Heliyon 10 (2024) e29979

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Evaluation of multitarget drugs on the expression of cocaine-induced locomotor sensitization in male rats: A comparative study

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ARTICLE INFO

Keywords:
Multitarget drugs
Cocaine
Locomotor activity
Locomotor sensitization
Pharmacotherapy

ABSTRACT

Purpose: — Cocaine use disorder (CUD) is a complex disease. Several studies have shown the efficacy of multitarget drugs used to treat CUD. Here we compare the efficacy of mirtazapine (MIR), pindolol (PIN), fluoxetine (FLX), risperidone (RIS), trazodone (TRZ), ziprasidone (ZPR), ondansetron (OND), yohimbine (YOH), or prazosin (PRZ), to reduce long-term cocaine-induced locomotor activity and the expression of cocaine-induced locomotor sensitization in rats.

Methods: — The study consists of four experiments, which were divided into four experimental phases. Induction (10 days), cocaine withdrawal (30 days), expression (10 days), and post-expression phase (10 days). Male Wistar rats were daily dosed with cocaine (10 mg/kg; i.p.) during the induction and post-expression phases. During drug withdrawal, the MIR, PIN, FLX, RIS, TRZ, ZPR, OND, YOH, or PRZ were administered 30 min before saline. In the expression, the multitarget drugs were administered 30 min before cocaine. After each administration, locomotor activity for each animal was recorded for 30 min.

During the agonism phase, in experiment four, 8-OH-DPAT, DOI, CP-809-101, SR-57227A, or clonidine (CLO) was administered 30 min before MIR and 60 min before cocaine. After each administration, locomotor activity for each animal was recorded for 30 min.

Results: —MIR, FLX, RIS, ZPR, OND, or PRZ attenuated the cocaine-induced locomotor activity and cocaine locomotor sensitization. PIN, TRZ, and YOH failed to decrease cocaine locomotor sensitization. At the optimal doses used, PIN, FLX, RIS, TRZ, ZPR, OND, YOH, or PRZ failed to attenuate long-term cocaine locomotor activation. MIR generated a decrease in cocaine-induced locomotor activity of greater magnitude and duration than the other multitarget drugs evaluated.

Conclusion: — At the optimal doses of multitarget drugs evaluated, MIR was the multitarget drug that showed the greatest long-term cocaine-induced behavior effects compared to other multitarget drugs.

1. Introduction

Cocaine use disorder (CUD) is considered a public health problem and is related to several health disorders [1]. Various studies have described the efficacy of numerous therapeutic approaches, ranging from detoxification to behavioral therapy, and the use of

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https://doi.org/10.1016/j.heliyon.2024.e29979
Received 4 September 2023; Received in revised form 17 April 2024; Accepted 18 April 2024
Available online 1 May 2024

drugs [2–4]. However, the results of the studies remain inconsistent. Furthermore, the clinical application of the drugs evaluated is limited, given that the studies report high attrition, relapse, morbidity, and mortality rates [5–7]. Thus, despite many efforts, no drug approved by the Food and Drug Administration (FDA) has yet been shown to be safe and effective in treating CUD [8,9].

Pioneering preclinical studies have shown that serotonin (5-HT) and norepinephrine (NE) are neurotransmission systems that provide modulatory regulation of the reinforcing effects of cocaine [10,11]. The studies suggested that 5-HT and NE receptors: 1) are important key modulators of the midbrain dopamine (DA) system, which is fundamental to the development of the psychostimulant locomotor effects [12,13], and 2) single or simultaneous activation of the different subtypes of NE and 5-HT receptors could provide new targets for the development of pharmacological treatments for CUD [14–16].

Rodent studies have shown that the administration of α_1 NE, 5-HT_{2A}, or 5-HT_3 receptor antagonists and/or α_2 NE, 5-HT_{1A}, or 5-HT_{2C} receptor agonists attenuated cocaine-induced locomotor sensitization and conditioned place preference [17–23], cocaine self-administration [24–29], Fos protein expression [30,31], and cocaine-induced increases in extracellular dopamine levels [32–34].

In humans, the picture is different. Studies have evaluated the efficacy of different drugs that incorporate single (one-target) or

Table 1
5-HT and NE multitarget drugs: Pharmacological GENERALITIES

Drug	Receptors	Interaction	K_i (nM)	Bibliography
Mirtazapine	5-HT_{2A}	Antagonist	6.3–69	38; 36
	5-HT_{2C}	Inverse Agonist	8.9–39	
	5-HT_3	Antagonist	8.1	
	NE-α_2	Antagonist	20	
	H_1	Antagonist	0.14–1.6	
Pindolol	5-HT_{1A}	Antagonist	15–81	43
	5-HT_{1B}	Antagonist	34–151	
	NE-$\beta_{1\text{-}2}$	Antagonist	0.52–2.6	
Fluoxetine	5-HT_{2A}	Antagonist	119	37; 46
	5-HT_{2C} SERT	Antagonist	118	
		Selective Serotonin Reuptake Inhibitors	1	
Trazodone	5-HT_{1A}	Partial Agonist	96–118	
	5-HT_{2A}	Antagonist	20–45	
	5-HT_{2B}	Antagonist	74–189	
	5-HT_{2C}	Partial Agonist	22–402	
	α_1	Antagonist	12–42	
	α_2	Antagonist	106–490	
	H_1	Weak Antagonist	220	
	SERT	Weak Selective Serotonin Reuptake Inhibitors	160	
Risperidone	D_1	Antagonist	244	41
	D_2	Antagonist	3.57	
	5-HT_{1B}	Antagonist	14.9	
	5-HT_{1D}	Antagonist	84.6	
	5-HT_{2A}	Inverse Agonist	0.17	
	5-HT_{2B}	Inverse Agonist	61.9	
	5-HT_{2C}	Inverse Agonist	12	
	NE-α_1	Antagonist	5	
	NE-α_2	Antagonist	16.5	
	H_1	Inverse Agonist	20.1	
Ziprasidone	D_2	Antagonist	4.8	40; 45
	5-HT_{1A}	Partial Agonist	2.5–76	
	5-HT_{1B}	Partial Agonist	0.99–4	
	5-HT_{1D}	Partial Agonist	5.1–9	
	5-HT_{2A}	Antagonist	0.08–1.4	
	5-HT_{2B}	Antagonist	27.2	
	5-HT_{2C}	Antagonist	0.72–13	
	NE-α_1	Antagonist	18	
	NE-α_2	Antagonist	160	
	H_1	Antagonist	15–130	
	SERT	Selective Serotonin Reuptake Inhibitors	112	
	NET	Selective Norepinephrine Reuptake Inhibitors	44	
Ondansetron	5-HT_3	Antagonist	0.87	44
Yohimbine	D_2	Antagonist	339	18
	5-HT_{1A}	Partial Agonist	346	
	5-HT_{1B}	Antagonist	19.9	
	5-HT_{1D}	Antagonist	44.3	
	5-HT_{2B}	Antagonist	143.7	
	NE-α_2	Antagonist	1.05	
Prazosin	NE-α_1	Antagonist	0.13–1	42; 47

simultaneous (multitarget) NE or 5-HT receptor antagonism or agonism in their mechanism of action. However, the results of the studies have been contradictory. Some studies have shown that the medications attenuate cocaine-reinforcing effects [35–37], and other studies have reported that the drugs cannot alter cocaine effects [38–42].

Given that CUD is a complex disease, multifactorial in its etiology, that shows clear associations with other diseases, its treatment presents problems of adherence, and drug resistance, which results in inconsistent therapeutic effects. 5-HT and NE receptors are important modulators of the behavioral effects of cocaine and important therapeutic targets. Then it would be important to compare the efficacy of some multitarget drugs 5-HT and NE, whose use has been reported in several studies (see Table 1) to propose the use of any of them for the long-term prevention of relapses in CUD treatment.

Thus, this study aimed to compare the efficacy of various 5-HT and NE multitarget drugs used in studies on cocaine-induced behavioral effects in rats [41,43–47] to reduce cocaine-induced hyperlocomotion. To determine the efficacy of each drug, three criteria were considered: it must 1) significantly reduce cocaine-induced locomotor activity, 2) attenuate the expression of cocaine-induced locomotor sensitization, and 3) demonstrate a long-term decrease in cocaine-induced locomotor activity.

We used locomotor sensitization as a tool to study the effects of multitarget drugs: mirtazapine (MIR), pindolol (PIN), fluoxetine (FLX), risperidone (RIS), trazodone (TRZ), ziprasidone (ZPR), ondansetron (OND), yohimbine (YOH), and prazosin (PRZ) on the cocaine locomotor sensitization. Behavioral sensitization has been implicated in the development of long-term neuroadaptive changes in the brain, which result in an increase in drug salience, locomotor activity, and compulsive drug-seeking behaviors [48,49].

2. Materials and methods

2.1. Animals

We used male Wistar rats weighing 250–280 g at the beginning of the study. They were housed in groups of four in standard plastic rodent cages (57 cm × 35 cm x 20 cm) in a colony room maintained at constant temperature (21 ± 2 °C) and humidity (40–50 %) on a 12:12-h light/dark cycle (lights on at 7:00 a.m.) for an acclimation period of 3 days, in which the animals had continuous access to rodent chow pellets and water, except during the experimental sessions. All experiments took place during the light phase of the light/dark cycle (between 9:00 a.m. and 7:00 p.m.). The Institutional Animal Care- and Bioethics Committee approved the procedures (CEI/C/IC092020/2006) in strict compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH).

2.2. Drugs

The Mexican government kindly donated cocaine hydrochloride (COC) under strict regulatory controls. All multitarget drugs used in experimental animals were kept under official surveillance (COFEPRIS- LC-0004-2003). MIR (Remeron, Schering-Plough-Organon-SANFER; Kenilworth, New Jersey; U.S.A.), PIN (Visken, Novartis; Basilea, Suiza), FLX (Fluoxac, Psicopharma; CDMX, México), RIS (Risperidona, AMSA; CDMX, México), TRZ (Sideril; Senosiain; CDMX, México), ZPR (Geodon; Pfizer; Nueva York, U.S.A.), OND (Nalisin, Cryopharma; CDMX, México), YOH (Sigma-Aldrich; St. Louis, MO, U.S.A.), and PRZ (Minipress, Pfizer; Nueva York, U.S.A.) were purchased after obtaining the required regulatory permission, as per official guidelines (COFEPRIS-2016, Mexico).

