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ORIGINAL INVESTIGATION

Adolescent cannabinoid exposure modulates the vulnerability to cocaine-induced conditioned place preference and DNMT3a expression in the prefrontal cortex in Swiss mice

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Abstract

Rationale *Cannabis sativa* is the most widely used drug by adolescents globally. The recreational use of synthetic cannabinoids by teenagers has also grown in recent years. Despite the wrong perception that exposure to these drugs does not cause harm, repeated exposure to cannabinoids at early stages of life compromises important maturation processes and brain development. Chronic early cannabinoid use has been related to a higher risk of psychiatric outcomes, including cocaine addiction. Evidence suggests that exposure to natural and synthetic cannabinoids during adolescence modifies molecular and behavioral effects of cocaine in adulthood. Responses to cocaine are regulated by epigenetic mechanisms, such as DNA methylation, in the brain's reward regions. However, the involvement of these processes in modulation of the vulnerability to the effects of cocaine induced by prior exposure to cannabinoids remains poorly understood.

Objectives Investigate whether exposure to the synthetic cannabinoid WIN55,212–2 during adolescence modulates anxiety- and depression-like behavior, memory, and cocaine reward in adult mice. We also evaluated whether exposure to cannabinoids during adolescence modulates the expression of enzymes that are involved in DNA methylation.

Results Exposure to WIN55,212–2 during adolescence did not alter anxiety- or depressive-like behavior. However, prior exposure to cannabinoids inhibited cocaine-induced conditioned place preference without modulating cocaine-induced hyperlocomotion, accompanied by an increase in expression of the enzyme DNA methyltransferase 3a (DNMT3a) in the prefrontal cortex.

Conclusions Our findings suggest that exposure to WIN55,212–2 during adolescence leads to changes in DNMT3a expression, and this pathway appears to be relevant to modulating the rewarding effects of cocaine.

Keywords Cannabinoids; Adolescence · Cocaine · Epigenetic · DNA methylation · DNMT3a

Introduction

Adolescence is a period of life when many individuals first begin experimenting with recreational drugs (Salmanzadeh et al. 2020). Marijuana (*Cannabis sativa*) is the most widely used illicit drug globally, especially among adolescents (Chadwick et al. 2013; United Nations Office on Drugs and Crime 2016). Similarly, synthetic cannabinoid products have been increasingly used by teenagers during the past several years (Loeffler et al. 2016; Paul et al. 2018). This period of life is a critical phase of brain development, characterized by intense neuronal maturation and rearrangement processes (Rubino and Parolaro 2016; Sturman and Moghaddam 2011). Despite the wrong perception that synthetic or natural cannabinoid use does not cause harm or risk, repeated exposures to these substances, especially at

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early stages of life, compromise maturation processes and brain development during this period, thereby increasing the risk of psychiatric outcomes in adulthood, including cocaine addiction (Hurd et al. 2014; Rubino and Parolaro 2016).

Clinical and epidemiological evidence shows that cannabinoid consumption during adolescence modulates vulnerability to the effects of cocaine (Fergusson et al. 2006; Kalayasiri et al. 2010). However, these studies did not clarify whether the relationship between prior cannabinoid exposure and later cocaine use disorder is caused only by prior cannabinoid use or whether other drug-related factors, such as concomitant psychiatric disorders and socioeconomic status, are also involved (Hurd et al. 2014; Kandel et al. 2006). Another limitation of epidemiological data is the impossibility to determine precise concentrations of cannabinoid exposure during this period because these concentrations are highly variable across drug products.

The use of experimental animal models is an important strategy to obtain direct insights into the relationship between early cannabinoid exposure and molecular and behavioral disruptions that promote vulnerability to the effects of cocaine (Renard et al. 2016; Rubino and Parolaro 2016). Preclinical studies reported that rats that were treated with Δ^9-tetrahydrocannabinol (THC), the main psychoactive component of cannabis, during adolescence exhibited an increase in cocaine-induced locomotor sensitization (Dow-Edwards and Izenwasser 2012; Melas et al. 2018). Similarly, animals that were exposed to a synthetic cannabinoid during puberty exhibited higher rates of cocaine self-administration (Higuera-Matas et al. 2008). Interestingly, modulation of the vulnerability to the effects of cocaine that is induced by prior exposure to cannabinoids appears to be multifaceted. Some studies did not find changes in cocaine-induced locomotion or cocaine self-administration in animals that were previously exposed to cannabinoids during adolescence (Friedman et al. 2019).

In addition to behavioral changes, animals with a history of cannabinoid exposure during adolescence exhibit alterations of molecular responses to cocaine. For example, permanent neuroadaptations in dopaminergic pathways, such modulation of the expression of both the dopamine transporter and dopamine receptors, were observed in animals that were previously treated with cannabinoids (Higuera-Matas et al. 2010). Similarly, dopaminergic neurons were significantly less responsive after prior cannabinoid exposure (Pistis et al. 2004).

Molecular and behavioral effects of cocaine are regulated by epigenetic mechanisms, such as histone modification and DNA methylation, within the brain's reward regions (Nestler 2014; Pierce et al. 2018). The regulation of histone modifications is promoted by a wide range of histone-modifying enzymes, including histone acetyltransferases (HACs) and histone deacetylases (HDACs) (Jenuwein and Allis 2001). DNA methylation is catalyzed by a family of enzymes called DNA methyltransferases (DNMTs) that consist of two main groups: DNMT1 and DNMT3 (Goll and Bestor 2005). Much evidence indicates that pharmacological and genetic modulation of the function of enzymes that are involved in histone modification and DNA methylation regulates responses to cocaine (Nestler 2014; Vaillancourt et al. 2017).

