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Effects of environmental enrichment on the incubation of cocaine craving

Claudia Chauvet [a], Steven R. Goldberg [b], Mohamed Jaber [a,c], Marcello Solinas [a,c,*]

[a] University of Poitiers, 1 Rue George Bonnet, 86022 Poitiers, France
[b] Preclinical Pharmacology Section, Behavioral Neuroscience Research Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services, Baltimore, MD 21224, USA
[c] INSERM U-1084, Experimental and Clinical Neurosciences Laboratory, Poitiers, France

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ABSTRACT

Recent studies have demonstrated that exposure to environmental enrichment (EE) during withdrawal periods reduces the risks of relapse to drug-seeking behavior. In this study, we investigated whether EE could prevent the development of time-dependent increases in cocaine-seeking behavior (incubation of craving). In addition, we investigated whether EE could eliminate already developed incubation and whether the effects of EE would last when enrichment is discontinued. For this, we allowed rats to self-administer cocaine for 10 daily 6 h sessions and measured cocaine-seeking 1, 30 and 60 days after the last self-administration session. In between these tests, rats were kept in forced abstinence and housed either in EE or standard environments (SE). Between day 30 and 60 of withdrawal, half of the rats in each group were maintained in their original environmental condition and the other half was switched to the other environmental condition. We found that exposure to EE prevents development of incubation of cocaine craving and eliminates already developed incubation. In addition, contrary to our expectations, when EE was discontinued, its positive effects on incubation of craving disappeared. These results indicate that EE can reduce cocaine seeking but only temporarily and questions the hypothesis that EE can permanently eliminate the neural consequences of exposure to drugs of abuse. Therefore, stimulating environments could have positive effects on the treatment of cocaine addiction only if they are maintained for long periods of abstinence that encompass the time-frame during which addicts are most vulnerable to relapse.

1. Introduction

Accumulating evidence suggests that life experiences play a major role in determining vulnerability to and severity of addiction (Kreek et al., 2002; Piazza and Le Moal, 1996). In particular, negative environmental conditions such as stress increase the vulnerability to addiction (Kreek et al., 2002; Piazza and Le Moal, 1996; Sinha, 2001). On the other hand, recent studies demonstrate that positive living conditions such as environmental enrichment (EE) can have not only preventive (Laviola et al., 2008; Solinas et al., 2009; Stairs and Bardo, 2009) but also therapeutic effects on drug addiction (Chauvet et al., 2009; Solinas et al., 2008, 2010; Thiel et al., 2009). In fact, exposing already "addicted" animals to EE during periods of forced withdrawal decreases drug-seeking behavior in both mice and rats (Chauvet et al., 2009; Solinas et al., 2008; Thiel et al., 2009). In particular, in drug self-administration procedures in rats, exposure to EE during 21, 30 or 90 days of forced abstinence produces significant decreases in cocaine-seeking behavior and in cue- and stress-, but not cocaine-, induced reinstatement (Chauvet et al., 2009; Thiel et al., 2009). In addition, this EE-induced reduction in cocaine seeking is associated with blunting of the activation of brain circuits involved in relapse in mice and rats (Chauvet et al., 2011; Thiel et al., 2010). Interestingly, similar results have been obtained by simply allowing rats to run on a wheel for 2 h per day (Lynch et al., 2010). These results clearly demonstrate that positive environmental factors are key elements in facilitating abstinence and preventing relapse and suggest that protracted abstinence is a window of opportunity for the treatment of drug addiction (Solinas et al., 2010).

In reinstatement models of relapse, it has been shown that drug-seeking behavior is relatively low after one or a few days of withdrawal but increases over time and remains elevated for up to two-three months of withdrawal (Grimm et al., 2001; Lu et al., 2004a, 2004b). This phenomenon named incubation of craving

* Corresponding author. INSERM U-1084, Experimental and Clinical Neurosciences Laboratory, Neurobiology and Neuropharmacology Addiction Group, University of Poitiers, 1 Rue George Bonnet, 86022 Poitiers, France. Tel.: +33 5 49 366343; fax: +33 5 49 454014.
E-mail address: marcello.solinas@univ-poitiers.fr (M. Solinas).

