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Relapse to cocaine use persists following **extinction** of drug-primed craving

Paul Girardeau^{1,2}, Sylvia Navailles^{1,2}, Audrey Durand^{1,2}, Caroline Vouillac-Mendoza^{1,2}, Karine Guillem^{1,2} and Serge H Ahmed^{1,2*}

¹Université de Bordeaux, Institut des Maladies Neurodégénératives, UMR 5293, 146 rue Léo-Saignat, F-33000 Bordeaux, France ; ²CNRS, Institut des Maladies Neurodégénératives, UMR 5293, 146 rue Léo-Saignat, F-33000 Bordeaux, France

*Correspondence to: Serge H. Ahmed, Ph.D.

Université de Bordeaux

Institut des Maladies Neurodégénératives, UMR CNRS 5293

146 rue Léo Saignât, 33076 Bordeaux, France

Email: serge.ahmed@u-bordeaux.fr

Phone: +33 557 571 566

Fax: +33 556 900 278

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ABSTRACT

Craving often precedes relapse into cocaine addiction. This explains why considerable research effort is being expended to try to develop anti-craving strategies for relapse prevention. Recently, we discovered using the classic reinstatement model of cocaine craving that the reinstating or priming effect of cocaine can be extinguished with repeated priming in rats – a phenomenon dubbed extinction of cocaine priming because it is thought to involve extinction of the conditioned interoceptive cues of the drug. Here we measured the effect of this extinction strategy on subsequent relapse-like behavior in rats (i.e., return to the pre-extinction pattern of cocaine self-administration once the drug is made again available after extinction). We found that **extensive extinction** of the conditioned priming effects of cocaine had no major impact on relapse-like behavior. This lack of effect occurred despite evidence for post-extinction loss of neuronal responses to cocaine priming in brain regions causally involved in cocaine reinstatement (i.e., the dorsomedial prefrontal cortex and the core of the nucleus accumbens). These findings suggest that the conditioned priming effects of cocaine can be dissociated from and are thus not essential for relapse-like behavior, and that extinction of these effects is unlikely to represent a viable approach to relapse prevention. Overall, these findings are in general agreement with previous neurobiological dissociation studies and with research on extinction of exteroceptive drug cues.

Key-words: addiction; relapse; cocaine; resistance to extinction; prelimbic cortex.

1. Introduction

Cocaine not only evokes intense pleasurable sensations but also induces an overwhelming desire or craving for more cocaine. This latter process is especially obvious in individuals with a diagnosis of cocaine use disorder and is thought to contribute, together with other factors, to precipitate or favor relapse after abstinence (Jaffe et al., 1989; Mahoney et al., 2007; Volkow et al., 2005). Cocaine-induced craving can be approached and studied in animals using the classic drug reinstatement model (Epstein and Preston, 2003; Epstein et al., 2006). In this model, operant responding for the drug (e.g., pressing a lever) is first extinguished by discontinuing drug reinforcement and then reinstated by drug priming (i.e., passive or non-contingent drug administration). Importantly, after drug priming, reinstated operant responding continues to be non-reinforced as during extinction and, therefore, is thought to be a behavioral expression of genuine drug seeking (Ahmed and Koob, 1997; Grimm et al., 2001). This standard animal model has been extensively used to study the neural correlates and substrates of cocaine seeking and to develop potential anti-craving interventions for relapse prevention (Kalivas et al., 2005; Schmidt et al., 2005; Shalev et al., 2002).

Using this model, we found that the reinstating effect of cocaine on previously extinguished cocaine seeking is not an unconditioned effect as it can itself be extinguished with repeated drug priming – a phenomenon dubbed extinction of drug priming to distinguish it from extinction of operant drug responding (Mihindou et al., 2011). Extinction of cocaine priming was also observed after escalation of cocaine self-administration in rats with extended drug access (Mihindou et al., 2011). Such extinction was largely unanticipated by most theories on the mechanisms underlying cocaine priming-induced reinstatement, except by the drug interoceptive conditioning hypothesis (Ahmed and Koob, 1997; Keramati et al., 2017;

Mihindou et al., 2011). According to this hypothesis, the priming effect of cocaine on reinstatement of cocaine seeking would result from an interoceptive drug conditioning mechanism whereby the interoceptive cues of cocaine are associated with the availability of cocaine reinforcement and become conditioned Pavlovian stimuli (Ahmed and Koob, 1997; Mihindou et al., 2011; Wang et al., 2013; Wise et al., 2008). During cocaine priming, perception of these conditioned cues would induce reinstatement of responding. In other words, according to this view, cocaine priming would be a special form of cue-induced reinstatement. In theory, the interoceptive cues of cocaine could reinstate responding *via* a simple stimulus-response mechanism, a goal-directed mechanism, or an incentive salience attribution mechanism. For instance, perception of these interoceptive cues after instrumental extinction could lead rats to expect, albeit falsely, that the cocaine outcome is again available for self-administration, thereby prompting them to reinstate responding. This goal-directed account of cocaine priming seems to be consistent with the critical role of the prelimbic cortex in cocaine-primed reinstatement (Capriles et al., 2003; McFarland and Kalivas, 2001; Vassoler et al., 2013) – a brain region that is also involved in goal-directed instrumental behavior (Gourley and Taylor, 2016; Killcross and Coutureau, 2003). Regardless of the underlying mechanisms, however, after repeated priming, these cues would progressively lose their conditioned effects, thereby explaining extinction of cocaine priming.