Recent studies evaluated whether exposure to cannabinoids during adolescence alters epigenetic mechanisms (Szutorisz and Hurd 2018). Alterations of histone modification processes were observed after adolescent cannabinoid exposure in different reward-related brain regions, including the hippocampus and prefrontal cortex. Furthermore, these changes correlated with behavioral alterations in adult animals (Prini et al. 2017a, 2017b; Tomasiewicz et al. 2012). Despite studies that demonstrate that exposure to cannabinoids during adolescence regulates histone modification processes, the influence of early cannabinoid exposure on DNA methylation remains poorly investigated.

Thus, the present study investigated whether exposure to the synthetic cannabinoid WIN55,212–2 during adolescence modulates anxiety- and depression-like behavior, memory, and responses to cocaine, in adult mice. We also evaluated whether exposure to cannabinoids during adolescence modulates the expression of enzymes that are involved in DNA methylation. We found that adolescent exposure to cannabinoids inhibited cocaine-induced conditioned place preference (CPP). This effect was unrelated to memory impairment, in which treatment with the cannabinoid receptor agonist did not alter memory performance in the novel object recognition (NOR) test.

Materials and methods

Subjects

Male Swiss mice (3 weeks old) were provided by our local animal facility (Ribeirão Preto Campus). The animals arrived in our department at least 1 week before they were treated with WIN55,212–2. During that period, the animals were kept in the Department of Pharmacology animal house in transparent polycarbonate boxes (20 cm × 12 cm × 30 cm; four animals per cage) with 2 cm of wood shavings. The room had a controlled temperature (24 °C ± 1 °C) and a 12-h/12-h light/dark cycle (lights on 6:00 AM). The animals had free access to food and water, except during testing. All animal protocols were approved by the Ethical Committee on Animal Experimentation for the Use of Animals of the University of São Paulo (protocol no. 15.1.536.60.8) and were conducted according to the National Council of Animal Experimentation Control, which complies with international laws and policies for animal experimentation.

Drugs

Cocaine hydrochloride (15 mg/kg, Merck, Kenilworth, NJ, USA) was dissolved in physiological saline. The cannabinoid receptor agonist WIN55,212–2 (3 mg/kg; Cayman Chemical, Ann Arbor, MI, USA) was dissolved in cremophor–ethanol–saline (1:1:18, v/v). The doses were based on previous studies (Gobira et al. 2018; Tomas-Roig et al. 2017), and the solutions were prepared immediately before use and injected intraperitoneally in a volume of 10 ml/kg.

Adolescent cannabinoid exposure

The experimental design of WIN55,212–2 treatment and behavioral testing during adolescence and adulthood is illustrated in Fig. 1. Adolescent cannabinoid exposure was conducted as previously described (Ellgren et al. 2007), with minor modifications. The animals arrived in the laboratory on postnatal day 21 (PND21). During the 3-week treatment period from PND28 to PND49, the animals received injections of WIN55,212–2 (3 mg/kg) every 3 days (for a total of eight injections), and the control group received a corresponding volume of vehicle (Ellgren et al. 2007). The behavioral tests were performed 2 weeks after the last injection. Importantly, to avoid the possible influence of stress that is caused by the behavioral tests, each set of behavioral experiments was conducted with independent groups of animals that were previously treated with WIN55,212–2 or vehicle.

Cocaine-induced hyperlocomotion

The locomotion experiments were conducted in a circular arena (40-cm diameter with 50-cm high Plexiglas wall). The animals were habituated to this open field for 20 min. They received an injection of cocaine (15 mg/kg) and were immediately returned to the open field. The distance travelled was analyzed for 20 min using AnyMaze software (Stoelting).

Conditioned place preference

Conditioned place preference was assessed in an acrylic box that consisted of two chambers of equal size (20-cm length × 15-cm width × 10-cm height) with doors (5 cm × 5 cm) that connected them to a central compartment (6-cm length × 15-cm width × 10-cm height). The lateral chamber walls had interspersed black and white stripes, and the floors consisted of removable metal surfaces. In one of the chambers (chamber A), the walls were painted with vertical stripes, and the floor consisted of a metal grid with parallel, equally spaced rods. The other chamber (chamber B) had walls that were painted with horizontal stripes and a metal floor with circular holes. The light intensity was the same among the three compartments. The CPP protocol was based on previous studies (Gobira et al. 2018; Yu et al. 2011). In the preconditioning phase (day 1), each mouse was placed in the central compartment, with the doors open. They could freely explore the apparatus for 15 min. The time spent in each compartment was recorded and automatically analyzed with AnyMaze software (Stoelting). In the conditioning phase (days 2–7), the animals were randomly assigned to the experimental treatments. They received cocaine injections on days 2, 4, and 6 and were immediately confined to one of the chambers (drug-paired side) for 30 min. On alternate days (days 3, 5, and 7), they were injected with saline and confined to the chamber's other compartment for 30 min. We used a counterbalanced design, in which each experimental group included animals that received cocaine or saline injections in chamber A and chamber B. Finally, on the test day (day 8), the mice were tested for the expression of cocaine-induced CPP under drug-free conditions that were identical to the preconditioning phase. The CPP score was calculated as the time spent in the drug-paired chamber minus the time spent in the saline-paired chamber. Animals that exhibited a strong preference for one compartment during the preconditioning phase were excluded from the study. A strong preference was considered when the animal spent more than 80% of the total time in one of the compartments.

