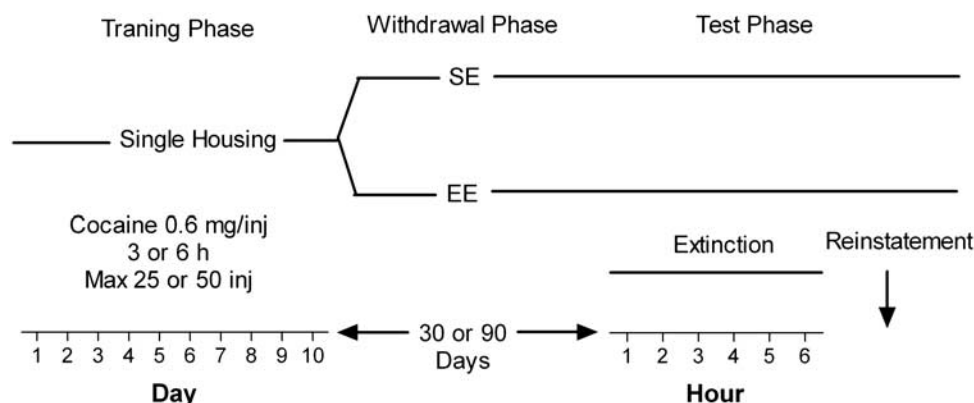
(light on at 7.00 AM). All experiments were conducted during the light phase in accordance to E.C. regulations for animal use in research (86/609/EEC).

### Housing Conditions

On arrival, rats were housed three per cage for about 1 week before intrajugular catheterization surgery. After surgery, rats were housed individually during the entire period of active cocaine self-administration to limit catheter loss because of reciprocal chewing. At the end of the last cocaine self-administration session, rats were pseudo-randomly divided into two groups, one housed in SE and the other in EE conditions, with similar levels of cocaine self-administration in the two groups. For SE, rats were housed in groups of three in cages sized 60 × 38 × 20 cm. For EE, rats were housed in groups of three in cages sized 80 × 50 × 100 cm. Each EE cage contained a house, a running wheel, three floors connected by ramps or tunnels and four toys that were changed once per week. For experiments 1, 2, 3, and 4, rats were housed in the animal facility in SE or EE housing conditions during a 30-day period of abstinence. For experiment 5, rats were kept abstinent from drug for 90 days in SE or EE housing conditions (see Figure 1 for a schematic representation of the experimental procedures). For experiment 6, rats were housed in SE or EE for eleven days during which they underwent extinction and reinstatement tests.

### Cocaine Self-Administration Apparatus

For cue- and cocaine-induced reinstatement experiments, we used Imetronic experimental chambers equipped with nose-pokes as operanda and controlled by Imetronic interfaces and software (Imetronic, Pessac, France; www.imetronic.com). As stress-induced reinstatement of drug seeking is more easily obtained using lever-press responding (Drs Yavin Shaham and Taco De Vries, personal communication), for stress-induced reinstatement



**Figure 1** Schematic representation of the experimental procedures. During the training phase, rats were singly housed and 10 cocaine self-administration sessions were conducted during which they could self-administer cocaine (0.6 mg/injection). For experiments 1, 2, 3, and 5 (Figures 2, 3, 4, and 6) session length was 6 h, whereas for experiment 4 (Figure 5) it was reduced to 3 h. The maximum number of injections was limited at 50 for 6 h session and to 25 for 3 h session. At the end of the last cocaine self-administration session, rats were assigned to standard environment (SE) or enriched environment (EE) conditions, assuring that two groups had similar levels of exposure to cocaine. During the withdrawal period, rats were housed three per cage in SE or EE in the animal facility. For experiments 1, 2, 3, and 4 (Figures 2, 3, 4, and 5), rats were kept abstinent for 30 days before reinstatement testing, whereas for experiment 5 (Figure 6), abstinence lasted for 90 days. At the end of the withdrawal period, on the same day, rats were subjected to one 6-h extinction session and, subsequently, to one or two 1-h reinstatement sessions (test phase).

experiments, we used Coulbourn experimental chambers that were kindly donated by the National Institute on Drug Abuse and were equipped with retractable levers as operanda and controlled by Coulbourn interfaces and Graphic State software (Coulbourn Instruments, Allentown, PA, USA; [www.coulbourn.com](http://www.coulbourn.com)).

### Cocaine Self-Administration Procedure

Ten cocaine self-administration sessions were conducted using a Fixed-Ratio 1 schedule of reinforcement. During daily sessions, a single response in the active nose-poke hole immediately delivered an i.v. injection of cocaine (0.6 mg/injection) and caused the house light to pulse for 5 s followed by a 5-s time-out. During this 5-s time-out period, the chambers were dark and responding had no programmed consequences. For experiments involving lever-press responding, conditions were similar but active lever-presses also resulted in retraction of the lever and delivery of the light plus tone stimuli. Responses in the inactive nose-poke hole or on the inactive lever were recorded but had no programmed consequences. Self-administration sessions lasted for 6 h in all experiments, except experiment 4 in which 3-h sessions were used.

### Extinction of Cocaine Self-Administration and Cocaine Seeking

After 30 or 90 days in either SE or EE housing conditions, rats were brought to the self-administration room and tested for extinction. For cue-induced reinstatement experiments, nose-pokes in the active or inactive hole during extinction sessions were recorded but had no programmed consequences. For all other experiments, active nose-pokes or active lever-presses during extinction sessions produced the same stimuli (light or light/tone and noise of the pump) that were earlier produced during cocaine self-administration sessions but syringes were removed from the injection pumps and cocaine was not delivered. The 6-h extinction session was divided into six 1-h extinction segments. The number of active nose-pokes or lever-presses was used as a measure of cocaine seeking.

### Cue-Induced Reinstatement

In cue-induced reinstatement tests, the presentation of conditioned stimuli (light and pump noise) was made contingent on active nose-pokes, as it was during cocaine self-administration sessions. A single 1 h cue-induced reinstatement session was conducted immediately after the extinction session.

### Stress-Induced Reinstatement

For stress-induced reinstatement experiments, saline (1 ml/kg i.p.) was injected immediately before the fifth and sixth hour of the extinction session to allow adaptation to stress effects because of injection manipulations. A single 1 h stress-induced reinstatement session was conducted immediately after the extinction session. Immediately before the stress-induced reinstatement test session, rats were injected with yohimbine (1.25 mg/kg i.p.). The 1.25 mg/kg

dose of yohimbine was chosen on the basis of earlier studies showing that this dose reliably induces reinstatement in rats (Shepard *et al*, 2004; Lee *et al*, 2004; Lê *et al*, 2005; Feltenstein and See, 2006; Ghitza *et al*, 2006; Kupferschmidt *et al*, 2009).