Recently, we suggested that extinction of drug priming could represent a potential cocaine exposure therapy for relapse prevention (Mihindou et al., 2011). The validity of this proposal ultimately depends, however, on whether the mechanisms involved in cocaine priming significantly overlap with those involved in cocaine self-administration when the drug is available. Previous research has shown that these mechanisms can be dissociated, at least partly, by some specific pharmacological or neurobiological interventions (Bachtell et al., 2008; Baker et al., 2003; Chauvet et al., 2015; Fletcher et al., 2002; Moussawi et al., 2011;

Peng et al., 2008; Pentkowski et al., 2010; Schroeder et al., 2010; Sun and Rebec, 2005). In addition, extinction of drug cues, albeit exteroceptive ones, has so far been relatively ineffective in preventing relapse in human addicts (Conklin and Tiffany, 2002). Thus, it is not clear whether and to what extent extinction of cocaine priming should impact relapse to cocaine self-administration.

The overarching goal of the present series of experiments was to directly assess this question. To this end, we developed a within-session, multiple drug priming procedure (i.e., 3 per session) to promote a rapid extinction of cocaine priming in rats with relatively long prior histories of intravenous cocaine self-administration (i.e., > 24 daily sessions) (see Section 2). Importantly, to avoid limitations due to the context-specificity of extinction (Conklin and Tiffany, 2002; Mihindou et al., 2011), this procedure was applied in the same environmental context where cocaine self-administration originally took place and where relapse-like behavior will occur. After extinction of cocaine priming, cocaine was again made available for self-administration under the same operant conditions as before extinction. Relapse-like behavior was then measured by comparing in the same individuals their pre- versus post-extinction patterns and rates of cocaine self-administration. To avoid confusion, the term relapse-like behavior is strictly defined here as a return to the pre-extinction pattern and rate of cocaine self-administration – an operational definition that is close to the clinical definition of relapse (Epstein and Preston, 2003; Miller et al., 2011). In contrast and as already defined above, the term reinstatement is exclusively used to refer to the reinstatement of non-reinforced cocaine seeking by cocaine priming.

2. Materials and methods

2.1. General Procedures

2.1.1. Initial cocaine self-administration training

In all experiments, young adult, male Wistar rats ($n = 171$, weighing 225-250 g at the beginning of experiments) were first habituated during 2 3-h daily sessions to the experimental chambers (see also Supplementary Data (Appendix A)). During habituation, no lever or light cue was presented, and rats were allowed to move freely and explore the cage. After habituation, rats were progressively trained to press a lever to self-administer cocaine intravenously (0.25 mg per injection) under a final fixed-ratio (FR) 3 schedule of reinforcement during 3-h daily sessions. The number of self-administration sessions before extinction of cocaine priming ranged from 24 to 64 depending on the experiment (see Section 2.2). All self-administration sessions began with extension of the operant lever and ended with its retraction after 3 h. No inactive lever was used in the present study. Intravenous delivery of cocaine began immediately after completion of the operant response requirement and lasted about 4 s. It was generally accompanied by illumination of the light cue above the lever for 20 s, except when indicated. Responses during the light cue were recorded but had no programmed consequence. All self-administration sessions were run 6-7 days a week.

2.1.2. Within-session multiple priming procedure for extinction of cocaine priming

Extinction of cocaine priming began 1 day after the last session of cocaine self-administration and was induced using a multiple drug priming variant of a within-session extinction-reinstatement procedure described previously (Ahmed and Cador, 2006; Mihindou et al.,

2013; Mihindou et al., 2011). Briefly, each session lasted 225 min and consisted of an initial 90-min period of extinction of operant responding during which completion of the FR3 continued to turn on the 20-s light cue above the operant lever (except when otherwise indicated) but was no longer reinforced by cocaine. After this initial period of 90 min, rats continued to be tested under operant extinction but received 3 1-mg priming doses of cocaine spaced 45 min apart. Each priming dose was generally administered non-contingently, except when otherwise mentioned (see Experiment 2). The dose of 1 mg was previously shown to induce a maximal priming effect on reinstatement of cocaine seeking in a previous study (Ahmed and Cador, 2006; Mihindou et al., 2011). Three priming doses of cocaine per session were used in an attempt to promote a rapid extinction of cocaine priming. Cocaine-induced reinstatement of operant responding was measured during the 45-min periods following each priming dose, and compared to non-primed responding measured during the 45-min period preceding the first priming dose. Depending on the experiment, the number of extinction sessions before relapse-like behavior testing ranged from 5 to 45 (see Section 3).

2.1.3. Post-extinction relapse-like behavior

One day after the last session of extinction of cocaine priming, cocaine was made again available for self-administration during 5-6 consecutive sessions under the same operant conditions as before extinction. No explicit cue announced post-extinction cocaine availability, except for the availability of cocaine reinforcement itself upon operant responding. In all experiments, the impact of extinction of cocaine priming on relapse-like behavior was assessed using a within-subject comparison of the pre- versus post-extinction patterns and rates of cocaine self-administration.

