# CB2 agonist mitigates cocaine-induced reinstatement of place preference and modulates the inflammatory response in mice

Oualid Abboussi[a], Zmarak Ahmad Khan[b], Hind Ibork[a], Simo S. Zulu[c], William Daniels[d], Khalid Taghzouti[a] and Tim G. Hales[b]



**Chronic exposure to cocaine is known to have profound effects on the brain, leading to the dysregulation of inflammatory signalling pathways, the activation of microglia, and the manifestation of cognitive and motivational behavioural impairments. The endocannabinoid system has emerged as a potential mediator of cocaine's deleterious effects. In this study, we sought to investigate the therapeutic potential of the cannabinoid CB2 receptor agonist, JWH-133, in mitigating cocaine-induced inflammation and associated motivational behavioural alterations in an in vivo model. Our research uncovered compelling evidence that JWH-133, a selective CB2 receptor agonist, exerts a significant dampening effect on the reinstatement of cocaine-induced conditioned place preference. This effect was accompanied by notable changes in the neurobiological landscape. Specifically, JWH-133 administration was found to upregulate Δ-FOSB expression in the nucleus accumbens (Nac), elevate CX3CL1 levels in both the ventral tegmental area and prefrontal cortex (PFC), and concurrently reduce IL-1β expression in the PFC and NAc among cocaine-treated animals. These findings highlight the modulatory role of CB2 cannabinoid receptor activation in altering the reward-seeking behaviour induced by cocaine. Moreover, they shed light on the intricate interplay between the endocannabinoid system and cocaine-induced neurobiological changes, paving the way for potential therapeutic interventions targeting CB2 receptors in the context of cocaine addiction and associated behavioural deficits. *Behavioural Pharmacology* 35: 26–35 Copyright © 2023 Wolters Kluwer Health, Inc. All rights reserved.**

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## Introduction

Cocaine is one of the most misused psychostimulant drugs with 0.4% of the global adult population having used cocaine at least once a year. This presents a major public health crisis with a high economic and social burden (Hurst, 2019) . Cocaine misuse is mainly attributed to its effects on the dopaminergic system. Cocaine inhibits the presynaptic dopamine transporter, preventing dopamine (DA) reuptake thereby increasing synaptic dopamine concentrations in the mesocorticolimbic system (Ritz *et al*., 1987) . It has been shown that cocaine increases the activity of neurons in the ventral tegmental area (VTA), which is a midbrain nucleus that mainly consists of dopamine-containing cell bodies implicated in motivation and reward prediction that results in dynamic DA release into the nucleus accumbens (Nac) (Steinberg *et al*., 2013; Saddoris *et al*., 2015; Hamid *et al*., 2016). Cocaine activation of the reward pathway leads to progressive, allostatic neuroadaptations of the VTA and altered neuroplasticity which subsequently contributes to prolonged cocaine-seeking behaviour (Ahmed *et al*., 2002). These neuronal and behavioural adaptations were shown to be mediated, at least in part, by the accumulation in the Nac of Δ-FOSB, which is a transcription factor upregulated following chronic cocaine exposure (Kelz *et al*., 1999; Harris *et al*., 2007). Δ-FOSB acts as a 'molecular switch' in the induction of the long-term effects of drug use and is considered a reliable biomarker of addiction (Nestler *et al*., 2001).

The neuroplastic changes observed in cocaine addiction have been associated with increased neuroinflammation (Liao *et al*., 2016; Sil *et al*., 2019). For instance, post-mortem studies on human cocaine addicts revealed elevated microglial activation (Little *et al*., 2009). Also, i*n vivo* studies showed that cocaine-induced microglial activation increases the release of proinflammatory cytokines (IL-1β, IL-6 and TNF-α) and chemokines, in particular, the fractalkine CX3CL1. A study on cocaine-addicted patients found that plasma IL-1β and the chemokine CX3CL1 concentrations were directly correlated with the severity of cocaine misuse and dependence (Araos

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*et al.*, 2015). Montesinos *et al.* (2020) reported that elevated levels of IL-1β were associated with increased CX3CL1 concentrations in the hippocampus of cocaine-treated mice. CX3CL1 is highly expressed in neurons and astrocytes (Hatori *et al.*, 2002; Montesinos *et al.*, 2020) and seems to play a major role in inflammation-mediated addiction.

The endocannabinoid system is implicated in the development of cocaine addiction and several studies suggest that cannabinoids may modulate cocaine-induced behavioural and neurochemical changes (Arnold, 2005; Lopes *et al.*, 2020). Cannabinoid drugs exert their effects mainly through the modulation of cannabinoid-1 (CB1) and cannabinoid-2 (CB2) G-protein-coupled receptors with CB1 receptors being primarily expressed in axon terminals and CB2 mainly in glia (Bie *et al.*, 2018; Lutz, 2022). This distribution positions CB receptors strategically for a role in modulation of the neural and inflammatory signalling (Rom and Persidsky, 2013).

Previous research has indicated that CB2 agonists, such as JWH-133 and β-caryophyllene, exert a regulatory influence on various aspects of cocaine action, including its reward-related behaviours and neurobiological alterations, underscoring the significance of exploring the neuronal CB2 receptor mechanism as a potential mediator of cocaine's effects (Xi *et al.*, 2011; dos Santos Barbosa *et al.*, 2023)

The stimulation of CB receptors has been shown to diminish the release of pro-inflammatory cytokines, iNOS and ROS via NF-κB pathway inhibition and to protect neurons from excitotoxicity (Donadelli *et al.*, 2011; Merighi *et al.*, 2012; dos-Santos-Pereira *et al.*, 2020). Additionally, cannabinoids induce an anti-inflammatory response, modulating CX3CL1 expression in astrocytes, and subsequently attenuating cocaine-induced rewarding effects (Linker *et al.*, 2020).