### Cocaine-Induced Reinstatement

For cocaine-induced reinstatement experiments, saline (1 ml/kg i.p.) was injected immediately before the fifth and sixth hour of the extinction session to allow adaptation to stress effects because of injection manipulations. Immediately after the extinction session, two consecutive 1 h cocaine-induced reinstatement sessions were conducted for experiments 3 and 4 by administering increasing doses of cocaine (3 and 6 mg/kg i.p.). A single 1 h cocaine-induced reinstatement session was conducted for experiments 5 with a cocaine dose of 6 mg/kg. In an attempt to magnify possible positive effects of EE on cocaine-induced reinstatement, we chose low doses of cocaine that reliably induce reinstatement in rats (Lu *et al*, 2004a).

### Between-Day Procedure for Extinction and Reinstatement

For this experiment (Figure 7), rats underwent self-administration training with nose-pokes as operanda as described earlier. At the end of the training, rats were assigned to SE or EE conditions and, approximately 18 h later, daily 3-h extinction sessions began for 10 consecutive days. On the 9th and 10th day of extinction, saline (1 ml/kg i.p.) was injected immediately before the extinction session to allow adaptation to stress effects because of injection manipulations. Subsequently, on the 11th day, rats were injected with a cocaine dose of 6 mg/kg and cocaine-induced reinstatement was monitored in a single 3-h session.

### Drugs

Cocaine HCl was purchased from the COOPER (Cooperation Pharmaceutique Française, France; [www.cooper.fr](http://www.cooper.fr)). Drugs were dissolved in sterile saline solutions (0.9%).

### Statistical Analysis

Differences in drug-seeking behavior and reinstatement were assessed by two-way or three-way ANOVA for repeated measures. For incubation of craving, differences were assessed by two-way ANOVA. Results showing significant overall changes were subjected to Student–Newman–Keuls post hoc test. Differences were considered significant when  $p < 0.05$ .

## RESULTS

### Self-Administration of Cocaine Before Assignment to SE or EE

For all experiments, rats were assigned to SE or EE at the end of the last cocaine self-administration session assuring that the two groups had similar basal levels of cocaine

self-administration. Therefore, for each experiment, the number of active and inactive responses (Figures 2a, 3a, 4a, 5a, and 6a) and the number of cocaine injections (Supplementary Figure 1) did not differ between future SE and future EE groups (see Supplementary Materials for detailed statistical analysis).

### Experiment 1: EE Reduces Cocaine Seeking and Cue-Induced Reinstatement

In extinction sessions performed in the absence of conditioned cues (lights and pump noise), both SE ( $n=10$ ) and EE ( $n=10$ ) rats showed a clear preference for the nose-poke hole compared with the inactive hole and drug-seeking behavior in both SE and EE rats extinguished within the 6-h session. However, drug seeking, measured as the number of active nose-pokes, was significantly lower in EE compared with SE rats (Figure 2b and c). Statistical analysis revealed a significant effect of Environment ( $F_{1,18}=7.92$ ,  $p<0.05$ ), of Time ( $F_{5,95}=48.10$ ,  $p<0.0001$ ), of Active Device ( $F_{1,18}=40.44$ ,  $p<0.0001$ ), and a Time  $\times$  Active Device interaction ( $F_{5,90}=18.41$ ,  $p<0.0001$ ).

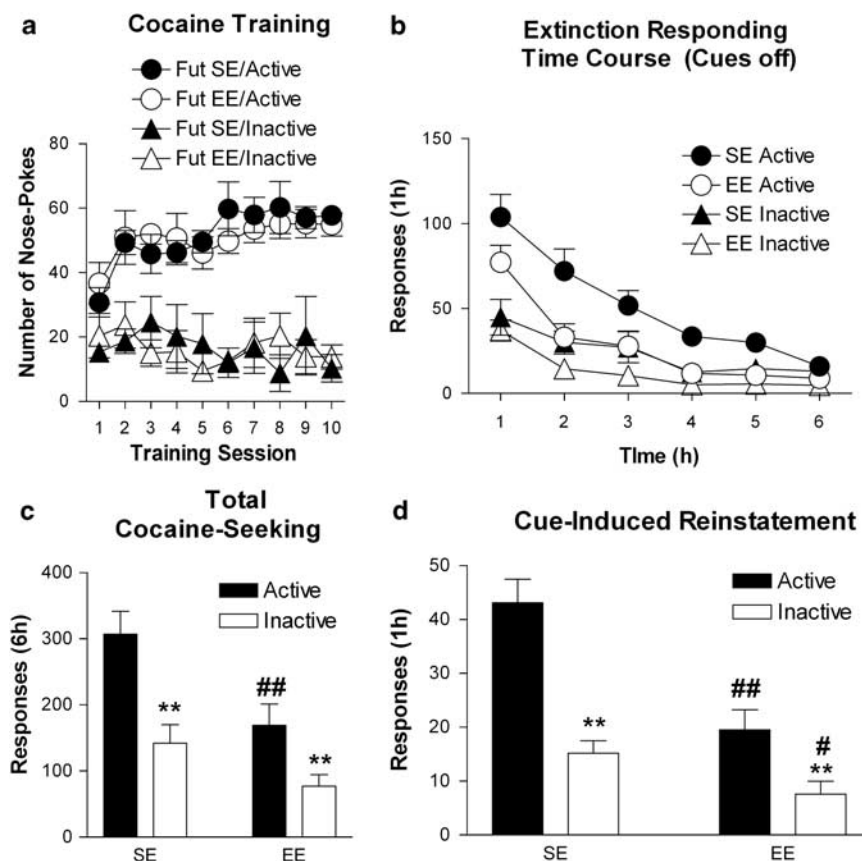
In reinstatement sessions, when presentation of conditioned cues was made contingent on active nose-poking, both SE and EE rats reinstated cocaine seeking. However, cue-induced reinstatement was significantly lower in EE

compared with SE rats (Figure 2d). Statistical analysis revealed a significant effect of Environment ( $F_{1,18}=15.53$ ,  $p<0.01$ ), of Active Device ( $F_{1,18}=60.26$ ,  $p<0.0001$ ), and an Environment  $\times$  Active Device interaction ( $F_{1,18}=9.74$ ,  $p<0.01$ ).

