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Research report

# Long-term loss of dopamine release mediated by CRF-1 receptors in the rat lateral septum after repeated cocaine administration

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## HIGHLIGHTS

- Activation of CRF-R1 in lateral septum (LS) increases dopamine extracellular levels.
- Repeated exposure to cocaine suppresses the increase in LS dopamine extracellular levels induced by CRF-R1 activation.
- Repeated exposure to cocaine transiently increases LS dopamine releasability.
- Suppression of LS dopamine release induced by CRF-R1 should have a significant impact on addictive behavior.

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## ABSTRACT

The lateral septum (LS) is a brain nucleus associated to stress and drug addiction. Here we show that dopamine extracellular levels in the lateral septum are under the control of corticotrophin releasing factor (CRF). Reverse dialysis of 1 μM stressin-1, a type 1 CRF receptor (CRF-R1) agonist, induced a significant increase of LS dopamine extracellular levels in saline-treated rats that was blocked by the co-perfusion of stressin-1 with CP-154526, a specific CRF-R1 antagonist. Repeated cocaine administration (15 mg/kg; twice daily for 14 days) suppressed the increase in LS dopamine extracellular levels induced by CRF-R1 activation. This suppression was observed 24 h, as well as 21 days after withdrawal from repeated cocaine administration. In addition, depolarization-induced dopamine release in the LS was significantly higher in cocaine-compared to saline-treated rats. Thus, our results show that the activation of CRF-R1 in the LS induces a significant increase in dopamine extracellular levels. Interestingly, repeated cocaine administration induces a long-term suppression of the CRF-R1 mediated dopamine release and a transient increase in dopamine releasability in the LS.



## 1. Introduction

Addiction is a chronic brain disorder characterized by the compulsion to continue seeking and using drugs despite adverse consequences on health and on social interactions. Evidence from the clinic, as well as from animal studies, supports a strong connection between stress and addiction [for review see 1, 2]. Stress increases likelihood of drug abuse in vulnerable individuals and acute stressful stimuli induce relapse in drug-experienced individuals. In addition, stress enhances the reinforcing properties of addictive drugs during the acquisition of drug self-administration [3,4].

Addictive drugs stimulate dopaminergic neurons of the ventral tegmental area (VTA) that project to several interconnected nuclei such as the accumbens, prefrontal cortex and lateral septum (LS) [5–9]. The dopaminergic pathway originated in the VTA and innervating the LS has been scarcely studied. Neurochemical evidence [10,11], as well as electrophysiological studies [17], suggest that the LS is a brain nucleus involved in drug reward. The role of LS in addictive behavior was originally evidenced by Olds and Milner [12] who showed that the LS is able to support self-stimulation indicating that is part of the reward system. Recently, it has been demonstrated that the LS is an important structure for processing contextual information associated to drugs of abuse and

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sending this information to other brain regions for the expression of reward-related behaviors [13,14]. The LS has also been related with other psychopathologies such as psychosis and anxiety, which are prevalent comorbidities in patients with drug abuse [for review see 15].

Studies in animal models of the addictive process have pointed to the corticotrophin releasing factor (CRF) system as a key player in the interaction between stress and drug abuse [1,16–21]. In this sense, electrophysiological studies have shown that CRF induces a concentration-dependent long-lasting potentiation of glutamatergic transmission between the lateral amygdala and central amygdala nuclei, which is enhanced after long- but not short-term cocaine withdrawal [22]. In the case of LS, repeated cocaine administration to rats induces an increase in CRF-mediated synaptic efficiency [17]. These authors suggested that this increase could be explained, at least in part, by a CRF receptor-mediated facilitation of LS glutamate release. There is also evidence suggesting a role of type-1 CRF receptors (CRF-R1) of the LS in stress-induced potentiation of cocaine reward [23]. Although there is evidence that dopamine (DA) extracellular levels in the LS are modified by the administration of drugs of abuse [10,11], the possibility that repeated cocaine exposure could induce neurochemical changes in LS dopaminergic innervation has not been studied.

Considering that the LS is involved in stress and drug abuse, a better understanding of the neurochemical changes produced by chronic cocaine administration should be useful to clarify the mechanisms underlying behavioral responses in the addictive process. Thus, the aim of the present work was to study the effect of stressin-1, a CRF-R1 agonist on LS DA extracellular levels in control and cocaine-repeatedly treated rats, both after short (24 h) and long-term (21 days) withdrawal.

## 2. Materials and methods

### 2.1. Animals and reagents

#### 2.1.1. Animals

Male Sprague-Dawley rats weighing 250–285 g were selected for the experiments. They were kept in a controlled environment with a 12-h light-dark cycle and at 21 °C room temperature. Food and water were provided *ad libitum*. All experimental procedures were approved by the Ethics Committee of Faculty of Biological Sciences of "Pontificia Universidad Católica de Chile" and follow the international guidelines (NIH Guide for the Care and Use of Laboratory Animals).

#### 2.1.2. Reagents

Cocaine HCl was donated by the National Institute on Drug Abuse (NIDA, Baltimore, USA). Stressin-1 and CP-154526 were purchased from Tocris Bioscience (Ellisville, MO, USA). Dopamine, EDTA and 1-octanesulfonic acid were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). All other reagents were of analytical grade.

### 2.2. Experimental procedure

Animals were divided in two experimental groups, saline controls and cocaine-treated rats. The repeated cocaine administration protocol consisted of injecting cocaine 15 mg/kg, i.p. twice daily for 14 days, as described by Liu et al. [17]. Daily administration of cocaine and saline solution was performed at the same hour in the morning and afternoon (beginning at 9:00 A.M. and 4:00 P.M.).