Therefore, the present study aimed to evaluate the potential of the CB2 agonist, JWH-133, to attenuate cocaine-induced behavioural changes, including reward-seeking behaviour, memory deficits, and locomotor stimulation, while also investigating the possible underlying molecular changes in Δ-FOSB, CX3CL1, and IL-1β expression.

## Methods

### Subjects

A total of 96 male C57BL/6 J mice (21 – 30 g, 7–8 weeks) were obtained from the central animal care facility of the Faculty of Sciences in Rabat, Morocco, and they were housed in groups of eight for 1 week in large transparent polycarbonate cages (38.2 × 22 cm surface × 15 cm height) on a 12-h-light/-dark cycle with unlimited access to food and water. After a 1-week acclimation period and until the end of experimentation, the 35-day-old mice were randomly assigned to two groups: (i) a cocaine group receiving cocaine hydrochloride of 89% purity (U. S. Pharmacopeia, CAS 53-21-4), dissolved in an isotonic saline solution (0.9% NaCl) and injected at a dose of 10 mg/kg in a volume of 10 mL/kg of body weight (i.p.); and (ii) a control group receiving saline (0.9% NaCl). All the experiments were conducted in the light phase, between 08:00 and 16:00, in an isolated, sound-attenuated room. Animal care and experimental procedures were conducted according to the guidelines from The European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Mohammed V University Animal Ethics Committee (Approval numbers: ESA19/107, ESE20/183).

### Conditioned place preference and reinstatement paradigm

The conditioned place preference (CPP) paradigm is used to observe the rewarding effects of drugs, where neutral contextual stimuli gain secondary reinforcing properties of the drug(Tzschentke, 2007).

The CPP apparatus consisted of a Perspex box separated into three distinct chambers: two main chambers (Chambers A and B) and one neutral chamber (Chamber C). Chambers A and B had different wall markings (horizontal vs. vertical black and white stripes) and flooring (grid rods vs. wire mesh). During all sessions, the mice were tracked using AnyMaze software (Stoelting Co) to record and analyse the time spent in each compartment. The CPP procedure consisted of three phases: habituation, conditioning, and testing. Extinction training and reinstatement followed the CPP procedure.

#### *Habituation (day 1)*

Mice were placed in the neutral chamber, with free access to both main chambers, and they were allowed to explore the apparatus for 15 min. Observation of the time spent in each chamber gave a 'pre-test' result. Animals that had a spontaneous individual preference for either of the main chambers (>65% of total time spent in one chamber) were excluded from the experiment. Thus, the cohort of mice used had no significant preference or aversion for either compartment, resulting in an unbiased place conditioning procedure (Tzschentke, 2007).

#### *Conditioning (days 2–11)*

The place conditioning lasted 10 days with morning and afternoon sessions. During the morning session of each day in this phase, all mice were injected with saline (0.9% i.p.) and were confined to the allocated chamber for 30 min. During the afternoon session, mice in the cocaine group were injected with cocaine (10 mg/kg i.p.), and mice in the saline group were again injected with saline (0.9% i.p.) and were confined to the opposite chamber during the morning session for 30 min. The cocaine conditioning dosage of 10 mg/kg was used based on pilot experiments investigating the dose sufficient to produce

CPP, and the dose and length of days for conditioning in C57BL/6 J mice were supported in the previous literature (Orsini *et al*., 2005). Drug compartment pairings were counterbalanced with even distribution of compartment allocations.

***Testing (day 12)***

24 h after the last conditioning session, mice were once again placed in the neutral chamber and had free access to both main chambers of the apparatus for 15 minutes. Observation of the time spent in each chamber gave a 'post-test' result. The difference in the 'pre-test' and 'post-test' results may be regarded as an index of drug-reward behaviour.

***Extinction (days 13–23)***

The extinction training began 24 h after the 'post-test' session. For the first 5 days of extinction training, the mice were placed in the neutral chamber and were allowed free access to both main chambers for 15 min per day with no cocaine or saline injections. This was followed by 5 days of daily injections of saline (0.9%) or JWH-133 at a dose of 10 mg/kg i.p in the animals' home cages. The dose of JWH-133 was determined in a pilot experiment to cause a significant decrease in cocaine consumption, and so this dose was used throughout. This dose of JWH-133 has been used to the same effect in a previous study (Xi *et al*., 2011). The extinction training was stopped when there was no longer preference for the cocaine-paired side.

***Reinstatement (day 24)***

Mice were injected with saline or cocaine (5 mg/kg i.p.) to test if the previous chamber preference could be reinstated. The group of mice receiving saline during conditioning and extinction were injected with saline during the reinstatement test to act as controls. The 5 mg/kg priming dose of cocaine was used following evidence from literature and in a pilot experiment investigating the dosage of cocaine sufficient to reinstate cocaine-induced CPP (Itzhak and Martin, 2002; Lu *et al*., 2004).

**Novel object recognition test**

The NOR assay studies the ability of rodents to recognise a novel object in a familiar environment and is a test of episodic-like memory (Antunes and Biala, 2011); (Cohen and Stackman Jr., 2015). This task evaluates the ability of the animal to discriminate between a novel and familiar object. The general apparatus used was the same as what would be used in an open-field test. Light levels were adjusted to 15 lux to avoid any stress related to bright light. Objects of various shapes were used ranging from 10 × 9 × 8 cm to 12 × 10 × 13 cm. The objects and apparatus were cleaned using 70% ethanol between trials to eliminate olfactory stimuli. The objects were randomised between each mouse and each group tested. The duration of approaching (<1 cm distance), touching, or sniffing the objects was scored. The discrimination index was calculated using the following: (time spent exploring the novel object – time spent exploring the familiar object)/total exploration time (Antunes and Biala, 2012). The NOR assay took place over 3 days and phases: familiarisation, acquisition, and retention.