2.1.4. *c-Fos immunohistochemistry*

We used c-Fos immunohistochemistry to check whether extinction of cocaine priming was associated with changes in neuronal responses to cocaine priming in brain regions critically involved in cocaine-induced reinstatement (see also Supplementary Data (Appendix A) and Experiment 10 below). Forty-five min after testing, rats were anesthetized and perfused transcardially with 4% formaldehyde. Then their brains were extracted, post-fixed, sliced, and processed for Fos immunohistochemistry analysis as described in details elsewhere (Mihindou et al., 2013; Navailles et al., 2015). Density of Fos-expressing cells (cells / mm²) was measured by a blind observer in both hemispheres of each rat and averaged from 3 sections covering the anterior-posterior extent of each region of interest. Brain regions of interest were defined here as regions previously reported to be consistently involved in cocaine-induced reinstatement. These regions include the anterior cingulate cortex, the prelimbic subdivision of the medial prefrontal cortex, and the core subdivision of the nucleus accumbens (Capriles et al., 2003; McFarland and Kalivas, 2001; Vassoler et al., 2013) (for exact anatomical coordinates, see (Navailles et al., 2015)). A more comprehensive Fos mapping of the neuronal correlates of extinction of cocaine priming will eventually be reported in a separate article. Here our goal is limited to testing if extinction of cocaine priming is also neurobiologically effective.

2.1.5. *in vivo prefrontal electrophysiology during extinction of cocaine priming*

To assess whether there was a temporal relationship between behavioral and neuronal changes during extinction of cocaine priming, we also used *in vivo* electrophysiology to record single-unit activity in the prelimbic subdivision of the medial prefrontal cortex. Cocaine is thought to reinstate cocaine seeking mainly via activation of prelimbic cortex neurons that project to the core of the nucleus accumbens (Stefanik et al., 2015). Here we sought to

directly demonstrate this activation *in vivo* and follow its evolution during repeated cocaine priming (see also Supplementary Data (Appendix A) and Experiment 10 below). Rats were implanted unilaterally under isoflurane anesthesia with arrays of 16 teflon-coated stainless steel microwires (MicroProbes Inc, Gaithersburg, MD) in the prelimbic cortex [AP: + 2.5 to + 4.2 mm, ML: 0.5 to 1.5 mm, and DV: - 4.0 mm relative to skull level]. Ten days after surgery, they were retrained to self-administer cocaine for 9 additional FR1 sessions before being tested for extinction of cocaine priming. Electrophysiological recordings were conducted during each daily extinction session. Within each session, prelimbic neuronal firing activity (in Hz) measured 45 min after cocaine priming (P) was compared to that measured during the preceding non-primed period of 45 min (NP). Neurons were recorded using commercial hardware and software (OmniPlex, Plexon, Inc, Dallas, TX) and classified into putative interneurons (n = 30) and pyramidal neurons (n = 160) according to the waveform spike width and average firing rate, as previously described (Guillem et al., 2010). Due to the small number of interneurons, data analysis was exclusively focused on putative pyramidal neurons. To count the number of individual neurons that changed their firing rate in response to cocaine priming during each session, we compared firing rate measured 25 min before and 25 min after cocaine priming using the Wilcoxon test.

2.2. Specific Experiments

2.2.1. Experiment 1: Effects of extinction of non-contingent priming cocaine on relapse-like behavior

This initial experiment was conducted as a pilot study in a group of rats (n = 20) from a previous, unrelated experiment. Before being exposed to repeated sessions of extinction of cocaine priming, these rats had been extensively tested for cocaine self-administration under

different conditions. In total, they self-administered cocaine during 64 daily sessions of at least 3h each whose 37 3-h sessions under a FR3 schedule of reinforcement. Extinction of cocaine priming was induced during 5 daily sessions using the triple priming procedure described above. Then one day after the last session of extinction, rats were tested for relapse-like behavior during 5 days as described above.

2.2.2. Experiment 2-10

To complement and better understand the outcomes of experiment 1, 9 additional experiments were conducted using parametric variants of the general procedures described above. To avoid repetition and ease reading, the principle of each of these experiments is outlined in relevant places in the Results section. A complete description of these experiments is provided in Supplementary Data (Appendix A).

3. Results

3.1. Experiment 1: Effects of extinction of non-contingent cocaine priming on relapse-like behavior

On the **first session of extinction**, all priming doses of cocaine reinstated responding above **the non-primed level of** responding but this effect rapidly decreased within-session with repeated priming (i.e., from **the first to the third priming dose**) ($F_{3,57} = 27.57, p < 0.01$) (Fig. 1a). A higher-resolution time course of the within-session extinction of cocaine priming is shown in Supplementary Fig. S1. After repeated session of extinction, there was a further extinction of cocaine priming (Priming x Session: $F_{3,57} = 17.58, p < 0.01$). On **session 5 of extinction**, all 3 priming doses of cocaine induced the same levels of reinstatement which were lower than those on **the first session of extinction** (Fig. 1a). To better capture the extinction of cocaine priming across sessions, the effects of **all 3 priming doses** were averaged for each rat and each session. After an initially large extinction, the average priming effect of cocaine rapidly levelled off at a residual level that was slightly, though significantly, above **the non-primed level of** responding, suggesting that maximum extinction of cocaine priming was achieved (Priming x Session: $F_{4,76} = 17.56, p < 0.01$) (Fig. 1b). Finally, the extinction of cocaine priming was behaviorally-specific because no significant change in cocaine-induced increased locomotion was observed within-session or between-session (Supplementary Fig. S2).

