Evaluation of the 5-HT2C receptor drugs RO 60-0175, WAY 161503 and mirtazepine in a preclinical model of comorbidity of cocaine addiction and depression

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Abstract

Epidemiological data indicate a high rate of comorbidity of depression and cocaine use disorder (CUD). The role of 5-HT_{2C} receptors in the mechanisms responsible for the coexistence of CUD and depression has not been investigated. Here, we combined bilateral olfactory bulbectomy (OBX), an animal model of depression, with intravenous cocaine self-administration and extinction/reinstatement in male rats to investigate two 5-HT_{2C} receptor agonists (Ro 60–0175 (RO) and WAY 161503 (WAY)) and the 5-HT_{2C}-receptor preferring antagonist mirtazapine (MIR; an antidepressant), with the goal of determining whether these drugs alter cocaine-induced reinforcement and seeking behaviors. Additionally, neurochemical analyses following cocaine self-administration and its abstinence period in the brain structures in OBX rats and SHAM-operated controls were performed.

Acute administration of RO reduced, while WAY non-significantly attenuated cocaine reinforcement in OBX and SHAM rats. Moreover, RO or WAY protected against cocaine-seeking behavior after acute repeated drug administration during extinction training in OBX and SHAM rats. By contrast, acutely administered MIR did not alter cocaine reinforcement in both rat phenotypes, while acute (but not repeated) pretreatment reduced cocaine seeking in OBX and SHAM rats. In neurochemical analyses, cocaine reinforcement increased 5-HT_{2C} receptor levels in the ventral hippocampus; this effect was enhanced by preexisting depression. The 10-daily cocaine abstinence from self-administration reduced 5-HT_{2C} receptor expression in the dorsolateral striatum but coexistence of depression and CUD enhanced local receptor expression. The present study is an extra recommendation to support current development of pharmacological strategies with drugs targeting the 5-HT_{2C} receptor for the treatment of comorbid depression and CUD.

1. Introduction

Depressive disorders represent one of the most common mental disorders and are associated with deterioration of social functioning and the quality of life. According to the World Health Organization, around 300 million of people suffer from this disease, what represents 4.4% of the world population. Women suffer from depression relatively more often and the occurrence of depression is not influenced by race or ethnicity (World Health Organization 2016). The main problems in the course of depression are mortality and limited access to an effective therapy. Annually, 850,000 people die as a result of suicide due to depressive disorders, which is probably the consequence of a wrong diagnosis and inadequate treatment (only 25% of patients have access to proper treatment and were correctly diagnosed) (World Health Organization 2016).

One-third of patients suffering from depression have been diagnosed with a substance use disorder (Daley and Douaihy 2006). Epidemiological and clinical data indicate a high rate of comorbidity of depression and substance abuse. Frequency of the coexistence of these disorders depends on the type of substance being abused: 54% patients with depression abuse opioids, 38% alcohol, and 32% psychostimulants (Kosten et al. 1998). In contrast, research conducted by Rounsaville et al. (1991),

relating only to cocaine use disorder (CUD), showed very high occurrence (60%) of this disorder among people suffering from depression. Primary depressive disorder leads to the use of psychostimulants (e.g. cocaine) to self-medicate the symptoms of depression. The 'self-medication' hypothesis was first described by Khantzian. According to its assumptions, patients with depressive disorders take cocaine to relieve symptoms of depression, hypomania or hyperactivity (Khantzian 1985, 1974).

Based on several preclinical and clinical observations, in both disorders serotonin (5-HT) receptors (mainly 5-HT_{2C} subtypes) play a crucial role (Cryan and Lucki 2000; Zaniewska et al. 2010; Post and Kalivas 2013). Human and animal research indicate that these receptors are implicated in depression. Thus, the clinically-approved atypical antidepressants mirtazapine and mianserin (Hayasaka et al. 2015) as well as agomelatine (with additional melatonin 1 and 2 receptor agonism properties; Millan et al. 2003, 2011; Millan 2005) have clear antagonistic 5-HT_{2C} receptor profiles (Jenck et al. 1994; Millan 2005; Ni and Miledi 1997; Pälvimäki et al. 1996; Kostyalik et al. 2014). Prolonged treatment with various antidepressants leads to adaptation with a reduction in the number of active 5-HT_{2C} receptors in the central nervous system (Moreau et al. 1993; Serretti et al. 2004; Van Oekelen et al. 2003). On the other hand, both 5-HT_{2C} receptor agonists and antagonists have been reported to have antidepressant properties in animal models of depression (Moreau et al. 1996; Cryan and Lucki 2000; Nic Dhonnchadha et al. 2003; for review see Filip et al. 2012; Kennett et al. 1994, 1996, 1997, 2000; Wood et al. 2001; Millan 2005; Harada et al. 2006) and desensitization of the 5-HT_{2C} in serotonin transporter KO in mice shows antidepressant-like effects (Prisco and Esposito 1995; Di Giovanni et al. 2006).

Considering CUD, 5-HT_{2C} receptors are valid therapeutic targets for treating various addictive processes. Since the early years of the 21st century, several laboratories have reported inhibitory effects of selective 5-HT_{2C} receptor stimulation towards brain stimulation reward, voluntary cocaine intake as well as reinforcing and cue reactivity in rodents and non-human primates (Katsidoni et al. 2011; Grottick et al. 2000; Fletcher et al. 2002, 2004, 2009, 2011; Neisewander and Acosta 2007; Cunningham et al. 2011; Burbassi and Cervo 2008; Swinford-Jackson et al. 2016; Manvich et al. 2012 a, b; Ruedi-Bettschen et al. 2015). In line with preclinical observations, abstinent cocaine users exhibit lower sensitivity to the effects of 5-HT_{2C} agonists (Lee and Meltzer 1994; Buydens-Branchey et al. 1997; Patkar et al. 2006; Anastasio et al. 2014). By using genetic and pharmacological tools, 5-HT_{2C} receptor blockade produces opposite effects on cocaine reinforcement and the reinstatement of drug seeking in rodents (Rocha et al. 2002; Fletcher et al. 2002; Pelloux et al. 2012) or in primates (Manvich et al. 2012a, b; see also Ruedi-Bettschen et al. 2015).

Epidemiological and clinical data indicate a high rate of comorbidity of depression and substance abuse. The role of 5-HT_{2C} receptors in the mechanisms responsible for the coexistence of CUD and depression has not been investigated. The purpose of this study is to clarify the role of these receptors in pharmacological mechanisms underlying the pathology and to evaluate the efficacy of several 5-HT_{2C} receptor ligands, including receptor agonists and an antidepressant mirtazapine in a preclinical model of these two diseases. Additionally, to clarify the results obtained for 5-HT_{2C} receptor ligands, we performed

neurochemical analyses following cocaine self-administration and its abstinence period in bulbectomized (OBX) rats and SHAM controls, in which receptor expression levels in the brain structures were linked to CUD and depression.

2. Materials And Methods

2.1. Animals

Male Wistar rats (Charles River, Germany; initial weight 280–300 g) were used. The animals were housed up to 5/cage (training procedures) or individually (self-administration procedures) in standard rodent cages at a room temperature of 22 ± 2°C and 45–65% humidity with a 12-h light–dark cycle (lights on at 6:00). The animals had free access to food (Labofeed pellets) and water during a 7-day habituation period. Following habituation, the rats were given limited access to water (no longer than 6 h) during the initial lever-press training sessions. All experiments were conducted during the light phase of the light – dark cycle (between 07:00 and 16:00) and were carried out in accordance with the European directive 2010/63/EU on the protection of animals used for scientific purposes and were approved by the AnimalCare and Use Committee of the Institute of Pharmacology, Polish Academy of Sciences in Krakow (no. 1261/2015). The initial experimental groups consisted of 7–10 rats each.

2.2. Drugs

Cocaine hydrochloride (Sigma-Aldrich, St. Louis, USA), mirtazapine (MIR, Kemprotec, UK), RO 60–0175 (RO, Tocris, Germany) and WAY 161503 (WAY, Tocris, Germany) were dissolved in sterile 0.9% NaCl. Cocaine was administered i.v. or i.p. in a volume of 0.1 ml/infusion or 1 ml/kg, respectively, whereas other drugs used were given i.p. in a volume of 1 ml/kg.

2.3. Behavioral procedure

2.3.1. Lever-press training and catheter implantation

After one-week acclimatization, the animals were deprived of water for 6 h and then were trained for 3 days to press a lever in 2-h daily sessions under the fixed ratio (FR) schedule 1 of water reinforcement. On the fourth day of the training protocol, the schedule of reinforcement was increased to FR3, through FR5 during next days. Two days after the training time under FR5 (food and water *ad libitum*), the rats were anesthetized with ketamine HCl (75 mg/kg, i.p., Bioketan, Biowet, Poland) and xylazine (5 mg/kg, i.p., Sedazin, Biowet, Poland) and were chronically implanted with a silastic catheter in the external jugular vein, according to the procedure devised by Frankowska et al. (2014). After the surgery, during recovery, the catheters were flushed daily with 0.1 ml of a heparinized saline solution (70 U/ml, Biochemie, Austria) or 0.1 ml of a cephazolin solution (10 mg/ml Biochemie GmbH, Austria). The convalescence of animals lasted 14 days before experiments began.