***Familiarisation (day 25)***

The animals were placed in the apparatus and were allowed to explore it for 5 min to become familiarised with the environment.

***Acquisition (day 26)***

Two objects (A1 and A2) were placed 17 cm from the sidewall of the apparatus in diagonal corners opposing each other. The mouse was gently placed in the corner of the apparatus facing the objects. The mouse was allowed to explore the objects freely for 10 min then it was removed from the apparatus and returned to its home cage.

***Retention (day 27)***

Twenty-four hours after the acquisition session, the mouse was returned to the NOR apparatus containing one familiar object from the acquisition session and one new object. The mouse was then allowed 5 min of exploration. During this stage, locomotor activity was also measured.

The duration of exploring each object and the locomotor activity of the mice were tracked using AnyMaze software (Stoelting Co).

**Western blot analysis**

Proteomic analysis was performed using Western blots to quantify the relative protein expression of Δ-FOSB, CX3CL1 and IL-1β. 24 h after the 'retention' session of the NOR test, the animals were sacrificed by cervical dislocation. Brain tissues were harvested and lysed in RIPA lysis buffer (w/v) (Sigma). Lysates were homogenised and centrifuged at 3500*g* for 10 min at 4°C. The supernatants were extracted, and their protein concentrations were determined using the Bradford assay method (Kruger, 2009). The samples were denatured for 5 minutes at 95°C. Equal amounts of protein (20 µg) were resolved by electrophoresis on a 10% sodium dodecyl-sulfate-polyacrylamide gel in a running buffer (Bio-Rad) at 200 V for 1 h. Proteins were transferred onto a nitrocellulose membrane using Trans-Blot Turbo (Bio-Rad). The membrane was blocked with blocking buffer (Li-COR) for 2 h, thereafter, the membrane was incubated with one of the following antibodies and left to incubate overnight: anti-Δ-FOSB mAb (1:200; sc-398595 Santa Cruz Biotechnology); Anti-CX3CL1 mAb (1:1000; ab-25088 Abcam); Anti-IL-1β antibody (1:500; ab-9722 Abcam) or antibody against β-ACTIN (1:5000 ab-8226 Abcam). The membrane was washed three times with 0.1M PBS-Tween for 10 min. Secondary antibodies

IRDye 800CW goat anti-rabbit and IRDye 680RD goat anti-mouse (LI-COR) (1:10000 diluted in blocking buffer) were added to the membrane and incubated for 2 h at room temperature. The membrane was washed three times with 0.1M PBS-Tween and washed once with PBS. The membrane was viewed on an Odyssey CLx LI-COR infrared fluorescence imaging system (Biosciences). The intensities of signals on the blots were normalised to β-ACTIN in a single channel using the LI-COR Odyssey Image Studio software (LI-COR Biosciences) (Fig. 1).

### Statistical analysis

Analysis was performed using the statistical package GraphPad Prism 8 (GraphPad Software, USA). Data normality was tested using the Shapiro-Wilk test. Comparisons of the NOR results and Δ-FOS B, IL-1β, TNF-α and CX3CL1 expression between groups at a single time point were analysed for statistical significance using two-way ANOVA with Bonferroni *post-hoc* test when appropriate. Two-way repeated measures ANOVA was used to analyse CPP data over time (pre-test vs. post-test). The level of significance was set at $P < 0.05$ and data were expressed as mean ± SEM.

## Results

### Cocaine evoked place preference

Data were analysed using two-way repeated measures ANOVA revealing a significant effect of treatment ($F_{(1, 54)} = 28.34$, $P < 0.001$), time (pre-test vs. post-test) ($F_{(1, 54)} = 34.62$, $P < 0.001$) and a significant interaction between these two factors ($F_{(1, 54)} = 31.75$, $P < 0.001$). Bonferroni's *post-hoc* analysis showed a significant increase in the time spent in the cocaine-associated chamber in animals treated with cocaine in comparison to animals treated with saline ($P < 0.05$) (Fig. 2).

### JWH-33 prevents cocaine-induced reinstatement of place preference.

The CPP pre-reinstatement test revealed a successful extinction of cocaine-induced CPP, as neither cocaine (10 mg/kg) conditioning or subsequent JWH-133 (10 mg/kg) treatment had an effect on the time spent by the animals in the cocaine-associated chamber ($F_{(1, 52)} = 0.09$, NS; $F_{(1, 52)} = 0.73$, NS, respectively, Fig. 3a). However, administration of cocaine at a dose of 5 mg/Kg induced CPP reinstatement in mice preconditioned with cocaine (10 mg/kg) ($F_{(1, 28)} = 6.32$, $P < 0.05$) as revealed by two-way ANOVA analysis. This cocaine-induced CPP-reinstatement effect was significantly attenuated by JWH-133 (10 mg/kg) treatment ($F_{(1, 28)} = 2.79$, $P < 0.01$, Fig. 3b).

### Cocaine and JWH-133 have no effect on episodic-like memory

Data analysis showed no significant effect of either cocaine-conditioning ($F_{(1, 28)} = 0.01$, NS) or of JWH-133 treatment ($F_{(1, 28)} = 2.45$, NS) or an interaction ($F_{(1, 28)} = 0.13$, NS) on novel object recognition as revealed by two-way ANOVA analysis (Fig. 4).