8-OH-DPAT (5-HT_{1A} receptor agonist), DOI (5-HT_{2A} receptor agonist), CP-809-101 (5-HT_{2C} receptor agonist), SR-57227A (5-HT3 receptor agonist), and Clonidine (Clo; α_2 adrenergic receptor agonist) were purchased from a commercial supplier (Sigma-Aldrich; St. Louis, MO, U.S.A.). All multitarget drugs were dissolved and diluted in a sterile saline solution (SAL; 0.9 % NaCl, Sigma Aldrich; St. Louis, MO, U.S.A.). The solutions were freshly prepared before their intraperitoneal (i.p.) administration to the animals. The pH was adjusted to seven. During the experiments, the solutions were maintained at 4 °C.

To determine if MIR, PIN, FLX, RIS, TRZ, ZPR, OND, YOH, or PRZ can prevent the effects of cocaine, the multitarget drugs were administered 30 min before cocaine or saline administration.

To determine if 8-OH-DPAT, DOI, CP-809-101, SR-57227A, or Clo, can prevent the MIR effects, the drugs were administered 30 min before MIR administration. The volume injected into the animals depended on their body weight (BW) in grams.

A technical limitation of this study is related to the loss of animals due to illness or physical damage that the animals could have due to the treatments. To avoid these situations, the following activities were carried out: To minimize tissue damage and avoid affecting the adequate absorption of the treatments, the following animal care measures were carried out: 1) the administration of the treatments did not occur in the same site, and the injection site was rotated clockwise; 2) a new needle was used each time an animal received an administration, and 3) veterinarians specialized in the management of minor species, rodents, carried out daily checks to verify the health of each animal. As measures of possible damage to the animal's health, constant reviews of the animal's weight, amount of food and water consumed by the animal, and condition of the coat were carried out.

To avoid stress induced by the experimental conditions, the animals lived in the room where the experiments were carried out. This allowed 1) to habituate the animals to the experimental environment and 2) avoid the increase in locomotor activity induced by novel signals or due to the movement of the animals from the animal housing areas (biotery) to the experimentation rooms. The experiment rooms have lighting, temperature, humidity, and sound control. The recording of the basal locomotor activity of the SAL controls was constantly evaluated to determine the possible effects of stress and served as a measure of the effectiveness of the experimental conditions described above.

2.2.1. Dose Selection

The determination of the optimal dose of COC was based on previous studies, that reported that 10 mg/kg of COC induces a robust increase in locomotor activity and behavioral sensitization [50] and does not cause seizures or lethality [50].

The optimal MIR dose (30 mg/kg) was that of previous studies [51–55]. They showed that ≥30 mg/kg MIR does not affect spontaneous locomotor activity or produce sedation in rats, nor does it induce weight gain [51,52]. Preclinical and human studies have reported that 30 mg/kg of MIR decreases cocaine-induced locomotor activity [53] and place preference [54].

The PIN, FLX, RIS, TRZ, ZPR, OND, YOH, or PRZ dose ranges were chosen to be below or at the upper end of the dose range at which each compound produces *in vivo* cocaine-induced locomotor activity antagonism in rodents [18,55–59]. The doses used for each drug were MIR (a-15, b-30, c-60 mg/kg/i.p.), PIN (a-5, b-10, c-20 mg/kg/i.p.), FLX (a-5, b-10, c-20 mg/kg/i.p.), RIS (a-0.05, b-0.5, c-2 mg/kg/i.p.), TRZ (a-1, b-2, c-5 mg/kg/i.p.), ZPR (a-1, b-4, c-10 mg/kg/i.p.), OND (a-0.2, b-1, c-4 mg/kg/i.p.), YOH (a-2.5, b-5, c-10 mg/kg/i.p.), and PRZ (a-0.5, b-1, c-3 mg/kg/i.p.).

8-OH-DPAT, DOI, CP-809-101, SR-57227A, or Clo, doses were selected based on previous animal studies [18,58,60–63], where it was found that 0.2 mg/kg of 8-OH-DPAT (5-HTR_{1A}), 0.3 mg/kg of DOI (5-HTR_{2A}), 1 mg/kg of CP-809-101 (5-HTR_{2C}), 3 mg/kg of SR-57227A (5-HTR_3), or 0.030 mg/kg of Clo (α_2 NER) were capable of selectively activating the 5-HT and NE receptors.

2.3. Behavioral sensitization procedure

2.3.1. Apparatus

For each animal, we assessed locomotor activity in transparent Plexiglass activity chambers (50 x 50 × 30 cm) connected to a PC. Each chamber had a 16x16 photocell beam array located 3 cm from the floor surface to scan locomotor activity (OMNIALVA, Instruments, Mexico). Photobeam interruptions were automatically quantified with OABiomed software (1.1) and analyzed afterward. We defined locomotor activity as the continuous horizontal locomotor activity performed by a rat, which generates the simultaneous interruption of several photo beams (OMNIALVA, Mexico).

2.3.2. Procedure

We estimated spontaneous locomotor activity with a standard protocol [53]. The animals were habituated to the activity chambers in three 30-min sessions and were randomly assigned to different pharmacological treatment groups. The rats were returned to their home cages after each experimental session had been completed.

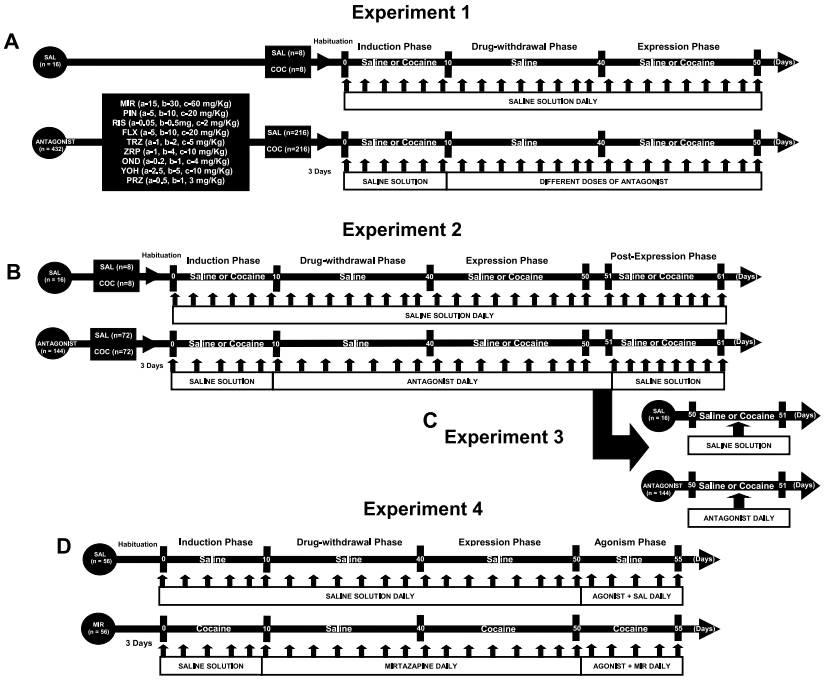

Fig. 1. Experimental timeline. Experiment 1 (A). The effect of the administration for 30 days during drug withdrawal of different doses of each drug on cocaine-induced locomotor activity was evaluated during the 10 days of the expression phase. Experiment 2 (B). The effect of the administration for 30 days during drug withdrawal of a fixed dose of each drug on cocaine-induced locomotor activity and cocaine locomotor sensitization was evaluated in the 10 days of the expression phase. The long-term effect of each of the multitarget drugs was evaluated in the post-expression phase. Experiment 3 (C). The effect of administration of each of the multitarget drugs on the duration of cocaine-induced locomotor activity was evaluated during the last day of the expression phase. Experiment 4 (D). The effect of dosing of 8-OH-DPAT, DOI, CP-809-10, or SR-57227A was evaluated during the antagonism phase.