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has been proposed as one contributing factor to long-lasting risks of relapse (Epstein et al., 2006) and it has been recently demonstrated in humans (Bedi et al., 2011). Incubation of craving is believed to result from neuroadaptations that occur during the first days of withdrawal and then last for several months (Grimm et al., 2001, 2003; Lu et al., 2004a; Lu et al., 2004b). Exposure to EE produces dramatic changes in the brain (Nithianantharajah and Hannan, 2006; Rosenzweig and Bennett, 1996; Solinas et al., 2010; van Praag et al., 2000) and decreases cue- and stress-induced reinstatement in animal models of relapse (Chauvet et al., 2009; Thiel et al., 2009), suggesting that exposure to EE could reduce incubation of cocaine craving.

To investigate the effects of EE on incubation of cocaine craving, we first allowed rats to self-administer cocaine for ten daily 6-h sessions and then tested them for cocaine-seeking behavior after one day of withdrawal. Starting on the subsequent day, half the rats were housed in standard environments (SE) and half in EE conditions for a 30-day period of withdrawal. After a test for incubation of cocaine craving on day 31, as evidenced by cocaine-seeking behavior, rats were further divided in two groups. Half remained in the same environmental condition and half were switched to the other condition for 30 days and then both groups were tested again for cocaine seeking. We found that EE reduces cocaine-seeking behavior regardless of the time of intervention (early or late abstinence). However, the positive effects of EE are lost if EE is discontinued. In a second experiment, we tested whether the effects of switching from one to the other environment could be achieved rapidly. For this, after the first 30-day period in SE or EE, rats were switched from their environment and tested for cocaine-seeking behavior after only 7 days in the new environment. Finally, rats were tested after 23 more days, i.e. approximately after 2 months of abstinence. We found that even a short exposure to EE produces positive effects on cocaine seeking but these effects are rapidly lost when EE is discontinued.

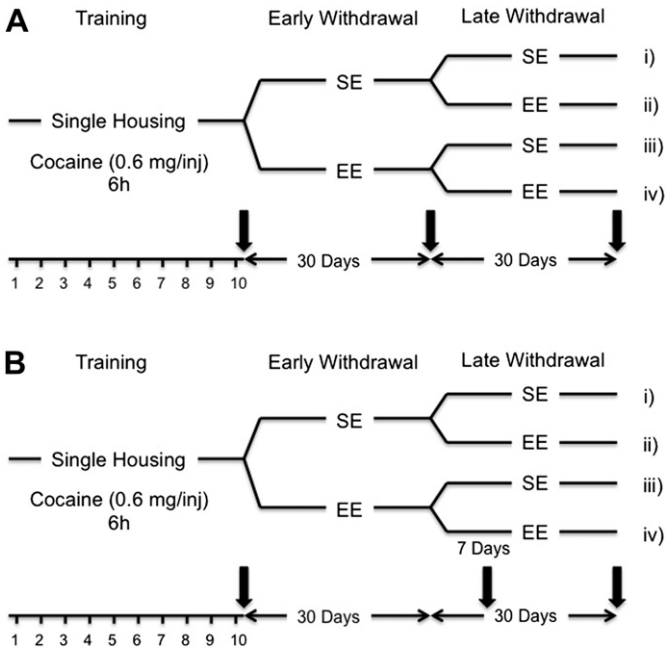

Fig. 1. Schematic representation of the experimental protocol used. A) Experiment 1 Singly housed rats were allowed self-administration of cocaine for 10 experimental sessions (Training). One day after the last training session, rats were tested for cocaine-seeking behavior and then they were housed in standard environments (SE) or environmental enrichment (EE) for 30 days (Early Withdrawal). On day 31, rats were tested again for cocaine-seeking behavior and then they were further separated and housed in SE or EE for another 30-day period: one half of the rats remained in the same environmental housing condition and the other half was switched the environmental condition to which they had not been previously exposed (Late Withdrawal). At the end of this period rats underwent a final test for cocaine-seeking behavior. B) Experiment 2. Experimental procedure was the same of experiment 1 until the end of the first period of withdrawal. At the end of this period, rats' environments were switched and tests for cocaine seeking behavior were conducted 7 days and 30 days after the switch. At the end of this period rats underwent a final test for cocaine-seeking behavior. Vertical arrows indicate tests for cocaine-seeking behavior. For both experiment 1 and 2, a total of 4 groups was obtained for the last test: i) SE–SE; ii) SE–EE; iii) EE–SE; and iv) EE–EE.

2. Materials and methods

2.1. Subjects and general experimental design

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 with E.C. regulations for animal use in research (86/609/EEC).