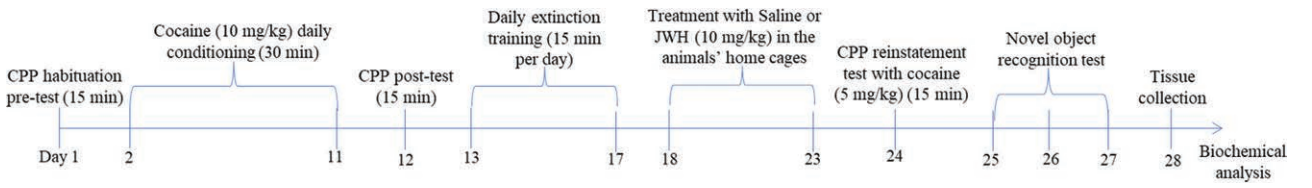
### Effect of cocaine and JWH-133 on locomotor activity during reinstatement of CPP

Injection of a priming dose of cocaine (5 mg/kg) following extinction of CPP causes locomotor stimulation. Two-way ANOVA analysis demonstrated a significant effect of cocaine exposure (cocaine vs. saline) ($F_{(1, 28)} = 14.67$, $P < 0.001$), no effect of JWH-133 ($F_{(1, 28)} = 1.02$, NS) and a significant interaction ($F_{(1, 28)} = 7.05$, $P < 0.05$) on total distance travelled (Fig. 5). Bonferroni's multiple comparisons test revealed that cocaine (5 mg/kg) stimulates locomotor activity more in animals preconditioned with cocaine in comparison to the control group ($P < 0.001$). This effect was successfully prevented by a pre-treatment with JWH-133 ( (Fig. 5). It is noteworthy that cocaine (i.p.) at a dose of 5 mg/kg does not affect locomotor activity in cocaine-naïve mice (Tang *et al*., 2005; Tilley *et al*., 2007; Harraz *et al*., 2021).

### Cocaine conditioning and JWH-133 treatment modulate Δ-FOSB expression in the nucleus accumbens

Two-way ANOVA analysis revealed a main effect of cocaine (5 mg/kg) administered to induce CPP reinstatement on Δ-FOSB expression ($F_{(1, 12)} = 8.77$, $P < 0.05$), no effect of JWH-133 ($F_{(1, 12)} = 0.89$, NS) and a significant interaction between these two factors ($F_{(1, 12)} = 6.84$, $P < 0.05$) in animals pre-treated with cocaine during the conditioning stage (Fig. 6). Acute administration

**Fig. 1**




Schematic representation of the overall experimental design.

of cocaine at a dose of 5 mg/kg significantly increased Δ-FOSB expression in the Nac in animals pre-treated with saline and not in animals who received JWH-133 (10 mg/kg) when compared to the control group that received only saline ($P = 0.01$, $P > 0.5$, respectively) (Fig. 6).

### Cocaine and JWH-133 treatment modulate CX3CL1 expression in the reward system of mice

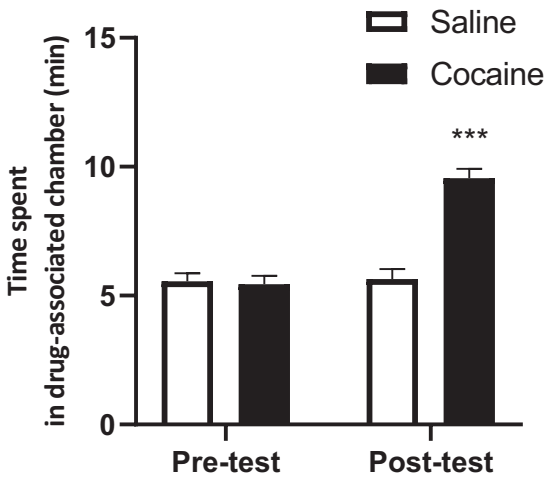
Two way-ANOVA analysis revealed a main effect of the subsequent administration of cocaine (5 mg/kg) on CX3CL1 expression in the VTA ($F_{(1, 28)} = 16.67$, $P < 0.001$, Fig. 7a), the prefrontal cortex (PFC) ($F_{(1, 28)} = 15.91$, $P < 0.001$, Fig. 7b) but not in the Nac ($F_{(1, 28)} = 2.27$, NS, Fig. 7c). Similarly, JWH-133 treatment had a significant effect in the VTA ($F_{(1, 28)} = 21.32$, $P < 0.001$, Fig. 7a) and the PFC ($F_{(1, 28)} = 5.08$, $P < 0.05$, Fig. 7b) but had no effect on CX3CL1 expression in the Nac ($F_{(1, 28)} = 2.27$, NS, Fig. 7c). Interaction between cocaine (5 mg/kg) and JWH-133 treatment was found only in the PFC ($F_{(1, 28)} = 10.16$, $P < 0.01$, Fig. 7b), but neither in the VTA ($F_{(1, 28)} = 0.29$, NS, Fig. 7a) nor the Nac ($F_{(1, 28)} = 2.41$, NS, Fig. 7a) of animals conditioned with cocaine (10 mg/kg).

Bonferroni *post-hoc* comparisons show that cocaine (5 mg/kg) administered to induce CPP reinstatement in cocaine (10 mg/kg)-conditioned animals significantly decreased CX3CL1 expression in the PFC in comparison to animals treated with saline ($P < 0.001$, Fig. 7b). However, JWH-133 treatment prevented this effect, as it increased CX3CL1 expression in the PFC in comparison to animals who received saline ($P < 0.01$, Fig. 7b).

