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# Mirtazapine impairs acquisition and reinstatement of cocaine-induced place preference in rats

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ABSTRACT

Exposure to cues previously associated with drug use and the environment can trigger intense craving and drug-seeking, often leading to relapse in individuals with substance use disorders. Several studies suggest that the decrease in the effects of the cues and the environment could help maintain abstinence from drug use in individuals abusing drugs. Mirtazapine, an antagonist of the noradrenergic (NE) $\alpha_2$ receptor and the 5-$HT_{2A/C}$ and 5-$HT_3$ receptors has demonstrated efficacy in reducing the rewarding effect of different drugs.

The purpose of the present study was to investigate whether the mirtazapine, blocks the acquisition and reinstatement of cocaine-induced conditioned place preference (CPP). In this study, 120 *Wistar* male rats were utilized and we use the CPP as a behavioral tool to measure the context-rewarding effect of an unconditioned stimulus such as cocaine. Mirtazapine was dosed for 30 or 60 consecutive days prior to treatment with cocaine or during the extinction phase. We found that dosing with mirtazapine for 30 consecutive days caused a time-related reduction in acquisition or reinstatement of preference for the cocaine-paired chamber. When the duration of treatment is increased (60 days), reductions in preference for the cocaine-paired chamber were potentiated.

These observations support its potential clinical anti-addictive properties against drugs.

## 1. Introduction

Cocaine addiction has become one of the most serious economic and public health problems (Shorter and Kosten, 2011). The exposure cues previously associated with drug use and the environment can trigger intense craving and drug-seeking, often leading to relapse in individuals with substance use disorders (Weiss et al., 2000; Cervo et al., 2003). Several studies suggest that the decrease in the salience of the effects of the cues and the environment, as well as associative learning that occurs between the reinforcing effects of drugs and associated contextual cues could help maintain abstinence in individuals abusing drugs (Ehrman et al., 1992; O'Brien et al., 1998).

An approach to the treatment of adverse effects of drug abuse disorders is the use of antidepressant drugs (Torrens et al., 2005). Have been reported numerous preclinical studies and clinical trials support the effectiveness of such antidepressive agents (Pani et al., 2011; Verrico et al., 2014; Mancino et al., 2014; Raby et al., 2014) to reduce the adverse effects of drug abuse. In addition, other studies have shown that some serotonergic or noradrenergic antidepressants have the capacity to modulate memory processes, such as the process of emotional or spatial memory consolidation (An et al., 2016; Burda-Malarz et al., 2014; Feltmann et al., 2015; Shilyansky et al., 2016), which could contribute to enhancement of their therapeutic potential to relieve cocaine use disorders.

Mirtazapine is an effective noradrenergic and specific serotoninergic antidepressant with pronounced early anxiolytic effects in patients with major depression (Croom et al., 2009).

Animal studies showed that mirtazapine administration decreases the reinstatement of methamphetamine self-administration and attenuates the expression of conditioned place preference (CPP) to morphine and methamphetamine in rats (Voigt and Napier, 2012; Voigt et al., 2011; Herrold et al., 2009; Graves and Napier, 2011).

Additionally, preclinical studies of our laboratory have reported that daily dosing of mirtazapine (30 mg/kg, ip) for 30 days during cocaine-extinction, significantly attenuates the induction and expression of locomotor sensitization to cocaine, decreases the duration of cocaine-induced locomotor effect (Barbosa-Méndez et al., 2017b), and reduced the cocaine self-administration (Barbosa-Méndez et al., 2017a)

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and reduced the toxic effect of sublethal doses of cocaine in rats (Salazar-Juárez et al., 2016). In addition, recently we reported that that minimal treatment time to reducing the cocaine effects is 30 days (Barbosa-Méndez et al., 2017b).

Collectively these data indicate that mirtazapine alters the behavioral effects of addictive drugs and reduces the salience of the drug-associated signals (Graves and Napier, 2011; Graves et al., 2012b).

Associative learning that occurs between the reinforcing effects of drugs and associated contextual cues can be modeled in rodents using the CPP. CPP measures the tendency to remain in a conditioned environment paired with an unconditioned stimulus such as cocaine (Tzschentke, 1998, 2007).

Since exposure to cues previously associated with drug use can trigger relapse in individuals with substance use disorder (Weiss et al., 2000; Cervo et al., 2003) and that several preclinical studies support the effectiveness of mirtazapine to attenuate the reinstatement of methamphetamine-induced self-administration and the expression of CPP (Voigt and Napier, 2012; Voigt et al., 2011; Herrold et al., 2009; Graves and Napier, 2011), then we hypothesized that the acquisition and reinstatement of the cocaine CPP can be altered by the chronic administration of mirtazapine. We found that chronic dosing with mirtazapine attenuated the acquisition and reinstatement of cocaine CPP.

The results of this study support the evidence that suggests that mirtazapine may be a pharmacological agent that merits further testing in clinical trials for the treatment of cocaine dependence.