Our general experimental design is schematized in Fig. 1. After 10 days of cocaine self-administration, rats were tested for cocaine-seeking behavior. The following day, rats were pseudo-randomly divided into two groups, one housed in SE and the other in EE conditions, with similar levels of cocaine-seeking behavior in the two groups, and remained abstinent for 30 days. For experiment 1 (Fig. 1A), at the end of this 30-day period, rats were again tested for cocaine-seeking behavior (first incubation test). The following day, rats from each group were again divided into two further groups: a group that remained in the same environmental conditions and a group that was switched to the experimental condition they had not experienced before. Assignment to different group at this stage was again based on cocaine seeking behavior but changes in triplets of rats in each cage (SE or EE) were avoided. A total of four groups was obtained: 1) the SE–SE group was housed for 30 days in SE, tested for cocaine-seeking behavior and then housed again for 30 days in SE; 2) the SE–EE group was housed for 30 days in SE, tested for cocaine-seeking behavior and then switched for 30 days to EE; 3) the EE–EE group was housed for 30 days in EE, tested for cocaine-seeking behavior and then housed again for 30 days in EE; 4) the EE–SE group was housed for 30 days in EE, tested for cocaine-seeking behavior and then switched for 30 days to SE. The logic of the four groups was the following: SE–SE and EE–EE were control groups and based on our previous results (Chauvet et al., 2009) were expected to have respectively high and low levels of cocaine-seeking behavior both after 30 and 60 days. The SE–EE group was meant to provide information on whether incubation of craving could be eliminated after it was already expressed. The EE–SE group was meant to provide information on whether EE could have a lasting effect on incubation of craving when EE was discontinued.

In experiment 2, after the initial 30-day period in EE or SE, rats were switched, without testing for cocaine seeking, to SE or EE and tested 7 and 30 days after this switch (see Fig. 1B). This experiment was designed to investigate whether onset and offset of the positive effects of EE would occur rapidly or would require several weeks. It should be noted that we did not perform a test after the 30-day period of enrichment to avoid testing under extinction conditions too many times, which can lead to decreases in drug-seeking behavior. In addition, we chose to test rats one week, rather than 1 day, after the switch of environmental conditions in order to avoid possible confounding effects of stress induced by the switch itself.

2.2. Housing conditions

Upon arrival rats were housed three per cage for about one week before intrajugular vein catheterization surgery as previously described (Solinas et al., 2003). After surgery, rats were housed individually during the entire period of cocaine self-administration in order to limit catheter loss due to reciprocal chewing. After the test for cocaine-seeking behavior, on day 1 of withdrawal, rats were housed in either SE or EE conditions, as detailed above. 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 small plastic house, a running wheel, three floors connected by ramps or tunnels, and four toys that were changed once per week. Rats were housed in the animal facility in SE or EE housing conditions for two consecutive periods of abstinence separated by one day with a test for cocaine-seeking behavior.

2.3. Cocaine self-administration apparatus and procedure

Experiments were performed in Imetronic experimental chambers equipped with nose-pokes as operanda and controlled by Imetronic interfaces and software (Imetronic, Pessac, France; www.imetronic.com). Ten cocaine self-administration sessions were conducted using a Fixed Ratio 1 (FR1) schedule of reinforcement, as previously described (Chauvet et al., 2009). During daily sessions, a single response in the active nose-poke hole immediately delivered an i.v. injection of cocaine

(0.6 mg/injection, Cooperation Pharmaceutique Francaise, France; www.cooper.fr) 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 consequence. Responses in the inactive nose-poke hole were recorded but had no programmed consequences. Self-administration sessions lasted for 6 h in all experiments. Cocaine-seeking behavior was investigated in 3-h extinction sessions. In these extinction sessions, active nose-pokes produced the same stimuli (light and noise of the pump) that were previously produced during cocaine self-administration sessions but syringes were removed from the injection pumps and cocaine was not delivered. The 3 h extinction session was divided into three 1 h extinction segments separated by 5 min intervals. The number of active nose-pokes was used as a measure of cocaine seeking.