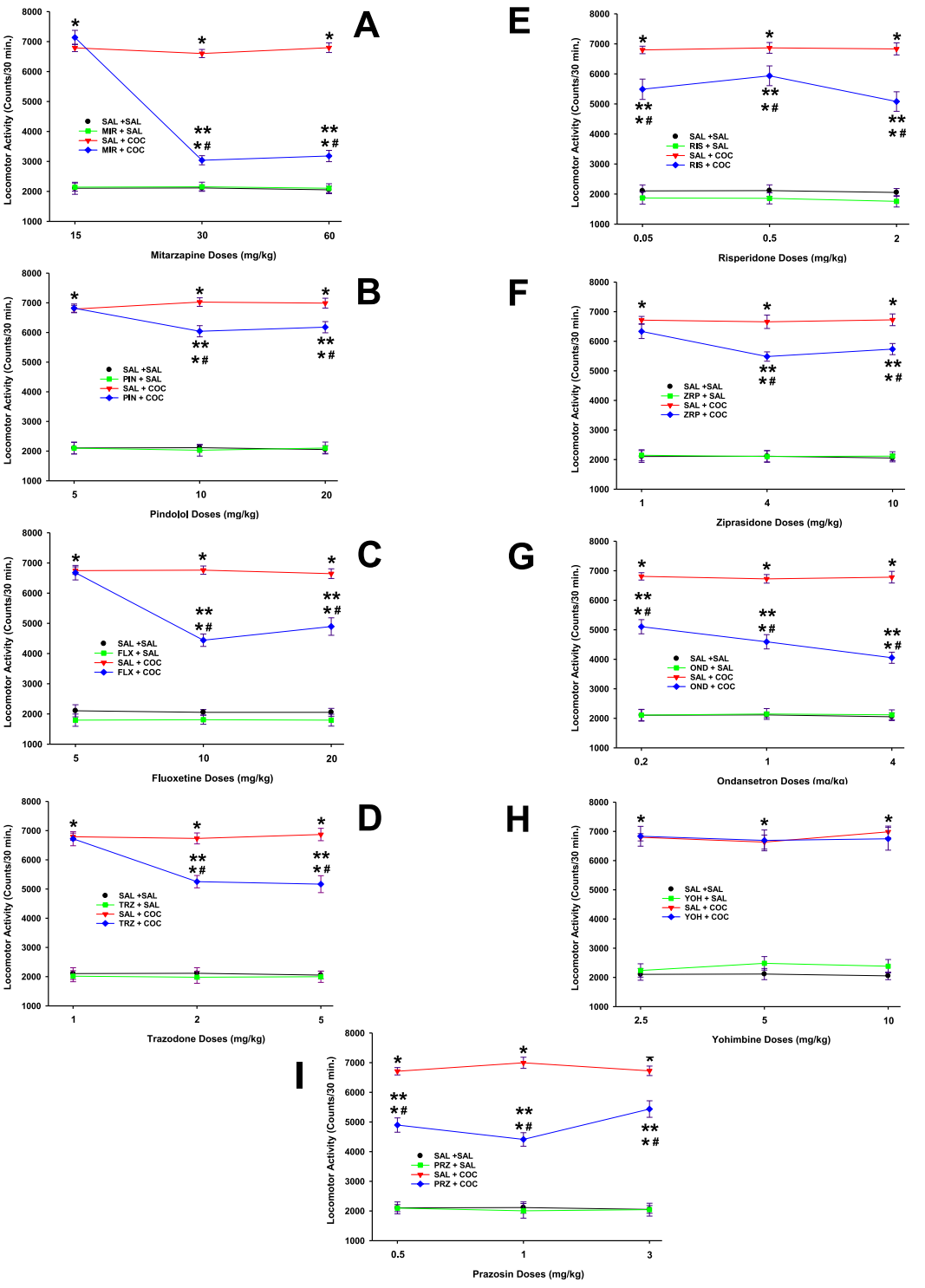

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Fig. 2. The (A) MIR-, (B) PIN-, (C) FLX-, (D) TRZ-, (E) RIS-, (F) ZPR-, (G) OND-, or (I) PRZ groups treated for 30 days during drug withdrawal had a dose-dependent decrease in cocaine-induced locomotor activity. **(H)** YOH failed to reduce the locomotor effect of COC. Three different doses were evaluated for each of the multitarget drugs and three groups were evaluated for each dose (SAL + SAL, SAL + COC, Drug + SAL, and Drug + COC). Mean locomotor activity (±S.E.M.) by group (n = 8 animals per group) during the 10 days of expression. *$p < 0.01$ significant effects of cocaine treatment on locomotor activity compared to the SAL + SAL groups. **$p < 0.01$ significant effects of the different doses of the multitarget drugs evaluated on locomotor activity compared to the SAL + COC group. $^{\#}p < 0.01$ significant effects between the different groups, as determined by two-way ANOVA followed by Tukey's tests.

2.4. Experimental procedures

The study used 720 male Wistar rats in five experiments. For Experiment 1, we used 448 animals further divided into 56 experimental groups (n = 8); for Experiments 2 and 3, we used 160 animals that were divided into 20 experimental groups (n = 8); and for Experiment 4, we used 112 animals in 14 groups (n = 8). Each experimental group received a different pharmacological treatment.

Each experiment was divided as follows: Phase I-Development of the induction of cocaine-induced locomotor sensitization. This phase was carried out to generate cocaine-induced neuroplasticity changes. Phase II- To determine if long-term administration of different multitarget drugs during the cocaine withdrawal phase decreases the expression of cocaine-induced locomotor sensitization. Phase III- To determine the effect of the administration of different multitarget drugs on the expression of cocaine sensitization (decrease drug relapses). Phase IV- To determine if the decrease induced by different multitarget drugs in the expression of sensitization to cocaine does not depend on the presence of the drug.

2.4.1. Experiment 1

2.4.1.1. Experimental phases. To determine the optimal dose of each of the evaluated multitarget drugs on cocaine-induced locomotor activity, the experiment was divided into three experimental phases. Phase I, or the cocaine-induction phase, lasted 10 days. Phase II, or the cocaine-withdrawal phase, lasted 30 days, and Phase III, or the cocaine-expression phase, lasted 10 days (Fig. 1A) (see Fig. 2).

To minimize the number of animals and given that the treatment of the animals in the SAL + SAL and SAL + COC groups consisted of the administration of SAL or COC, in a fixed dose, the SAL + SAL and SAL + COC groups were used as controls in each of the sessions in which the effect of each of the doses of the drug was evaluated. All treatments were administered once a day.

2.4.1.2. Experimental procedure. After three days of habituation, the SAL + SAL group received SAL (9 % NaCl, i.p.), during the three phases. The SAL + COC group received COC (10 mg/kg, i.p.) daily during the induction and expression phases. During the cocaine-withdrawal phase, COC was withdrawn, and the groups received daily SAL only.

The DRUG + SAL (MIR, PIN, FLX, RIS, TRZ, ZPR, OND, YOH, PRZ) groups received SAL (9 % NaCl, i.p.) daily during the induction phase. During cocaine withdrawal and expression, the rats received MIR (15, 30, 60 mg/kg/i.p.), PIN (5, 10, 20 mg/kg/i.p.), FLX (5, 10, 20 mg/kg/i.p.), RIS (0.05, 0.5, 2 mg/kg/i.p.), TRZ (1, 2, 5 mg/kg/i.p.), ZPR (1, 4, 10 mg/kg/i.p.), OND (0.2, 1, 4 mg/kg/i.p.), YOH (2.5, 5, 10 mg/kg/i.p.), and PRZ (0.5, 1, 3 mg/kg/i.p.), 30 min before administration of either SAL or COC (10 mg/kg, i.p.).

In this experiment, the dose of COC is fixed (10 mg/kg, i.p.), but the doses of the multitarget drugs were different. In such a way, the groups treated with 15, 30, or 60 mg/kg of mirtazapine; with 5, 10 or 20 mg/kg of pindolol; with 5, 10 or 20 mg/kg of fluoxetine; with 0.05, 0.5 or 2 mg/kg of risperidone; with 1, 2 or 5 mg/kg of trazodone; with 1, 4 or 10 mg/kg of ziprasidone; with 0.2, 1 or 4 mg/kg of ondansetron; with 2.5, 5 or 10 mg/kg of yohimbine, and with 0.5, 1 or 3 mg/kg of prazosin received a fixed dose of COC (10 mg/kg) daily during the induction phase. During cocaine withdrawal and expression, the rats received 15, 30, or 60 mg/kg of mirtazapine; 5, 10, or 20 mg/kg of pindolol; 5, 10, or 20 mg/kg of fluoxetine; 0.05, 0.5 or 2 mg/kg of risperidone; 1, 2 or 5 mg/kg of trazodone; 1, 4 or 10 mg/kg of ziprasidone; 0.2, 1 or 4 mg/kg of ondansetron; 2.5, 5 or 10 mg/kg of yohimbine, and 0.5, 1 or 3 mg/kg of prazosin respectively, 30 min before administration of either SAL or COC (10 mg/kg, i.p.). After each administration, locomotor activity for each animal was recorded for 30 min (Fig. 1A).

2.4.2. Experiment 2

2.4.2.1. Experimental phases. Once the optimal doses to antagonize cocaine-induced locomotor activity were determined, Experiments 2 and 3 were carried out using only the optimal doses of each of the multitarget drugs**.** To minimize the number of animals used in the study and since experiment 3 is an extension of experiment 2, the same animals were used for experiments 2 and 3.

To compare the efficacy of the optimal dose of each of the multitarget drugs evaluated on cocaine-induced locomotor activity, cocaine locomotor sensitization, and the long-term effect of each drug in rats, the experiment was divided into four experimental phases. Phase I, or the cocaine-induction phase lasted 10 days. Phase II, or the cocaine-withdrawal phase, lasted 30 days. Phase III, or the cocaine-expression phase, lasted 10 days. Lastly, Phase IV, or the post-expression phase, lasted 10 days (Fig. 1B). All treatments were administered once a day.

2.4.2.2. Experimental procedure. The SAL + SAL group received SAL (9 % NaCl, i.p.), during the four phases. The SAL + COC group received SAL 30 min before administration of COC (10 mg/kg, i.p.) daily during induction, expression, and post-expression. During the cocaine-withdrawal phase, cocaine was withdrawn, and the groups received daily SAL only.

The MIR + SAL, PIN + SAL, FLX + SAL, RIS + SAL, TRZ + SAL, ZPR + SAL, SAL + OND, YOH + SAL, and PRZ + SAL groups received SAL (9 % NaCl, i.p.) during the induction and the post expression phases. During cocaine withdrawal and expression, the rats received MIR (30 mg/kg, i.p.), PIN (10 mg/kg, i.p.), FLX (10 mg/kg, i.p.), RIS (2 mg/kg, i.p.), TRZ (2 mg/kg, i.p.), ZPR (4 mg/kg, i.p.), OND (4 mg/kg, i.p.), YOH (5 mg/kg, i.p.), and PRZ (1 mg/kg, i.p.), 30 min before administration of the SAL solution.

The MIR + COC, PIN + COC, FLX + COC, RIS + COC, TRZ + COC, ZPR + COC, OND + COC, YOH + COC, and PRZ + COC groups received COC daily during the induction, and the post expression phases, 30 min before administration of the SAL. During cocaine withdrawal, the rats received MIR, PIN, FLX, RIS, TRZ, ZPR, OND, YOH, and PRZ, 30 min before administration of the SAL. Instead, in the expression phase, the rats received MIR, PIN, FLX, RIS, TRZ, ZPR, OND, YOH, and PRZ, 30 min before administration of COC (10 mg/kg, i.p.). After each administration, locomotor activity for each animal was recorded for 30 min (Fig. 1B).

2.4.3. Experiment 3

2.4.3.1. Experimental phases. Experiment 3 evaluated the effect of different multitarget drugs on the duration of cocaine-induced locomotor activity. It included three phases: cocaine induction (10 days), cocaine withdrawal (30 days), and cocaine expression (1 day). All treatments were administered once a day.