2.3.2. Olfactory bulbectomy

The removal of the olfactory bulbs was carried out carried out in parallel with the catheter implantation. A midline incision was made from approximately 1 cm posterior to 1 cm anterior to the bregma. Burr holes (2 mm diameter) were then carefully drilled through the skull, approximately 7 mm anterior to the bregma and 2 mm on either side of the midline. The olfactory bulbs were then gently removed by suction and care was taken to avoid damage to the cortex. The burr holes were filled with hemostatic sponges and the wound was closed with surgical thread under aseptic conditions. SHAM animals were treated in a similar manner, in addition to the removal of the olfactory bulbs. The rats were given an analgesic for 5 days; meloxicam (0.2 mg/ml; vol. 0.2 ml/rats, s.c.; Boehringer Ingelheim, Germany). During recovery, the rats were handled every day to eliminate any aggressiveness that would otherwise develop (Kelly et al. 1997; Leonard and Tuite 1981). Verification of the correctness of bulbectomy was carried out at the end of behavioral experiments.

2.3.3. Maintenance of cocaine self-administration

Following the recovery, the rats were retrained to press the lever according to FR5 schedule for water reinforcement in a 2-h session. The next day, they began to press the lever for cocaine reinforcement during 2-h daily sessions (6 days/week). The light in the experimental cage was on during each session. OBX and SHAM groups were trained to self-administer cocaine at a dose of 0.5 mg/kg/infusion with FR5 schedule of reinforcement. Each completion of the schedule by pressing the active lever resulted in a 5-s injection of cocaine and a 5-s presentation of the stimulus complex that consisted of the activation of the white stimulus light directly above the active lever and a tone from the generator (2000 Hz; 15 dB above the ambient noise level). Twenty seconds after each injection (time-out period), the next injection was impossible. During this time the responses were recorded, but there were no programmed consequences. Pressing the inactive lever was recorded, but never resulted in cocaine delivery.

To analyze the expression of the 5-HT_{2C} receptor, the part of cohort that underwent self-administration (at least 14 days) was sacrificed immediately following the session (6 rats/group). In pharmacological studies, separate groups of OBX and SHAM rats (6–10 rats/group) were pretreated acutely with WAY (1–3 mg/kg) 15 min, RO (3–10 mg/kg) 20 min and MIR (5–20 mg/kg) 30 min before cocaine self-administration sessions. The test sessions were separated by at least two to three baseline days of cocaine self-administration.

2.3.4. Extinction training

After cocaine self-administration (at least 16 days), separate groups of rats that were trained to self-administer cocaine at a level that met the acquisition criterion (active lever presses for an average of 3 consecutive days and a standard across those 3 days that varied by 15% or less; Frankowska et al., 2014) were used in the extinction/reinstatement tests. The rats were tested for the response reinstatement induced by either a non-contingent priming injection (10 mg/kg cocaine, i.p.) or a conditioned cue (the tone + light) that had previously been paired with cocaine self-administration. Active lever presses during the reinstatement tests resulted in an intravenous injection of saline only.

One cohort of OBX and SHAM rats was sacrificed immediately following the last session of extinction (biochemical analysis).

2.3.5. Reinstatement of cocaine seeking

The other cohort of OBX and SHAM rats (n = 6–11) was acutely pre-treated with MIR (2.5–20 mg/kg), WAY (0.1-1.0 mg/kg), RO (0.3–3.0 mg/kg) or the corresponding vehicles before the reinstatement test sessions. Each rat underwent only one type of reinstatement procedure, in which either cocaine (10 mg/kg, i.p.) or the cue was presented in a maximum of 4 tests. The order of the injections was balanced according to a Latin square design, and the drug combinations were given in a randomized order. The test sessions were separated by at least two to three extinction sessions.

Separate groups of OBX and SHAM rats (6–10 rats/groups) were chronically pretreated with MIR (10 mg/kg), WAY (1.0 mg/kg), RO (1.0 mg/kg) or the corresponding vehicle during extinction training and then cocaine or cue-induced reinstatement tests were performed. The test sessions were separated by at least two to three extinction sessions. The doses of WAY and RO were selected based on the acute drug effects.

2.4. Western blot

Brain structures were homogenized in a homogenization buffer (1 mM HEPES, 0.1 M DTT, 0.1 mM EGTA (pH 7.2–7.8), COMPLETE and sterile water) using a homogenizer ball (Bioprep-24, Allsheng, China) (10 s at 10000 rpm) and were then denatured for 2 min at 85°C, 2 min in ice, 5 min at 85°C, and finally 2 min in ice. The bicinchoninic acid assay (BCA) protein assay kit (Serva, Germany) was used for protein determination. Protein samples (40 mg) were resolved in 4–15% SDS polyacrylamide gels and transferred to a polyvinylidene difluoride (PVDF) membrane. Membranes were blocked in 3% non-fat dry milk, and separate sets of membranes were probed with rabbit anti-5-HT_{2C} monoclonal antibody (1:1000; Abcam, UK). Antibodies were validated using the 5-HT_{2C} receptor peptide (Abcam, UK) The expressions of 5-HT_{2C} receptors were evaluated relative to anti-glyceraldehyde 3-phosphate dehydrogenase antibody (GAPDH), using rabbit anti-GAPDH polyclonal antibody (1:1500, sc-25778, Santa Cruz Biotechnology, USA). Blots were washed and incubated with goat anti-rabbit secondary antibody (1:6000; 926- 68071; Li-cor, USA) and visualized with fluorescence detection Odyssey Clx (Licor, USA). The analysis was performed using the Image Studio v.2.1. All data were expressed as % of control.

2.5. Statistical analysis

Data from the Western blot were analyzed using the GraphPad PRISM 5.0 (GraphPad software, San Diego, USA). Statistical analyses covered i) a two-way analysis of variance (ANOVA) with surgical condition (OBX or SHAM) and treatment (vehicle or cocaine) as factors or ii) t-Student test. Group differences after significant ANOVAs were analyzed by the post hoc Newman- Keuls. The criterion for a statistically significant difference was set at $p < 0.05$.

Statistical analyses for data from the behavioral experiments were performed using mixed analyses of variance (ANOVA) for the following factors under the specific protocols: i) cocaine self-administration with acute drug treatment: surgical condition (SHAM and OBX), pre-treatment (vehicle and drug), lever (active and inactive), and their interaction; or surgical condition (SHAM and OBX), pre-treatment (vehicle and drug), and their interaction; ii) extinction with repeated drug treatment: surgical condition (SHAM and OBX), session (from 1 to 9), pre-treatment (vehicle and drug), and their interaction; iii) reinstatement tests with acute drug treatment: surgical condition (SHAM and OBX), lever (active and inactive), reinstatement (extinction and cue or cocaine), and their interaction; or surgical condition (SHAM and OBX), pre-treatment (vehicle and drug), lever (active and inactive), and their interaction; iv) reinstatement following repeated drug treatment during extinction: surgical condition (SHAM and OBX), pre-treatment (vehicle and drug), lever (active and inactive), and reinstatement (extinction and cue or cocaine), and their interaction.

Post hoc Newman-Keuls tests were used to analyze the differences between the group means. Animals which did not complete the self-administration acquisition/maintenance, extinction criteria or did not maintain extinction criteria between tests were excluded from the tests and the data analysis. The criterion for a statistically significant difference was set at $p < 0.05$.

3. Results

3.1. Behavioral experiments

3.1.1. Cocaine self-administration

Figure 1A shows the effects of the acute WAY administration in OBX and SHAM rats on the active and inactive lever presses and on the number of cocaine infusions. A three-way ANOVA for the surgical condition × pretreatment × lever did not show differences between the groups treated with WAY ($F(2,74) = 0.10$ $p = 0.91$), and the analysis inside each experimental group treated with WAY did not indicate a difference in the cocaine reinforcement (a two-way ANOVA: $F(2,34) = 1.50$ $p = 0.24$ and $F(2,40) = 1.10$ $p = 0.34$, respectively).

Acute treatment with WAY did not alter the number of cocaine infusions in both animal groups, as shown by a two-way ANOVA for the surgical condition × pre-treatment interaction in OBX ($F(2,17) = 1.60$ $p = 0.23$) and SHAM groups ($F(2,20) = 0.93$ $p = 0.40$); the observed effect was independent of surgical manipulation ($F(3,44) = 0.24$ $p = 0.86$).

The numbers of active and inactive lever presses and the number of cocaine infusions for OBX and SHAM animals after RO administration are shown in Fig. 1B. A three-way ANOVA for the surgical condition × pretreatment × lever interaction did not show a difference between the types following RO treatment ($F(2,66) = 0.48$ $p = 0.62$), what means that the administration of RO had a similar effect on the number of presses on the active lever in both groups of animals. Pre-treatment with RO in a dose-dependent manner decreased the number of presses of the active, but not the inactive lever in both

groups of rats. Individual two-way ANOVA analyses for OBX and SHAM groups indicted a significant effect for the pre-treatment × lever interaction ($F(2,30) = 5.54$ $p < 0.01$ and $F(2,36) = 16.66$ $p < 0.001$, respectively).

Removal of the olfactory bulbs did not affect the number of cocaine infusions after acute administration of RO ($F(2,33) = 1.21$ $p = 0.34$). A one-way ANOVA for each group showed a significant effect of RO on the cocaine reinforcement in OBX ($F(4,28) = 3.85$ $p < 0.05$, 3–10 mg/kg) and SHAM rats ($F(2,18) = 21.67$ $p < 0.001$), but only at a dose of 10 mg/kg (Fig. 1B).