2.4. Food reinforcement experiments

In order to rule out non-specific "fatigue" effects of EE on the ability of rats to perform operant responding, we performed an experiment in which we tested whether EE would reduce responding even when a non-drug reinforcer (food pellet) was actually delivered (i.e. not under extinction conditions). For the food self-administration training, rats were put on a light restriction diet (approximately 15 g per day) that resulted in an initial 10 % weight loss and then to a stable weight of approximately 350 g. They were then trained to press a lever for food under a fixed ratio 1 (FR1) schedule in sessions that lasted 30 min or until 100 pellets were obtained. 18 sessions were run to allow all rats to obtain 100 food pellets within the 30 min. We then divided the rats in two groups, one that was housed in SE and the other that was housed in EE for 30 days in the animal facility. During this period, and until the end of the experiment, rats were fed ad libitum. After one month in SE or EE, rats were put back in the operant chambers and had again the possibility to respond to obtain food under the same FR1 schedule used for training during one single 30-min session.

2.5. Statistical analysis

Differences in drug seeking behavior were assessed by two-way or three-way ANOVA for repeated measures. For incubation of craving after one month, differences were assessed by two-way repeated measure ANOVA with environment (EE and SE) and day (1 and 30 days) as factors. For incubation of craving after switching environments, differences were assessed by two-way repeated measure ANOVA with environmental history (SE–SE, SE–EE, EE–EE and EE–SE) and time (1, 30 and 60 days or 1, 37 and 60) as factors. Results showing significant overall changes were subjected to Student-Newman–Keuls post-hoc test. For food reinforcement experiments, differences were assessed by unpaired, two-tail Student t-test. Differences were considered significant when $p < 0.05$.

3. Results

3.1. Cocaine self-administration training and cocaine seeking behavior after 1 day of withdrawal

All rats were allowed to self-administer cocaine under a FR1 schedule of reinforcement. For both experiment 1 and 2, cocaine self-administration was similar in all experimental groups in the number of injections received and in the number of active or inactive nose-pokes (data not shown). In addition, because this measure was used *a posteriori* to equitably assign rats to different experimental groups, cocaine seeking after 1 day of withdrawal did not differ between rats that were assigned to SE or EE conditions (Figs. 2–4).

3.2. Experiment 1

Cocaine seeking was measured in extinction sessions in which conditioned cues (lights and pump noise) were presented contingently after active nose-pokes but cocaine injections were not delivered (Fig. 2). In SE rats, consistent with previous reports, cocaine seeking was significantly higher after 30 days than after 1 day of withdrawal (Grimm et al., 2001; Lu et al., 2004a, 2004b). In contrast, we found that exposure to EE during the 30 days of withdrawal significantly reduced incubation of cocaine craving. Statistical analysis revealed a significant effect of environment ($F_{1,36} = 15.25$, $p < 0.01$), of day ($F_{1,36} = 55.30$, $p < 0.0001$) and an environment × day interaction ($F_{1,36} = 16.50$, $p < 0.01$).

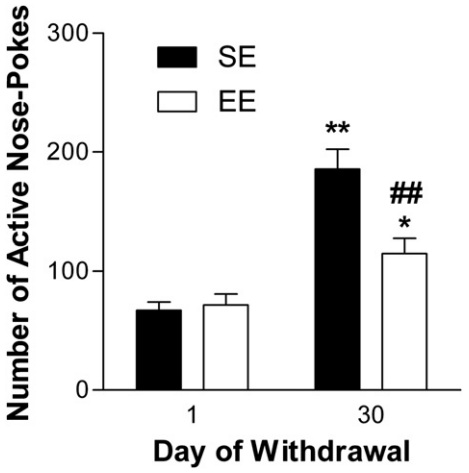

Fig. 2. Exposure to EE during early stages of withdrawal reduces incubation of craving. Total cocaine seeking behavior on day one and day 30 of withdrawal from cocaine self-administration as measured by the number of active nose-pokes during 3 h sessions. Note that incubation of craving was found in both SE and EE groups but it was significantly higher in SE compared to EE rats. Two-way ANOVA for repeated measures followed by post-hoc Student-Newman–Keuls's test: * and **, $p < 0.05$ and $p < 0.01$ Day 1 vs. Day 30; ##, $p < 0.01$ vs. SE control.