### Cocaine and JWH-133 treatment modulate IL-1β expression in the reward system of mice

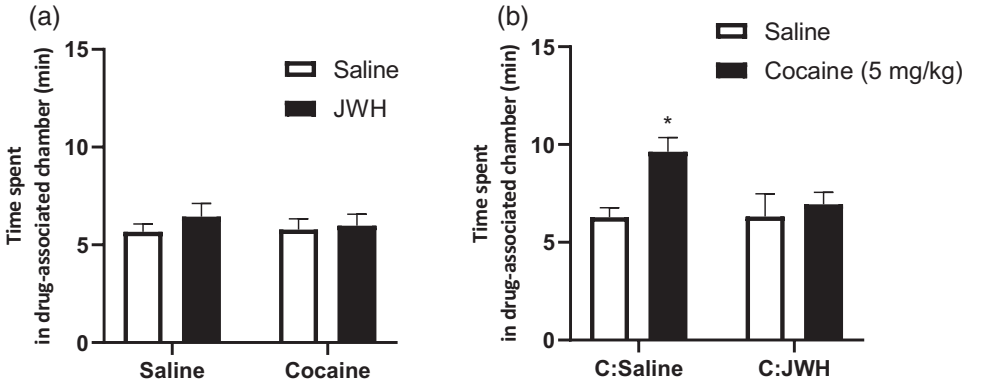
The administration of cocaine (5 mg/kg) following cocaine conditioning (10 mg/kg) significantly affected IL-1β expression in the PFC ($F_{(1, 28)} = 7.15$, $P < 0.05$) and the Nac ($F_{(1, 28)} = 7.84$, $P < 0.01$) but had no impact on the VTA ($F_{(1, 28)} = 0.24$, NS). JWH-133 also had a significant effect in the PFC ($F_{(1, 28)} = 4.30$, $P < 0.05$) and the Nac ($F_{(1, 28)} = 18.94$, $P < 0.001$) but not in the VTA ($F_{(1, 28)} = 0.17$, NS) (Fig. 8). A significant interaction between the acute administration of cocaine (5 mg/kg) and JWH-133 treatment was only observed in the Nac when analysing the data with a two-way ANOVA ($F_{(1, 28)} = 10.66$, $P < 0.01$) but not in the PFC ($F_{(1, 28)} = 3.396$,

**Fig. 2**



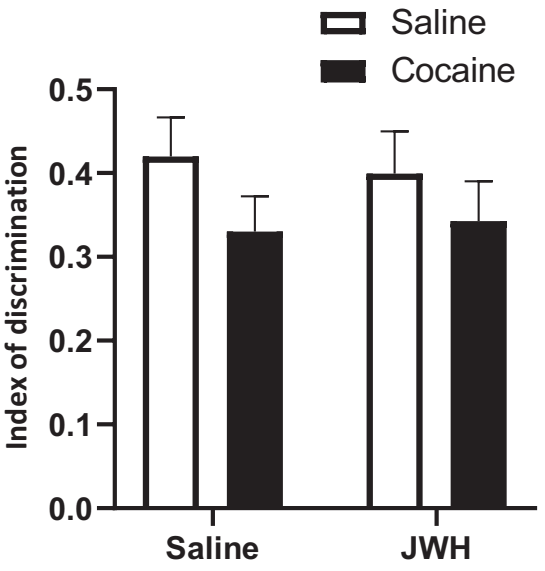

Cocaine evoked place preference. Place preference in mice expressed as the time spent in the drug-associated chamber in pre-test and post-test after 10 days of saline (0.9%) or cocaine (10 mg/kg) conditioning (n = 24 in the control group and n = 32 in the cocaine group). ***$P < 0.001$ vs. cocaine during the pre-test (Bonferroni *post-hoc* test). Data presented as mean ± SEM.

**Fig. 3**



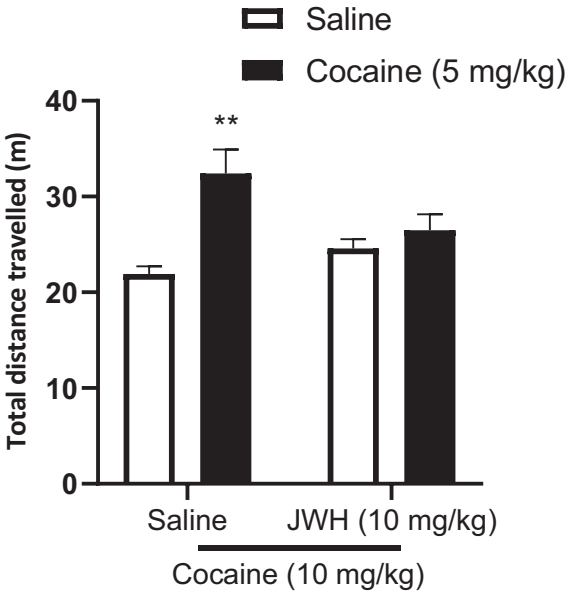

Extinction and reinstatement of cocaine (10 mg/kg) induced conditioned place preference. (a) Time spent in each chamber by mice that received saline/saline (n = 12) or saline/JWH (n = 12) and cocaine/saline (n = 16) or cocaine/JWH (n = 16). (b) Reinstatement by a priming injection of cocaine (5 mg/kg) in animals treated with JWH-133 (10 mg/kg). Time spent in each chamber by mice receiving saline/saline (n = 8) or saline/JWH (n = 8). *$P < 0.001$ vs. saline/saline (Bonferroni *post-hoc* test). Data presented as mean ± SEM.

**Fig. 4**




Effects of cocaine and subsequent acute intraperitoneal administration of JWH-133 on episodic-like memory. Data are presented as mean ± SEM (n = 8 per group). No significant effect of JWH-133 using two-way ANOVA and Bonferroni *post-hoc* test.