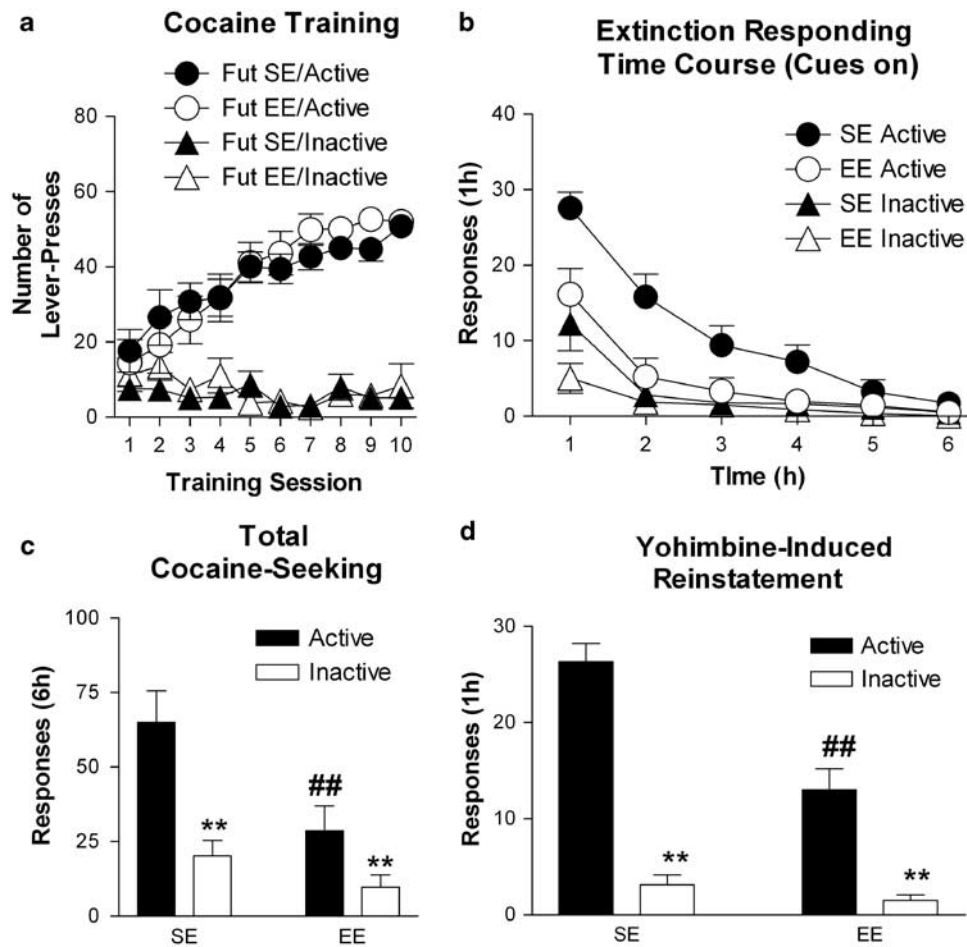
### Experiment 2: EE Reduces Cocaine Seeking and Stress-Induced Reinstatement

In extinction sessions, both SE ( $n=9$ ) and EE ( $n=8$ ) rats showed a clear preference for the active lever compared with the inactive lever and drug-seeking behavior in both SE and EE rats extinguished within the 6-h session. However, drug seeking, measured as the number of active lever-presses, was significantly lower in EE compared with SE rats (Figure 3b and c). Statistical analysis revealed a significant effect of Environment ( $F_{1,15}=7.01$ ,  $p<0.05$ ), of Time ( $F_{5,75}=55.89$ ,  $p<0.0001$ ), of Active Device ( $F_{1,15}=39.64$ ,  $p<0.0001$ ), an Environment  $\times$  Time interaction ( $F_{5,75}=4.37$ ,  $p<0.05$ ), an Environment  $\times$  Active Device interaction ( $F_{1,15}=14.19$ ,  $p<0.01$ ), a Time  $\times$  Active Device interaction ( $F_{5,75}=85.73$ ,  $p<0.0001$ ), and an Environment  $\times$  Time  $\times$  Active Device interaction ( $F_{5,75}=5.72$ ,  $p<0.01$ ).

When the pharmacological stressor yohimbine (1.25 mg/kg i.p.) was injected immediately before the reinstatement session, both SE and EE rats reinstated cocaine seeking.



**Figure 2** Effects of environmental enrichment on cue-induced reinstatement. Basal level of self-administration (a), time course of extinction (b), total cocaine seeking during extinction (c), and cue-induced reinstatement (d) in rats housed in standard environments (SE) or enriched environments (EE) during a 30-day abstinence period. Two-way ANOVA for repeated measures followed by *post hoc* Student–Newman–Keuls test: \*\* $p<0.01$  vs compared with active nose-poke; ## $p<0.01$  vs compared with SE control.



**Figure 3** Effects of environmental enrichment on stress-induced reinstatement. Basal level of self-administration (a), time course of extinction (b), total cocaine seeking during extinction (c), and stress-induced reinstatement (d) in rats housed in standard environments (SE) or enriched environments (EE) during a 30-day abstinence period. Note that immediately before the fifth and sixth hour of extinction, rats were administered saline (1 ml/kg). Reinstatement was triggered by administration of the pharmacological stressor yohimbine (1.25 mg/kg i.p.). Two-way ANOVA for repeated measures followed by *post hoc* Student–Newman–Keuls test: \*\* $p < 0.01$  vs active nose-poke; ## $p < 0.01$  vs SE control.

However, stress-induced reinstatement was significantly lower in EE compared with SE rats (Figure 3d). Statistical analysis revealed a significant effect of Environment ( $F_{1,15} = 19.01$ ,  $p < 0.01$ ), of Active Device ( $F_{1,15} = 146.29$ ,  $p < 0.0001$ ), and an Environment X Active Device interaction ( $F_{1,15} = 16.67$ ,  $p < 0.01$ ).