The day after the test at day 30, one half of the rats housed in each environment were switched to the other environment whereas the other half was kept in the same environment (see Fig. 1). After 30 more days (a total of 60 days of withdrawal), rats were tested again for cocaine-seeking behavior. Rats kept in SE (SE–SE) once again showed increased cocaine-seeking behavior compared to day 1 (although slightly lower than on day 30). Conversely, rats kept in EE (EE–EE) showed levels of cocaine-seeking behavior similar to day 1 (Fig. 3). These results are generally in agreement with our previous results showing that one or three months of EE reduce cocaine seeking and cue- and stress-induced reinstatement (Chauvet et al., 2009).

Importantly, SE–EE rats that showed incubation of craving during the first test for cocaine-seeking showed low levels of

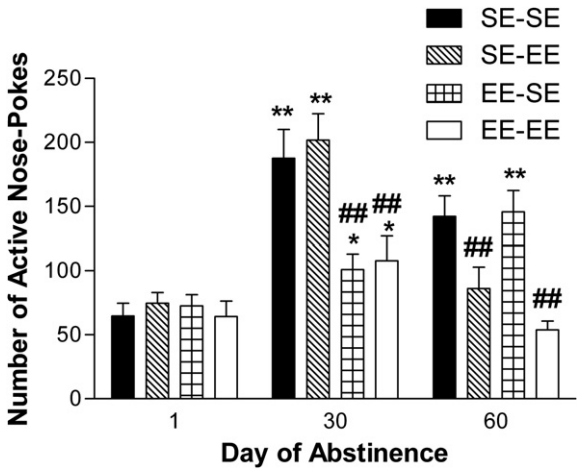

Fig. 3. Early or late exposure to EE reduces incubation of craving but switch from EE to SE eliminates the beneficial effects of EE. Total cocaine seeking at day 1, day 30 and day 60 (30 + 30) of withdrawal from cocaine self-administration as measured by the number of active nose-pokes during 3 h sessions in i) SE–SE, ii) SE–EE, iii) EE–SE and iv) EE–EE rats. Note that incubation of craving was low when exposure to EE immediately precedes tests for cocaine seeking and was high when SE precedes tests for cocaine seeking. Two-way ANOVA for repeated measures followed by post-hoc Student-Newman–Keuls's test: * and **, $p < 0.05$ and $p < 0.01$ compared to day 1; ##, $p < 0.01$ compared to respective environmental controls. Data at day 30 derive from the data shown in Fig. 2.

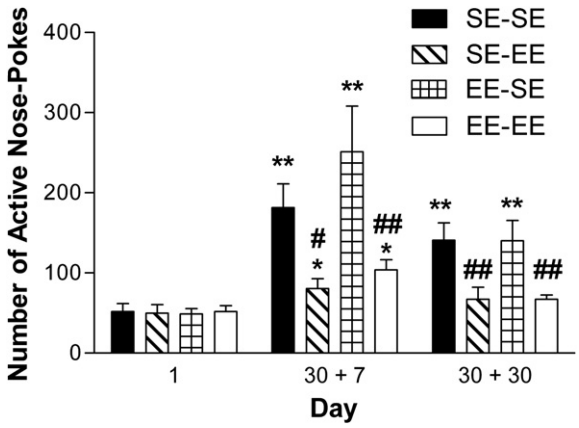

Fig. 4. Rapid onset and offset of the positive effects of EE on cocaine seeking behavior. Total cocaine seeking at day 1, day 30 + 7 and day 60 (30 + 30) of withdrawal from cocaine self-administration as measured by the number of active nose-pokes during 3 h sessions in i) SE–SE, ii) SE–EE, iii) EE–SE and iv) EE–EE rats. Two-way ANOVA for repeated measures followed by post-hoc Student-Newman–Keuls's test: * and **, $p < 0.05$ and $p < 0.01$ compared to day 1; # and ##, $p < 0.05$ and $p < 0.01$ compared to respective environmental controls.

cocaine-seeking behavior on the second test that were comparable to levels of cocaine-seeking on day 1 (Fig. 3). Interestingly, and rather unexpectedly, EE–SE rats that showed reduced incubation of craving during the first test for cocaine seeking, showed increased levels of cocaine-seeking behavior on the second test that were comparable to those of rats that were always kept in SE (Fig. 3). Statistical analysis revealed a significant effect of the environmental history ($F_{3,68} = 5.82$, $p < 0.01$), of time ($F_{2,68} = 35.13$, $p < 0.0001$), and environmental history × time ($F_{6,68} = 8.87$, $p < 0.0001$).