On the day after extinction of cocaine priming, rats were given again access to cocaine for self-administration as before extinction to measure relapse-like behavior. Overall, no effect on relapse-like behavior was observed. The pre- versus post-extinction patterns of cocaine

self-administration were nearly identical (Fig. 1c). Though some rats were slightly slower to reinitiate cocaine self-administration within the first post-extinction session, they nevertheless readily re-expressed the same pattern of self-administration as before extinction once it was initiated. This was confirmed by a quantitative analysis of the rate of cocaine self-administration (i.e., total number of injections in the session divided by the time elapsed since initiation of cocaine self-administration). Clearly, after extinction of cocaine priming, rats took cocaine at a rate identical to or slightly higher than pre-extinction (average of the last 3 pre-extinction sessions), from post-extinction session 1 onward ($F_{5,95} = 7.31$, $p < 0.01$) (Fig. 1d).

3.2. Experiment 2: Effects of extinction of response-contingent cocaine priming on relapse-like behavior

The absence of effect on relapse-like behavior despite extinction of cocaine priming may be related to the passive, involuntary nature of our multiple-priming procedure. To test this, a separate group of rats ($n = 20$) was given voluntary control over cocaine priming. Briefly, after an initial 45-min period of extinction, each **priming period**, including the **non-primed period** preceding the **first priming period**, was self-initiated by the rats (see Supplementary Data (Appendix A)). Overall, as with non-contingent cocaine priming, self-priming effectively reinstated responding above **the non-primed level of** responding (session 1 to 5: $F_{1,19} > 18.00$, $p < 0.01$; session 6: $F_{1,19} = 5.93$, $p < 0.05$) and this effect decreased considerably, though not completely, with repeated session ($F_{5,95} = 31.59$, $p < 0.01$) (Supplementary Fig. S3). This between-session extinction of cocaine self-priming was not associated with a change in cocaine-induced increased locomotion ($F_{5,95} = 0.58$). Finally, despite extinction of cocaine self-priming, there was no significant impact on relapse-like behavior post-extinction

($F_{6,14} = 1.26$). The rate of cocaine self-administration returned to the pre-extinction level as early as the first post-extinction session onwards (Supplementary Fig. S3).

3.3. Experiment 3: Effects of extensive extinction of cocaine priming on relapse-like behavior

In the two previous experiments, despite a large extinction of cocaine priming, there remained a small residual effect of cocaine priming. To test if this residue could be totally extinguished with additional sessions of extinction to prevent relapse-like behavior, another group of rats ($n = 18$) were tested for a total of 24 sessions. These sessions were organized in 4 extinction blocks of 6 sessions each that alternated with 4 relapse blocks of 6 sessions of cocaine self-administration. This was done to follow any change in vulnerability to relapse-like behavior with increased sessions of extinction. There was clear evidence for a large extinction of cocaine priming with repeated sessions and across blocks of extinction (Priming \times Session: $F_{23,391} = 3.74$, $p < 0.01$) (Fig. 2a). Interestingly, there was some evidence for reconditioning of cocaine priming between extinction blocks (Session [1 versus 6] \times Block: $F_{3,51} = 7.17$, $p < 0.01$) (Fig. 2a, b). Specifically, the effect of cocaine priming on session 1 of extinction block 2 increased above that of session 6 of block 1 (Fig. 2b). However, this reconditioning effect was short-lived. It was not observed during the following blocks of extinction (Fig. 2b), indicating a stable retention of extinction of cocaine priming across blocks. On the last 3 sessions of the final block of extinction, the priming effect of cocaine was very low compared to the first session of block 1 ($F_{3,51} = 9.78$, $p < 0.01$) but was still significantly above **the non-primed level of** responding (Fig. 2a,c). Overall, there was no effect on relapse-like behavior between blocks of extinction ($F_{24,408} = 1.44$) (Fig. 2d). The pre- versus post-extinction patterns of cocaine self-administration remained unchanged between blocks of extinction (Supplementary Fig. S4).

3.4. Experiment 4: Effects of saline substitution on residual cocaine priming after extinction

Apparently, the priming effect of cocaine cannot be entirely extinguished, even after extended extinction testing. This prompted us to investigate the underlying mechanisms of the post-extinction residual effect of cocaine priming. We first showed in a separate group of rats ($n = 13$) that this effect was not attributable to the intravenous infusion procedure itself as it completely disappeared when cocaine was replaced with saline – its vehicle – all else being equal ($F_{3,36} = 14.57, p < 0.01$) (Fig. 3a).

3.5. Experiment 5: Effects of light cue conditions on residual cocaine priming after extinction

We next tested an eventual role for the response-contingent light cue above the operant lever in the residual effect of cocaine priming. To this end, rats ($n = 18$) from experiment 3 were tested during 21 additional sessions of extinction under 3 light cue conditions: **light cue present; no light cue available; and house light cue present** (i.e., a novel diffuse light from the cage roof never associated with cocaine). Overall, there was a main effect of sessions, indicating an influence of the light cue condition on the residual effect of cocaine priming ($F_{20,340} = 2.6, p < 0.01$) (Fig. 4a). Subsequent analysis of data averaged across all sessions with the same light cue condition revealed that removal of the light cue above the operant lever abolished the residual effect of cocaine priming ($F_{2,34} = 4.33, p < 0.05$) (Fig. 4b). Importantly, this effect was probably due to some unconditioned visual properties of the light cue because the residual effect of cocaine re-emerged **when a novel house light cue was presented after responding** (Fig. 4b).