2.4.3.2. Experimental procedure. All animals were subjected to three daily habituation sessions. The MIR + SAL, PIN + SAL, FLX + SAL, RIS + SAL, TRZ + SAL, ZPR + SAL, SAL + OND, YOH + SAL, and PRZ + SAL groups were administered the treatments described in the experiment above, daily during the induction and cocaine-withdrawal phases (Fig. 1C). The MIR + COC, PIN + COC, FLX + COC, RIS + COC, TRZ + COC, ZPR + COC, OND + COC, YOH + COC, and PRZ + COC groups received COC daily during induction. In the cocaine-withdrawal phase, animals received MIR, PIN, FLX, RIS, TRZ, ZPR, OND, YOH, and PRZ, 30 min before administration of SAL. In the expression phase, the rats of the MIR + COC, PIN + COC, FLX + COC, RIS + COC, TRZ + COC, ZPR + COC, OND + COC, YOH + COC, and PRZ + COC groups received MIR, PIN, FLX, RIS, TRZ, ZPR, OND, YOH, and PRZ, respectively, 30 min before the administration of COC (10 mg/kg, i.p.). Upon completion of the cocaine-expression phase, all rats were placed in the testing box for 30 min, with no treatment, to determine baseline responses. After each animal/group had received its treatment, locomotor activity was recorded every 30 min for 240 min.

2.4.4. Experiment 4

2.4.4.1. Experimental phases. To evaluate the participation of each of the 5-HT and/or NE receptors that determine the pharmacological profile of MIR on the mirtazapine-induced decrease in cocaine-induced locomotor activity**,** the experiment comprised four experimental phases. The cocaine-induction phase lasted 10 days. Phase II, or the cocaine-withdrawal phase, lasted 30 days. Phase III, or the cocaine-expression phase, lasted 10 days. Lastly, Phase IV, or the agonist phase, lasted 5 days (Fig. 1D). All treatments were administered once a day.

2.4.4.2. Experimental procedure. During the induction, cocaine-withdrawal, expression, and antagonism phases, the SAL + SAL and SAL + COC groups were administered the treatments described in Experiment 2. The SAL + MIR, SAL + 8-OH-DPAT, SAL + DOI, SAL + CP-809-10, SAL + SR-57227A, and SAL + Clo groups received SAL (9 % NaCl, i.p.) during the induction, cocaine-withdrawal, and expression phases.

During the agonism phase, the SAL + MIR, SAL + 8-OH-DPAT, SAL + DOI, SAL + CP-809-10, SAL + SR-57227A, and SAL + Clo groups received MIR (30 mg/**kg**), 8-OH-DPAT (0.2 mg/kg), DOI (0.3 mg/kg), CP-809-10 (1 mg/kg), SR-57227A (3 mg/kg), and Clo (0.030 mg/kg) 30 min before SAL.

The MIR + COC, MIR + 8-OH-DPAT + COC, MIR + DOI + COC, MIR + CP-809-10 + COC, MIR + SR-57227A + COC, and MIR + Clo + COC groups received SAL 30 min before administration of COC daily during the induction phase. During cocaine withdrawal and expression, the rats received MIR 30 min before administration of SAL or COC (10 mg/kg, i.p.), respectively.

During the agonism phase, COC + MIR + 8-OH-DPAT, COC + MIR + DOI, COC + MIR + CP-809-10, COC + MIR + SR-57227A, and COC + MIR + Clo received 8-OH-DPAT (0.2 mg/kg), DOI (0.3 mg/kg), CP-809-10 (1 mg/kg), SR-57227A (3 mg/kg), and Clo (0.030 mg/kg) 15 min before MIR, and MIR 30 min before COC (10 mg/kg, i.p.). After each administration, locomotor activity for each animal was recorded for 30 min (Fig. 1D).

2.5. Statistical analysis

Data are expressed as the means ± S.E.M. Locomotor activity was measured by counting beam breaks during the testing session. For the graphic representation, in Experiment 1, the mean of the 10 days of the expression phase was used. In Experiment 2, the mean of the 10 days of the induction, expression, and post-expression phases was used for the graphic representation. For Experiment 4, the graphical representation used the mean of the 5 days of the agonism phase.

Experiment 1. For experiment 1, we used a two-way analysis of variance (ANOVA), with groups (SAL + SAL, SAL + COC, SAL + Drug [MIR, PIN, FLX, RIS, TRZ, ZPR, OND, YOH, PRZ], COC + Drug [MIR, PIN, FLX, RIS, TRZ, ZPR, OND, YOH, PRZ]) and dose (a, b, c mg/kg), as the between-subjects factors.

Experiment 2. To determine the effect of each drug on cocaine-induced locomotor activity, the mean cocaine-induced locomotor

activity of the 10 days within the expression phase was analyzed. To determine the effect of each of the multitarget drugs on cocaine locomotor sensitization, the mean cocaine-induced locomotor activity of the last 5 days of the induction phase was compared versus the mean locomotor activity of the first 5 days of the expression phase (comparison between phases). To determine the long-term effect on cocaine-induced locomotor activity, during the post-expression phase the drug administration was stopped, and the cocaine-induced locomotor activity shown by each group was compared versus the cocaine-induced locomotor activity shown by the COC group, within the post-expression phase.

To determine the effect of the different multitarget drugs on cocaine-induced locomotor activity during the expression phase, in Experiment 2, we used a two-way ANOVA, with experimental groups and treatment (antagonist) as the between-subject factors, followed by a post hoc analysis. In addition, to analyze the effect of the different multitarget drugs on the expression of locomotor sensitization, a three-way ANOVA was used, with groups, treatment, and phase (induction and expression) as the between-subject factors. To determine the long-term effect of the different multitarget drugs on cocaine-induced locomotor activity during the post-expression phase we used a two-way ANOVA, with experimental groups and treatment (antagonist) as the between-subject factors.

Experiment 3. For Experiment 3, the study used a two-way ANOVA, with experimental groups (SAL or COC) and treatment (antagonist) as the between-subject factors, followed by a post hoc analysis. To determine the time (minutes; it takes for the cocaine-induced locomotor activity to reach baseline), data were analyzed by two-way repeated-measures ANOVA, with days as the repeated measures. The locomotor activity in the SAL group was the baseline value. For our purposes, locomotor activity induced by cocaine returned to the baseline level when the post hoc test found no significant differences between each group and the SAL group.

Experiment 4. For Experiment 4, the study used a two-way ANOVA with experimental groups (SAL and MIR) and treatment (agonist), as the between-subject factors, followed by a post hoc analysis. When there was a significant F value in the interaction, a post hoc analysis of differences between groups was performed, as well as an additional Tukey test. For the statistical analysis, the SAL + SAL and SAL + agonists groups were considered as the control groups of the experiment. The statistical significance level was set at $p < 0.05$.

3. RESULTS

3.1. Experiment 1

To determine the optimal dose of each of the multitarget drugs evaluated to attenuate cocaine-induced locomotor activity during the expression phase of locomotor sensitization, we measured the effect of different drug doses on locomotor activity induced by a fixed dose of COC (10 mg/kg) (see Table 1).

Two-way ANOVA found significant differences in the Group × dose interaction (Table 2). The post hoc test found no difference in basal locomotor activity in the SAL + SAL and SAL + drugs groups evaluated. In contrast, COC at a dose of 10 mg/kg significantly increased locomotor activity in all cocaine groups evaluated (Supplementary Material; Table 1 A-I).

Table 2
TWO-WAY ANOVA result: EXPERIMENT 1.

Drugs	Factors	Two-Way ANOVA
Mirtazapine	A) Group	$F(3, 96) = 1687.439$; $p < 0.01$
	B) Doses	$F(2, 96) = 165.028$; $p < 0.01$
	A × B Interaction	$F(6, 96) = 152.465$; $p < 0.01$
Pindolol	A) Group	$F(3, 96) = 2177.412$; $p < 0.01$
	B) Doses	$F(2, 96) = 7.435$; $p < 0.01$
	A × B Interaction	$F(6, 96) = 4.373$; $p < 0.01$
Risperidone	A) Group	$F(3, 96) = 1943.275$; $p < 0.01$
	B) Doses	$F(2, 96) = 4.747$; $p < 0.01$
	A × B Interaction	$F(6, 96) = 5.614$; $p < 0.01$
Fluoxetine	A) Group	$F(3, 96) = 990.478$; $p < 0.01$
	B) Doses	$F(2, 96) = 21.960$; $p < 0.01$
	A × B Interaction	$F(6, 96) = 18968$; $p < 0.01$
Trazodone	A) Group	$F(3, 96) = 1198.525$; $p < 0.01$
	B) Doses	$F(2, 96) = 15.129$; $p < 0.01$
	A × B Interaction	$F(6, 96) = 12.233$; $p < 0.01$
Ziprasidone	A) Group	$F(3, 96) = 1569.823$; $p < 0.01$
	B) Doses	$F(2, 96) = 6.381$; $p < 0.01$
	A × B Interaction	$F(6, 96) = 3.841$; $p < 0.01$
Ondansetron	A) Group	$F(3, 96) = 1022.371$; $p < 0.01$
	B) Doses	$F(2, 96) = 5.106$; $p < 0.01$
	A × B Interaction	$F(6, 96) = 4.917$; $p < 0.01$
Yohimbine	A) Group	$F(3, 96) = 1319.297$; $p < 0.01$
	B) Doses	$F(2, 96) = 0.038$; $p = 0.963$
	A × B Interaction	$F(6, 96) = 0.710$; $p = 0.643$
Prazosin	A) Group	$F(3, 96) = 1139.765$; $p < 0.01$
	B) Doses	$F(2, 96) = 6.637$; $p < 0.01$
	A × B Interaction	$F(6, 96) = 4.503$; $p < 0.01$

The Tukey's test found significant differences in cocaine-induced locomotor activity exhibited by the groups treated with 30 or 60 mg/kg of MIR; with 10 or 20 mg/kg of PIN; with 10 or 20 mg/kg of FLX; with 0.05, 0.5 or 2 mg/kg of RIS; with 2 or 5 mg/kg of TRZ; with 4 or 10 mg/kg of ZPR; with 0.2, 1 or 4 mg/kg of OND and with 0.5, 1 or 3 mg/kg of PRZ compared to the SAL + COC group (Supplementary Material; Table 1A-I).

However, the post hoc test found no differences (Supplementary Material; Table 1A-1) in the cocaine locomotor effect shown by the COC + $MIR_{.15\ mg}$, COC + $PIN_{.5\ mg}$, COC + $FLX_{.5\ mg}$, COC + $TRZ_{.1\ mg}$, COC + $ZPR_{.1\ mg}$, COC + $YOH_{.2.5\ mg}$, COC + $YOH_{.5\ mg}$, and COC + $YOH_{.10\ mg}$ groups regarding that shown by the SAL + COC group (Fig. 2A–I).