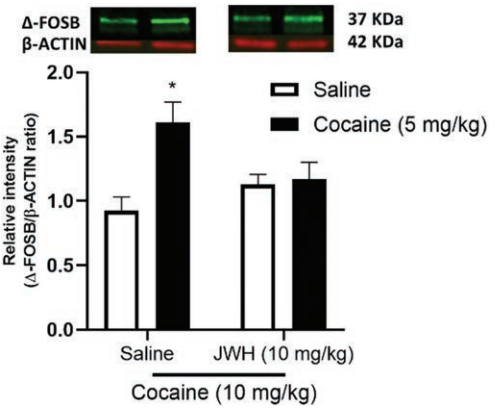
**Fig. 5**




Locomotor stimulation by cocaine during reinstatement of CPP is abolished by JWH-133. Acute injection of cocaine (5 mg/kg) stimulated locomotor activity during reinstatement of CPP in mice administered saline (0.9%), but not those administered JWH-133 (10 mg/kg). Data are presented as mean ± SEM. $^{**}P < 0.05$ in comparison to the saline/saline group. Bonferroni *post-hoc* test (n = 8 per group).

NS) or the VTA ($F_{(1, 28)} = 0.042$, NS). Bonferroni post-hoc analysis revealed a significant increase in IL-1β expression in the Nac following CPP-reinstatement with

**Fig. 6**




Cocaine-evoked enhancement of Δ-FosB in the nucleus accumbens is abolished by CB2 receptor antagonism. Effects of acute administration of cocaine (5 mg/kg) on Δ-FosB expression in the Nac in animals treated with cocaine during the conditioning period and injected with either JWH-133 (10 mg/kg) or saline during the extinction period. Data are presented as mean ± SEM, $^{*}P < 0.05$ in comparison to the saline/saline group. Bonferroni *post-hoc* test (n = 8 per group).

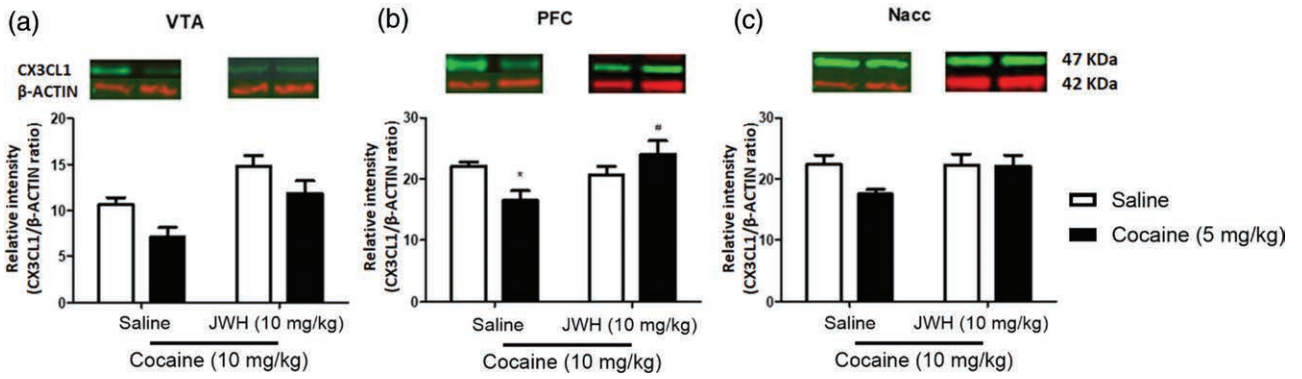
a 5 mg/kg dose of cocaine, compared to the saline group ($P < 0.01$) in animals conditioned with a 10 mg/kg dose of cocaine. This effect induced by the acute administration of cocaine (5 mg/kg) was significantly attenuated by JWH-133 treatment ($P < 0.001$). However, levels of IL-1β did not change following JWH-133 treatment in the animals that received saline instead of cocaine (5 mg/kg) during the reinstatement of cocaine-induced CPP when compared to saline-administered mice (NS) (Fig. 8).

## Discussion

This study aimed to investigate the modulatory effect of a selective CB2 receptor agonist, JWH-133, on the neurochemical and behavioural changes induced by cocaine in mice. Our results showed that JWH-133 attenuated the reinstatement of cocaine-induced CPP in mice without affecting their episodic memory. Additionally, JWH-133 increased Δ-FOSB expression in the Nac, elevated CX3CL1 levels in the VTA and PFC, and reduced IL-1β levels in the PFC and Nac in cocaine-treated animals. While CB2 receptors were initially believed to be predominantly expressed in peripheral tissues, recent studies have demonstrated their presence in the central nervous system, particularly in the mesocorticolimbic dopamine pathway, in both glial and neuronal cells, and their functional modulation of motivational and motor behaviour (Zhang *et al.*, 2014; Jordan and Xi, 2019). Consequently, CB2 receptor agonists have been suggested as potential treatments for drug addiction, relapse, and cravings (Onaivi *et al.*, 2008; Yang *et al.*, 2012).