3.3. Experiment 2

We then investigated whether the effects of EE would be lost rapidly after discontinuation of EE or whether several weeks of housing in SE would be necessary to eliminate the positive effects of EE on incubation of craving. We again found that animals that were always housed in EE (EE–EE) consistently showed reduced cocaine-seeking behavior compared to animals that were always housed in SE (SE–SE). In addition, we found that rats housed in EE and then switched to SE show levels of cocaine-seeking behavior similar to control SE–SE rats as early as one week after the switch (Fig. 4). Conversely, rats that were housed in SE and then switched to EE showed beneficial effects of EE already one week after the switch (Fig. 4). Both effects were still present one month after the switch (Fig. 4). Statistical analysis revealed a significant effect of the environmental history ($F_{3,52} = 4.70$, $p < 0.01$), of time ($F_{2,52} = 21.63$, $p < 0.0001$), and environmental history × time ($F_{6,52} = 5.41$, $p < 0.0001$).

3.4. Effects of one-month exposure to EE on active food taking behavior

All rats were allowed to self-administer food under a FR1 schedule of reinforcement. Food self-administration was similar in all experimental groups with all rats receiving the maximum number of food pellets within the 30 min of the session (Fig. 5A). Importantly, after one month in EE or SE, levels of food taking (Fig. 5B) and the rates of responding in this test session were similar between SE and EE (Fig. 5C).

4. Discussion

In this study, we investigated the effects of EE on incubation of craving, a phenomenon characterized by time-dependent increases in cocaine-seeking behavior (Epstein et al., 2006; Grimm et al., 2001; Lu et al., 2004b). In addition to the typical approach of verifying the effects of environmental manipulations on a first test for drug-seeking behavior (Conrad et al., 2008; Lu et al., 2005, 2009), we also conducted a second test at a later time-point after switching environmental conditions. This allowed us to obtain further insights into the nature of the effects of EE and into the incubation phenomenon in general. We found that 1) exposure to EE either in early or late phases of withdrawal reduces cocaine-seeking behavior; 2) the effects of EE are temporary, i.e. if EE is discontinued incubation of craving can be expressed at later stages of abstinence; 3) the onset and offset of the positive effects of EE are rather rapid. These results support the hypothesis that positive environmental conditions are keys for facilitating abstinence and reducing the risks of relapse (Chauvet et al., 2009; Solinas et al., 2008, 2010; Thiel et al., 2009) but also suggest that, in order to be effective, EE needs to be provided continuously over long periods of withdrawal. In addition, our findings suggest that, similar to other neuroadaptive drug-induced phenomena such as behavioral sensitization (Kalivas and Stewart, 1991; Robinson et al., 1998; Vanderschuren and Kalivas, 2000), two phases can be distinguished in incubation of craving: 1) the development or induction of

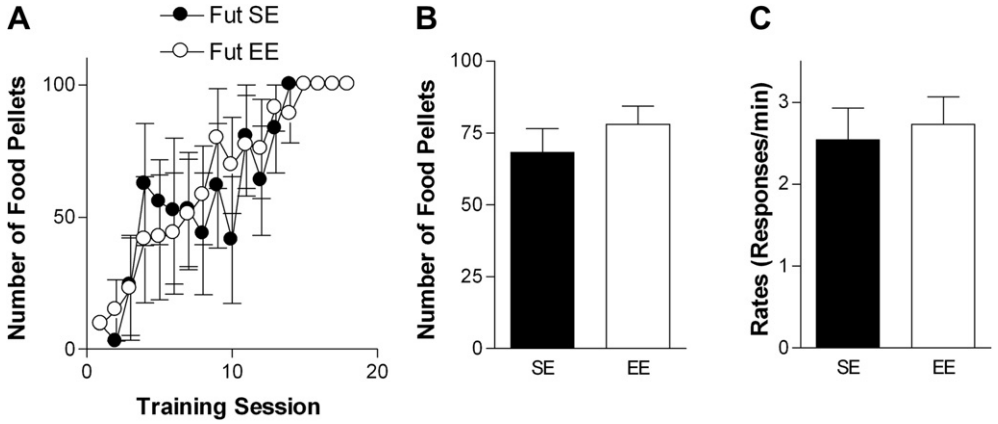

Fig. 5. EE does not reduce active food taking under a fixed ratio 1 (FR1) schedule. A) Training for food self-administration in rats ($n = 5$ per group) that were later exposed to SE (Fut SE) or EE (Fut EE). In the last four sessions, all rats obtained the maximum 100 pellets within the 30-min session. B) Number of pellets obtained and C) Rates of responding after one month in SE or EE during a test session in which operant responding was reinforced by food pellet under the same FR1 schedule used during training.

incubation of craving phase, which takes place during early periods of withdrawal and appears insensitive to EE, and 2) the expression of incubation of craving phase, which appears after several days of abstinence, lasts for several months of withdrawal, and is sensitive to EE.