Novel object recognition test

The NOR test was performed as described previously, with minor modifications (Alaghband et al. 2017; Rodrigues da Silva et al. 2020). The animals initially underwent a habituation session in a Plexiglas circular arena (40-cm

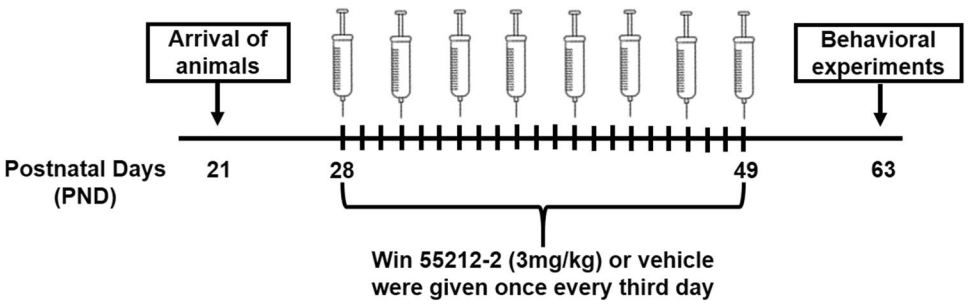

Fig. 1 Schematic representation of the experimental design. The adolescent exposure experiment began on PND28. During the 3-week treatment period from PND28 through PND49, the animals received WIN55,212–2 (3 mg/kg) every 3 days (for a total of eight injections). The control group received a corresponding volume of vehicle

diameter × 40-cm height) for 15 min. Twenty-four hours later, each animal underwent an acquisition trial. Twenty-four hours later, they underwent a test trial. In the acquisition trial, the mice were placed in the experimental apparatus with two identical objects and allowed to explore these objects for 10 min. In the test trial, one copy of the familiar object and one novel object were placed in the same locations as during the acquisition trial. All combinations and locations of objects were used in a balanced manner to reduce potential bias that can be caused by a preference for particular locations or objects. The familiar and novel objects were 8 cm high, were too heavy to be displaced by the animals, and had different shapes, colors, and textures. All of the acquisition and test trials were video-recorded and manually scored by researchers who were blind to the animals' treatments. The videos were analyzed with regard to total exploration of the objects, and the discrimination index was calculated (discrimination index = [time exploring novel object − time exploring familiar object]/[time exploring novel object + time exploring familiar object]), which was used to assess recognition memory (Alaghband et al. 2017; Rodrigues da Silva et al. 2020).

Forced swim test

The forced swim test (FST) was performed similarly to Sartim et al. (2019). The mice were individually placed in a cylinder (18-cm diameter) with water at a depth of 10 cm for 6 min and allowed to swim freely. The water temperature was maintained at 24 °C ± 1 °C. Behavior was recorded, and the time spent immobile (i.e., when the animal performed only movements that were required to keep its head above the water) was recorded during the last 4 min of the test. The same experimenter analyzed all animals blind to treatment to avoid individual bias. The water was changed after each trial to avoid the influence of alarm substances.

Elevated plus maze

The elevated plus maze (EPM) consisted of two opposite open arms (30 cm × 10 cm) that were constructed of wood and crossed perpendicularly by two closed arms (30 cm × 10 cm, with 30-cm high walls with no roof). The apparatus was elevated 50 cm above the floor. The EPM was placed in a sound-attenuated, temperature-controlled (23 °C) room that was illuminated by one 40-W fluorescent light that was placed 1.5 m away from the apparatus. AnyMaze software (Stoelting) was used for the behavioral analysis, which included the time spent on the open arms, the number of entries into the open arms, and the number of entries into the closed arm. Each session lasted 5 min. The data are expressed as percentages of entries into the open arms and the time spent on the open arms ([open arm entries/time]/[open + closed arm entries/time] × 100) during 5-min sessions. The number of entries into the closed arms, which represents a measure of locomotor activity, was also recorded (Campos et al. 2013). After each trial, the maze was cleaned with an alcohol solution and dried.

Tissue collection

Tissue was collected immediately after the CPP test. Following rapid decapitation, brains were removed, and the prefrontal cortex and hippocampus were dissected, rapidly frozen on dry ice, and stored at − 80 °C until use.

Protein preparation and Western blot assay

Frozen samples of the prefrontal cortex and hippocampus were lysed and homogenized in RIPA buffer (catalog no. R0278, Sigma-Aldrich, St. Louis, MO, USA), plus protease (catalog no. P2714, Sigma-Aldrich, St. Louis, MO, USA), and phosphatase (catalog no. P5726, Sigma-Aldrich, St. Louis, MO, USA) inhibitors, followed by centrifugation at 14,000 × *g* for 30 min at 4 °C. The supernatant was collected, and total protein levels were measured by the Bradford method, with each sample analyzed in triplicate. The samples were stored at − 80 °C until the moment of Western blot analysis, which was performed according to Casarotto et al. (2015). Briefly, each sample was calculated to contain 80 µg of protein in a total volume of 10 µl per well. This volume was achieved by adding Laemmli buffer and RIPA buffer to the sample. Protein in each sample was separated according to mass by electrophoresis in an 8% polyacrylamide gel, followed by transfer to polyvinylidene fluoride membranes. The membranes were then treated with Ponceau's reagent (catalog no. P3504, Sigma-Aldrich, St. Louis, MO, USA) to ensure the equal transfer of protein in the membranes. Afterward, the membranes were blocked with 5% bovine serum albumin solution in TBST buffer for 1 h and then incubated overnight at 4 °C with anti-Dntm3a primary antibody (1:500, catalog no. sc-20703, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-GAPDH antibody as the standard (1:2000, catalog no. sc25778, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The membranes were washed for 5 min in TBST and incubated with secondary antibody (horseradish peroxidase–conjugated anti-mouse IgG, 1:2000, catalog no. 7076, Cell Signaling Technologies, Inc., Danvers, MA, USA) for 1 h at room temperature. The membranes were then washed three times with TBS for 5 min each, followed by three washes with TBST for 5 min each. The membranes were revealed by the calorimetric method using a commercial kit (catalog no. NEL300001EA, PerkinElmer) and scanned with an HP Scanjet3800. The optical density of each sample was determined using Image Studio Lite 5.2 (Li-COR). After