As shown in Table1 A-1, in supplementary material, the post hoc test found no difference in cocaine-induced hyperactivity between the groups treated with 30 or 60 mg/kg of mirtazapine; with 10 or 20 mg/kg of pindolol; with 10 or 20 mg/kg of fluoxetine; with 0.05, 0.5 or 2 mg/kg of risperidone; with 2 or 5 mg/kg of trazodone; with 4 or 10 mg/kg of ziprasidone; with 0.2, 1 or 4 mg/kg of ondansetron and with 0.5, 1 or 3 mg/kg of prazosin (Fig. 2A–I).

Since no differences were found between the intermediate and higher doses in each of the multitarget drugs, we used the intermediate dose as the optimal dose in each of the multitarget drugs evaluated in Experiments 2 and 3.

3.2. Experiment 2

As shown in supplementary material, during the induction (Supplementary Material Table 2), expression (Supplementary Material Table 2), and post-expression (Supplementary Material; Table 4) phase, the three-way ANOVA found significant differences in the

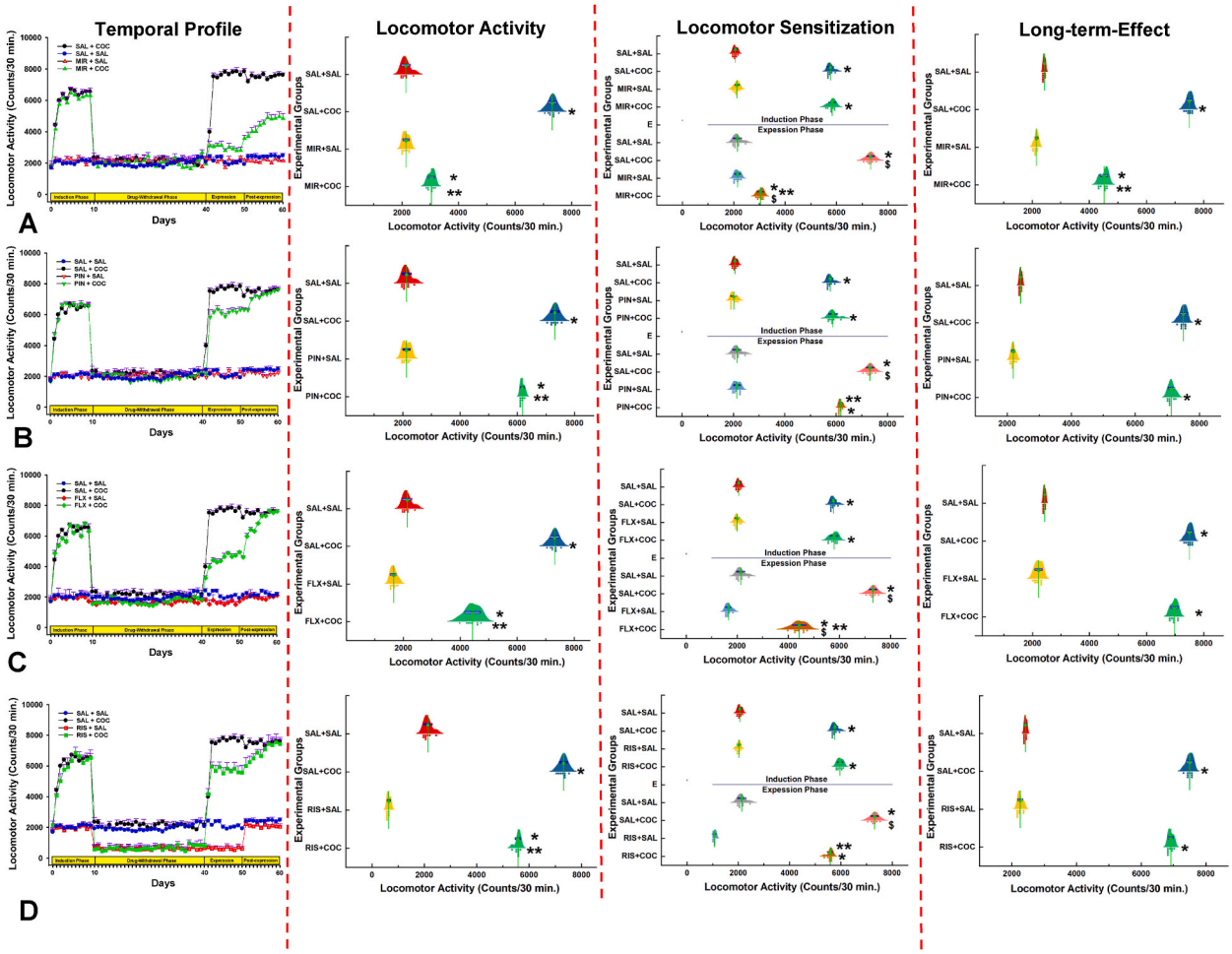

Fig. 3. (A) MIR (30 mg/kg i.p.) administered for 30 days during drug withdrawal attenuates cocaine-induced locomotor, and cocaine sensitization and leads to a long-term attenuation of cocaine sensitization. In contrast, (B) PIN, (C) FLX, or (D) RIS, decreased cocaine-induced locomotor activity and cocaine sensitization but did not decrease the expression of locomotor sensitization in the long term. Mean locomotor activity (±S.E.M.) by group (n = 8 animals per group) *$p < 0.01$ significant effects of cocaine treatment on locomotor activity compared to the SAL + SAL groups. **$p < 0.01$ significant effects of different multitarget drugs on locomotor activity compared to the SAL + COC group, $^{\S}p < 0.01$ significant effects between the induction and expression phase, as determined by two-way ANOVA followed by Tukey's tests.

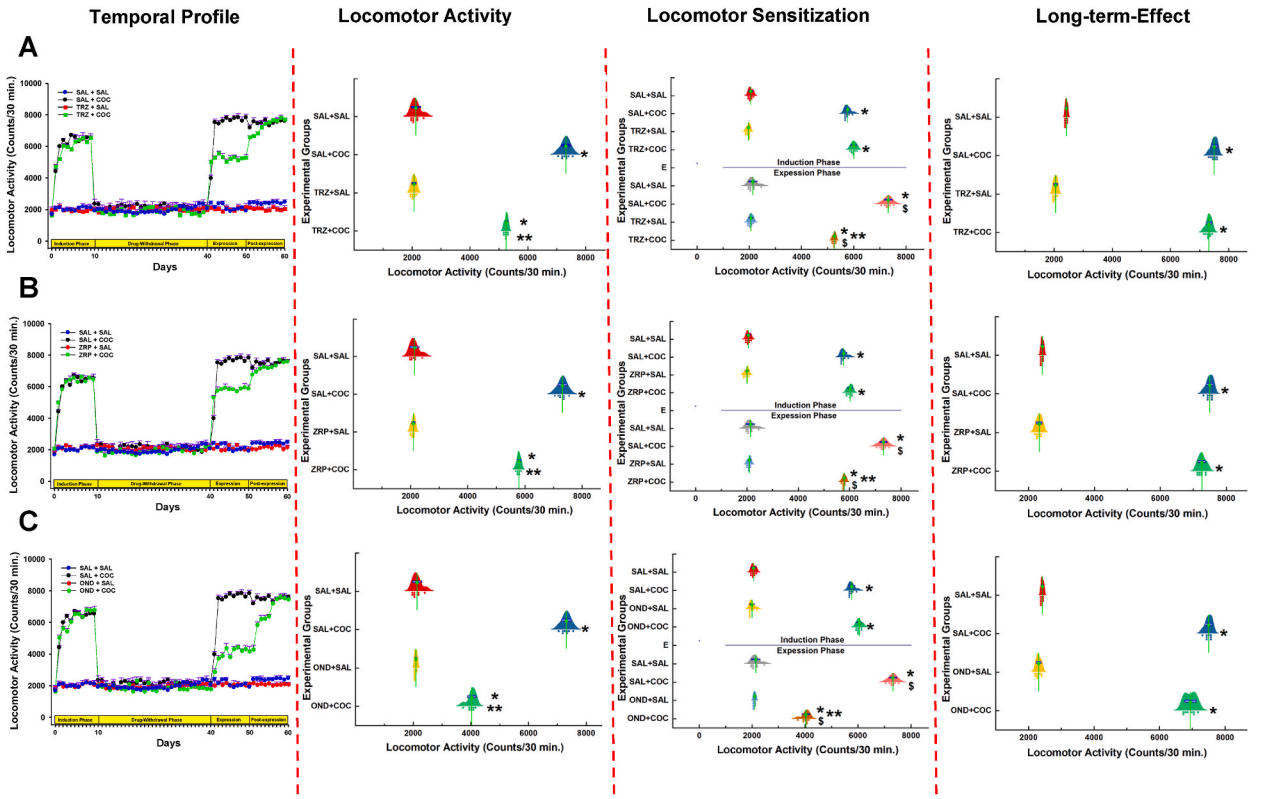

Fig. 4. (A)TRZ, (B) ZPR, or (**C)** OND, decreased cocaine-induced locomotor activity and cocaine sensitization but did not decrease the expression of locomotor sensitization in the long term. Mean locomotor activity (±S.E.M.) by group (n = 8 animals per group) *p < 0.01 significant effects of cocaine treatment on locomotor activity compared to the SAL + SAL groups. **p < 0.01 significant effects of different multitarget drugs on locomotor activity compared to the SAL + COC group, §p < 0.01 significant effects between the induction and expression phase, as determined by two-way ANOVA followed by Tukey's tests.

interaction between groups X treatment X days.

The post-hoc test showed no significant increases in locomotor activity between the SAL + SAL, SAL + MIR, SAL + PIN, SAL + FLX, SAL + TRZ, SAL + ZPR, SAL + OND, and SAL + PRZ groups during the induction, expression, and post expression phases (Fig. 3A–D, 4A-C, 5A-B). However, the SAL + RIS and SAL + YOH groups showed a decrease and increase, respectively, in locomotor activity compared to the locomotor activity shown by the SAL + SAL, SAL + MIR, SAL + PIN, SAL + FLX, SAL + TRZ, SAL + ZPR, SAL + OND, and SAL + PRZ groups in each of the experimental phases (Supplementary Material; Table 5 A-I).

As shown in Fig. 3A–D, 4A-C, and 5A-B, 10 mg/kg COC significantly increased locomotor activity during the induction, expression, and post-expression phases.