### 2.3. In vivo microdialysis and analysis of dialysate samples

#### 2.3.1. Microdialysis

Twenty-four hours or 21 days after the last cocaine or saline injection, microdialysis experiments were performed. Rats were deeply anesthetized with choral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic apparatus (Stoelting, Wood Dale, MA). Corporal temperature of the rats was maintained at 37 °C with an electrical blanket controlled by a thermostat. A quarter of the initial dose of choral hydrate was given every hour to maintain the rat anesthetized during the course of the experiments. Concentric brain microdialysis probes (Microdialysis Probe, CMA-12, 100,000 Daltons cut-off, Stockholm, Sweden) were implanted in the LS as previously described [10]. The following coordinates, according to the atlas of Paxinos and Watson [24] were used: LS: 0.2 mm anterior to bregma, 0.8 lateral and 5.8 mm ventral. The microdialysis probe was perfused with Krebs-Ringer's phosphate buffer plus bovine serum albumin 0.01% (KRP-BSA) at a flow rate of 2 μL/min, using a Harvard infusion pump (Model 22; Dover, MA). Rats were subjected to one of three microdialysis protocols: (1) After a stabilization period of 90 min, three perfusion samples of 10 min each were collected in 3 μL of perchloric acid (0.2 M). At 30 min, the KRP-BSA solution was changed for 10 min to KRP-BSA-110 mM $K^+$. Between 40 and 70 min, the KRP-BSA solution was again perfused through the microdialysis probe. (2) After a stabilization period of 90 min, two perfusion samples of 10 min each were collected in 3 μL of perchloric acid (0.2 M). At 20 min, the KRP-BSA solution was changed to KRP-BSA containing 1 μM stressin-1 (a selective CRF-R1 agonist) that was perfused intra-LS for additional 50 min. (3) During the stabilization period (90 min) and throughout the experiment (50 min) KRP-BSA containing 1 μM CP-154526 (a selective CRF-R1 antagonist) was perfused. After the stabilization period, two perfusion samples of 10 min each were collected in 3 μL of perchloric acid (0.2 M). At 20 min and until the end of the experiment, the KRP-BSA-CP-154526 solution was changed to KRP-BSA-CP-154526 plus 1 μM stressin-1. All perfusion samples were maintained on ice during the experiment and stored at −80 °C until analysis. At the end of each experiment, rats were sacrificed and brains quickly removed and stored in formalin. Brain sections of 50 μm were stained with cresyl violet to verify probe location under microscope.

#### 2.3.2. Dopamine analysis

HPLC-electrochemical determination of DA was performed as described previously [25]. Briefly, 10 μL of dialysates were injected into a HPLC system with the following configuration: an isocratic pump (model PU-2080 Plus, Jasco Co. Ltd., Tokyo, Japan), a UniJet microbore column (MF-8949, BAS, West Lafayette, IN) and an amperometric detector (set at 650 mV, 0.5 nA; model LC-4C, BAS, West Lafayette, IN). The mobile phase, containing 0.1 M $NaH_2PO_4$, 1.2 mM 1-octanesulfonic acid, 1 mM EDTA and 4.0% (v/v) $CH_3CN$ (pH adjusted to 2.8) was pumped at a flow rate of 80 μL/min. The retention time for DA was 10.0 min and the detection limit was 0.1 fmol/μL.

### 2.4. Statistical analysis

One-way ANOVA followed by Newman–Keuls post hoc test was used to determine significant differences between each point of microdialysis experiments (Figs. 1 and 2). Two-way ANOVA was used to compare the effect of stressin-1 and $K^+$-depolarization in control saline rats versus cocaine-treated rats. Mann–Whitney *t*-test was used to determine significant differences between stimulated values in each group of rats (50 min points in Fig. 1A and B). The statistical analyses were carried out with GraphPad Prism v5.0 (GraphPad Software, San Diego, CA).

## 3. Results

### 3.1. In vivo microdialysis

Reverse dialysis of stressin-1 in LS induced a significant increase in LS DA extracellular levels in saline control rats, either 24 h (Fig. 1A; $[F(6,21) = 14.10,\ p < 0.0001]$; by one-way ANOVA) or 21 days (Fig. 1B; $[F(6,14) = 4.501, p = 0.0096]$; by one-way ANOVA) after the last saline injection. In contrast, stressin-1 did not change LS DA extracellular levels in cocaine repeatedly treated rats, either 24 h (Fig. 1A) or 21 days (Fig. 1B), after the last cocaine injection. In contrast, reverse dialysis of stressin-1 in LS did not induce an increase in LS DA extracellular levels in cocaine-treated rats, either 24 h (Fig. 1A) or 21 days (Fig. 1B) after last cocaine injection. A two-way ANOVA analysis comparing saline control rats with cocaine-treated rats showed a significant effect of cocaine treatment at 24 h $[F(1,56) = 23.39, p < 0.0001]$ and after long-term withdrawal $[F(1,42) = 68.81,\ p < 0.0001]$. Thus, repeated cocaine treatment provoked a significant long-lasting suppression of LS DA release induced by stressin-1. No changes on basal extracellular levels of DA in the LS were observed after short or long-term cocaine withdrawal (Table 1).

Next, we tested whether the complete suppression of the response towards the effect of stressin-1, observed 24 h and 21 days after cocaine withdrawal, was due to a decrease in LS DA releasability. To this end, we determined depolarization-induced release of LS DA by perfusing 110 mM $K^+$ through the microdialysis probe. $K^+$-depolarization induced a statistically significant increase of LS DA release in saline (Fig. 1C; $[F(6,56) = 5.746, p = 0.0001]$; by one-way ANOVA) and in cocaine repeatedly treated rats (Fig. 1C; $[F(6,77) = 39.01, p < 0.0001]$; by one-way ANOVA), 24 h after cocaine withdrawal. Surprisingly, at this short-term cocaine withdrawal,