The lack of significant effects after acute administration of MIR in OBX and SHAM rats was demonstrated with a three-way ANOVA ($F(2,182) = 0.69$ $p = 0.57$; Fig. 1C). The inner analysis for each experimental group treated with MIR did not indicate a difference in the cocaine reinforcement. MIR (5–20 mg/kg) did not alter the numbers of lever responses in OBX or SHAM rats (a two-way ANOVA: $F(3,88) = 2.30$ $p = 0.08$ and $F(3,94) = 0.14$ $p = 0.92$, respectively).

Acute treatment with MIR did not alter the number of cocaine infusions in both animal groups, as shown by a two-way ANOVA for the surgical condition × pre-treatment interaction in OBX ($F(3,47) = 0.37$ $p = 0.77$) and SHAM groups ($F(3,44) = 1.60$ $p = 0.33$). The observed response was independent of surgical manipulation ($F(3,91) = 0.89$ $p = 0.45$).

3.1.2. Extinction training

After cocaine self-administration, an extinction training was introduced to all animals, without the drug or a drug-associated cue. Figure 2 shows the effects of the repeated administration of WAY, RO or MIR, in OBX and SHAM rats on the active and inactive lever presses during extinction training.

A mixed repeated measures ANOVA did not show a significant effect of the repeated administration of WAY (1 mg/kg) or VEH during extinction training in OBX and SHAM rats (the surgical condition × session × pre-treatment × lever interaction ($F(8,464) = 0.68$ $p = 0.71$). Similarly, a three-way repeated measures ANOVA for each group separately did not indicate a significant effect for the session × pre-treatment × lever interaction in OBX ($F(8,256) = 1.30$ $p = 0.24$) and SHAM rats ($F(8,208) = 3.40$ $p = 0.92$)(Fig. 2A).

Repeated administration of RO (1 mg/kg) during extinction training in SHAM and OBX rats did not cause changes in the number of lever presses ($F(8,256) = 0.52$ $p = 0.84$ and $F(8,208) = 0.19$ $p = 0.99$, respectively). Moreover, the effect of the session × pre-treatment × lever interaction was the same in both groups of animals ($F(8,454) = 0.48$ $p = 0.87$) (Fig. 2B).

In like manner, the repeated administration of MIR (10 mg/kg) or VEH did not significantly vary lever responses between experimental groups during the extinction training $F(8,608) = 0.74$ $p = 0.65$). The inner three-way repeated measures ANOVA for each group did not indicate a significant effect for the session × pre-treatment × lever interaction in OBX ($F(8,272) = 1.18$ $p = 0.31$) and SHAM rats ($F(8,304) = 0.99$ $p = 0.44$)(Fig. 2C).

3.1.3. Cue-induced reinstatement of seeking behavior – acute administration

The results for the WAY experiment following presentation of the cocaine-associated cue (tone + light) to OBX and SHAM groups are shown in Fig. 3A. A two-way ANOVA for the self-administration procedure × lever interaction showed a decrease in the number of active lever presses after 10 days extinction training in SHAM and OBX groups, as compared to cocaine self-administration ($F(1,26) = 193.02$ $p < 0.001$ and $F(1,28) = 66.56$ $p < 0.001$, respectively), regardless of the surgical condition ($F(1,54) = 1.01$ $p = 0.31$).

A significant main effect was observed for the reinstatement (cue) × lever interaction ($F(1,54) = 0.63$ $p = 0.43$), and a post hoc analysis revealed an increase ($p < 0.001$) in the number of active lever presses during the reinstatement of the drug-seeking behavior in rats that had previously self-administered cocaine (0.5 mg/kg/infusion).

A three-way ANOVA did not reveal an effect for the surgical condition × pre-treatment × lever interaction ($F(3,104) = 0.42$ $p = 0.74$), what indicates that there was no significant difference between OBX and SHAM rats in the effects of WAY. An individual two-way ANOVA analysis for SHAM and OBX rats showed that pre-treatment with WAY (0.1–1 mg/kg) significantly decreased the cue-induced reinstatement in SHAM (pre-treatment × lever interaction: $F(3,54) = 3.04$ $p < 0.05$) and OBX ($F(3,50) = 5.90$ $p < 0.05$) animals, what proves that pretreatment with WAY effectively altered the reinstatement of the drug seeking behavior (Fig. 3A).

For the RO experiment, the results for the cue-induced reinstatement of cocaine seeking in OBX and SHAM animals are shown in Fig. 3B. A two-way ANOVA showed a significant reduction in active lever presses after 10 days of extinction training under both surgical conditions (OBX: $F(1,24) = 37.67$ $p < 0.001$; SHAM: $F(1,24) = 74.61$ $p < 0.001$). A two-way ANOVA revealed a significant effect for the reinstatement (cue) × lever interaction in both SHAM ($F(1,24) = 13.60$ $p < 0.01$) and OBX ($F(1,24) = 39.89$ $p < 0.001$) groups, regardless of the surgical condition ($F(1,46) = 1.21$ $p = 0.27$).

A three-way ANOVA (the surgical condition × pre-treatment × lever interaction) did not show an effect for acute pre-treatment with RO (0.1-1 mg/kg) ($F(3,90) = 0.37$ $p = 0.76$). An individual analysis with a two-way ANOVA in OBX or SHAM rats showed that pre-treatment with RO significantly decreased the cue-induced reinstatement in OBX (pre-treatment × lever: $F(3,44) = 4.15$ $p < 0.01$) and SHAM (pre-treatment × lever: $F(3,46) = 5.90$, $p < 0.01$) animals, what indicates that pre-treatment with RO effectively changed the reinstatement of the drug seeking behavior (Fig. 3B).

The effects of the MIR experiment, following presentation of the cocaine-associated cue, are shown in Fig. 3C. A two-way ANOVA revealed a significant effect for the self-administration procedure × lever interaction in both OBX ($F(1,28) = 24.93$ $p < 0.001$) and SHAM ($F(1,28) = 15.34$ $p < 0.001$) rats; a reduction in active lever presses after 10 days of extinction was found in both types of rats.

The conditional cue induced an increase ($p < 0.001$) in the number of active lever presses during the reinstatement of the drug-seeking behavior in OBX (a two-way ANOVA for reinstatement (cue) × lever interaction: $F(1,28) = 28.51$ $p < 0.001$) and SHAM rats ($F(1,28) = 16.18$ $p < 0.001$); the observed effect was independent of the surgical condition ($F(1,68) = 0.22$ $p = 0.64$).

During cue induced reinstatement a significant reduction in the drug-seeking behavior in OBX and SHAM rats was observed, following acute administration of MIR (a two-way ANOVA: $F(3,56) = 24.37$ $p < 0.001$ and $F(3,56) = 7.36$ $p < 0.001$). Post hoc analyses showed that a significant reduction in the drug seeking behavior occurred in both groups following administration of MIR at doses 2.5–10 mg/kg in OBX rats and only for 5 and 10 mg/kg in SHAM rats. The effect of pretreatment with MIR on OBX and SHAM rats was regardless of the surgical condition ($F(3,112) = 1.33$ $p = 0.23$).

3.1.4. Cocaine-induced reinstatement of seeking behavior – acute administration

Figure 4A shows the effects of acute WAY administration on cocaine-induced reinstatement in OBX and SHAM groups. A 10-day extinction training led to the reduction in active lever presses ($p < 0.001$), more than inactive lever presses, compared to the last cocaine self-administration session in both experimental groups (Fig. 4A) (a two-way ANOVA for the self-administration procedure × lever interaction: OBX $F(1,20) = 39.68$ $p < 0.001$; SHAM $F(1,24) = 17.45$ $p < 0.001$). A reduction in active lever presses after 10 days of extinction was found in both group.

A three-way ANOVA did not indicate an effect for the surgical condition × pretreatment × lever interaction ($F(3,84) = 2.32$ $p = 0.08$), what indicates that there was no significant difference between OBX and SHAM rats in the effects of WAY. A two-way ANOVA analysis demonstrated a significant effect for the pre-treatment × lever interaction in SHAM and OBX rats ($F(3,44) = 8.02$ $p < 0.001$ and $F(3,40) = 2.92$ $p < 0.05$, respectively). Post hoc analyses showed that a significant reduction in the drug seeking behavior occurred in both groups following administration of WAY, for all doses (0.3-3 mg/kg) in OBX rats and in SHAM rats only for the dose of 3 mg/kg.

Similarly to the RO experiment, a two-way ANOVA showed a significant reduction in the active lever presses after 10 days of extinction training for both types of surgery condition (OBX: $F(1,24) = 37.68$ $p < 0.001$; SHAM: $F(1,24) = 74.61$ $p = < 0.001$), regardless of the surgical condition ($F(1.48) = 0.54$ $p = 0.46$) (Fig. 4C). Administration of cocaine at a dose of 10 mg/kg (i.p.) resulted in an increase in the responses to the active lever in OBX ($F(1,24) = 53.98$ $p < 0.001$) and SHAM groups ($F(1,24) = 19.26$ $p = < 0.001$). A three-way ANOVA did not indicate an effect for the surgical condition × pretreatment × lever interaction ($F(3,93) = 0.78$ $p = 0.51$), what shows that there was no significant difference between OBX and SHAM rats in the effects of RO. A two-way ANOVA analysis demonstrated a significant effect for the pre-treatment x lever interaction in SHAM and OBX rats ($F(3,48) = 7.07$ $p < 0.001$ or $F(3,45) = 7.29$ $p < 0.001$, respectively) (Fig. 4B).