In the induction phase, the post hoc test did not find significant differences in the cocaine-induced locomotor activity shown by the COC + MIR, COC + PIN, COC + FLX, COC + RIS, COC + TRZ, COC + ZPR, COC + OND, COC + YOH, or COC + PRZ groups (Supplementary Material; Table V A-I) compared to the SAL + COC group (Fig. 3A–D; 4A-C; 5A-B).

3.2.1. *Cocaine-induced locomotor activity*

To compare the efficacy of the optimal dose of each of the multitarget drugs evaluated on cocaine-induced locomotor activity, during the expression phase the two-way ANOVA revealed differences between groups and treatments ($F(1.200) = 67.296$ $p < 0.001$). As shown in Table V A-I, in supplementary material, the post hoc test found differences in cocaine-induced locomotor activity in the COC + MIR, COC + PIN, COC + FLX, COC + RIS, COC + TRZ, COC + ZPR, COC + OND, and COC + PRZ groups compared to the SAL + COC groups. However, Tukey's test found no difference between the COC + YOH and SAL + COC groups (Fig. 3A–D; 4A-C; 5A-B).

3.2.2. *Cocaine locomotor sensitization*

To determine if the administration of each of the multitarget drugs altered the expression of cocaine locomotor sensitization (Fig. 3A–D; 4A-C; 5A-B), three-way ANOVA ($F(1.420) = 38.485$ $p < 0.001$) found differences in the group X treatment × phase interaction. Tukey's test found significant differences in the cocaine-induced locomotor activity shown during the induction phase compared to that shown in the expression phase in the SAL + COC and COC + YOH groups (Supplementary Material; Table VI A-I). The post hoc test found a decrease in cocaine-induced locomotor activity during the induction phase compared to that shown during the

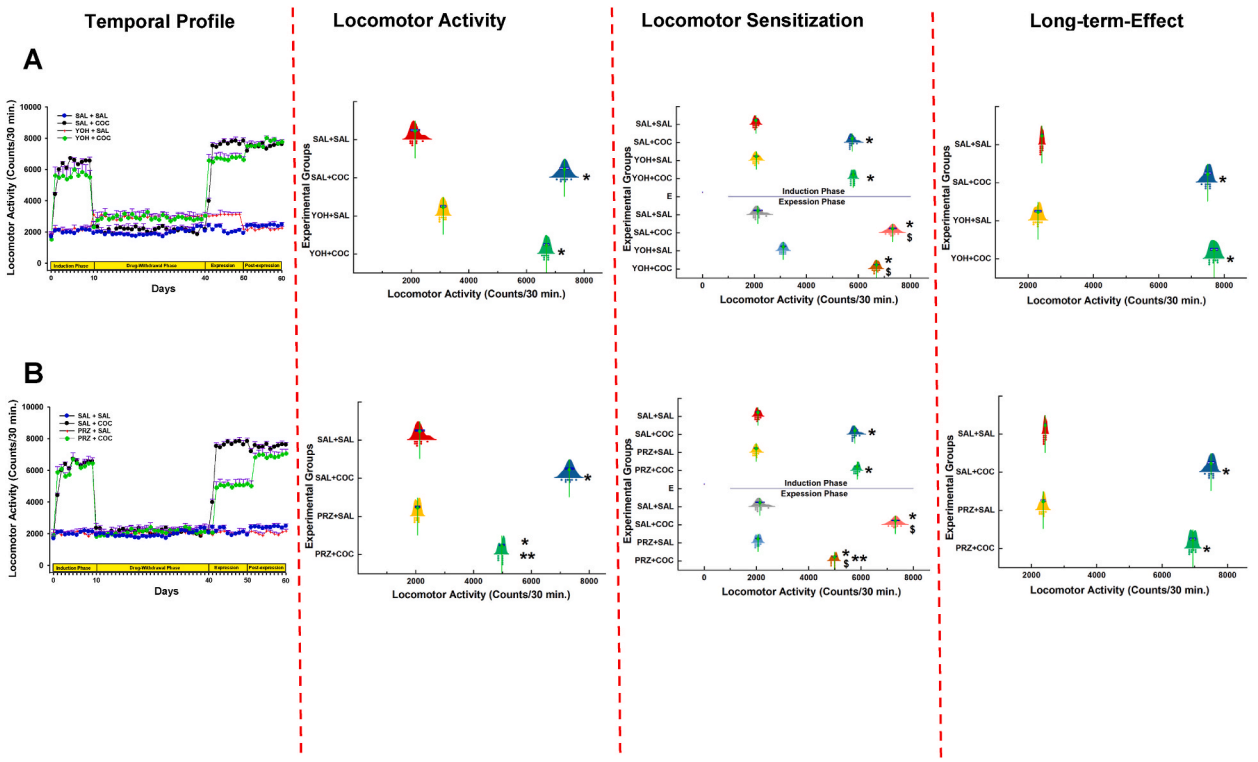

Fig. 5. (**B**) PRZ, decreased cocaine-induced locomotor activity and cocaine sensitization but did not decrease the expression of locomotor sensitization in the long term. In contrast, (**A**) YOH, did not attenuate cocaine-induced locomotor activity, or cocaine sensitization and had no long-term effect on locomotor activity. Mean locomotor activity (±S.E.M.) by group (n = 8 animals per group) *p < 0.01 significant effects of cocaine treatment on locomotor activity compared to the SAL + SAL groups. **p < 0.01 significant effects of different multitarget drugs on locomotor activity compared to the SAL + COC group, $^{\$}$p < 0.01 significant effects between the induction and expression phase, as determined by two-way ANOVA followed by Tukey's tests.

expression phase in the COC + MIR, COC + FLX, COC + TRZ, COC + PRZ, and COC + OND groups. However, Tukey's test did not find differences in locomotor activity between the induction and expression phases in the COC + PIN, COC + RIS, and COC + ZPR groups (Supplementary Material; Table VI A-I).

3.2.3. *Long-term effect*

To compare the long-term effect of each multitarget drug, during the post-expression, the two-way ANOVA revealed differences between groups and treatments ($F(1.\ 200) = 37.735$ $p < 0.001$). Tukey's test did not reveal significant differences in the cocaine-induced locomotor activity shown by the groups treated with PIN, FLX, RIS, TRZ, ZPR, OND, YOH, or PRZ compared to the group treated with cocaine (Supplementary Material Table 6 A-I). However, the statistical analysis found differences between the COC + MIR and SAL + COC groups (Fig. 3A–D; 4A-C; 5A-B).

3.3. *Experiment 3*

Daily administration of SAL, MIR, PIN, FLX, RIS, TRZ, ZPR, OND, YOH, or PRZ did not alter the duration of locomotor activity ($p = 0.99$).

As shown in Fig. 6A–I, a dose of COC (10 mg/kg) significantly increased the duration of locomotor activity (Supplementary material; Table 8 A-I), reaching its maximum level 30 min after drug administration. The locomotor activity in the SAL + COC group decreased 150 min after injection.

In contrast, chronic treatment with MIR, FLX, RIS, TRZ, OND, and PRZ significantly reduced the duration of locomotor activity. PIN, ZPR, and YOH did not decrease the duration of locomotor activity (Fig. 6A–I).

Cocaine-induced locomotor activity in the MIR + COC, FLX + COC, RIS + COC, TRZ + COC, OND + COC, and PRZ + COC groups achieved maximum levels of activity 30 min after drug administration and were considerably different from the SAL + COC ($p < 0.0001$) group. In contrast, the PIN + COC, ZPR + COC, and YOH + COC groups achieved maximum levels of activity 30 min after drug administration, which was not different from the maximum level of activity shown by the SAL + COC ($p = 0.94$) group. Locomotor activity in the MIR + COC group decreased rapidly at 90 min after drug administration ($p = 0.85$). In contrast, the locomotor activity shown by the FLX + COC, OND + COC, and PRZ + COC groups and by the RIS + COC ($p = 0.91$) and TRZ + COC ($p = 0.88$) groups