scanning, the membranes were washed with 100% methanol for 5 min, followed by TBST to remove the chromogenic reagent, and incubated again overnight with anti-Dnmt3b antibody (1:500, catalog no. sc-20704, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Subsequent secondary antibody incubation, band acquisition, and image analysis were performed as previously described (Casarotto et al. 2015). The optical density of each analyzed band was normalized to its corresponding GAPDH value and is expressed as a percentage of control. The vehicle-cocaine group was the control for the WIN55,212–2-cocaine group.

Statistical analysis

The animals were randomized to the different experimental treatments. Behavior in the EPM, FST, and NOR test and the molecular data were analyzed using Student's *t*-test. Drug-induced CPP was analyzed by comparing CPP scores using analysis of variance (ANOVA) followed by the Bonferroni post hoc test. The data are expressed as mean ± SEM. Values of $p < 0.05$ were considered statistically significant.

Results

Adolescent cannabinoid exposure did not alter anxiety- or depressive-like behavior

Figure 2A–C shows that treatment with the cannabinoid receptor agonist WIN55,212–2 during adolescence did not alter any of the parameters that were analyzed in the EPM. Student's *t*-test revealed no difference between groups in the percentage of entries into the open arms ($t_{14} = 0.82$, $p = 0.42$). No difference was found in the percentage of time spent on the open arms ($t_{14} = 0.04$, $p = 0.96$). No effect on the number of closed arm entries was found, suggesting that the treatment did not affect basal motor activity ($t_{14} = 0.02$, $p = 0.98$). Treatment with WIN55,212–2 during adolescence did not alter behavior in the FST (Fig. 2D). Student's *t*-test revealed no difference in immobility time between the groups that received WIN55,212–2 and saline during adolescence ($t_{15} = 0.44$, $p = 0.67$), suggesting that this treatment did not induce a depressive-like effect in these animals.

Adolescent cannabinoid exposure did not alter memory in the novel object recognition test

Figure 3 shows that adolescent cannabinoid exposure did not affect memory in the NOR test. The two-way ANOVA revealed no effect of treatment ($F_{1,30} = 0.015$, $p > 0.05$) or time ($F_{1,30} = 0.151$, $p > 0.05$) and no treatment × time interaction ($F_{1,30} = 0.089$, $p > 0.05$), indicating that there were no significant changes in the exploration of identical objects during the acquisition trial in both experimental groups (Fig. 3A). Student's *t*-test revealed no difference in discrimination between animals that received WIN55,212–2 and vehicle ($t_{15} = 0.50$, $p = 0.62$; Fig. 3B). The groups did not differ in the total time exploring the novel objects ($t_{15} = 0.12$, $p = 0.89$; Fig. 3C), suggesting that this treatment did not impair memory processes in the NOR test.

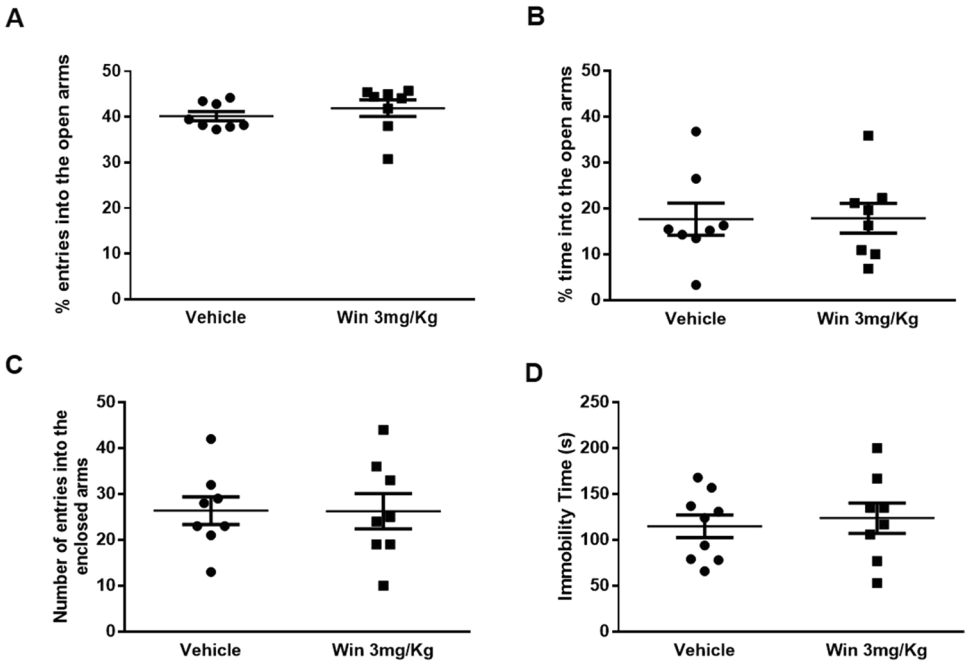

Fig. 2 Effects of adolescent cannabinoid exposure in the elevated plus maze (**A**–**C**) and forced swim test (**D**). Early exposure to WIN55,212–2 did not alter the percentage of entries into the open arms (**A**), percentage of time spent on the open arms (**B**), or the number of entries into the closed arms (**C**). Treatment with WIN55,212–2 did not alter immobility time (**D**). The data are expressed as mean ± SEM. $n = 8$–9/group