3.6. Experiment 6: Effects of cocaine on operant responding for a novel light cue after extinction of cocaine priming

The effect of a novel light cue (i.e., house light) was further confirmed in an independent group of rats ($n = 13$) that were trained to self-administer cocaine self-administration with no response-contingent light cue (Supplementary Fig. S5).

3.7. Experiment 7: Effects of cocaine on operant responding for a novel light cue in naïve rats

To rule out an eventual stimulus generalization effect, we tested the effects of cocaine on operant responding for a light cue in a group of initially drug- and operant-naïve rats ($n = 28$). During testing, they received increasing i.v. doses of cocaine during access to an operant lever that turned on a light cue above it for 20 s under a FR1 procedure. Cocaine dose-dependently increased responding when a response-contingent light cue was present but not when it was absent (Dose x Cue: $F_{3,81} = 4.23$, $p < 0.01$) (Fig. 3b). The latter findings demonstrate that the residual effect of cocaine priming observed previously does not in fact reflect a residue of cocaine seeking *per se* but a drug potentiation of some unconditioned reinforcing effects of light itself, whatever they may be.

3.8. Experiment 8: Effects of extinction of cocaine priming **to the maximum extent feasible on relapse-like behavior**

In experiments 3 and 5, the same rats ($n = 18$) received a total of 45 sessions of extinction of cocaine priming. Interestingly, during the last two sessions of extinction, there was no longer evidence for a residual effect of cocaine priming ($F_{1,17} = 0.00$) (Fig. 4a). We took advantage of **this maximal extinction** to measure its effect on relapse-like behavior. However, there was again no significant impact on relapse-like behavior ($F_{2,34} = 1.30$) (Fig. 4c) (see Supplementary Fig. S4 for representative pre- and post-extinction patterns of cocaine self-administration).

3.9. Experiment 9: Effects of extinction of cocaine priming on relapse-like behavior under a more demanding procedure of cocaine self-administration

In all previous experiments, a relatively low level of effort (i.e., FR3) was required to obtain cocaine during both maintenance of and relapse-like behavior. This factor may contribute to explain why extinction of cocaine priming had no major impact on relapse-like behavior. To test this hypothesis, an independent group of rats ($n = 8$) were trained to self-administer cocaine under a more demanding FR procedure that also allowed us to measure the motivation for the drug (Hursh and Silberberg, 2008; Koffarnus and Woods, 2012; Lenoir and Ahmed, 2008). Briefly, the FR requirement for obtaining cocaine was increased quasi-exponentially within-session, from FR1 to FR81. Overall, despite an initial decrease in intake and motivation on the first post-extinction session of cocaine self-administration, rats nevertheless fully recovered their initial performance as early as the second post-extinction session onward (Supplementary Fig. S6 and S7).

3.10. Experiment 10: Neuronal correlates of extinction of cocaine priming

3.10.1. *c-Fos* immunohistochemistry

Since there was no evidence for a major impact of extinction of cocaine priming on relapse-like behavior, we controlled for possible evidence for extinction-resistant neurobiological responses to cocaine in brain regions causally involved in cocaine-induced reinstatement using *c-Fos* immunohistochemistry (see Supplementary Data (Appendix A)). These regions include the anterior cingulate cortex, the prelimbic subdivision of the medial prefrontal cortex, and the core subdivision of the nucleus accumbens (Capriles et al., 2003; McFarland and Kalivas, 2001; Vassoler et al., 2013) (for exact anatomical coordinates, see (Navailles et al., 2015)). This experiment involved a total of 4 different subgroups of rats ($n = 4-5$ per subgroup) with different histories of extinction and different treatments during final testing: subgroup V-V was repeatedly primed with vehicle during extinction and tested with vehicle; subgroup V-C was repeatedly primed with vehicle during extinction and tested with cocaine; subgroup C-V was repeatedly primed with cocaine during extinction and tested with vehicle; and finally, subgroup C-C was repeatedly primed with cocaine during extinction and tested with cocaine. As expected, during final testing, cocaine priming induced a large reinstatement effect in rats V-C, but not in rats C-C, compared to control rats that were tested with vehicle (i.e., rats V-V and C-V) (Group x Subgroup x Period interaction: $F_{1,15} = 15.72$, $p < 0.01$) (Fig. 5a). At the neuronal level, cocaine priming induced a large increase in Fos expressing-cells in all brain regions of interest in rats V-C compared to control rats (Group x Subgroup interaction: $F_{1,15}$ values > 6.20 , p values < 0.05) (Fig. 5b-d). These neuronal responses to cocaine priming were lost in rats C-C as they were not significantly different from those measured in control rats receiving vehicle (Fig. 5b-d). Representative Fos immunohistochemistry examples are shown in Supplementary Fig. S8.