### Experiment 3: EE Reduces Cocaine Seeking but Not Cocaine-Induced Reinstatement

In extinction sessions performed in the presence of conditioned cues, both SE ( $n = 10$ ) and EE ( $n = 11$ ) rats showed a clear preference for the active nose-poke hole compared with the inactive hole and drug-seeking behavior in both SE and EE rats extinguished within the 6-h session. However, drug seeking was significantly lower in EE compared with SE rats (Figure 4b and c). Statistical analysis revealed a significant effect of Environment ( $F_{1,19} = 8.96$ ,  $p < 0.01$ ), of Time ( $F_{5,95} = 48.11$ ,  $p < 0.0001$ ), of Active Device ( $F_{1,19} = 41.98$ ,  $p < 0.0001$ ), and a Time  $\times$  Active Device interaction ( $F_{5,95} = 11.78$ ,  $p < 0.0001$ ).

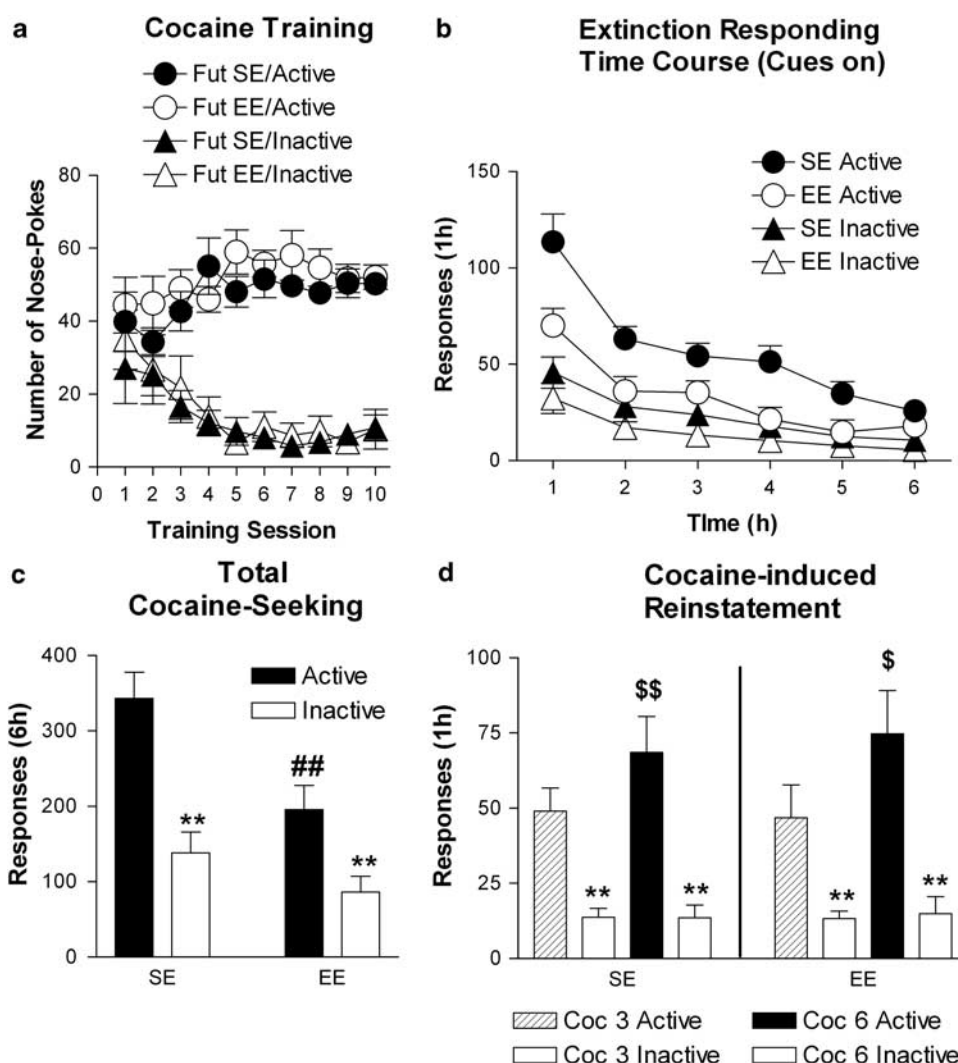
When cocaine (3 or 6 mg/kg i.p.) was injected immediately before the reinstatement session, both SE and EE rats

reinstated cocaine seeking. Surprisingly, cocaine-induced reinstatement was similar in EE and SE rats regardless of the cocaine dose (Figure 4d). Statistical analysis revealed a significant effect of Active Device ( $F_{1,18} = 40.10$ ,  $p < 0.0001$ ), of Dose ( $F_{1,18} = 19.78$ ,  $p < 0.01$ ), and an Active Device  $\times$  Dose interaction ( $F_{1,18} = 11.97$ ,  $p < 0.01$ ).

### Experiment 4: The Lack of Effects of EE on Cocaine-Induced Reinstatement Does Not Depend on the Level of Cocaine Exposure

As we had found earlier that cocaine-induced reinstatement of an extinguished CPP in mice was prevented by EE (Solinas *et al*, 2008), we were surprised by the lack of effect of EE on cocaine-induced reinstatement of drug seeking in this study. Therefore, we designed a series of manipulations to rule out the possibility that differences in the experimental procedures may have masked the positive effects of EE in the present experiments.

First, we investigated whether our cocaine self-administration procedure had rendered rats too ‘addicted’ for EE to produce its effects by reducing the cocaine (0.6 mg/injection) self-administration sessions to 3 h instead of 6 h



