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Research report

# Corticotropin-releasing factor receptor type-2 is involved in the cocaine-primed reinstatement of cocaine conditioned place preference in rats

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## HIGHLIGHTS

- Changes of CRFR2 expression in mPFC, HP and DS in cocaine-induced and extinct CPP rats.
- Effects of local blockade of CRFR2 on reinstatement of cocaine seeking behavior.
- CRFR2 is involved in relapse to cocaine-intake in a brain region-specific manner.

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## ABSTRACT

Here we explored the in vivo role of brain corticotropin-releasing factor receptor type-2 (CRFR2) in cocaine-primed reinstatement of drug seeking. Conditioned place preference (CPP) procedure was used to assess the acquisition, extinction and reinstatement of cocaine-seeking behavior in rats. First, expressions of CRFR2 were shown to be affected in a brain region-specific manner within cocaine-induced CPP and cocaine-extinct CPP models. Bilateral blockade of CRFR2 in the dorsal portion of the medial prefrontal cortex (mPFC), or hippocampus (HP) was partially inhibited, but in the dorsal striatum (DS) did not affect, the cocaine-primed reinstatement of cocaine CPP.



## 1. Introduction

The successful treatment of drug addiction has been challenged by the high rate of relapse. Relapse to cocaine use can be triggered by re-exposure to cocaine, drug-associated cues, or stressors. The molecular mechanisms underlying the compulsive cocaine craving behaviors remain unclear. Corticotropin-releasing factor (CRF) system has been highly implicated in cocaine relapse [1–6]. Among the two types of CRF receptors, the role of CRF receptor type-1(CRFR1) in drug abuse has been well studied [7,8], whereas the role of CRFR2 in cocaine abuse is less clear. CRFR2 differs from CRFR1 in its endogenous ligands and brain distribution [9–12]. Therefore, CRFR2 may exhibit different pharmacological profiles and play different roles in cocaine abuse, as compared to CRFR1. Although systemic blockade of CRFR2 activity does not affect the reinstatement of cocaine-seeking behavior after extinction [5], recent studies have suggested that CRFR2 signaling in certain brain areas may contribute to cocaine-induced neuroplasticity, and ultimately lead to cocaine relapse. For instance, selective blockade of CRFR2 activity in ventral tegmental area (VTA) suppressed relapse to cocaine [13], whereas activation of CRFR2 activity in VTA mimicked footshock-induced reinstatement of cocaine seeking behavior [14]. Activation of CRFR2 in prefrontal cortex was reported to potentiate cocaine-induced enhancement of EPSCs [15]. Chronic cocaine administration was able to switch CRFR2-mediated depression to facilitation of glutamatergic transmission in rat lateral septums

*Abbreviations:* Ast2B, astressin2-B; CPP, conditioned place preference; CRF, corticotropin-releasing factor; CRFR1, corticotropin-releasing factor type-1 receptor; CRFR2, corticotropin-releasing factor type-2 receptor; DS, dorsal striatum; HP, hippocampus; mPFC, medial prefrontal cortex.

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[16]. Taken together, these results demonstrate that the roles of CRFR2 in cocaine addiction are likely to be in a brain region-specific manner.

We previously explored the roles of CRFR2 in development of cocaine-induced electrophysiological plasticity [17,18]. We found that selective blockade of CRFR2 activity inhibited cocaine withdrawal-enhanced long-term potentiation (LTP) in hippocampus (HP) slices, and also attenuated CRF-enhanced LTP in corticostriatal slices from cocaine withdrawal rats. There is evidence from both human and animal studies that hippocampal and corticostriatal circuits contribute to cocaine addiction [19–21]. Within these circuits, hippocampus (HP), medial prefrontal cortex (mPFC), and dorsal striatum (DS) are three important neural substrates for the development of cocaine relapse [22–26]. We hypothesized that CRFR2 in these brain regions are involved in the development of cocaine relapse. As an extension of our previous observations and to further test this hypothesis, here we assessed whether CRFR2 in HP, mPFC, and DS regions play a role in development of cocaine relapse. Conditioned place preference (CPP) paradigm has been widely used to study the rewarding effects of addictive drugs. In this study, CPP procedure was used to assess the acquisition, extinction and reinstatement of cocaine-seeking behavior in rats. So far, reports on the expression of CRFR2 in cortex remain controversial among studies [12,27–29], and there is little evidence for the presence of CRFR2 in striatum. Thus, the first aim of this study is to examine expressions of CRFR2 in HP, mPFC and DS regions under physiological conditions and during acquisition and extinction of cocaine CPP. Next, the roles of CRFR2 in all three regions in the cocaine-primed reinstatement of cocaine CPP were explored by using a selective CRFR2 antagonist, astressin2-B [30–32].

## 2. Material and methods

All experiment procedures were performed in accordance with the Nanjing Medical University Guide for the Care and Use of Laboratory Animals, China, and the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23). All efforts were made to minimize the number of animals used and to minimize their suffering.