For the acute MIR administration experiment, the effect of the 5-HT_{2C} agonist on cocaine-induced reinstatement in OBX and SHAM groups is shown in Fig. 4C. A two-way ANOVA for the self-administration procedure × lever interaction showed a significant reduction in active lever presses after 10 days of extinction in both experimental groups (OBX $F(1,32) = 23.86$ $p < 0.001$; SHAM $F(1,36) = 95.16$ $p < 0.001$); regardless of the surgical condition ($F(1,68) = 0.12$ $p = 0.73$).

Regarding the cocaine-induced seeking behavior, measured as increases of active lever-responses ($p < 0.001$), a two-way ANOVA revealed an effect for the cue-induced reinstatement of seeking behavior in both experimental groups (OBX: $F(1,30) = 31.37$ $p < 0.001$ and SHAM: $F(1,36) = 20.00$ $p < 0.001$).

When MIR (2.5–20 mg/kg) was administered before placing rats in the experimental cage, a marked reduction in the number of active lever-responses induced by cocaine was observed (a two-way ANOVA: OBX $F(4,74) = 3.81$ $p < 0.001$; SHAM $F(4,78) = 4.08$ $p < 0.01$). A post hoc test demonstrated a reduction in active lever presses in rats treated with 10 and 20 mg/kg doses of MIR for OBX and in SHAM rats only for 20 mg/kg. A three-way ANOVA did not indicate an effect for the surgical condition × pretreatment × lever interaction ($F(4,150) = 0.14$ $p = 0.97$), what demonstrates that there was no significant difference between OBX and SHAM rats in the effects of MIR.

3.1.5. Cue-induced reinstatement of cocaine seeking behavior – repeated drug administration during extinction training

After10-daily administration of vehicle or WAY (1 mg/kg), during the extinction-training period, rats were tested for the response reinstatement induced by a cocaine-associated cue (Fig. 5A). As shown by a mixed ANOVA for the surgical condition surgical condition × pretreatment × reinstatement (cue) × lever interaction, repeated WAY administration did not alter the cue-induced reinstatement for either type of surgery condition ($F(1,116) = 0.17$ $p = 0.68$; Fig. 5A).

In the SHAM-operated animals, rats after repeated WAY administrations during extinction training reacted differently on cue-induced reinstatement ($F(1,52) = 15.09$ $p < 0.001$). A post hoc analysis showed a significant increase in the number of active lever presses ($p < 0.001$) in the group receiving the vehicle, while in the group chronically treated with WAY a significant reduction in the cue-induced relapse ($p < 0.001$) was observed, as compared to the vehicle group. In OBX group, the differences were also observed in the response on cue-induced reinstatement between rats receiving repeated doses of WAY or VEH ($F(1,64) = 14.24$ $p < 0.05$) (Fig. 5A). As demonstrated by the post hoc analysis, WAY administered chronically significantly attenuated the cocaine-induced relapse ($p < 0.001$) (Fig. 5A).

In like manner, a mixed ANOVA for both types of surgery condition did not show a significant effect of the surgical condition × pretreatment × reinstatement (cue) × lever interaction ($F(1,116) = 0.01$ $p = 0.94$) (Fig. 5B), what proves that pretreatment with RO (1 mg/kg) had the same effect for both surgery conditions. However, for these conditions, a three-way ANOVA showed a significant effect of the reinstatement (cue) × lever × pre-treatment interaction in SHAM ($F(1,52) = 12.16$ $p < 0.001$) and OBX

(F(1,64) = 5.49 p < 0.05) rats. A post hoc analysis showed a significant reduction in the cue-induced relapse for both surgery conditions (p < 0.001) after chronic administration of RO.

As shown by a mixed ANOVA for the surgical condition surgical condition × pretreatment × reinstatement (cue) × lever interaction (F(1,145 = 1.26 p = 0.26), the reinstatement of seeking behavior induced by a cue was relevant following either VEH or MIR (10 mg/kg) pretreatment in both OBX or SHAM rats (Fig. C). Moreover, inner analyses for each group did not reveal a significant difference between treatment with VEH or MIR, suggesting that rats responded similarly to the cue (OBX: F(1,68) = 2.28 p = 0.14; SHAM: F(1,76) = 0.25 p = 0.62).

3.1.6. Cocaine-induced reinstatement of cocaine seeking behavior – repeated drug administration during extinction training

Following 10 days of VEH or WAY administration, during the extinction training, SHAM and OBX rats were tested for the response reinstatement induced by cocaine (10 mg/kg, i.p., Fig. 5A). A mixed ANOVA for both surgery conditions did not reveal a significant effect of repeated administration of WAY during extinction training for the pretreatment × surgical condition × cocaine × lever interaction (F(1,116) = 0.02 p = 0.90), what indicates that the surgery condition, OBX and SHAM, did not correlate with a behavioral response to a priming cocaine injection (Fig. 5B). A separate three-way ANOVA for SHAM (F(1,51) = 2.31 p = 0.13) and OBX (F(1,64) = 2.73 p = 0.10) rats showed no significant effect of WAY on the cocaine-induced reinstatement (Fig. 5B).

After 10 days of repeated treatment with the vehicle or RO, during the extinction training, SHAM and OBX rats were tested for the response reinstatement induced by cocaine (10 mg/kg, i.p.). For both types of surgery condition, a mixed ANOVA did not reveal a significant effect for the pretreatment × surgical condition × cocaine × lever interaction (F(1,116) = 0.14 p = 0.71), what provides evidence that OBX and SHAM rats responded similarly to RO pretreatment. A separate analysis for SHAM (F(1,52) = 1.37 p = 0.25) and OBX (F(1,64) = 2.87 p = 0.09) rats indicated no significant effect of RO on the cocaine-induced reinstatement (Fig. 5B).

Repeated administration of VEH or MIR did not change behavioral responses during reinstatement of seeking behavior, represented as the increase of lever presses in experimental groups (a mixed ANOVA for the pretreatment × surgical condition × reinstatement (cocaine) × lever interaction: F(1,144) = 0.06 p = 0.80)(Fig. 5C). Further, an individual analysis revealed that the cocaine-induced reinstatement was similar in both groups (OBX: F(1,76) = 0.12 p = 0.73; SHAM: F(1,68) = 0.57 p = 0.45).

3.2. The expression of the 5-HT_{2C} receptor

Removal of the olfactory bulbs induced an increase in the 5-HT_{2C} receptor expression only in the ventral hippocampus (t=-2,43; df = 10; p < 0,05) (Fig. 6A). Cocaine self-administration *per se* did not change 5-HT_{2C} receptor expression in all examined rat brain structures (Fig. 6A and Table 1).

Table 1
5-HT_{2C} receptor expression in the several brain structures in bulbectomized and SHAM control rats following cocaine self-administration and yoked saline delivery.

Brain structure	$PFCX_{PL}$	$PFCX_{IL}$	FCX	vHIP	dHIP	DLS	DMS	NAC	BLA	CER
F(1,20)=	0.01	2.40	0.28	1.20	3.34	1.82	0.47	0.11	0.61	0.90
p=	0.91	0.14	0.60	0.28	0.08	0.19	0.49	0.11	0.61	0.90

$PFCX_{IL}$ – infralimbic prefrontal cortex, $PFCX_{PL}$ – prelimbic prefrontal cortex, FCX – frontal cortex, vHIP – ventral hippocampus, dHIP – dorsal hippocampus, DLS – dorsolateral striatum, DMS – dorsomedial striatum, NAC – nucleus accumbens, BLA – basolateral amygdala, CER – cerebellum. Two-way ANOVA.

A two-way ANOVA showed significant changes in the 5-HT_{2C} receptor expression in SHAM and OBX groups after 10 days extinction in rats self-administering cocaine or the vehicle group in the frontal cortex ($F(1,20) = 5,42$; $p < 0,05$) and dorsomedial striatum ($F(1,20) = 39,88$; $p < 0,001$). In OBX vehicle group, an increase in the 5-HT_{2C} receptor expression was shown in the frontal cortex ($t=-2,64$; $df = 10$; $p < 0,05$), as compared to SHAM vehicle group. In OBX cocaine group, there was a decrease in the 5-HT_{2C} receptor expression in the frontal cortex ($t = 3,35$; $df = 10$; $p < 0,01$) and an increase in the dorsomedial striatum ($t=-7,01$; $df = 10$; $p < 0,001$), compared to OBX vehicle group (Fig. 6B).

4. Discussion

The comorbidity of depression and CUD has become a serious medical and social problem, while the relationship between these two disorders and their potential mechanisms is still not understood. Since numerous studies have suggested that both disorders share common mechanisms and anatomical pathways, in this paper we focused on the association between addiction and depression, highlighting the potential mediating role of 5-HT_{2C} receptors in this comorbidity *via* the regulation of behavioral effects *in vivo* and changes in the signaling *ex vivo*.