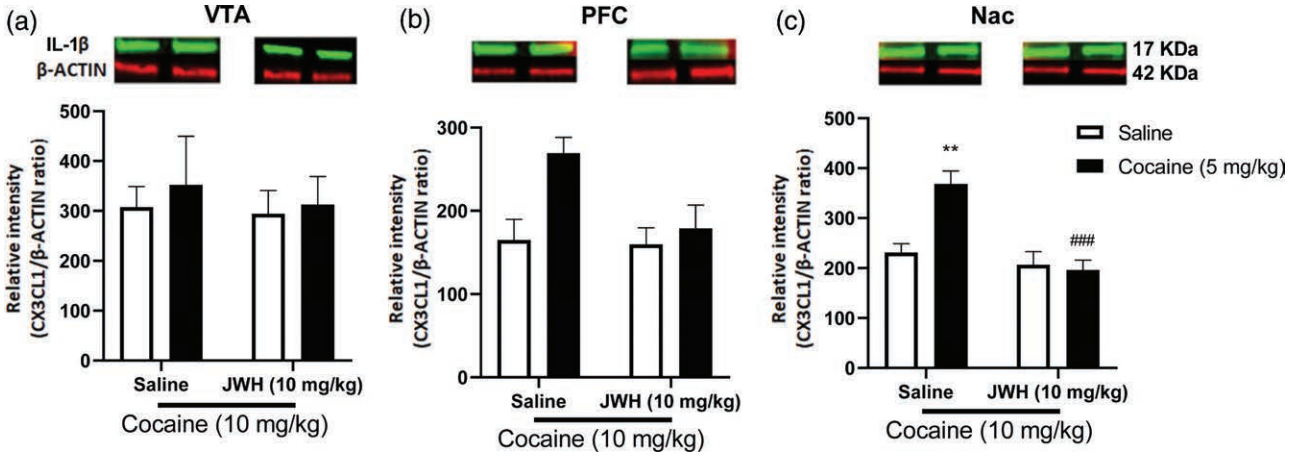
In our experiments, cocaine produced a robust preference for the drug-paired compartment and, at a lower dose, evoked reinstatement of CPP after

**Fig. 7**




JWH-133 prevents CX3CL1 downregulation in the reward system following CPP-reinstatement with cocaine. Cocaine (5 mg/kg) reduced CX3CL1 expression in A, the ventral tegmental area (VTA), B, the prefrontal cortex (PFC) and C, in the nucleus accumbens (Nac) in animals conditioned with cocaine (10 mg/kg). This effect was significant in the PFC. Data are presented as mean ± SEM, $^{*}P < 0.05$ in comparison to Saline/Saline group; $\#P < 0.05$ in comparison to Saline/cocaine group. Bonferroni post-hoc test (n = 8 per group).

**Fig. 8**




JWH-133 prevents cocaine-induced upregulation of IL-1β expression in the reward system following CPP-reinstatement with cocaine (5 mg/kg) in animals conditioned with cocaine (10 mg/kg). The impact of cocaine (5 mg/kg) treatment on IL-1β expression A, in the ventral tegmental area (VTA), B, in the prefrontal cortex (PFC) and C, in the nucleus accumbens (Nac). Data are presented as mean ± SEM, $^{**}P < 0.01$ in comparison to saline/saline group; $^{\#\#\#}P < 0.001$ in comparison to C: Saline/Cocaine group. Bonferroni post-hoc test (n = 8 per group).

extinction, corroborating earlier studies (Shippenberg and Heidbreder, 1995; Ródenas-González *et al*., 2021). Cocaine-induced CPP reinstatement was inhibited by JWH-133 at a dose of 10 mg/kg which abolished both chamber preference and cocaine-induced locomotor activity. We should note that JWH-133 had no effect on locomotion in the absence of cocaine, which excludes the possibility of an unspecific inhibitory effect of JWH-133 on locomotion. These data extend previous studies that showed that activation of CB2 receptors by JWH-133 (Delis *et al*., 2016) or β-caryophyllene (Al Mansouri *et al*., 2014) reduces the reinforcing effect of cocaine in the CPP. Notably, it has been demonstrated that JWH-133 administered systemically or directly infused into the Nac decreases cocaine-elevated dopamine in rodent Nac (Xi *et al*., 2011; Zhang *et al*., 2017). These JWH-133 effects appear to depend on the activation of the CB2 receptors; as they were inhibited by pre-administration of AM630, a CB2 antagonist (Galaj *et al*., 2020). Accordingly, electrophysiological evidence of brain CB2 receptors modulation *ex vivo* and *in vivo* showed that CB2 receptor agonists may reduce mesocorticolimbic dopaminergic neurone firing (Zhang *et al*., 2017, 2019). Thus, the effects of JWH-133 on the mesocorticolimbic dopaminergic function could explain the general effects of JWH-133 on the inhibition of CPP reinstatement with cocaine and on cocaine-evoked locomotor activity and give further support to the presence of functional CB2 receptors in the brain.

Given that CPP relies on preserved neural circuits responsible for declarative memory, and considering the involvement of the endocannabinoid system in memory consolidation, contextual memory, and spatial learning as previously reported (Cheer *et al*., 2000; Prus *et al*., 2009; Huston *et al*., 2013; Lopes *et al*., 2020), we examined the cognitive effect of JWH-133. Our results showed that cocaine (10 mg/kg) conditioning followed by extinction and cocaine (5 mg/Kg) reinstatement did not alter the performance of mice in the novel object recognition test. In contrast, previous studies showed that prolonged cocaine consumption causes deficits in object recognition memory in rats (Briand *et al*., 2008). The difference in results may have been due to the cocaine conditioning period being shorter in our study, insufficient to cause significant long-term changes in memory. Alternatively, the long extinction period (10 days), may have negated any short-term effects of cocaine conditioning on episodic-like memory. Similarly, treatment with the JWH-133 had no effect on the episodic-like memory which indicates that the prevention of reinstatement of cocaine-induced CPP by the CB2 agonist was not due to an altered cognitive function. Also, the apparent lack of a JWH-133 cognitive effect does not depend on altered motor activity since it did not reduce spontaneous locomotion. Therefore, the effect of the CB2 agonist was likely specific to the pathways involved in the cocaine reinforcement.