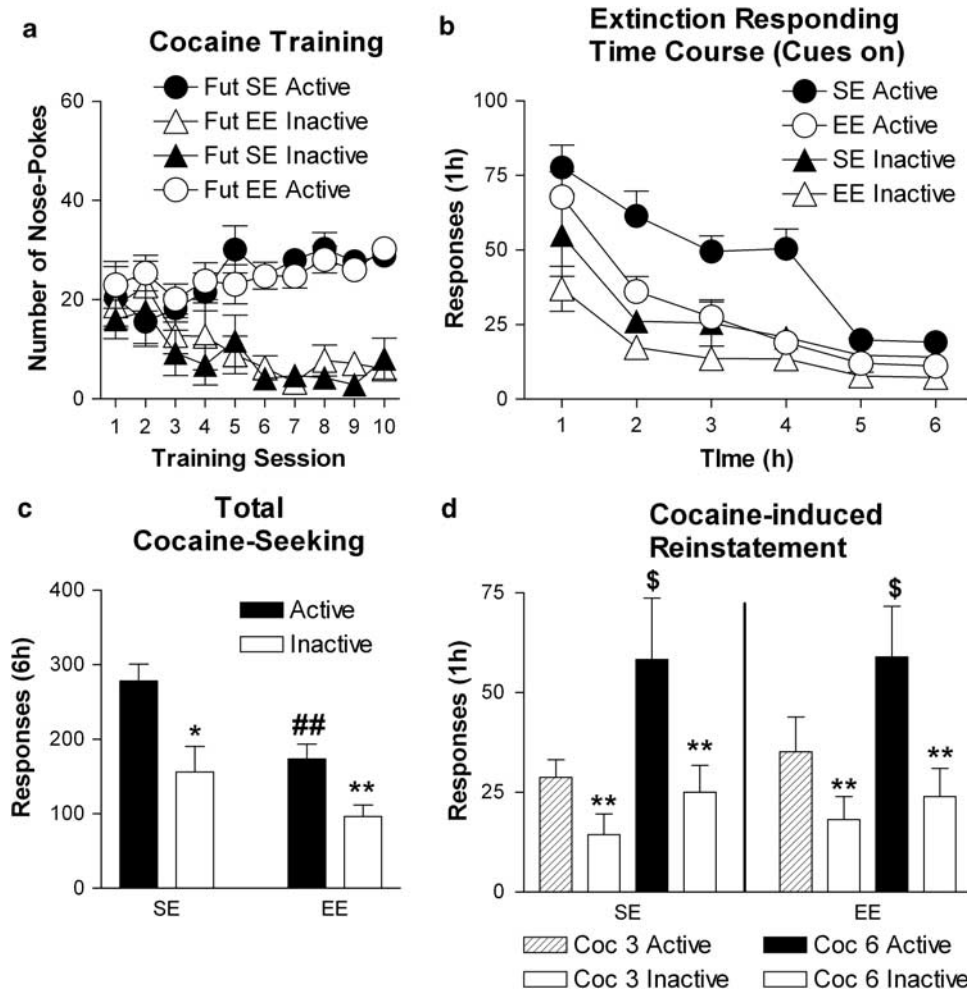
**Figure 4** Effects of environmental enrichment on cocaine-induced reinstatement in rats with 6 h access to cocaine. Basal level of self-administration (a), time course of extinction (b), total cocaine seeking during extinction (c), and cocaine-induced reinstatement (d) in rats housed in standard environments (SE) or enriched environments (EE) during a 30-day abstinence period. Note that immediately before the fifth and sixth hour of extinction, rats were administered saline (1 ml/kg). Reinstatement was triggered by administration of cocaine (3 and 6 mg/kg i.p.). Two-way ANOVA for repeated measures followed by *post hoc* Student–Newman–Keuls test: \*\* $p < 0.01$  vs active nose-poke; ## $p < 0.01$  vs SE control; \$\$\$ $p < 0.05$  and  $0.01$  vs cocaine 3 mg/kg.

for 10 consecutive days before assigning rats to SE or EE housing conditions. Consistent with the other self-administration experiments, during extinction sessions, both SE ( $n = 7$ ) and EE ( $n = 8$ ) rats showed a clear preference for the active nose-poke hole and drug-seeking behavior in both SE and EE rats extinguished within the 6-h session. However, drug seeking was significantly lower in EE compared with SE rats (Figure 5a and b). Statistical analysis revealed a significant effect of Environment ( $F_{1,13} = 8.88$ ,  $p < 0.05$ ), of Time ( $F_{5,65} = 47.11$ ,  $p < 0.01$ ), of Active Device ( $F_{1,13} = 29.25$ ,  $p < 0.0001$ ), and a Time  $\times$  Active Device interaction ( $F_{5,65} = 9.22$ ,  $p < 0.0001$ ).

In addition, similar to what was found with 6 h cocaine self-administration sessions, cocaine-induced reinstatement was similar in EE and SE rats that had self-administered cocaine during the 3-h sessions, regardless of the cocaine dose (Figure 5c). Statistical analysis revealed a significant effect of Active Device ( $F_{1,13} = 8.72$ ,  $p < 0.05$ ) and of Dose ( $F_{1,13} = 33.00$ ,  $p < 0.0001$ ).

#### Experiment 5: The Lack of Effects of EE on Cocaine-Induced Reinstatement Does Not Depend on the Duration of EE

As a second step, we investigated whether a longer period of EE was necessary for EE to produce its effects on cocaine-induced reinstatement. After the 10 sessions of cocaine self-administration, rats were separated into SE or EE housing conditions and were then kept abstinent for 90 instead of 30 days. Once again, in extinction sessions, both SE ( $n = 4$ ) and EE ( $n = 5$ ) rats showed a preference for the active nose-poke hole and drug-seeking behavior in both SE and EE rats extinguished within the 6-h session. Again, drug seeking was significantly lower in EE compared with SE rats (Figure 6a and b). Statistical analysis revealed a significant effect of Environment ( $F_{1,7} = 17.78$ ,  $p < 0.01$ ), of Time ( $F_{5,35} = 34.11$ ,  $p < 0.0001$ ), an Environment  $\times$  Time interaction ( $F_{5,35} = 2.88$ ,  $p < 0.05$ ), and a Time  $\times$  Active Device interaction ( $F_{5,35} = 3.11$ ,  $p < 0.05$ ).



**Figure 5** Effects of environmental enrichment on cocaine-induced reinstatement in rats with 3 h access to cocaine. Basal level of self-administration (a), time course of extinction (b), total cocaine seeking during extinction (c), and cocaine-induced reinstatement (d) in rats housed in standard environments (SE) or enriched environments (EE) during a 30-day abstinence period. In this experiment, rats self-administered cocaine for 3 h instead of 6 h for 10 days before the abstinence period. Note that immediately before the fifth and sixth hour of extinction, rats were administered saline (1 ml/kg). Reinstatement was triggered by administration of cocaine (3 and 6 mg/kg i.p.). Two-way ANOVA for repeated measures followed by *post hoc* Student–Newman–Keuls test: \*\*\* $p < 0.05$  and 0.01 vs active nose-poke; ## $p < 0.01$  vs SE control; \$ $p < 0.05$  vs cocaine 3 mg/kg.

In addition, similar to what was found in rats kept abstinent in their respective environmental condition for 30 days, in rats housed in EE and SE for 90 days, cocaine-induced reinstatement was similar (Figure 6c). Statistical analysis revealed only a significant effect of Active Device ( $F_{1,7} = 7.34$ ,  $p < 0.05$ ).