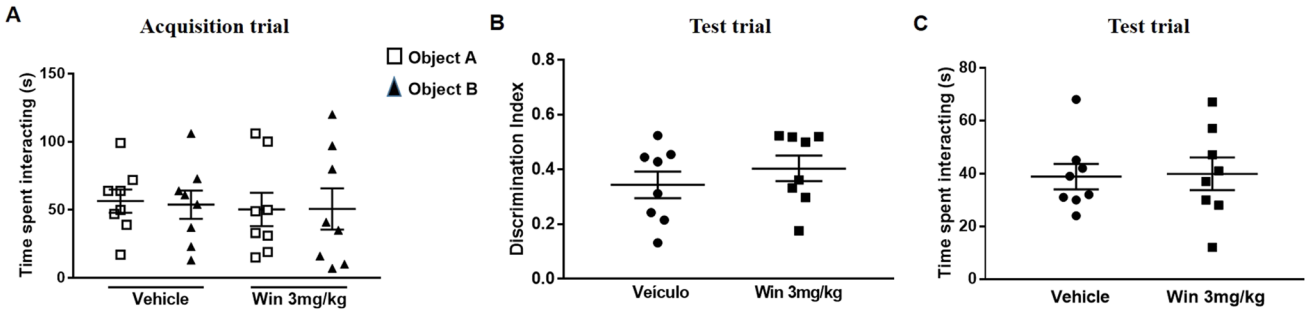

Fig. 3 Effects of adolescent cannabinoid exposure in the novel object recognition test. Treatment with the cannabinoid receptor agonist WIN55,212–2 did not alter the exploration of two identical objects in the acquisition trial (**A**). Mice that were treated with WIN55,212–2 exhibited a similar preference for the novel object as the vehicle-treated group in the test trial (**B**). The groups did not differ in the time spent interacting with the novel objects in the test phase (**C**). The data are expressed as mean ± SEM. $n = 8$/group

Adolescent cannabinoid exposure impaired cocaine-induced conditioned place preference

Figure 4A shows that treatment with the cannabinoid receptor agonist WIN55,212–2 during adolescence impaired cocaine-induced CPP. The two-way ANOVA revealed significant effects of treatment ($F_{1,16} = 6.85$, $p < 0.05$) and time ($F_{1,16} = 8.31$, $p < 0.05$) and a significant treatment × time interaction ($F_{1,16} = 5.27$, $p < 0.05$). The Bonferroni post hoc test showed, in animals that were exposed to vehicle during adolescence, that cocaine at a dose of 15 mg/kg induced a preference for the compartment where cocaine was administered ($p < 0.05$), reflected by an increase in the time spent in this compartment. In animals that were exposed to WIN55,212–2 during adolescence, this response was not observed ($p > 0.05$). These data suggest that exposure to cannabinoids, during this stage of development, impairs cocaine-induced CPP. Figure 4B shows the total time spent in the central chamber of the CPP apparatus in the pre- and postconditioning phases. The two-way ANOVA revealed significant effects of treatment ($F_{1,16} = 13.82$, $p < 0.05$) and time ($F_{1,16} = 17.54$, p 0.05) and a significant treatment × time interaction ($F_{1,16} = 36.09$, $p < 0.05$). The Bonferroni post hoc test showed that cocaine at a dose of 15 mg/kg decreased the time spent in the central compartment in the vehicle group compared with animals that were exposed to WIN55,212–2 during adolescence($p < 0.05$). Importantly, two animals were excluded from this analysis (one from each group) because they exhibited a strong preference for a particular compartment during the preconditioning phase.

Adolescent cannabinoid exposure increased DNMT3a expression in the prefrontal cortex

Figure 5 shows the expression of DNMT3a and DNMT3b in the prefrontal cortex and hippocampus in adult animals that had a history of cannabinoid exposure during adolescence and that were submitted CPP test. Student's *t*-test revealed an increase in the expression of DNMT3a in the prefrontal cortex in animals that were previously treated with WIN55,212–2 ($t_{15} = 2.81$, $p = 0.013$). In contrast, DNMT3b expression in the prefrontal cortex was unaffected ($t_{15} = 0.27$, $p = 0.86$). Similarly, prior cannabinoid exposure did not affect the expression of DNMT3b in the hippocampus ($t_{15} = 0.13$, $p = 0.90$), with no differences in DNMT3a expression in this region ($t_{15} = 0.06$, $p = 0.94$).