Here, behavioral investigation revealed for the first time that acute administration of RO significantly, and WAY non-significantly (a reduction in active lever pressing and cocaine infusions by 34% and 46%, respectively) attenuated the reinforcing properties of self-administered cocaine, while MIR did not change this behavioral response in OBX rats. The same inhibitory effects of RO and WAY were observed in SHAM animals and they reflect previous observations regarding RO (Grottick et al. 2001), WAY (Cunningham et al. 2011) and lorcaserin, another 5-HT_{2C} receptor-preferring agonist (Gannon et al. 2018). The similarity of behavioral results for OBX and SHAM groups following the administration of 5-HT_{2C} receptor agonists indicate that reinforcing activity of cocaine is directly associated with these receptors, while a depression-like phenotype is diffusely related. Our *ex vivo* analyses also showed that cocaine self-administration raised the expression of the 5-HT_{2C} receptor protein in the ventral hippocampus in OBX and SHAM animals, what may indicate that it acts as a molecular target controlling cocaine reinforcement. This finding extends a previous observation with local (intra-ventral tegmental area, intra-amygdala or intra-

medial prefrontal cortex (mPFCX)) infusions of 5-HT_{2C} receptor agonists that reduce the above cocaine behavior (Pentkowski et al. 2010; Fletcher et al. 2004; Pockros-Burgess et al. 2014). On the other hand, tonic activation of 5-HT_{2C} receptors does not alter reinforcing effects of cocaine, as MIR altered neither the number of active lever pressing nor the number of cocaine infusions. This finding extents previous observations from our laboratory, since the 5-HT_{2C} preferring-receptor antagonist SDZ SER-082 – in contrast to 5-HT_{2A} receptor antagonism – also did not change cocaine reinforcement in rats (Filip 2005).

In the next set of experiments we verified whether any 5-HT_{2C} receptor ligand given acutely (before a reinstatement test) or repeatedly (during extinction sessions) could diminish cocaine seeking, what may prevent addicts to take drugs. We found that not only acute pretreatment with 5-HT_{2C} receptor agonists but also acute MIR administration reduced cocaine seeking behavior evoked by cue- or cocaine priming in both SHAM and OBX animals. This report for the first time indicated efficacy of RO and WAY in effective inhibition of recurrence of cocaine-seeking behavior in OBX rats, what means that 5-HT_{2C} receptor agonism demonstrates the potential clinical utility to reduce cocaine seeking enhanced by co-existing depression. What is more, similar findings were delivered by other authors, where RO and WAY attenuated increases of active-lever presses for a conditioned stimulus previously associated with cocaine self-administration or the priming effect of an acute cocaine injection following systemic (Grottick et al. 2001; Burbasi and Cervo 2008; Fletcher et al. 2009), intra-nucleus accumbens or intra-mPFCX (but not intra-ventral tegmental area) microinjections (Katsidoni et al. 2011; Pockros-Burgess et al. 2014; Pentkowski et al. 2010). As indicated in more recent studies, selective 5-HT_{2C} receptor activation decreased drug-seeking behavior also in nonhuman primate models of cocaine abuse (Berro et al. 2017; Ruedi-Bettschen et al. 2015). In a separate randomized controlled trial, lorcaserin delayed intravenous choices and decreased craving under some conditions in regular cocaine users, however, some positive subjective effects of cocaine were enhanced by lorcaserin (Pirtle et al. 2019). Although 5-HT_{2C} receptor agonism is linked to sedation and reduction in motor behaviors, such nonspecific impairment of behaviors related to inhibition of relapse is not likely to depend on RO or WAY. In fact, RO doses used in this study were much lower than those which weaken motor functions in the rotarod test (Grottick et al. 2001), while both agonists had no significant effect on the number of inactive lever presses during the reinstatement of seeking behavior, what indicates the specificity of its action and also natural reward (Grottick et al. 2001; Hewitt et al. 2002). The anti-relapse effects of RO and WAY were blocked by 5-HT_{2C} receptor antagonists, what proves that the observed changes, such as inhibited recurrence of cocaine-seeking behavior, were dependent on the stimulation of these receptors (Grottick et al. 2000; Burbasi and Cervo. 2008). Since 5-HT_{2C} receptor antagonists per se did not reduce cocaine seeking behaviors (Filip 2005; Bubasi and Cervo 2008), the inhibitory response of MIR is of special interest. It should be underlined that this drug, apart from 5-HT_{2C} receptors, also targets5-HT_{2A} and 5-HT_3 receptors (Anttila and Leinonem 2001). The latter pharmacological targets are engaged in the control of cocaine seeking behaviors, as shown in laboratory animals using selective 5-HT_{2A} (Filip 2005; Nic Dhonnchadha et al. 2009; Pockros et al. 2011; Sholler et al. 2019) and 5-HT_3 (Zhou et al. 2019) receptor antagonists. MIR also enhances noradrenergic neurotransmission via antagonism of the central α2-adrenergic autoreceptors and heteroreceptors

(Anttila and Leinonem 2001), however their role in cocaine relapse has not been investigated so far and is recommended for future research. Hence, 5-HT_{2C} agonists, by elimination of animals behavioral response on the contingent cue, become important modulators of subsequent behavior.

Our findings also demonstrate that repeated administration of 5-HT_{2C} agonists – RO and WAY – during extinction training resulted in a significant inhibition of the reinstatement of cocaine-seeking behavior induced by the presentation of the conditioned stimulus, while a weaker (non-significant) reduction in the reinstatement after administration of a priming dose of cocaine in both groups, SHAM and OBX, was observed. The different effects of chronic administration of 5-HT_{2C} agonists during extinction training on the reinstatement of seeking behavior induced by different stimuli (cue vs. cocaine) may be due to the involvement of, among others, separate regions of rat (sub)brain. For example, the prelimbic PFCX and infralimbic cortex play an opposite role in the reinstatement induced by the conditioned cue (Tavares et al. 2009). Furthermore, lesion of the medial part of the orbitofrontal cortex enhances the reinstatement of seeking-behavior induced by the priming dose of cocaine (Fuchs et al. 2004), while pharmacological inactivation or lesion of the lateral orbitofrontal cortex inhibited exploratory seeking behavior for cocaine after the presentation of the conditional stimulus (Fuchs et al. 2004; Gallagher et al. 1999; Lasseter et al. 2011).

Secondly, a pharmacological analysis with drug microinjections demonstrated that stimulation of the 5-HT_{2C} receptor in the prelimbic and infralimbic subregions (but not in the anterior cingulate cortex) of the mPFCX or in the central amygdala (but not in the basolateral amygdala) suppressed reinstatement of cocaine-seeking behavior, an effect reversed by co-infusion of a selective 5-HT_{2C} receptor antagonist (Pentkowski et al. 2010; Pockros-Burgess et al. 2014). Thirdly, there is only one report showing variation in the 5-HT_{2C} receptor expression in different brain areas during high cocaine cue reactivity in humans and animals (Anastasio et al. 2014). The latter authors indicated that enhancement in drug-associated lever presses reinforced by the discrete cue complex was correlated with lower 5-HT_{2C} receptor protein expression in the mPFCX and blunted sensitivity to the suppressive effects of WAY, a selective 5-HT_{2C} agonist (Anastasio et al. 2014). In this context our present neurochemical analyses revealed that cocaine abstinence with extinction training evoked a significant decrease in the dorsomedial striatum only. To check whether this change contributes to cue- or cocaine-related drug seeking, further pre- and post-training microinjections of 5-HT_{2C} agonists are needed.

As shown above, MIR given acutely reduced cocaine seeking but its repeated administration – at a dose effectively blocking cocaine actions – during extinction failed to inhibit cue or the reinstatement of cocaine-seeking behavior. This finding is in direct contrast with previous observations, where daily dosing of MIR (30 mg/kg, i.p.) for 30 days significantly attenuated the induction of cocaine-induced locomotor sensitization (Barbosa-Méndez et al. 2017a, 2021; Salazar-Juárez et al. 2016) and attenuated the re-acquisition of cocaine-seeking responses in rats (Barbosa-Méndez et al. 2017b, 2018). Additionally, it should be stressed that chronic dosing of MIR (30 mg/kg, ip) during cocaine withdrawal reduced depression- and anxiety-like behaviors that characterize cocaine withdrawal in rats (Barbosa Mendez and

Salazar-Juarez 2019). Since in those and the present studies observations were made at different periods of cocaine abstinence, and different time of drug administration as well as MIR doses were applied, it could justify the occurrence of differences.

In the current study, we reported that the development of a depressive status by removing the olfactory bulbs produced significant and time-dependent increases in 5-HT_{2C} receptor levels in the rat ventral hippocampus and frontal cortex, as compared to SHAM saline controls. The increased protein expression of these receptors is likely to be responsible for the intensification of primary depressive disorders in OBX rats, and thus may lead to an increase of the reinstatement of cocaine-seeking behavior observed in these animals (Frankowska et al. 2014; Jastrzebska et al. 2016). In neurochemical analyses we also demonstrated significant enhancement of 5-HT_{2C} receptors localized in the dorsomedial striatum in animals co-expressing depression and CUD. The latter brain area mediates the acquisition and performance of cocaine-seeking behavior (Zapata et al. 2010) and pharmacological activation of the dorsomedial striatal 5-HT_{2C} receptors leads to a reduction in dopaminergic neurotransmission (Hasler et al. 2008; Nestler and Carlezon 2006). A reduction in the expression of the 5-HT_{2C} receptor protein level in the dorsomedial striatum mediates the restoration of normal dopaminergic transmission (Lex and Hauber 2010; Pacchioni et al. 2011). Furthermore, an increase in 5-HT_{2C} receptor protein expression in the dorsomedial striatum in laboratory animals is associated with anxiety (Millan 2005, 2006; Serretti and Smeraldi 2004) and depressive disorders (Gardiner and Du 2006; Moreau et al. 1996; Ribases et al. 2008).