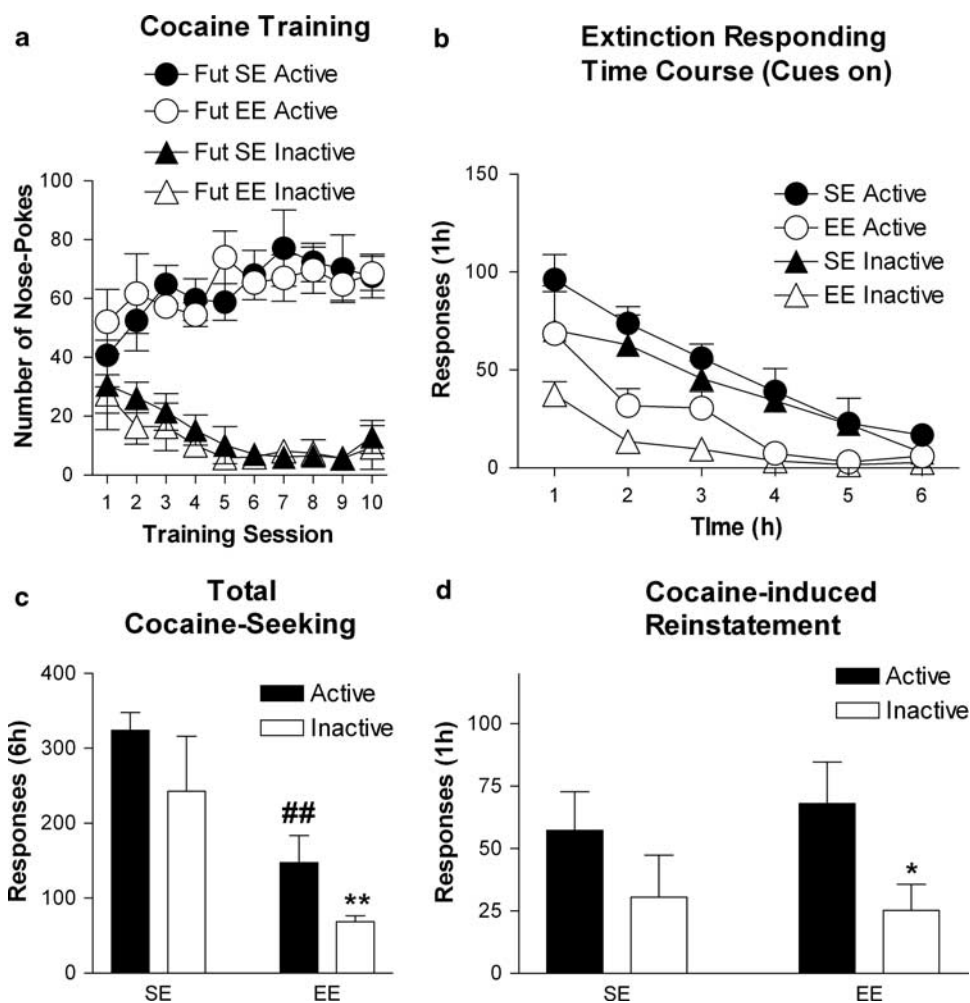
#### Experiment 6: EE Does Not Attenuate Cocaine-Induced Reinstatement in a Between-Day Procedure of Extinction and Reinstatement

In our earlier study in mice with CPP procedures, 10 extinction sessions and one reinstatement session were performed over an 11-day period (Solinas *et al*, 2008). To determine whether these procedural differences in extinction conditions might be responsible for the lack of effects of EE on cocaine-induced reinstatement of drug seeking, we used a similar extinction procedure in rats that had self-administered cocaine. Thus, immediately after the last day of cocaine self-administration, rats were housed in SE

( $n = 8$ ) or EE ( $n = 8$ ) conditions and, approximately 18 h later, daily 3 h extinction sessions began. Extinction of cocaine seeking was faster and more pronounced in EE than compared with SE rats, even when they were in the EE for <24 h (Figure 7a) but once again, cocaine-induced reinstatement was similar between SE and EE rats (Figure 7b).

#### DISCUSSION

In these experiments, we found that exposure to EE during long periods of abstinence from cocaine self-administration consistently reduces drug seeking and attenuates reinstatement induced by conditioned cues and stress. On the other hand, reinstatement induced by experimenter-administered cocaine was not altered by EE under a variety of experimental conditions. These results suggest that EE can reduce the risks of relapse to cocaine addiction but also



**Figure 6** Effects of 90 days of environmental enrichment on cocaine-induced reinstatement. Basal level of self-administration (a), time course of extinction (b), total cocaine seeking during extinction (c), and cocaine-induced reinstatement (d) in rats housed in standard environments (SE) or enriched environments (EE). In this experiment, the abstinence period and the exposure to SE or EE was increased to 90 days. Note that immediately before the fifth and sixth hour of extinction, rats were administered saline (1 ml/kg). Reinstatement was triggered by administration of cocaine (6 mg/kg i.p.). Two-way ANOVA for repeated measures followed by *post hoc* Student–Newman–Keuls test: \*\*\* $p < 0.05$  and  $0.01$  vs active nose-poke; ## $p < 0.01$  vs SE control.