### 2.1. Animals

Male Sprague-Dawley rats (300–330 g, $n = 308$) were used in this study. Rats were maintained on a reverse 12 h light/dark cycle with ad libitum access to food and water.

### 2.2. Surgery

Rats were anesthetized with chloral hydrate (514 mg/kg, i.p.) and placed in a stereotaxic apparatus. Stainless-steel guide cannula (23-gauge; Plastics One Inc., USA) were bilaterally implanted 1 mm above the hippocampus (HP), the dorsal striatum (DS), or medial prefrontal cortex (mFPC). The coordinates for the CA1 area of HP (Paxinos, 1986) were AP, −4.2 mm from bregma; ML, ±2.2 mm from midline; and DV, −2.5 mm from skull surface. The DS coordinates were AP, +0.7 mm from bregma; ML, ±2.0 mm from midline; and DV, −3.6 mm from skull surface. The implantation of cannulae into mPFC were performed at a 30° angle from vertical to avoid the superior sagittal sinus, and the coordinates were AP, +2.2 mm from bregma; ML, ±2.3 mm from midline; and DV, −3.1 mm from skull surface. The cannula was affixed to the skull with screws and dental cement. A stylet was placed into the guide cannula to allow the guide cannula to maintain patency. The rats were allowed to recover for 7 days after surgery. We checked the injection tracks when we collected samples and after behavioral tests. We discarded the samples and data from the rats present in incorrect injection positions.

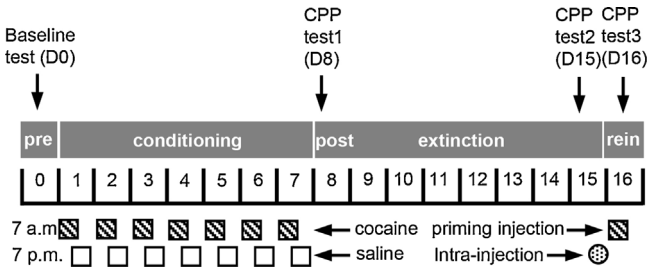


**Fig. 1.** Timeline of the CPP tests and drug treatment.

### 2.3. Drug treatment

Cocaine hydrochloride (Qinghai Pharmaceutical, China) was dissolved in sterile saline and was injected (10 mg/kg, i.p.) during CPP training (day 1–7) or 10 min before CPP test 3 (as shown Fig. 1). The local activities of CRFR2 were selectively blocked by bilateral intra-mPFC (0.3 μl/side), intra-HP (0.8 μl/side) or intra-DS (1 μl/side) injections of Astressin2_B (Ast2B) at doses of 0 ng/side (vehicle), 200 ng/side, 400 ng/side, 800 ng/side or 1.6 μg/side. On the afternoon of day 15, Ast2B was dissolved in artificial CSF (aCSF, vehicle) and was injected bilaterally over a period of 15 min with Hamilton syringes. The injection needle was kept in place of 1 mm deep from guide cannula for an additional 10 min to allow drug diffusion.

### 2.4. Conditioned place preference (CPP)

CPP was conducted in an apparatus constructed of three chambers (72 cm × 25 cm × 32 cm, Zhenghua Biologic Apparatus, China). The two larger side chambers (30.5 cm × 25 cm × 32 cm each) differ in their walls (black or black with white stripes) and floors (stainless-steel mesh or stainless-steel bars). The smaller middle chamber (11 cm × 25 cm × 32 cm) has gray wall with a smooth PVC floor. The three distinct chambers are separated by removable guillotine doors. Time spent in each chamber was recorded by means of infrared beam crossings which are located in the walls of each chamber.

The place preference procedure consisted of three phases: pre-conditioning phase (baseline preference), conditioning (CPP training), and post-conditioning test (CPP test). The detailed CPP timeline of this study is shown in Fig. 1. During pre-conditioning phase (day 0) the rats were free to explore the three chambers for 15 min. Time spent in each chamber was calculated by computer and recorded as baseline data. Rats that spent more than 500 s in one chamber were dismissed from testing. CPP training (day 1–7) was performed for 7 consecutive days with twice daily injections. The first injection was performed in the morning with either administration of cocaine hydrochloride (10 mg/kg, i.p., Qinghai Pharmaceutical, China) or saline (0.5 ml/kg, i.p.), and the rats were confined to one conditioning side chamber (drug-paired chamber) for 45 min after the injection and then returned to their home cages. The second injection was performed in the afternoon with administration of saline (0.5 ml/kg, i.p.), and the rats were confined to the other side chamber (non-drug-paired chamber) for 45 min and then returned to their home cages. In CPP test phase, the rats freely moved throughout the apparatus for 15 min, exactly as in the pre-conditioning phase. CPP test 1 was carried out on day 8. After the CPP test 1, rats were subjected to extinction of cocaine conditioning. During the extinction period, all rats were free to access the three chambers for 15 min each day without any injections. Following