The present study is an extra recommendation to support current development of pharmacological strategies for the treatment of comorbid depression and CUD with drugs targeting the 5-HT_{2C} receptor. Treatments based on manipulations of 5-HT_{2C} receptors successfully inhibit the rewarding properties of cocaine as well as the reinstatement of seeking-behavior induced by conditional and unconditional stimuli. These results point to the special significance of 5-HT_{2C} receptor agonists as a potential pharmacotherapy against CUD.

Declarations

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Contribution:

JJ, MFr, – planned all behavioral and biochemical experiments, performed surgery, behavioral experiments and analyses, were involved in interpreting experimental data and wrote the manuscript.; AS, RP – performed surgery and behavioral experiments, collected raw data; JJ, IS, MHM – performed biochemical experiments; JJ, IS – performed analyses and interpreted data from biochemical experiments; EP – was involved in designing behavioral experiments, and participated in revising the

article; MF – was involved in designing and supervising behavioral and biochemical experiments and participated in writing and revising the manuscript.

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Figures

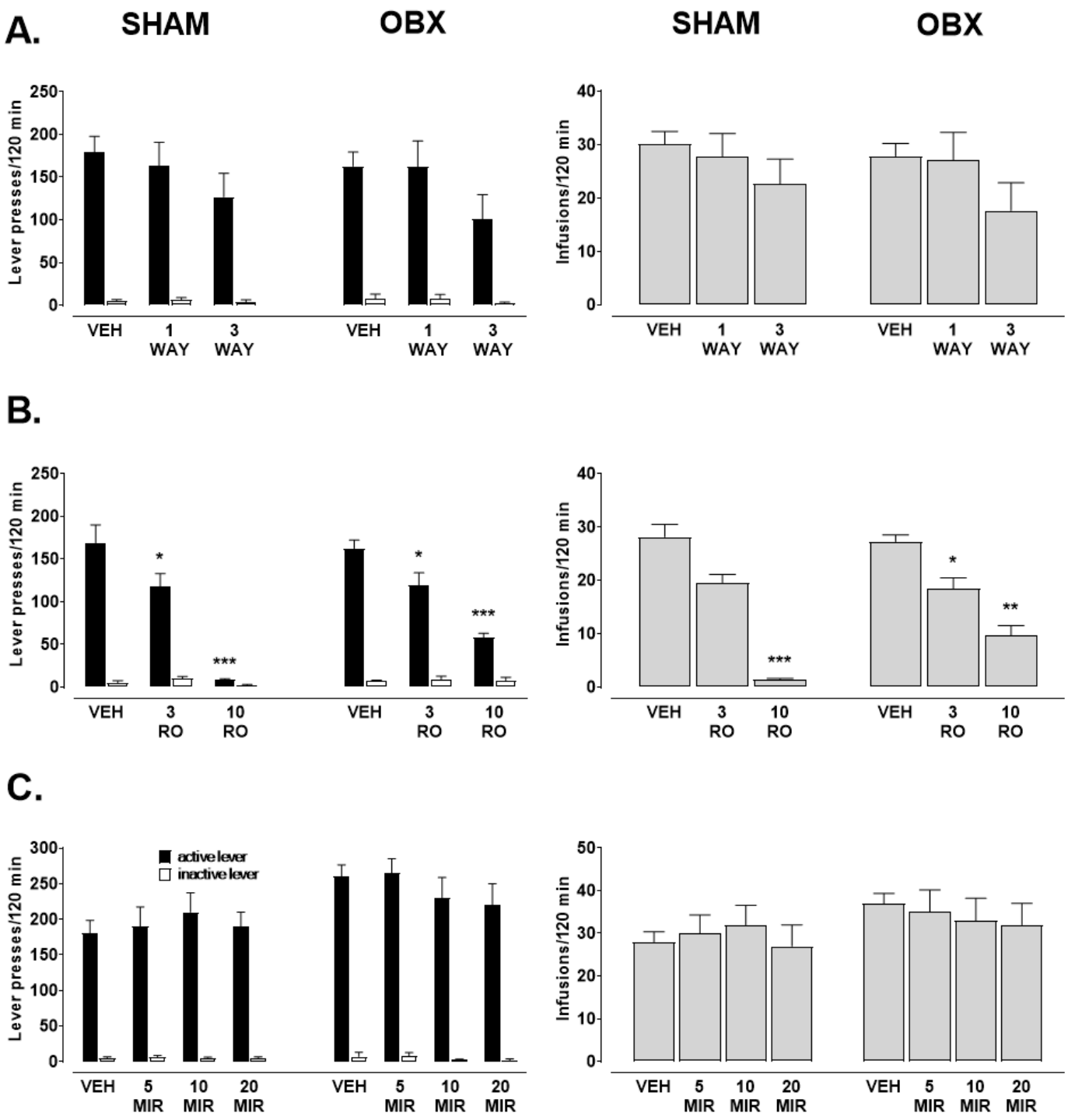

Figure 1

Effects of acute WAY 161503 (WAY; 1 and 3 mg/kg, i.p., **A**), RO 60-0175 (RO; 3 and 10 mg/kg, i.p., **B**) or mirtazapine (MIR, 5-20 mg/kg, i.p., **C**) treatment on the maintenance of cocaine (0.5 mg/kg/infusion) self-administration under the FR5 schedule of reinforcement in the bulbectomized (OBX) and control SHAM-operated rats. The numbers of active and inactive lever presses as well as cocaine infusions are expressed as the means (±SEM) of the data from 6 to 10 rats/group. VEH – vehicle. *p<0.05, **p<0.01, ***p<0.001 versus vehicle (VEH) (Newman-Keuls test).

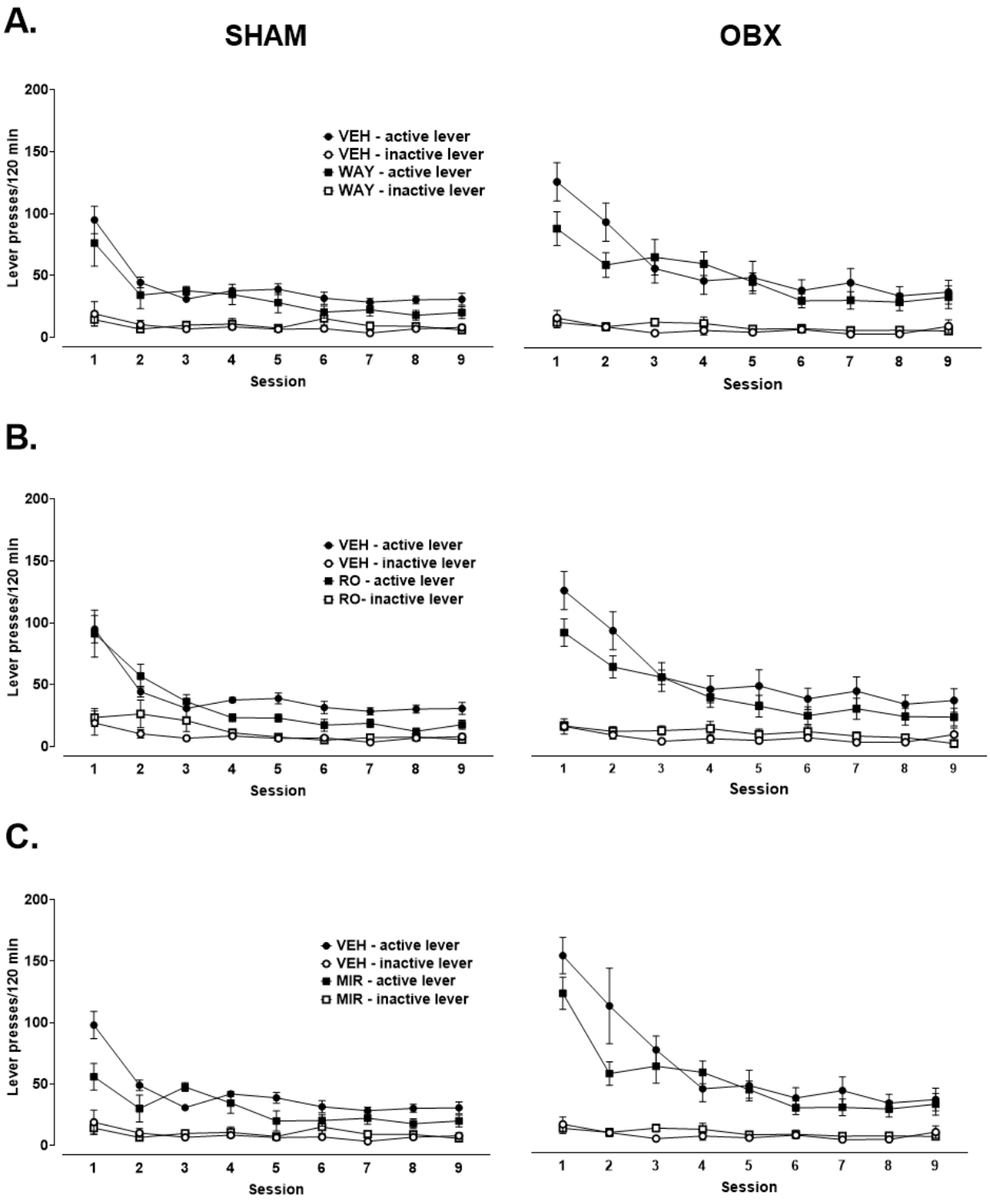

Figure 2

Effects of repeated treatment with WAY 161503 (WAY; 1 mg/kg, i.p., **A**), RO 60-0175 (RO; 1 mg/kg, i.p., **B**) and mirtazapine (MIR, 10 mg/kg, i.p., **C**) or the corresponding vehicle (VEH) injected daily during the

extinction training in the bulbectomized (OBX) and SHAM-operated rats. The results are expressed as the group means (±SEM) from 6 to 10 rats/group.