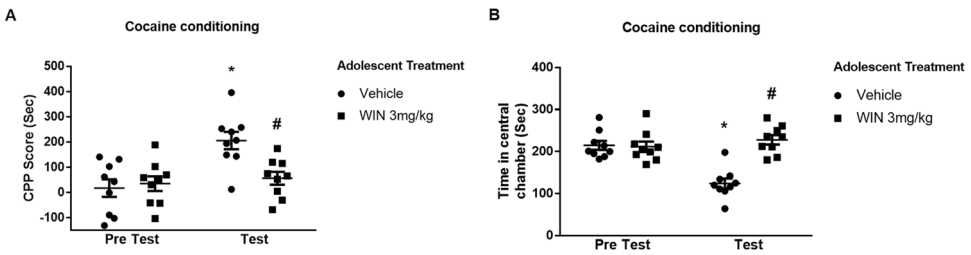

Fig. 4 Effect of adolescent cannabinoid exposure on cocaine-induced conditioned place preference. (**A**) Early exposure to WIN55,212–2 impaired cocaine-induced CPP. (**B**) Total time spent in the central chamber of the CPP apparatus during the pre- and postconditioning phases in each treatment group. The data are expressed as mean ± SEM. *$p < 0.05$, significantly different from the vehicle group in the preconditioning phase; #$p < 0.05$, significantly different from the vehicle group in the test phase. $n = 9$/group

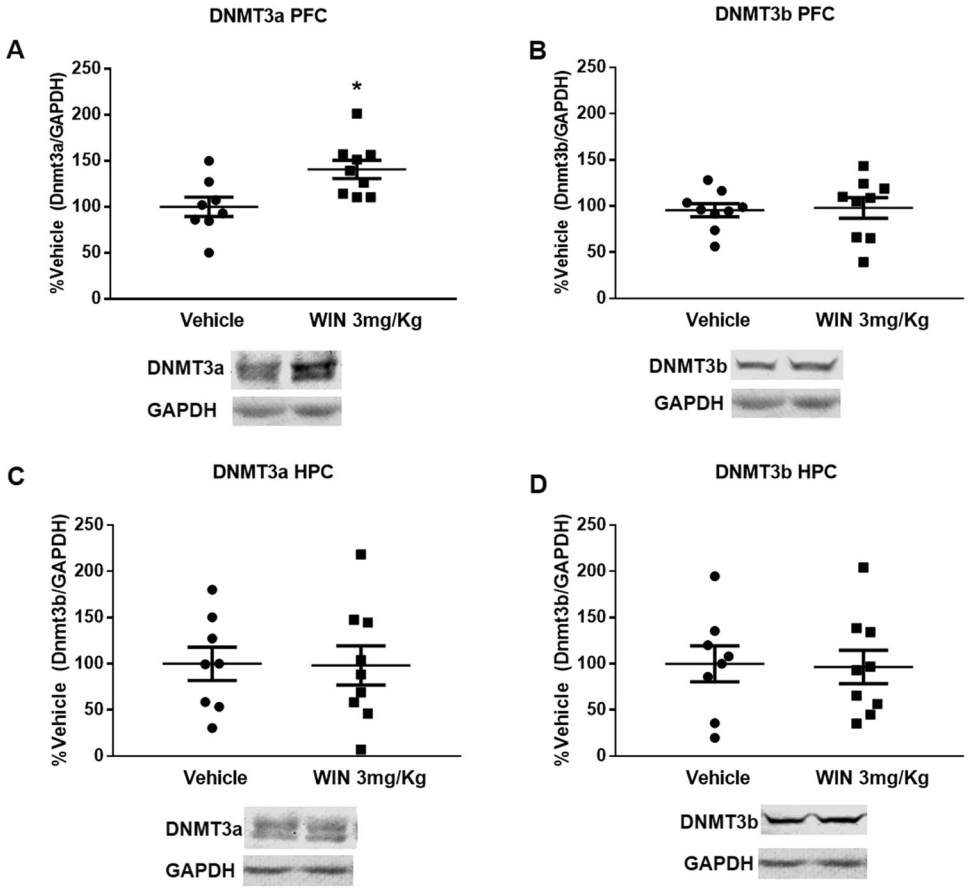

Fig. 5 Effect of adolescent cannabinoid exposure on DNMT3a and DNMT3b expression in the prefrontal cortex (PFC) and hippocampus (HPC). Early exposure to WIN55,212–2 increased the expression of DNMT3a (**A**) but not DNMT3b (**B**) in the PFC. Neither vehicle- nor WIN55,212–2-treated mice exhibited a difference in the expression of DNMT3a or DNMT3b in the HPC (**C**, **D**). The data are expressed as mean ± SEM. *$p < 0.05$, significantly different from the vehicle group. $n = 8$–9/group

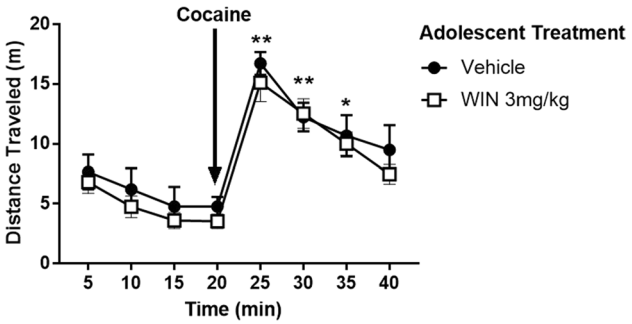

Fig. 6 Effect of adolescent cannabinoid exposure on cocaine-induced hyperlocomotion. Early exposure to WIN55,212–2 did not alter basal locomotion or cocaine-induced hyperlocomotion. The data are expressed as mean ± SEM. *$p < 0.05$, significantly different from each group before the cocaine injection. $n = 8$/group

Adolescent cannabinoid exposure did not alter cocaine-induced hyperlocomotion

Figure 6 shows that treatment with the cannabinoid receptor agonist WIN55,212–2 during adolescence did not influence cocaine-induced hyperlocomotion. The two-way ANOVA revealed a significant effect of time ($F_{1,12} = 31.15$, $p < 0.05$) but no effect of treatment ($F_{1,12} = 0.89$, $p > 0.05$) and no treatment × time interaction ($F_{1,12} = 0.22$, $p > 0.05$). Because the ANOVA indicated no significant interactions with adolescent treatment, post hoc comparisons between adolescent treatment groups were not probed further. The post hoc analyses revealed that the cocaine-induced increase in locomotor activity was observed 5–15 min after the cocaine injection in each adolescent treatment group.