Relapse to drug use is one of the hallmarks of addiction and one of its most intriguing aspects (Epstein et al., 2006; Hyman et al., 2006; O'Brien, 2005). Incubation of craving, and especially the increased sensitivity to drug-associated cues, is believed to play an important role in relapse after protracted abstinence (Epstein et al., 2006). In agreement with previous studies (Conrad et al., 2008; Grimm et al., 2001; Lu et al., 2004a, 2004b, 2005), in rats housed in SE, cocaine seeking was low after 1 day of withdrawal but increased after 30 days and stayed elevated for at least 2 months. Exposure to EE, whenever it was provided chronically prior to the test, reduced, but did not completely block, cocaine seeking. In a recent paper, it was reported that EE did not affect incubation of cocaine craving (Thiel et al., 2011). However, in our opinion, the discrepancy between that study and ours is only apparent and mostly due to experimental procedures. In fact, Thiel et al. did not measure cocaine seeking at day 1 and, thus, it was not possible to determine and quantify precisely the incubation effect. In contrast, because we first measured basal cocaine seeking and then divided rats in SE and EE, we could isolate and quantify the incubation effect. Therefore, the present results extend previous findings of the restorative effects of enrichment, or simple physical exercise, on cocaine addiction (Chauvet et al., 2009; Lynch et al., 2010; Solinas et al., 2008, 2010; Thiel et al., 2009) and suggest that positive and stimulating environmental conditions may be central in reducing the risks of relapse.

Exposure to EE has been shown to reduce seeking also for natural rewards such as sucrose (Grimm et al., 2008). Therefore, EE appears to have a rather general effect on the ability of conditioned reinforcers to sustain drug-seeking behavior in the absence of actual reinforcement. On the other hand, incubation of food-seeking behavior has been shown to last only 30 days (Grimm et al., 2003), whereas incubation of drug-seeking behavior lasts up to 90 days after interruption of active self-administration (Chauvet et al., 2009; Grimm et al., 2003). Therefore, the effects of enrichment may be particularly evident and strong especially in the case of drug addiction in humans for which conditioned stimuli remain powerful triggers of relapse for months and even years.

Whereas operant responding under extinction, i.e. in the absence of actual delivery of the reward, is reduced by EE both for food and drugs (Chauvet et al., 2009; Grimm et al., 2008; Thiel et al., 2011, 2009), operant responding that is reinforced by food delivery is not altered by EE. The fact that EE also does not block drug-induced reinstatement may also indicate that when operant responding is associated with the effects of the primary reinforcer, EE is not capable of reducing seeking behavior. Therefore, it appears that the effects of EE are somewhat specific to conditioned reinforcement whereas unconditioned reinforcement appears unaffected by EE. Importantly, this is true only when animals are exposed to EE only after self-administration of drugs because if EE is provided before drug self-administration, especially during early periods of life, EE reduces active self-administration as well as the risks of relapse (Gipson et al., 2011; Stairs et al., 2006).

Incubation of craving is believed to result from neuroadaptations that occur during the first days of withdrawal and last for several months (Conrad et al., 2008; Grimm et al., 2001; Lu et al., 2004a, 2004b, 2005). In this theoretical framework, incubation would consist of a development phase during which these neuroadaptations take place, and a later phase when they would become stable. A corollary of this idea is that if these neuroadaptations were blocked at their initiation phase, then incubation would never develop. This hypothesis is indeed supported by a recent study showing that blockade of GDNF in the ventral tegmental area, during the first days of withdrawal, blocks the expression of incubation of craving at later time points (Lu et al., 2009). Whereas our initial hypothesis was that EE would prevent the development of incubation of craving, the temporary effects of EE in the present experiments indicates that EE does not block the neuroadaptations that take place during withdrawal periods but, rather, prevents the expression of cocaine seeking. Indeed, it appears that EE acts as a brake on drug-seeking behavior but when EE is discontinued, the mechanisms underlying increased cocaine seeking are rapidly revealed. In this sense, incubation of craving would resemble other neuroadaptive phenomena, such as behavioral sensitization, in which development and expression appear to be two clearly distinct phases (Robinson et al., 1998; Vanderschuren and Kalivas, 2000).