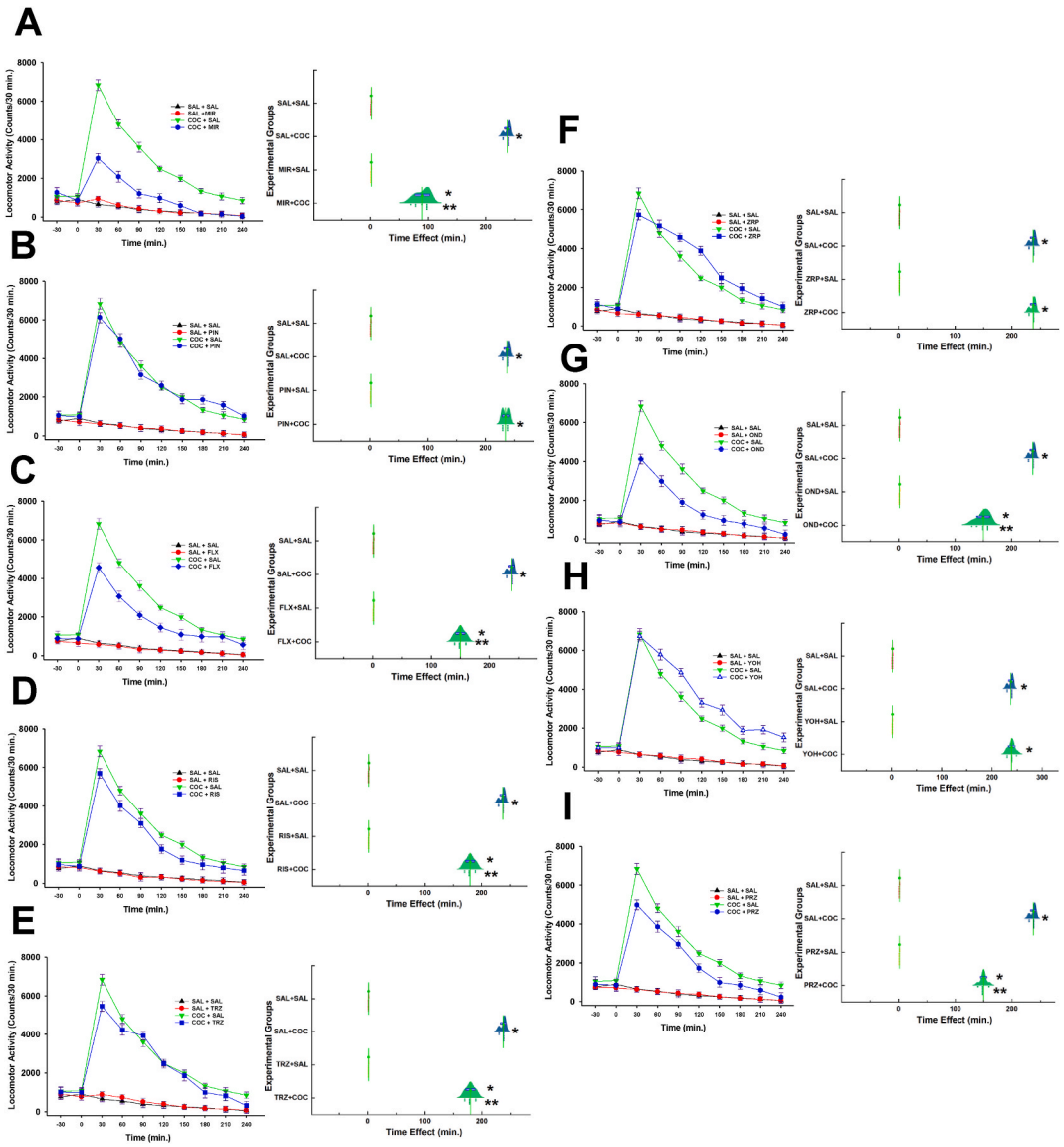

Fig. 6. Treatment with (A) MIR, (C) FLX, (D) RIS, (E) TRZ, (G) OND, or (I) PRZ, decreased the duration of the cocaine-induced locomotor effect. In contrast, (B) PIN, (F) ZPR, or (**H**) YOH, did not attenuate the cocaine-induced locomotor effect. Time profile of the effect of treatments on locomotor activity and the mean duration (±S.E.M.) of the locomotor effect induced by the treatments during the testing phase. *$p < 0.01$ Significant effects of cocaine treatment on locomotor activity compared to the SAL + SAL groups. **$p < 0.01$ significant effects of MIR on locomotor activity compared to the SAL + COC group, as determined by two-way ANOVA followed by Tukey's tests.

decreased 150 and 180 min after drug administration, respectively. The locomotor activity in the PIN + COC ($p = 0.95$), ZPR + COC ($p = 0.92$), and YOH + COC ($p = 0.98$); groups decreased 240 min after drug administration (Fig. 6A–I).

Statistical analysis found significant differences (two-way ANOVA; group × treatment interaction; $F(1, 176) = 1073.436$ $P < 0.0001$) in the duration of locomotor activity in the groups treated with MIR, FLX, RIS + COC, TRZ + COC, OND + COC, and PRZ + COC compared to the SAL + COC group (Supplementary Material; Table 9 A-I). Tukey's test revealed differences in locomotor duration in the MIR + COC group compared to the PIN + COC ($p < 0.0001$), FLX + COC ($p < 0.0002$), RIS + COC ($p < 0.0002$), TRZ + COC ($p < 0.0002$), ZPR + COC ($p < 0.0001$), OND + COC ($p < 0.0002$), YOH + COC ($p < 0.0001$), and PRZ + COC ($p < 0.0002$) groups. Our statistical analyses, however, did not find differences between the SAL + COC and the PIN + COC ($p = 0.98$), ZPR + COC ($p = 0.97$), and YOH + COC ($p = 0.96$) groups (Fig. 6A–I).

3.4. Experiment 4

As shown in Tables 9 and in supplementary material, during the agonism phase, the three-way ANOVA found significant differences

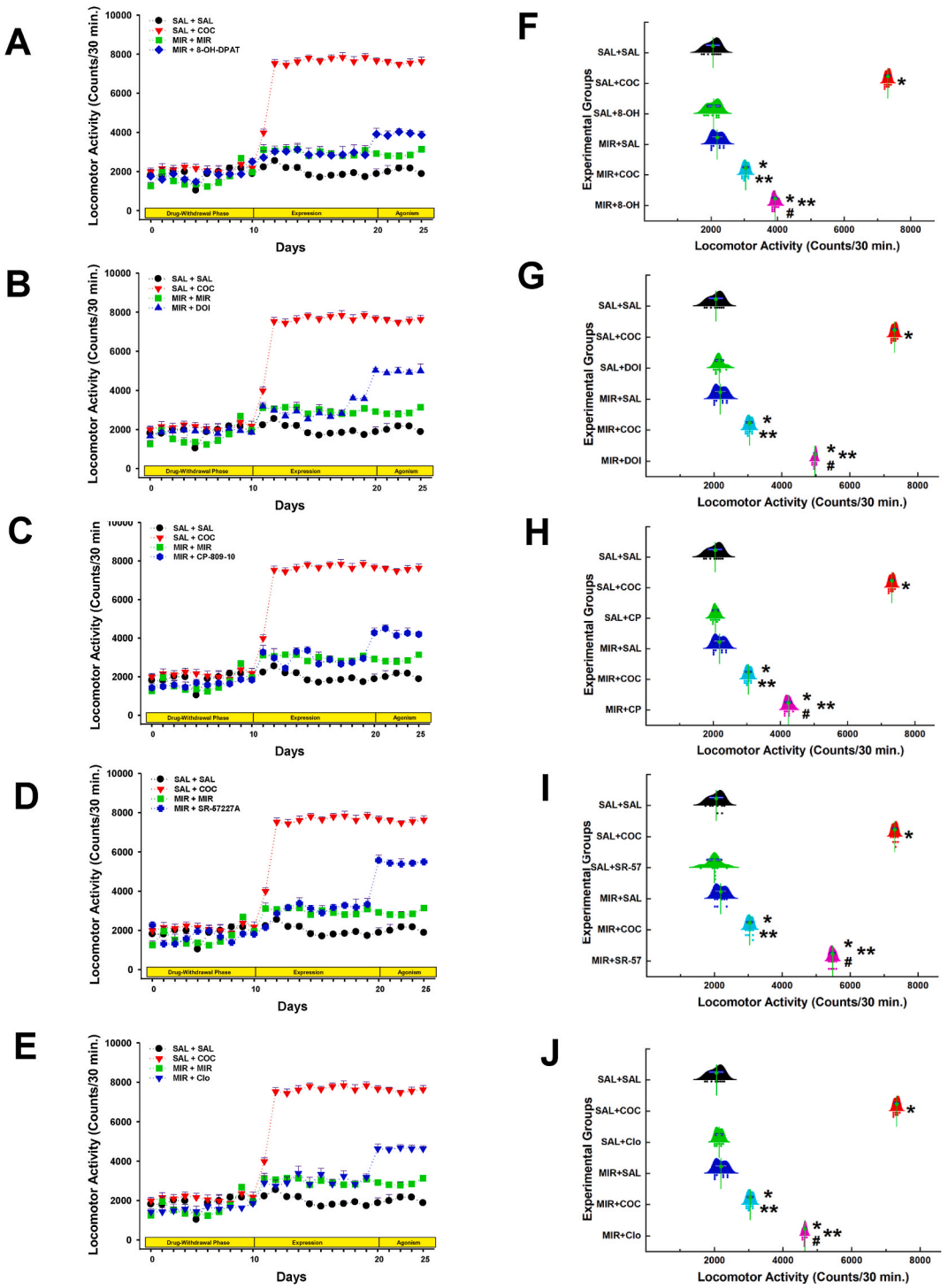

Fig. 7. Dosing of 8-OH-DPAT, DOI, CP-809-10, or SR-57227A (agonist) blocks the effect of MIR on cocaine sensitization. Time profile of the effect of treatments (A–E) and the mean locomotor activity (±S.E.M.) by group (n = 8 animals per group) during the 5 days of agonism (**F-J**). *$p < 0.01$ significant effects of 8-OH-DPAT, DOI, CP-809-10, or SR-57227A on locomotor activity compared with the SAL + SAL group. **$p < 0.01$ significant effects of 8-OH-DPAT, DOI, CP-809-10, or SR-57227A on locomotor activity compared to the SAL + COC group. $^{\#}p < 0.01$ significant effects of 8-OH-DPAT, DOI, CP-809-10, or SR-57227A on locomotor activity compared to the MIR + COC group, as determined by two-way ANOVA followed by Tukey's tests.

in the interaction between groups X Treatment X days.

Statistical analysis (two-way ANOVA; Groups × Treatment interaction, $F(1, 112) = 1805.321$ $p < 0.0001$) found differences in cocaine-induced locomotor activity shown by the SAL + COC group regarding the locomotor activity shown by the MIR + COC, MIR + 8-OH-DPAT + COC, MIR + DOI + COC, MIR + CP-809-10 + COC, MIR + SR-57227A + COC, and MIR + Clo + COC groups (Supplementary Material; Table 11 A-I).

The post hoc test revealed differences in cocaine-induced locomotor activity in the MIR + COC group compared to the MIR + 8-OH-DPAT + COC, MIR + DOI + COC, MIR + CP-809-10 + COC, MIR + SR-57227A + COC, and MIR + Clo + COC groups (Supplementary Material; Table 11 A-I). Tukey's test revealed differences between the MIR + 8-OH-DPAT + COC ($p < 0.003$), MIR + DOI + COC ($p < 0.002$), MIR + CP-809-10 + COC ($p < 0.002$), MIR + SR-57227A + COC ($p < 0.001$), and MIR + Clo + COC ($p < 0.003$) groups (Fig. 7A–J).

4. Discussion

We found that the repeated administration of 10 mg/kg of COC led to an enhancement of cocaine-induced locomotor activity during the expression phase compared to the control (SAL pretreatment). This result is in line with previous studies, which show that the stimulant effect of COC on locomotor activity increases with daily administration [50,53].

Various studies in rodents have shown the effect of different doses of MIR, PIN, FLX, RIS, TRZ, ZPR, OND, YOH, or PRZ on cocaine-induced locomotor activity [64–69]. The studies showed a dose range of 30–60 mg/kg of MIR, 10–20 mg/kg PIN, 10–20 mg/kg FLX, 0.05–2 mg/kg RIS, 2–5 mg/kg TRZ, 4–10 mg/kg ZPR, 0.2–4 mg/kg OND, and 0.5–3 mg/kg PRZ, could decrease cocaine locomotor hyperactivity [41,70–73]. Our results are in line with these studies. The administration of MIR, PIN, FLX, RIS, TRZ, ZPR, OND, or PRZ in the previously described dose ranges generated a significant attenuation of cocaine-induced locomotor activity.