## 2. Material and methods

### 2.1. Animals

Male *Wistar* rats weighing 250–280 g at the beginning of the study were utilized. Four rats were housed per standard plastic rodent cage (57 cm × 35 cm × 20 cm) in a colony room maintained both at a regulated temperature (21 ± 2 °C) and at 40–50% humidity under a 12:12-h light/dark cycle (lights on at 7:00 a.m.). The animals had continuous access to rodent chow pellets and water except during experimental sessions. All experiments were conducted during the light phase of the light/dark cycle (between 9:00 a.m. and 3:00 p.m.). Procedures were approved by the Institutional Animal Care and Bioethics Committee in strict accordance with National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

### 2.2. Drugs

Cocaine hydrochloride (97% purity) was kindly donated by the Mexican government under strict regulatory permission and with official surveillance of the drugs employed in experimental animals (COFEPRIS- LC-0004–2003). Cocaine hydrochloride and mirtazapine (REMERON, Schering-Plough-Organon) were dissolved in sterile saline solution (0.9% NaCl, Sigma-Aldrich); both solutions were freshly prepared before their intraperitoneal (i.p.) administration to the animals. During the experiment, the solutions were maintained at − 20 °C. Saline (0.9% NaCl) was used as the control in all experiments. Mirtazapine (30 mg/Kg) was administered 30 **min** prior to cocaine or saline administration. The volume injected into each animal depended on its body weight (BW): BW (g)/100 ml. The optimum dose of mirtazapine used in the study (30 mg/kg) was chosen based on previous observations that showed that the dosage of ≥ 30 mg/Kg of mirtazapine does not affect spontaneous locomotor activity (Salazar-Juárez et al., 2016), does not produce sedation and does not induce weight gain (Salazar-Juárez et al., 2017; Bittolo et al., 2016) in rats. Administration of mirtazapine was performed in their home cages; the following treatment was administered at 30 **min** and the animals were immediately placed inside center compartment of the place preference device.

### 2.3. Place preference procedure

#### 2.3.1. Apparatus

Four identical, conditioned place-preference boxes (OMNIALVA, Instruments, México) were used for the experiments. Each was placed in a sound-attenuating ventilated box. Each CPP box consisted of two equally sized conditioning chambers (60 cm × 60 cm × 60 cm) separated by a neutral central area (20 cm × 60 cm × 60 cm). Both conditioning chambers are connected through an opening that could be closed with a guillotine door. One of the conditioning chambers was black, had a smooth floor, and was more dimly lighted (4.5 lx), while the other was white, had a rough floor, and was more strongly lighted (60 lx) in order to balance chamber preference. The neutral chamber was gray and had a smooth floor. The CPP is expressed as the time spent in the drug-paired compartment. Time spent in each chamber was recorded using a computerized video tracking system (OAVid Reg 12, OMNIALVA, Instruments; México).

#### 2.3.2. Procedure

The CPP procedure consisted of six phases as follows: pre-conditioning phase (baseline preference); conditioning (CPP training); post-conditioning test; extinction phase; re-conditioning test, and place-preference test (CPP test). The detailed CPP timeline for each study is depicted in each figure.

Prior to placement of the animals in the testing chambers, all rats were injected with saline solution intraperitoneally (i.p.) for 3 consecutive days in their home cages. These home-cage injections were done in order to habituate the rats to injections and to reduce the stress that this procedure entails.

The CPP procedure began with a pre-conditioning phase (day 0) when all animals were injected with saline (i.p.) and placed inside center compartment directly at the open doorway of the place-preference apparatus. During this session, the rats could freely explore the three chambers for 30 **min**, and they were then removed and returned to their home cages. Time spent in each compartment was recorded as baseline data. Rats spending > 60% of their time in any single chamber during the pre-conditioning phase were excluded from the study and not subjected to further procedures in order to maintain minimal contribution of novelty-seeking or extreme bias to the procedures. Rats that remained ≤ 60% of their time in any single chamber were randomly assigned to different experimental groups, depending upon the pharmacological treatment administered.

This pre-conditioning phase was followed by a conditioning phase consisting of 12 consecutive daily sessions. For all of these sessions, the doorway between compartments was closed. The cocaine (10 mg/Kg) was administered i.p., and the rats were immediately confined to sided with the rough floor and more strongly lighted (drug-paired chamber) for 30 **min**. This conditioning procedure was repeated once every other day for a total of 6 cocaine conditioning days. On alternating days, rats were injected with saline i.p. and placed in the other side chamber (non-drug-paired chamber) for 30 **min**. The order of cocaine-saline conditioning was counterbalanced across rats. After each experimental session was completed, the animals were returned to their home cages.

The post-conditioning test (CPP test) was performed 24 h after the last day of the conditioning procedure and was conducted with the rats in a drug-free state (day 13). For testing, animals were placed in the center of the apparatus with access to all compartments for 30 min, exactly as during the pre-conditioning phase. Time spent in each compartment was recorded.

During the extinction phase, all rats were free to access the three chambers for 30 **min**, each day. In each session, the rats were administered saline, both in the drug-paired and saline-paired compartment. At 10, 20 and 30 days of extinction the time spent in each compartment was recorded. When all rats spent the same amount of time in the drug-paired chamber as they did in the non-drug-paired chamber, complete extinction was established.