3.10.2. *in vivo* prefrontal electrophysiology during extinction of cocaine priming

Thus, extinction of cocaine priming was also associated with an extinction of the neuronal responses to cocaine in brain regions critically involved in reinstatement, notably in the prelimbic cortex. To obtain a glimpse of the dynamic of this neuronal extinction, we recorded *in vivo* single-unit neuronal activity in the prelimbic cortex of a separate subgroup rats ($n = 3$) during extinction of cocaine priming (see Fig. 6a for electrode placements). During the first extinction session, cocaine priming induced a robust reinstatement effect (Fig. 6b) which was accompanied by a rapid and prolonged increase in neuronal activity in the prelimbic cortex that concerned most recorded neurons (i.e., 64 %) (Fig. 6c,d). This finding provides direct *in vivo* evidence for the hypothesis that cocaine priming induced reinstatement of cocaine seeking via increased neuronal activity in the prelimbic cortex. It also further validates the interpretation that increased Fos expression in this brain region reflects increased neuronal activity. With repeated priming, however, cocaine-induced increased neuronal activity in the prelimbic cortex progressively decreased to **the non-primed level of responding** (Priming x Session: $F_{4,116} = 8.62$, $p < 0.01$) (Fig. 6f). This gradual neuronal adaptation paralleled in time the decrease in the reinstating effects of cocaine (Priming x Session: $F_{4,8} = 11.04$, $p < 0.01$) (Fig. 6e). Overall, this study reveals that extinction of the behavioral effects of cocaine on reinstatement of cocaine seeking was paralleled in time by a dramatic extinction of its effects on neuronal activity in the prelimbic cortex.

Finally, despite extinction of cocaine priming **to the maximum extent feasible** after a total of 45 sessions of extinction, rats from experiment 5 continued nevertheless to exhibit a relatively high level of non-primed responding on the last 2 sessions of extinction ($F_{17,289} = 13.30$, $p < 0.01$), especially at session onset (Fig. 7a) and at a cumulative rate sufficient to meet rapidly several times the FR3 requirement for cocaine reinforcement ($F_{17,289} = 11.21$,

$p < 0.01$; comparison with 3: all t_{17} values > 3.00 , all p values < 0.01) (Fig. 7b). Clearly, this behavior insured that rats will rapidly detect cocaine when it was again available for self-administration after extinction of cocaine priming, thereby probably explaining rapid relapse-like behavior. Importantly, this extinction-resistant behavior was not a side-effect of repeated cocaine priming because a similar behavior was also exhibited by control rats that were repeatedly primed with saline during extinction after cocaine self-administration (Fig. 7c-d: Responses: $F_{17,170} = 12.27$, $p < 0.01$; Cumulative responses: $F_{17,170} = 15.10$, $p < 0.01$; comparison with 3: all t_{10} values > 3.23 , all p values < 0.01).

4. Discussion

The present study demonstrates that extinction of cocaine priming, whether administered contingently or non-contingently, has no major effect on subsequent relapse-like behavior in rats. Importantly, this lack of effectiveness could not be due to the context-specificity of extinction of cocaine priming (Mihindou et al., 2011) since relapse-like behavior occurred in the same environmental context. The lack of effectiveness of extinction of cocaine priming on subsequent relapse-like behavior could not also be attributed to a residual effect of cocaine priming. When such a residue was observed at the behavioral level, it was found to result from a cocaine potentiation of unconditioned light-seeking behavior – a finding that confirms previous research with other psychostimulants (Berlyne, 1969; Glow and Russell, 1973; Gomer and Jakubczak, 1974; Kiernan, 1965) – and not from cocaine seeking *per se*. In addition, at the neurobiological level, extinction of cocaine priming was associated with a loss of neuronal responses to cocaine in brain regions critically involved in cocaine-induced reinstatement, such as the prelimbic cortex and the nucleus accumbens core (Capriles et al., 2003; McFarland and Kalivas, 2001; Vassoler et al., 2013). Notably, in the prelimbic cortex, loss of neuronal responses to cocaine paralleled in time the extinction of its behavioral effects on reinstatement of cocaine seeking. In fact, even **when cocaine priming was extinguished to the maximum extent feasible** at the behavioral level, it had no major impact on relapse-like behavior. An effect of extinction of cocaine priming was only observed when cocaine was available for self-administration under more demanding conditions. However, this effect was only modest and short-lived. In all experiments conducted in the present study, once rats found that cocaine was again available for self-administration after extinction, they immediately recovered their initial pre-extinction self-administration behavior as if no extinction had occurred.

Overall, these findings demonstrate that the mechanisms underlying cocaine-primed reinstatement are not essential for relapse-like behavior and are dissociable from the mechanisms underlying cocaine self-administration. This behavioral dissociation was particularly evident in rats from experiment 3 that quickly relapsed after each block of extinction despite evidence for retention of extinction of cocaine priming across blocks. During the last blocks, the same individual rats no longer responded to the priming effects of cocaine but nevertheless continued to self-administer cocaine at the same rate and according to the same pattern as before extinction. Lack of responses to the priming effects of cocaine co-existed, within the same individuals, with an unchanged cocaine self-administration behavior, demonstrating that the former does not play a significant causal role in the latter. This within-subject behavioral dissociation is in fact in general agreement with previous between-subject neurobiological dissociation studies and is thus not surprising. As mentioned in the Introduction, these studies have shown that neural interventions that reduce or block cocaine-primed reinstatement have no effect on cocaine self-administration (Bachtell et al., 2008; Baker et al., 2003; Chauvet et al., 2015; Fletcher et al., 2002; Moussawi et al., 2011; Peng et al., 2008; Pentkowski et al., 2010; Schroeder et al., 2010; Sun and Rebec, 2005). Taken together, these findings suggest that though extinction of interoceptive drug cues is a robust phenomenon, it does not seem to represent a promising approach to prevent relapse, contrary to our initial proposal (Mihindou et al., 2011). This conclusion is also consistent with previous failures with exteroceptive cue extinction procedures (Conklin and Tiffany, 2002). Whether this conclusion can also be extrapolated to other strategies that also target the priming effects of cocaine or related cues is currently unknown, mainly because, apart from rare exceptions (Luo et al., 2015), the effects of these strategies on relapse-like behavior have not been tested yet.