Discussion

The present study provided evidence that exposure to WIN55,212–2 during adolescence did not alter anxiety- and depressive-like behavior in the EPM and FST, respectively. We detected no alterations of memory performance in the NOR test, suggesting that cannabinoid exposure during adolescence did not impair memory in our protocol. However, we found that prior exposure to WIN55,212–2 inhibited subsequent cocaine-induced CPP without affecting cocaine-induced hyperlocomotion. This effect was accompanied by an increase in the expression of DNMT3a in the prefrontal cortex.

Adolescence is a critical period of brain development, during which different neurotransmitter systems undergo developmental changes, including the endocannabinoid system (Schneider 2008; Sturman and Moghaddam 2011). Activity of the endocannabinoid system, including its receptors and endogenous ligands, appears to be highest around puberty onset (Rubino and Parolaro 2016). The regular use of exogenous cannabinoids during this specific developmental period might have important consequences on processes that are modulated by this system, such as emotional responses (Rubino and Parolaro 2016; Schneider 2008). The endocannabinoid system is largely involved in anxiety- and depression-related behavior. Exposure to cannabinoids in adolescent rodents might lead to the dysregulation of these responses (Moreira and Lutz 2008; Rubino and Parolaro 2016). Adult rodents with a history of cannabinoid exposure during adolescence exhibit the exacerbation of anxiety-like behavior (Renard et al. 2017a; Schneider et al. 2008). Similarly, exposure to cannabinoids during adolescence promoted depressive-like behavior (Bambico et al. 2010; De Gregorio et al. 2020). Corroborating our present findings, the absence of alterations of these emotional responses following cannabinoid exposure has also been reported (Abush and Akirav 2013; Cadoni et al. 2015).

These differences might be related to rodent sex differences. Rubino et al. (2008) reported that early exposure to cannabinoids led to a depression-like phenotype only in female animals. This distinct behavioral phenotype was further supported by biochemical parameters of depression exclusively in females (Rubino et al. 2008). The specific period of cannabinoid exposure can also influence these differential effects on emotional responses. For example, anxiogenic effects were observed mainly following cannabinoid exposure between PND28 and PND40, whereas the absence of alterations of anxiety or even a decrease in anxiety-like behavior was observed when adolescent exposure occurred after PND40 (Renard et al. 2016). In the present experimental protocol, the animals were treated during early and later periods of adolescence. Thus, this treatment regimen could have masked eventual modifications of anxiety-like behavior. Moreover, in the present study, the mice were treated with the cannabinoid receptor agonist every 3 days, representing low-to-moderate cannabinoid exposure (Ellgren et al. 2007; Hurd et al. 2014). This intermittent protocol is different from other studies in which the drug was given once or twice daily at increasing doses, which might also explain the lack of effects on emotional responses that was observed in our study.

We also observed no differences in the time exploring novel objects between groups. This finding disagrees with earlier reports of long-term deleterious effects on cognition after early exposure to THC (Rubino et al. 2009b; Zamberletti et al. 2014). In addition to the influence of rodent sex differences and the specific period of cannabinoid exposure, another possible factor that might explain these distinct behavioral responses are differences in the pharmacological profile of cannabinoid agonists that are used in different studies. For example, WIN55,212–2 acts as a full cannabinoid receptor agonist, whereas THC acts only as a partial agonist of cannabinoid receptors (Breivogel and Childers 2000; Govaerts et al. 2004). Equally important, THC and WIN55,212–2 undergo dramatically different metabolic breakdown and have different pharmacological half-lives (Agu et al. 2006; Grotenhermen 2003; Klausner and Dingell 1971). These distinct pharmacological and pharmacokinetic characteristics might contribute to the behavioral discrepancies between the present study and previous studies of THC.

To investigate whether intermittent cannabinoid exposure in adolescence modulates cocaine-induced reward, we performed the CPP paradigm. Interestingly, we found that early exposure to cannabinoids impaired cocaine-induced CPP. These findings do not support the gateway drug hypothesis, which proposes that adolescent cannabinoid exposure predisposes individuals to the use of heavier drugs (Kandel and Kandel 2015). In contrast to our results, other preclinical studies corroborate the gateway hypothesis, pointing to an increase in the vulnerability to effects of other drugs in animals that are previously treated with cannabinoids (Hurd et al. 2014; Renard et al. 2016). For example, chronic THC exposure during adolescence increased heroin and morphine self-administration in adult rats (Biscaia et al. 2008; Ellgren et al. 2007; Tomasiewicz et al. 2012). Similarly, an increase in the vulnerability to rewarding effects of opioids following adolescent cannabinoid exposure has been demonstrated in adult rodents in the CPP paradigm (Cadoni et al. 2015).