The fact that already developed incubation of craving could be blocked by late exposure to EE indicates that incubation of craving is not an inevitable consequence of chronic drug-taking but rather that its expression depends dramatically on external factors. Importantly, these results support the idea that protracted abstinence is a window of opportunity for therapeutic interventions for addiction and that intervention could be successful in decreasing, at least temporarily, drug-seeking behavior at different stages of abstinence. Therefore, it may be possible to successfully treat addiction not only during the first days after interruption of active drug-taking behavior (Lu et al., 2009) but also later on when incubation of craving is already developed.

The effects of EE in reducing drug-seeking behavior take place rather rapidly and, in this study, they were found after one single week of enrichment. In addition, previous work has shown that even one day of EE may reduce cocaine-seeking behavior (Chauvet et al., 2009; Thiel et al., 2011). Because of the presence of a running wheel and the possibility to perform ad libitum physical activity, it could be possible that fatigue effects played a role in the effects of EE on cocaine seeking. However, in addition to the fact that our rats had minimal running activity, as already reported (Grimm et al., 2008), the fatigue effects are unlikely to explain the reduced operant responding of EE for cocaine or food because rats housed for 30 days in EE were able to show intense responding when their operant behavior was reinforced by food.

We recently discussed several bio-behavioral mechanisms that may explain the positive effects of EE on cocaine-seeking behavior (Solinas et al., 2010). One possibility is that chronic cocaine self-administration induces long-lasting neuroadaptations that are responsible for the persistent risks of relapse and that EE eliminates them. The present results suggest that EE does not eliminate cocaine-induced neuroadaptations but, rather, induces temporary states that render rats less reactive to cocaine-related stimuli. In our recent review article, we proposed a unified theoretical framework in which EE can be seen as a functional opposite of stress, with EE ameliorating the negative "emotional state" of rats making them less susceptible to relapse (Solinas et al., 2010). A recent paper found that exposure to EE increases basal levels of cortisosterone in Sprague–Dawley but blunts its response to an acute stress in terms of increases over the baseline and speeds up the return to basal levels (Konkle et al., 2010). In addition, withdrawal from cocaine self-administration is associated with elevated corticosterone levels and that are not found in rats housed in EE (Thiel et al., 2011). The present results showing a temporary effect of EE are also compatible with our anti-stress hypothesis of EE (Solinas et al., 2010). In this scenario, cocaine-exposed rats left in SE would be in a negative emotional state in which they would be vulnerable to relapse when exposed again to contexts and cues predictive of cocaine. In contrast, cocaine-exposed rats left in EE would be in

a positive emotional state in which they are less sensitive to motivational influences of drug-related cues and less impulsive and inflexible in their behavior, which would allow them to refrain from drug-seeking behavior. However, this positive emotional state would be rapidly lost when EE is discontinued and risks of relapse would consequently increase. As discussed by McLellan et al. (2000), the reappearance of the symptoms, once the treatment is discontinued, is not peculiar of addiction but it is common to other chronic disorders such as type 2 diabetes, hypertension and asthma.

In summary, we found that housing animals in EE decreases cocaine-seeking behavior, preventing the expression of increased cocaine-seeking behavior that is normally found after weeks or months of withdrawal. We also found that the effects of EE are not permanent and that incubation of cocaine craving can be expressed if enrichment is discontinued. Therefore, positive environmental conditions during periods of abstinence may help treat, but do not "cure", cocaine addiction. On the other hand, incubation of cocaine craving is a time-dependent phenomenon that spontaneously dissipates after several months of withdrawal (Lu et al., 2004a). Therefore, if EE is kept throughout the periods of higher vulnerability to relapse, incubation of craving might never be expressed. If these results were translated to human conditions, it would be expected that positive environmental conditions may prove beneficial if they are provided long enough to cover the periods of withdrawal when reactivity to cocaine cues is highest (Epstein et al., 2006). In other terms, environmental enrichment could "bridge" addicts from times when relapse is more likely to periods in which pharmacological, and behavioral and cognitive therapy could be more effective and lead to effective treatment of long-term risks of relapse.

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