Interestingly, we found that even after prolonged instrumental extinction and **extensive** extinction of cocaine priming, rats did not cease to respond entirely. They continued to

respond, mainly at the beginning of each extinction session, at a weak, albeit sufficient, level to obtain at least one dose of cocaine (i.e., had the drug been available). This persistent behavior suggests that though rats learn that cocaine is likely to be no longer available during extinction, they nevertheless continue to respond as if they were checking each day a possible return of drug availability. In wild habitats, when a particular source of reward is depleted, it is generally not permanently. It may eventually be replenished (e.g., a patch with earth worms may be replenished by the arrival of new worms). In other words, things may and do change (McFarland, 1977; Stephens et al., 2007), and thus it always pays to continue to explore, at least briefly, the environment. In the present study, persistent responding was clearly adaptive as it allowed rats to rapidly detect the post-extinction return of cocaine availability. A quick perusal of the relevant literature reveals that persistent responding is in fact one of the most reproducible, albeit largely overlooked, outcomes of drug reinstatement and extinction studies (e.g., all articles on cocaine-induced reinstatement cited in the References). This persistent behavior is not entirely surprising, however, if one considers that it is relatively costless in comparison to its potentially relatively large payoff (i.e., discovery of return of cocaine availability) and also that it occurs in an impoverished drug setting that typically lacks behavioral alternatives to drug use. Of particular interest, recent research has shown that access to other competing nondrug options during drug access can lead many rats to stop drug use (Ahmed, 2010, 2017). Thus, the significance and relevance of persistent extinction responding in the standard drug setting, though robust, may be of limited value to understand vulnerability to relapse in humans who typically have access to a rich array of nondrug alternative behaviors. Current research is beginning to explore more directly this important issue (Ahmed, 2010; Caprioli et al., 2015; Venniro et al., 2017).

Finally, the present study also provides some new insights about the neural mechanisms of cocaine-primed reinstatement of drug seeking. Previous research has amply demonstrated that the dorsomedial prefrontal cortex, including its anterior cingulate and prelimbic

subdivisions, plays a causal role in cocaine-primed reinstatement of cocaine seeking, notably through its top-down projections to the core of the nucleus accumbens (Capriles et al., 2003; McFarland and Kalivas, 2001; Stefanik et al., 2013; Vassoler et al., 2013). The present study is generally consistent with this conclusion. In addition, the fact that cocaine no longer activated this top-down prefrontal-accumbens pathway after repeated priming suggests that its recruitment is probably a conditioned response to the interoceptive stimuli of cocaine priming, as hypothesized here. One important virtue of this interpretation is that it explains cocaine-primed reinstatement as an instance of a more general mechanism of conditioned cue- or context-induced cocaine seeking (Ahmed and Koob, 1997; Crombag et al., 2008; Mihindou et al., 2011). In all cases, the starting point is the perception of conditioned drug cues or contexts. The only difference would be the interoceptive *versus* exteroceptive sensory modality of the conditioned cues or contexts and, perhaps, the different subcomponents of the prefrontal-accumbens pathway that they probably recruit. What the conditioned interoceptive stimuli of cocaine priming are exactly, where they are processed in the brain, and how the output of this processing leads to activation of the prefrontal-accumbens pathway that controls reinstatement of cocaine seeking remain important unanswered questions for future research.

Declaration of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at the Journal website.

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Figure captions

Fig. 1 Extinction of cocaine priming. (a) Within- and between-session extinction of the effect of cocaine priming on non-reinforced responding (mean \pm s.e.m.). After an initial non-primed period of 90 min, rats received intravenously 3 non-contingent priming doses of 1-mg cocaine (P1 to P3) spaced 45 min apart. The effect of each priming dose of cocaine was compared to the non-primed level of responding (NP) measured during the 45-min period preceding the first priming dose (P1). #, different from the non-primed level of responding (NP) during sessions 1 and 5 of extinction; *, different from the first priming dose (P1); °, different from session 1 of extinction ($p < 0.01$). (b) Extinction of the average effect of cocaine priming (i.e., $P = \text{average of the 3 primings}$) across sessions (mean \pm s.e.m.). #, different from the non-primed level of responding (NP); *, different from session 1 of extinction ($p < 0.01$). (c) Pre- versus post-extinction temporal patterns of cocaine injections in 3 representative rats. Pre-extinction patterns were obtained on the last session of self-administration preceding extinction. Post-extinction patterns were obtained on the first session of relapse-like behavior after extinction. Each vertical tick represents an i.v. injection of 0.25-mg cocaine under a FR3 procedure of self-administration. (d) Pre- versus post-extinction rate of cocaine self-administration (mean \pm s.e.m.). The pre-extinction rate was obtained by averaging the last 3 sessions of self-administration that preceded extinction of cocaine priming. *, different from pre-extinction ($p < 0.01$).