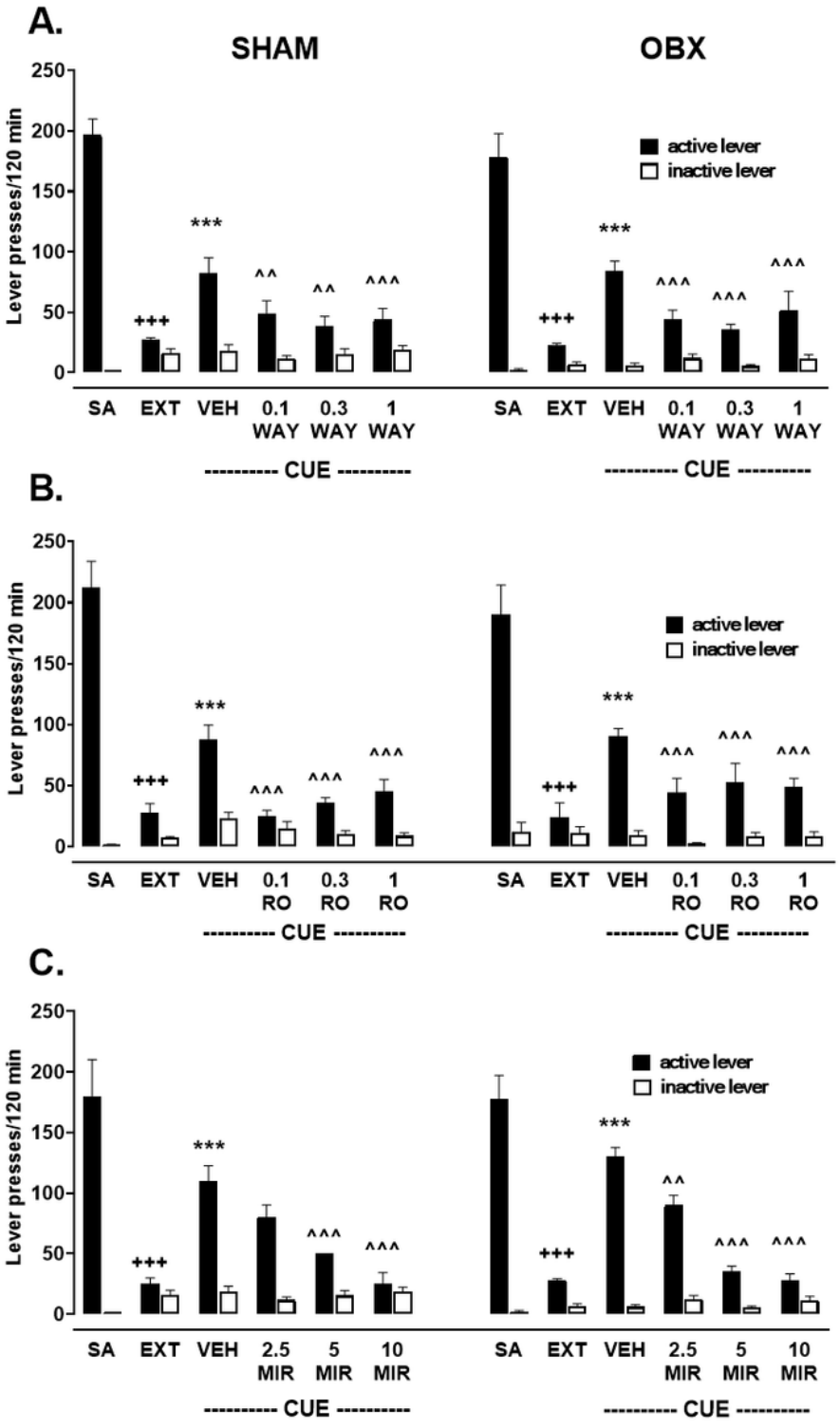

Figure 3

Effects of acute WAY 161503 (WAY; 0.1–1 mg/kg, i.p., **A**), RO 60-0175 (RO; 0.1–1 mg/kg, i.p., **B**) or mirtazapine (MIR, 2.5-10 mg/kg, i.p., **C**) treatment on the reinstatement of the cocaine-seeking behavior induced by the cue (CUE; tone + light) in the bulbectomized (OBX, right panels) and SHAM-operated rats (left panels). The numbers of active and inactive lever presses are expressed as the means (±SEM) of the data from 6 to 10 rats/group. ***p<0.001 versus extinction (EXT); ^^^p<0.001 versus vehicle (VEH); $^{+++}$p<0.001 versus cocaine self-administration (SA) (Newman-Keuls test).

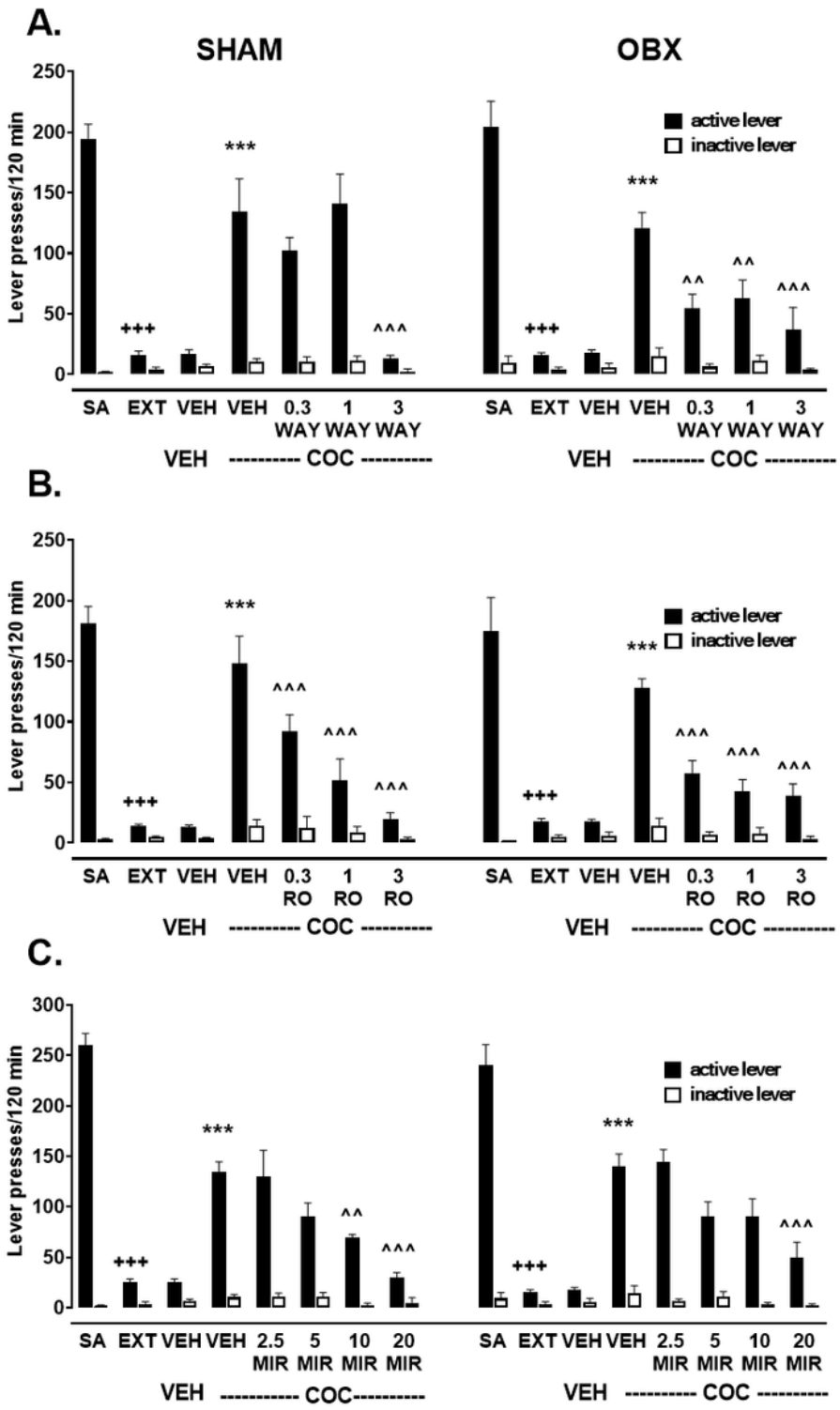

Figure 4

Effects of acute WAY 161503 (WAY; 0.3-3 mg/kg, i.p., **A**), RO 60-0175 (RO; 0.3-3 mg/kg, i.p., **B**) and mirtazapine (MIR, 2.5-20 mg/kg, i.p., **C**) or the corresponding vehicle (VEH) treatment on the cocaine-induced (COC, 10 mg/kg, i.p.) reinstatement of cocaine-seeking behavior in the bulbectomized (OBX, right panels) and SHAM-operated rats (SHAM, left panels). The numbers of active and inactive lever presses are expressed as the means (±SEM) of the data from 6 to 11 rats/group. ***p<0.001 versus extinction

(EXT); ^^p<0.01, ^^^p<0.01 versus VEH + COC; $^{+++}$p<0.001 versus cocaine self-administration (SA) (Newman-Keuls test).