On the other hand, JWH-133 prevented the increase in Δ-FOSB in the Nac mirroring its preventive effect of cocaine-induced reinstatement, as elevated expression of Δ-FOSB is necessary for plasticity changes in the Nac and subsequent cocaine-related behaviour. Although this result does not necessarily imply that the Nac is the direct site of action of this drug, it strengthens the case for the CB2 agonist attenuating the specific neurochemical changes induced by cocaine, as Δ-FOSB is considered a 'master control protein' in the development and maintenance of addiction (Robison and Nestler, 2011). Previous studies show that increased expression of Δ-FOSB in transgenic mice is associated with increased response to the rewarding and locomotor-activating effects of cocaine ((Iris Chen *et al*., 2011 ; Kelz *et al*., 1999). However, it is important to note that the effect of cannabinoid drugs on cocaine-naïve mice was not measured in our study, so it could be that CB2 activation decreases Δ-FOSB expression in the Nac regardless of prior cocaine exposure.

As CX3CL1 expression may have a neuroprotective role, its decrease in cocaine conditioning and reversal with JWH-133 treatment could be an underlying mechanism by which CB2 agonism had its behavioural effects. Relevant studies suggest that cocaine exerts its behavioural stimulant effects by eliciting neural autophagy *in vitro* and *in vivo* and showed that autophagy inhibition impairs cocaine-CPP in mice (Guo *et al*., 2015; Harraz *et al*., 2021; Kim *et al*., 2021). Cocaine-induced autophagy degrades transporters for dopamine in the Nac (Harraz *et al*., 2021). Moreover, it has been shown that autophagy could alter CX3CL1 expression by neurons and consequently change microglial inflammatory activity (Su *et al*., 2016; He *et al*., 2019). This suggests that the JWH-133 effect might also be attributed to its downregulation of autophagy (Angelina *et al*., 2022). Unfortunately, most studies on the implication of the endocannabinoid system in autophagy have been performed in tumoral models (Lee *et al*., 2021; Angelina *et al*., 2022). Thus, further studies are needed to establish how cannabinoidergic signalling is implicated in autophagy in models of cocaine-induced toxicity and behavioural changes.

CX3CL1 acts as a 'find-me' signal released by neurons undergoing apoptosis and autophagy(Sokolowski *et al*., 2014) . Modulation of CX3CL1 expression alters the effects of cocaine, as this chemokine increases microglial clearance of damaged neurons and upregulates antioxidant enzymes (such as heme oxygenase-1) within microglia (Noda *et al*., 2011). Montesinos *et al*. (2020) showed that repeated cocaine treatments in mice had a structure-related effect; it increases CX3CL1 expression in the hippocampus and decreases the expression in the PFC and striatum, hence, highlighting the complex effects of cocaine on CX3CL1 in different brain regions. CX3CL1 is expressed in two forms: a transmembrane-anchored protein and a soluble isoform. It is the soluble isoform of this protein that ameliorates microglial activation and proinflammatory cytokine release (Morganti *et al*., 2012). In the present study, a potential limitation was that the distinct isoforms of CX3CL1 were not separately assessed.

Cocaine conditioning also increased IL-1β expression, which was also reversed by CB2 agonism. Cocaine may be causing neuroinflammation by preventing clearance of apoptotic neurons due to downregulation of CX3CL1, promoting the microglial release of pro-inflammatory cytokines and limiting antioxidant production. However, there is a lack of literature investigating the effects of cocaine on CX3CL1 signalling (Montesinos *et al*., 2020). Previous studies have shown that cocaine interacts with the Toll-like receptor 4 (TLR4), the immunosurveillance receptor complex located on microglia to induce neuroinflammatory response and IL-1β is suggested to serve as a final effector in cocaine rewarding effects (Periyasamy *et al*., 2018). It has also been shown that disruption of this interaction attenuates cocaine-induced increases in Nac extracellular dopamine (Northcutt *et al*., 2015) . Thus, the reversal of the neuroinflammatory effects of cocaine by the CB2 agonist, particularly in the PFC, may explain its decrease in cocaine-seeking behaviour, as the PFC is particularly implicated in compulsive behaviours observed in addiction (Goldstein and Volkow, 2010). Complementarily, the rewarding effect

of cocaine observed in the CPP was associated with altered CX3CL1 concentration, particularly in the VTA and the PFC which suggests that CX3CL1 signalling might be implicated in cocaine-induced CPP and its associated inflammatory response. This hypothesis is supported by previous reports on the role of CX3CL1 in the regulation of glial activation and subsequent release of inflammatory cytokines, such as TNF-α and IL-1β (Souza *et al*., 2013; Liu *et al*., 2015).

In conclusion, the present study demonstrates that CB2 receptor modulation affects the behavioural and neurobiological changes induced by cocaine. CB2 receptor modulation should be investigated further as a potential therapeutic mechanism to attenuate cocaine reward-seeking behaviour. Future studies may investigate the effects of cocaine conditioning in CX3CR1 knockout mice, to determine the complex role played by CX3CL1 in cocaine-induced neuroinflammation. Also studying the effects of CB2 agonists in $CX3CR1^{-/-}$ cocaine-conditioned mice would provide insight into the importance of the role of CX3CL1 in mediating cannabinoid effects. Furthermore, specific CB2 agonists and antagonists may be used in CPP-reinstatement models, to gain an understanding of the precise roles of these receptors in the development of the reinforcing effects of cocaine.

## Acknowledgements

### Conflicts of interest

There are no conflicts of interest.

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