Fig. 2 Relapse-like behavior after extensive extinction of cocaine priming. (a) Extinction of the average effect of cocaine priming across 4 extinction blocks of 6 sessions (Ext 1-6) (mean \pm s.e.m.). Blocks of extinction alternated with blocks of 6 relapse sessions. #, different from the non-primed level of responding (NP); *, different from session 1 of block 1 ($p < 0.01$). (b) Average effect of cocaine priming during sessions 1 and 6 of extinction across

blocks. *, different from all the last sessions (i.e., session 6 of extinction) of all blocks ($p < 0.01$). (c) Extinction of the effect of all 3 priming doses of cocaine across sessions. #, different from the non-primed level of responding (NP) during session 1 of block 1 (Ext 1) and the last 3 stable sessions of block 4 (Ext 4-6); *, different from session 1 of extinction (Ext 1) ($p < 0.01$). (d) Pre- versus post-extinction rate of cocaine self-administration across the 4 relapse blocks that alternated with the 4 extinction blocks (mean \pm s.e.m.).

Fig. 3 Factors involved in the residual effect of cocaine priming. (a) Effect of the intravenous administration procedure. After extinction of cocaine priming, cocaine was replaced by saline all else being equal. Saline substitution was replicated 3 times during 3 sessions that alternated with regular sessions of cocaine extinction. Regardless of the priming dose (i.e., P1 to P3), substituting cocaine with saline was associated with a loss of effect on responding. #, different from the non-primed level of responding (NP); *, different from cocaine ($p < 0.01$). (b) Effect of increasing doses of intravenous cocaine on lever press responding for a 20-s light cue in naïve rats. #, different from the no light cue condition; *, different from dose 0 ($p < 0.01$).

Fig. 4 Cocaine potentiation of operant responding for a light cue. (a) Effects of different light cue conditions on the average effect of cocaine priming. The following three light conditions were tested for at least 4 sessions each (indicated by an horizontal grey bar above the curve): Lever light, response-contingent presentation of the light cue above the lever that was previously associated with cocaine; No light, no response-contingent light cue is presented; House light, response-contingent presentation of a novel house light cue. (b) Average effects across all testing sessions of the different light cue conditions. *, different from the non-primed level of responding (NP) ($p < 0.01$). (c) Pre- versus post-extinction rate of cocaine self-administration (mean \pm s.e.m.).

Fig. 5 Neuronal correlates of extinction of cocaine priming. (a) Between-subject evidence for extinction of cocaine priming. Four subgroups of rats ($n = 4-5$) with different histories of extinction received either vehicle or cocaine during final testing. Subgroup V-V was repeatedly primed with vehicle during extinction and tested with vehicle; subgroup V-C was repeatedly primed with vehicle during extinction and tested with cocaine; subgroup C-V was repeatedly primed with cocaine during extinction and tested with vehicle; and finally, subgroup C-C was repeatedly primed with cocaine during extinction and tested with cocaine. The effect of each priming dose of vehicle or cocaine was compared to non-primed responding (NP) measured during the 45-min period preceding priming. *, different from all other conditions ($p < 0.01$). Fos expression in the anterior cingulate cortex (b), the prelimbic cortex (c) and the nucleus accumbens core (d). Data are the mean (\pm s.e.m.) density of Fos-positive (Fos+) cells. *, different from control subgroups ($p < 0.05$).

Fig. 6 Neuronal activity correlates of extinction of cocaine priming in the prelimbic cortex. (a) Electrode placements within the prelimbic cortex. Each black dot represents the tip of an electrode. (b) Within-session time course of the effect of cocaine priming (1 mg, i.v.) on non-reinforced operant responding during the first session of extinction ($F_{17,37} = 3.32$, $p < 0.01$). #, different from the non-primed level of responding (NP) ($p < 0.01$). (c) Representative example of a prelimbic neuron that increases its firing activity during cocaine priming. (d) Mean (\pm s.e.m.) within-session time course of prelimbic firing rate (Hz). Prelimbic neuronal activity increased after cocaine priming (white circles) above the non-primed level of responding (NP) measured 45 min before (black circles) ($F_{17,510} = 11.37$, $p < 0.01$). (e) Extinction of the effect of cocaine priming on non-reinforced responding across sessions (mean \pm s.e.m.). The effect of cocaine priming (P) was compared to the non-primed level of responding (NP) measured during the 45-min period preceding priming. (f) Mean firing rate (Hz) of neurons in the prelimbic cortex across repeated priming sessions (mean \pm s.e.m.). The effect of cocaine priming (P) on prelimbic neuronal activity was compared to the non-

primed activity (NP) measured during the 45-min period preceding priming. #, different from the non-primed level of activity (NP); *, different from session 1 ($p < 0.01$).

Fig. 7 Continued non-primed responding after extinction of cocaine or saline priming. (a) Within-session time course of non-primed responding after extensive extinction of the priming effect of cocaine in rats that received a total of 45 sessions of extinction. *, different from the first 5 min ($p < 0.01$); (b) Cumulative non-primed responding in the same conditions as in (a). #, different from FR3 ($p < 0.01$). (c) Within-session time course of non-reinforced responding on the last (i.e., 10th) session of extinction in saline-primed rats. *, different from the first 5 min ($p < 0.01$). (d) Cumulative non-primed responding in the same conditions as in (c). #, different from FR3 ($p < 0.01$).