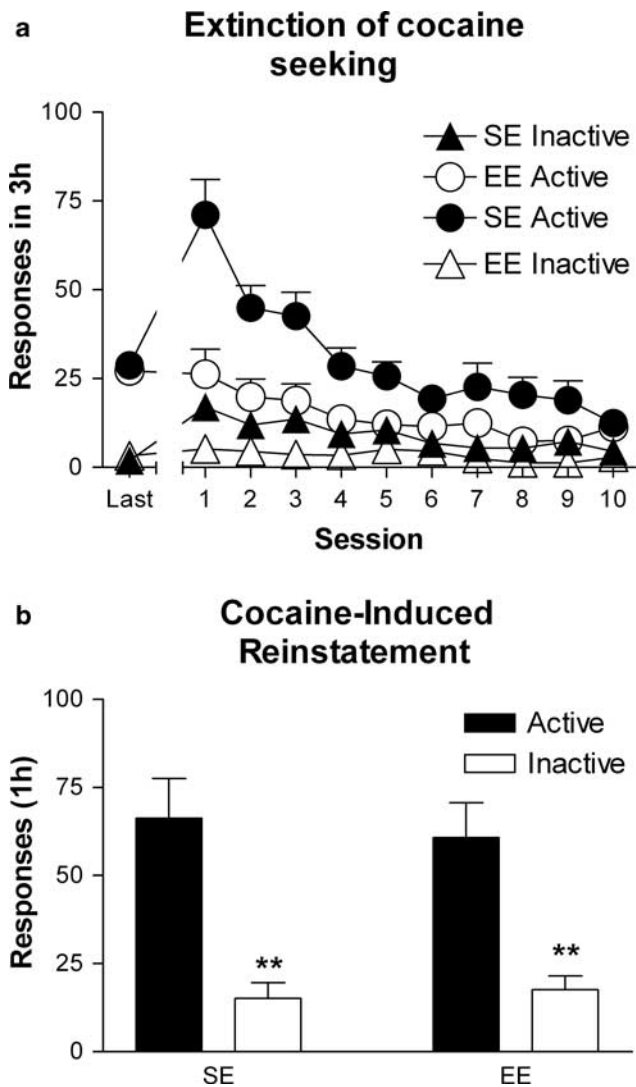
point out to the fact that exposure to earlier abused drugs may overcome the protection provided by EE.

In the last decades, several studies have shown that EE conditions potentiate cognitive functions (for review see Rosenzweig and Bennett 1996; van Praag *et al*, 2000) and can protect the brain against neurological and neurodegenerative disorders (Nithianantharajah and Hannan 2006; Laviola *et al*, 2008). In addition, EE conditions influence the behavioral and neurochemical effects of drug abuse and may provide protection against the development of drug addiction (Bardo *et al*, 2001; Carroll *et al*, 2009; El Rawas *et al*, 2009; Jessor and Jessor 1980; Solinas *et al*, 2009; Stairs and Bardo 2009). Although earlier studies had focused on the preventive effects of EE, we have recently shown that EE can also have ‘curative’ effects on cocaine addiction (Solinas *et al*, 2008). In addition, Grimm *et al* (2008) have recently reported that craving for food is also reduced by exposure to EE during periods of withdrawal (Grimm *et al*, 2008). Similarly, in this study, we found that cocaine seeking during extinction sessions was reduced in each of our experiments, regardless of the operanda used (nose-poke or

lever-press), the presence or absence of conditioned stimuli, the level of earlier cocaine exposure (3 or 6 h self-administration sessions), and the duration of exposure to EE during abstinence (30 or 90 days). Altogether, these findings strongly support the hypothesis that EE have significant beneficial effects on cocaine addiction.

Once cocaine-seeking behavior was extinguished, we were able to reinstate cocaine seeking by exposing animals to conditioned cues, to an experimenter-administered dose of cocaine or to the pharmacological stressor yohimbine (de Wit and Stewart 1981; Epstein *et al*, 2006; Shalev *et al*, 2002). Yohimbine is a drug with a mixed pharmacological profile. In fact, although its best-characterized mechanism of action is the blockade of  $\alpha$ -2 noradrenergic and the increase in noradrenergic cell firing and release (Aghajanian and VanderMaelen, 1982; Abercrombie *et al*, 1988), it has also been shown to bind to serotonin and dopamine receptors and to increase the activity of dopamine systems (Millan *et al*, 2000; Tanda *et al*, 1996). Yohimbine causes anxiety-like symptoms in humans and animals (Bremner *et al*, 1996a; Bremner *et al*, 1996b) and induces reinstatement





**Figure 7** Effects of environmental enrichment on cocaine-induced reinstatement in a between-day procedure of extinction and reinstatement. Number of active nose-pokes in the first 3 h of the last 6-h session of training (left part) and extinction of cocaine seeking in 10 consecutive 3-h sessions (a) in rats housed in standard (SE) or enriched environments (EE). Cocaine-induced reinstatement in SE and EE rats (b) during a single 1-h reinstatement session on the day after the last day of extinction. Note that immediately before the 9th and 10th session of extinction, rats were administered with saline (1 ml/kg). Reinstatement was triggered by administration of the cocaine (6 mg/kg i.p.). Two-way ANOVA for repeated measures followed by *post hoc* Student–Newman–Keuls test: \* $p < 0.05$  vs active nose-poke. \*\* $p < 0.01$

of extinguished drug seeking in rats and this effect appears to be more robust than that induced by footshock, a nonpharmacological stressor commonly used in rodent research (Shepard *et al*, 2004; Lê *et al*, 2005; Ghitza *et al*, 2006). Therefore, the use of yohimbine as a pharmacological stressor in reinstatement procedures has become increasingly popular (Shepard *et al*, 2004; Lee *et al*, 2004; Lê *et al*, 2005; Feltenstein and See, 2006; Ghitza *et al*, 2006; Kupferschmidt *et al*, 2009). Importantly, EE was able to significantly reduce reinstatement induced by contextual cues and stress. Given that exposure to conditioned cues, and stressful situations appear to be powerful triggers of

relapse in humans (Epstein *et al*, 2009), this suggests that environmental stimulation during periods of abstinence may be an important factor in decreasing the risks of relapse.

On the other hand, EE did not alter cocaine-induced reinstatement. The finding that a single manipulation (exposure to EE in this case) affects different forms of reinstatement differently is not uncommon (see Shalev *et al*, 2002 for review). For example, cocaine craving as measured by responding during extinction and cue-induced reinstatement increase over time, whereas cocaine-induced reinstatement does not (Grimm *et al*, 2001; Lu *et al*, 2004a). Given our earlier findings obtained in mice with CPP procedure (Solinas *et al*, 2008), it was unexpected that cocaine-induced reinstatement was not blocked by EE. Therefore, we performed a series of manipulations to exclude the possibility that differences in experimental procedures were responsible for this lack of effect. We found that the lack of effect of EE on cocaine-induced reinstatement was not due to of the level of cocaine exposure (ie, the rats were not too 'addicted'), nor to the duration of exposure to the EE (ie, 30 and 90 days of EE had similar effects on cocaine seeking). This discrepancy may be explained by differences in species or differences in the underlying behavior measured by self-administration vs CPP procedures (Bardo and Bevins 2000; Sanchis-Segura and Spanagel, 2006). Notwithstanding these differences, our self-administration data suggest that pharmacological stimulation of the brain by cocaine may overcome the protection provided by EE. However, given that cocaine administration generally follows a voluntary act in humans (Epstein *et al*, 2006), our data on cocaine seeking during extinction and cue- and stress-induced reinstatement indicate that stimulating environments may be powerful factors in facilitating abstinence and reducing the risks of relapse.