Based on the above, we determine that 30 mg/kg MIR, 10 mg/kg PIN, 10 mg/kg FLX, 2 mg/kg RIS, 2 mg/kg TRZ, 4 mg/kg ZPR, 4 mg/kg OND, or 1 mg/kg PRZ were the optimal doses that significantly decreased cocaine-induced locomotor activity. In contrast, the 5 mg/kg YOH dosage failed to decrease cocaine-induced locomotor activity.

Regarding cocaine locomotor sensitization, our results show that the administration of MIR, PIN, FLX, RIS, TRZ, ZPR, OND, or PRZ in the previously described dose ranges generated a significant attenuation of cocaine locomotor sensitization. The results are consistent with previous results, which showed that, at the dose mentioned above, MIR [50,53], FLX [71,74], RIS [56], ZPR [75,76], OND [64,77], and PRZ [78–80] reduce hyperactivity induced by COC; decrease cocaine self-administration and attenuate the induction and expression of cocaine sensitization.

Regarding the effect of PIN and TRZ, our results differed from those reported by other researchers, wherein PIN and TRZ did not affect the expression of locomotor sensitization to cocaine [67].

With YOH, our results are consistent with previous reports. These studies reported that 5 mg/kg YOH increased cocaine-induced locomotor activity during the induction and expression of locomotor sensitization, as well as cocaine self-administration [18,81].

However, compared to the decrease induced by PIN, FLX, RIS, TRZ, ZPR, OND, and PRZ, the magnitude of the mirtazapine-induced effect on cocaine-induced locomotor activity was significantly greater, and importantly, the effect was long-term.

This observation is in line with previous results from our laboratory, in which MIR dosing induced a long-term attenuation of cocaine- and nicotine-induced locomotor activity [53,82]. With PIN, FLX, RIS, TRZ, ZPR, OND, or PRZ, the decrease in cocaine-induced locomotor activity depended on the presence of the PIN, FLX, RIS, TRZ, ZPR, OND, or PRZ.

Thus, the results of the study suggest that MIR probably generated neuroplasticity changes at the cellular, neurochemical, or molecular level through the simultaneous effect on NE and 5-HT receptors. Evidence that supports this hypothesis indicates that chronic administration (21 days) of MIR enhances neural plasticity by modulating the activity of neurotrophic pathways, increasing the expression of brain-derived neurotrophic factor (BNDF), nerve growth factor (NGF), neurotrophin-3 (NT-3), and glial cell-derived neurotrophic factor (GNDF) [83–85]. Other studies have mentioned that MIR increases the activity of the $alpha_1$-adrenergic [86] system and alters the expression of dopaminergic [87] receptors. Together, the changes described above could explain the long-term effect of MIR on cocaine locomotor hyperactivity. However, future studies are required to evaluate these hypotheses.

Several studies have concluded that behavioral responses to COC peaked within 30 min of injection, and at 240 min after injection, the locomotor activity reached the basal levels [50,88,89]. We found similar results. Cocaine-induced locomotor activity reached its maximum peak at 30 min and, by 240 min after administration, the activity returned to its basal level.

We have widely reported that the dosage of MIR decreased the length of the locomotor effect induced by COC [50,53], which is consistent with what was observed in this study.

Instead, we found that PIN, ZPR, and YOH failed to decrease the length of cocaine-induced locomotor activity. However, FLX, RIS, PRZ, TRZ, and OND could decrease the length of cocaine-induced locomotor activity. To our knowledge, these are the first results related to the effect of FLX, RIS, TRZ, OND, and PRZ on the length of cocaine-induced locomotor activity. However, once again, the magnitude of the decrease in the MIR effect on the length of the cocaine motor effect was greater than that shown by FLX, RIS, TRZ, OND, and PRZ.

As mentioned above, MIR has a unique multitarget pharmacological profile [90–93], that includes antagonist activity at the α_2 noradrenergic receptor and the serotonin 5-HT_{2A} and 5-HT_3 receptors [92,94], as well as the inverse agonist properties of the serotonin 5-HT_{2C} receptor [95] and serotonin 5-HT_{1A} receptor agonist [96]. Thus, the decrease in cocaine-induced locomotor activity, in the expression of locomotor sensitization, as well as the decrease in the length of locomotor activity produced by the daily dosage of MIR could be caused by the simultaneous blocking of serotonin 5-$HT_{2A/C}$ and the 5-HT_3 receptors and by the activation of serotonin 5-HT_{1A} receptors. This supports the hypothesis that some subtypes of NE and the 5-HT receptors could be used as important therapeutic targets

to treat CUD.

Preclinical studies have shown that female rodents show larger locomotor responses to cocaine than males [97–99] and acquire cocaine self-administration faster than males [100,101]. We showed that MIR decreased cocaine-induced locomotor activity. The expression of locomotor sensitization was compared to the other multitarget drugs evaluated, in male Wistar rats. We previously reported that MIR could decrease cocaine locomotor sensitization in male and female rats [102]; however, a limitation of the study is that the effect of the other multitarget drugs in female rats was not evaluated.

On the other hand, various studies have reported that during cocaine withdrawal an increase in stress levels is observed and it has been suggested that the stress produced by cocaine withdrawal is a major trigger for relapse and increases drug-seeking and locomotor activity [103,104]. A limitation of the study was not evaluating the effect of stress-induced cocaine withdrawal on the effect induced by each of the multitarget drugs on cocaine-induced locomotor activity.

Pioneering studies have shown that MIR has a very similar affinity for the different receptors that make up its pharmacological profile [90,92,93,105]. We found that blocking or activating each of the MIR binding sites with agonists did not cause a sudden increase in cocaine-induced locomotor activity. This suggests that since MIR shows a similar affinity for its different binding sites [90,92,93], it would explain why the administration of specific agonists did not suddenly increase cocaine-induced locomotor activity. Therefore, the mirtazapine-induced effects on locomotor activity and expression of behavioral sensitization could be because of the joint action of MIR on the α_2 NE, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_3 receptors and not to the preferential action of MIR on one of the receptors. This would support its activity as a multitarget antidepressant drug.

On the other hand, MIR is also a potent antagonist of histamine H_1 receptors, which has been associated with increased sedation [90,92]. However, several studies in humans have shown that a lower initial dose of MIR ($\leq$15 mg/kg) provides potent histaminergic blockade that induces clear sedation and sleepiness [106–108], whereas a higher initial MIR dose ($\geq$30 kg) is associated with decreased sedative antihistaminergic activity due to increased noradrenergic transmission [109,110]. Other studies indicate that mirtazapine-induced sedation decreases over time [111]. In animals, we found that dosing of 30 mg/kg or more of MIR generates effects temporary sedatives (produced sedation within minutes and only in the first few days of administration) [52]. The above observations suggest that the participation of H_1 receptors in the mirtazapine-induced decrease in cocaine-induced increase in locomotor activity is limited; however, an important limitation of the study was not having evaluated the participation of histamine H_1 receptors in the overall effect of MIR.

In summary, CUD is a complex disease involving many factors [112,113]. Some have suggested that multitarget drugs could be useful in the treatment of CUD [113,114], which may require drugs with complex multitarget pharmacological profiles, such as MIR [91], that integrate the simultaneous NE or 5-HT receptor antagonism or agonism into their pharmacological profile. We have shown the efficacy of MIR on behavioral effects induced by cocaine in rodents and humans [36,50,53,55]. In addition, other authors have reported that chronic dosing of MIR alters the expression of dopamine D_1 and D_2 receptors, increases the expression of BNDF mRNA, and increases the expression of α_1 NE receptors [115,116]. Together, the results described above show that MIR acts simultaneously on the α_2 NE, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_3 receptors, showing a similar affinity for each of them and by generating neuroadaptations in the dopaminergic system.

Thus, the results of this study suggest that under the dosing schedule used in this study (30 mg/kg of MIR/30 days during drug withdrawal), MIR can have sustained inhibitory effects on the cocaine-induced behavioral effects and supports its use, primarily, in long-term relapse prevention (maintenance of long-term abstinence) in the treatment of patients who abuse cocaine. However, previous reports suggest that mirtazapine showed efficacy in reducing drug cravings and mood disorders [[36,38]], which suggests that MIR could also be used in clinical trials for the treatment of drug craving and mood disorders. However, a limitation of these studies was the lack of long-term evaluations.

In this sense, clinical studies have evaluated the effect of antidepressants, antipsychotics, and other drugs on CUD [35–42]. These studies mainly evaluated the immediate effect of the multitarget drug dosage on various clinical variables (drug craving, drug use, mood pathologies, etc.) characteristic of CUD, reporting, on many occasions, positive results. However, these studies generally lack long-term evaluations (drug effect monitoring). Thus, the results of this study suggest that future clinical trials with MIR or other multitarget drugs should include a long-term follow-up stage (maintenance of long-term abstinence) of the drug effect, to consider it as a real option as a new pharmacological treatment against CUD.

5. Conclusions

These results suggest that multitarget drugs with a pharmacological profile based on simultaneous antagonism and agonism of 5-HT and NE receptors are an excellent option for treating CUD, and MIR is a good example of a model multitarget drug with an ad hoc pharmacological profile to treat CUD.

Ethical Approval

The Institutional Animal Care approved the procedures (CICUAL/0016/2006) and Bioethics Committee (CEI/C/IC092020/2006), in strict compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH).

Conflict interest Disclosure

The authors declare that there are no conflicts of interest.

Funding

This study was carried out thanks to grants INP-2000-2040 and CI-IC092020.2 and support from CONADIC and the Gonzalo Río Arronte Foundation.

Availability of data and materials

Has data associated with your study been deposited into a publicly available repository? Response: The data that support the findings of this study are available from the corresponding author upon reasonable request. https://u.pcloud.link/publink/show?code=kZeFWY0ZOgylj3XWHVuT0vtP4d6h9umhcE2X.

CRediT authorship contribution statement

Susana Barbosa-Méndez: Supervision, Methodology, Investigation. **Alberto Salazar-Juárez:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29979.

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