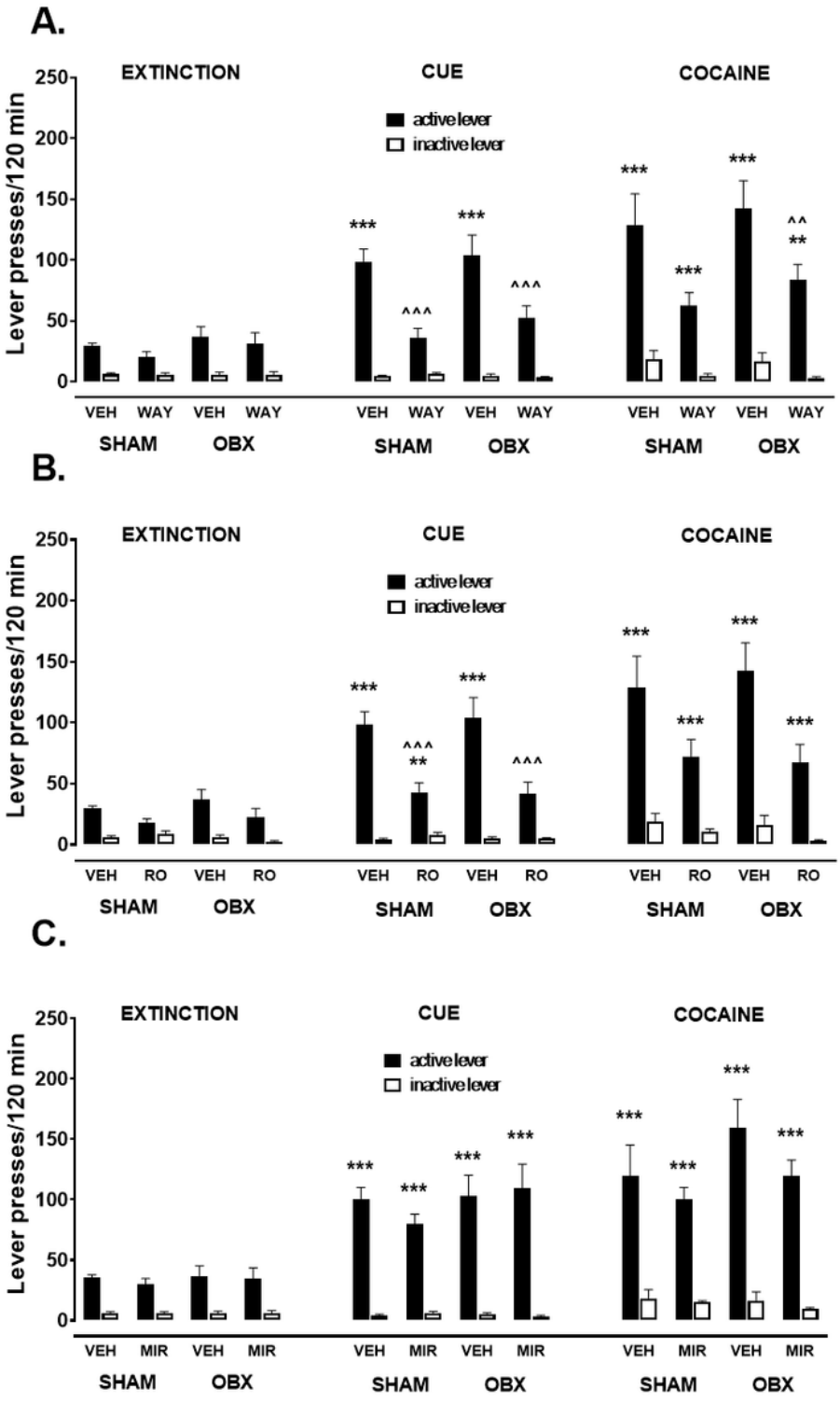

Figure 5

Effects of repeated WAY 161503 (WAY, **A**), RO 60-0175 (RO, **B**), mirtazapine (MIR, **C**) or corresponding vehicle (VEH) treatment during extinction training on the reinstatement of the cocaine-seeking behavior. MIR (10 mg/kg, i.p.), WAY (1 mg/kg, i.p.), RO (1 mg/kg, i.p.), or VEH were injected daily during the extinction training in the bulbectomized (OBX) and SHAM-operated control rats. The reinstatement of the cocaine-seeking behavior was initiated using the cue or cocaine (10 mg/kg, i.p.). The results are expressed as the group means (±SEM) from 6 to 10 rats/group. **p<0.01, ***p<0.001 versus corresponding group during extinction; ^^p<0.01, ^^^p<0.001 versus corresponding group treated with VEH during cue or cocaine relapse (Newman-Keuls test).

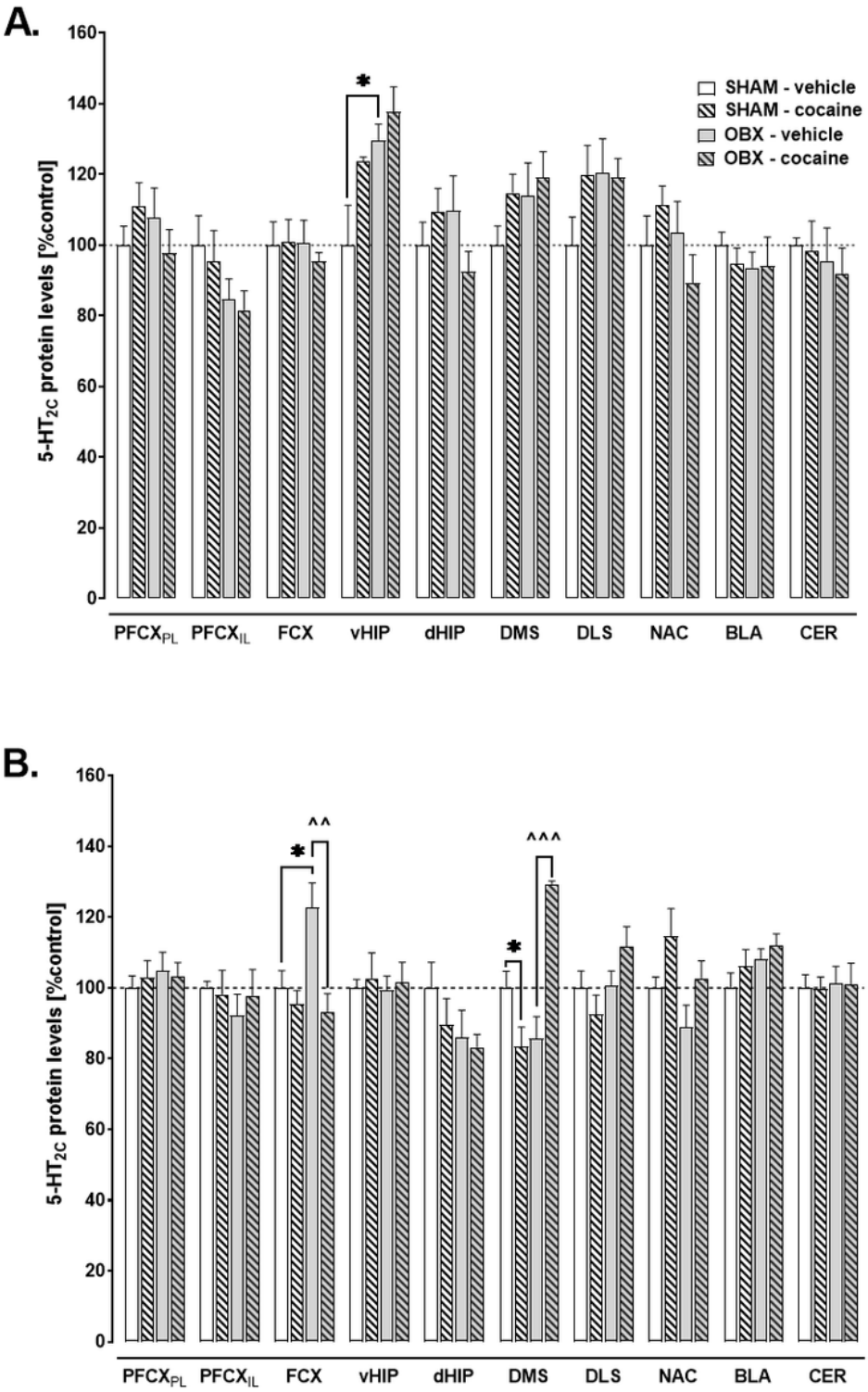

Figure 6

Changes in the expression of 5-HT_{2C} receptors in brain structures during cocaine self-administration in bulbectomized (OBX) and SHAM-operated rats that underwent extinction training. $PFCX_{IL}$ – infralimbic prefrontal cortex, $PFCX_{PL}$ – prelimbic prefrontal cortex, FCX -frontal cortex, vHIP – ventral hippocampus, dHIP – dorsal hippocampus, DLM – dorsolateral striatum, DMS – dorsomedial striatum, NAC – nucleus accumbens, BLA – basolateral amygdala, CER – cerebellum. All data are expressed as mean±SEM from

6 rats/group and presented as % of control. *p<0.05 versus SHAM-vehicle (control), **^^**p<0.01; **^^^**p<0.001 versus OBX-vehicle.