The mechanisms through which EE could reduce cocaine seeking and reinstatement are yet to be determined. Loss of cocaine memories does not seem to be a likely mechanism because the same EE rats that show consistent reductions in drug seeking during extinction sessions show drug seeking similar to SE rats after administration of cocaine, suggesting that memories of cocaine remain intact in EE rats. A well-known effect of EE is to increase learning (for review see Rosenzweig and Bennett, 1996; van Praag *et al*, 2000). On the basis of these results, Grimm *et al* (2008) suggested that EE rats learn faster in extinction that their actions are not rewarded and, therefore, quit responding earlier than SE controls. Alternatively, EE could have made cocaine-related stimuli less salient and rats could have been less motivated to respond for them. EE has been also shown to decrease impulsivity in rats (Hellemans *et al*, 2005; Perry *et al*, 2008). As impulsivity has been shown to be associated with high risks of relapse (Economidou *et al*, 2009; Lê *et al*, 2008; Perry *et al*, 2008), it could also be speculated that positive effects of EE on cocaine seeking and cue- and stress-induced reinstatement are mediated by decreases in impulsivity. Psychostimulant addiction appears to be associated with deficits in decision making and other cognitive tasks such as behavioral inflexibility and perseverative behaviors that may have an important function in relapse (Verdejo-García and Bechara, 2009; Stalnaker *et al*, 2009; Ersche *et al*, 2009). Therefore, EE may have acted by reversing these cocaine-induced deficits.

Stress can have marked influences on motivated behaviors. For example, acute stress increases the reactivity to rewards and precipitates reinstatement of extinguished drug seeking (Goeders, 2003; Shalev *et al*, 2002). On the other hand, chronic stress has been reported to decrease (Willner, 2005; Matthews and Robbins, 2003) or increase (Goeders, 2003; Carr, 2002; Koob and Kreek, 2007; Miczek *et al*, 2008) the reactivity to rewards depending on the intensity, the duration, and the type of stressor applied. In particular, exposure to chronic unpredictable mild stress or to repeated maternal separation produces a depression-like phenotype that is associated with reduction in the reinforcing effects of sucrose and of psychostimulants (Willner, 2005; Matthews and Robbins, 2003). In contrast, chronic food restriction, repeated uncontrollable foot-shock and social defeat increase the rewarding effects of drugs and/or of intracranial self-stimulation (Carr, 2002; Goeders, 2003; Koob and Kreek, 2007; Miczek *et al*, 2008). Exposure to EE prevents and reverses the behavioral and neurochemical consequences of the exposure to stressors (Laviola *et al*, 2008). Therefore, one important mechanism that could underlie the positive effects of EE is its antistress effects. This hypothesis is supported by our earlier findings in mice showing that EE decreased behavioral sensitization, whereas stress (social isolation) increased it (Solinas *et al*, 2008). In addition, the finding that EE reduces reinstatement induced by a pharmacological stressor are also in favor of this hypothesis. However, it should be noticed that in the present experiments rats were tested in extinction that is rats were not given the possibility to self-administer cocaine after the exposure to EE or SE conditions. Therefore, the modulation of the reinforcing effects of cocaine by stress cannot be responsible for the beneficial effects of EE on cocaine-seeking behavior. Indeed, our data on cocaine-induced reinstatement suggest that the motivational effects of cocaine are unaltered in EE rats. Thus, if EE produced its beneficial effects through an antistress effect, this would be achieved by reducing the ability of stress to facilitate responding for stimuli associated with cocaine (Goeders and Clampitt, 2002).

EE could also have reduced cocaine seeking and reinstatement through an antidepressant-like effect (Laviola *et al*, 2008). Consistent with this hypothesis, chronic administration of the antidepressant fluoxetine, like chronic exposure to EE, reduces the motivation to respond for cocaine-paired cues but has no effect on cocaine-induced reinstatement (Baker *et al*, 2001). Finally, other mechanisms, such as EE-induced hippocampal neurogenesis (van Praag *et al*, 2000) or chromatin rearrangement (Fischer *et al*, 2007; Pizzorusso *et al*, 2007; Sweatt 2009), could have a function in the positive effects of EE on cocaine addiction. Further studies are necessary to better define the neurobiological mechanisms underlying the effects of EE.

Quite unexpectedly, we found that rats housed in EE for <24 h show faster extinction of cocaine-seeking behavior than rats housed in SE (Figure 7). However, it is quite unlikely that the aforementioned behavioral and neurobiological mechanisms produced by chronic EE (learning, neurogenesis, antistress effects, etc) could take place in such a short time and it is more likely that different mechanisms are responsible for this rapid effect. For example, it has been shown earlier that exposures to environmental enrichment

for 3 and 6 h produce changes in gene expression in the cortex of mice that are qualitatively different from those of longer 14 days exposure (Rampon *et al*, 2000). Indeed, the effects of such a short exposure to EE could be due to several factors that we are not able to point out at the moment including exposure to novelty, stress, and physical activity. Although these results should not be confused with the effects of chronic environmental enrichment, they highlight the fact that EE can have a variety of effects that need to be better understood.

In conclusion, environmental stimulation facilitates abstinence and reduces the risks of relapse in rats. These results highlight how environmental conditions of people seeking to quit drug-taking may contribute to the successful outcome of their efforts. As recently discussed elsewhere, these preclinical results in animals are in agreement with human studies in which different forms of stimulation have been shown to be beneficial for the treatment of addiction. (Schnabel 2009; Setlow 2008). Therefore, the use of environmental stimulation together with pharmacological treatment and behavioral therapy may be of fundamental importance in the success of drug addiction treatment programs.

## ACKNOWLEDGEMENTS

We thank N Thiriet and D Belin for their helpful discussion and NIDA/NIH for donation of the Coulbourn cages. This work was supported by Centre National pour la Recherche Scientifique, University of Poitiers, Mission Interministérielle de la Lutte contre les Drogues et la Toxicomanie (MILDT-INSERM, 2006–2007 and MILDT-INSERM-INCA 2007–2009), Région Poitou Charentes, and the Intramural Research Program of the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, Baltimore, Maryland. C Chauvet is a recipient of a PhD fellowship from the French Ministry of Research.

## DISCLOSURE

The authors declare that, except for income received from their primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interests.

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