Although the gateway hypothesis has been consistent for some drugs, such as opioids, the impact of early cannabinoid exposure on the subsequent sensitivity to psychostimulants has been controversial (Hurd et al. 2014; Renard et al. 2016). For example, no differences were observed in amphetamine-related behaviors later in life in animals that were treated with cannabinoids during adolescence (Ellgren et al. 2004). Similarly, Friedman et al. (2019) did not observe alterations of the acquisition of cocaine self-administration in rats that were treated with THC during adolescence. However, when a lower dose of cocaine was administered, THC-exposed rats exhibited an increase in self-administration (Friedman et al. 2019). Additionally, treatment with the cannabinoid receptor agonist CP55,940 increased cocaine self-administration in female rats, but no such effects were observed in male rats (Higuera-Matas et al. 2008).

This evidence suggests that both rodent sex differences and psychostimulant dose are factors that can modify the vulnerability to cocaine-related responses that are induced by cannabinoid exposure during adolescence. Despite the limitations of the present study, in which we used only

male animals and tested only one dose of cocaine, other studies corroborate our findings. Rats that were exposed to WIN55,212–2 during adolescence exhibited lower acquisition of cocaine self-administration than controls, in which only 50% of the animals expressed this behavior (Kononoff et al. 2018). Moreover, Panlilio et al. (2007) reported that under a progressive-ratio schedule, in which the response that was required to obtain cocaine increased exponentially with each injection, cocaine-seeking behavior significantly decreased in rats that were previously exposed to THC. Altogether, these findings suggest that early exposure to cannabinoids inhibits the sensitivity to cocaine reward.

Behavioral alterations that are caused by adolescent cannabinoid exposure are supported by synaptic and neuronal changes in brain regions that are important to cocaine reward, such as the prefrontal cortex and hippocampus, areas that undergo dramatic modifications during adolescence (Realini et al. 2009; Renard et al. 2018). Among changes that are induced by early cannabinoid exposure in the hippocampus are a reduction of *N*-methyl-D-aspartate receptor expression, a decrease in total dendritic length and number, and a reduction of spine density (Rubino et al. 2009b). Cannabinoid exposure during adolescence induces γ-aminobutyric acid hypofunction in the adult prefrontal cortex, reduces synaptophysin and postsynaptic protein-95 expression, and reduces the ability to maintain normal synaptic plasticity (Renard et al. 2017b; Rubino et al. 2009a).

These long-lasting hippocampal and cortical modifications that are triggered by adolescent cannabinoid exposure might be influenced by epigenetic mechanisms. The disruption of epigenetic programming in these brain regions has been associated with early cannabinoid exposure (Prini et al. 2017a, 2017b). Indeed, rats that were previously exposed to cannabinoids exhibited hippocampal hypermethylation at a specific intragenic region (Tomas-Roig et al. 2017). An increase in histone acetylation in the hippocampus was observed in animals with a history of cannabinoid exposure during adolescence (Prini et al. 2017a). Similarly, an increase in histone acetylation and a decrease in HDAC6 levels, an enzyme that is responsible for removing acetyl groups, were observed in the prefrontal cortex in adult animals that were previously exposed to cannabinoids (Prini et al. 2017b; Scherma et al. 2020). Furthermore, these epigenetic changes were correlated with alterations of some behaviors in adult rodents, including the vulnerability to cocaine-related responses (Prini et al. 2017b; Scherma et al. 2020).

Epigenetic modifications are involved in behavioral alterations in adulthood that are triggered by adolescent exposure to cannabinoids. Therefore, we evaluated the expression of DNMT3a and DNMT3b in the hippocampus and prefrontal cortex in mice that were previously exposed to cannabinoids. We found that WIN55,212–2-exposed animals exhibited higher DNMT3a expression in the prefrontal cortex than animals that were treated with vehicle. DNA methylation machinery, including methyltransferases, is dysregulated in brain reward pathways after cocaine exposure (Vaillancourt et al. 2017). The pharmacological inhibition of this epigenetic process markedly enhanced cocaine-induced CPP (LaPlant et al. 2010). Furthermore, Tian et al. (2012) reported a decrease in DNA methylation in the prefrontal cortex in mice submitted to cocaine-induced CPP (Tian et al. 2012). Similarly, treatment with a methyl donor intensified DNA methylation and inhibited both molecular and behavioral effects of cocaine (Tian et al. 2012). This evidence suggests that DNA methylation negatively regulates cocaine reward. Corroborating this hypothesis, the increase in DNMT3a expression in animals that were previously exposed to cannabinoids in the present study was accompanied by the inhibition of cocaine-induced CPP. Consistent with the involvement of DNMT3a that was observed in the present study, viral-mediated DNMT3a overexpression was reported to inhibit cocaine-related responses, further supporting our findings (LaPlant et al. 2010).

Finally, we evaluated the impact of early cannabinoid exposure on the cocaine-induced increase in locomotor activity. We did not observe alterations of basal locomotion or the sensitivity to cocaine-induced hyperlocomotion in WIN55,212–2-treated animals compared with vehicle-treated animals during adolescence. Similarly, Friedman et al. (2019) found no effect of adolescent THC exposure on the locomotor-stimulating effects of cocaine following acute or repeated administration. These findings indicate that early cannabinoid exposure modulates cocaine reward without altering psychostimulant proprieties of the drug.

Conclusion

In the present study, we found that intermittent exposure to cannabinoids during adolescence did not alter anxiety- or depression-like behaviors or memory. In contrast, we found that exposure to cannabinoids during adolescence modified the vulnerability to cocaine-induced CPP. This effect was accompanied by an increase in expression of the enzyme DNMT3a in the prefrontal cortex. In conclusion, exposure to cannabinoids in adolescence might disrupt the normal developmental pattern of DNA methylation, leading to changes in the vulnerability to cocaine reward.

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Declarations

Conflict of interest The authors declare no competing interests.

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