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Cannabidiol prevents disruptions in sensorimotor gating induced by psychotomimetic drugs that last for 24-h with probable involvement of epigenetic changes in the ventral striatum

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ABSTRACT

Cannabidiol (CBD), a major non-psychotomimetic component of the *Cannabis sativa* plant, shows therapeutic potential in several psychiatric disorders, including schizophrenia. The molecular mechanisms underlying the antipsychotic-like effects of CBD are not fully understood. Schizophrenia and antipsychotic treatment can modulate DNA methylation in the blood and brain, resulting in altered expression of diverse genes associated with this complex disorder. However, to date, the possible involvement of DNA methylation in the antipsychotic-like effects of CBD has not been investigated. Therefore, this study aimed at evaluating in mice submitted to the prepulse inhibition (PPI) model: i) the effects of a single injection of CBD or clozapine followed by AMPH or MK-801 on PPI and global DNA methylation changes in the ventral striatum and prefrontal cortex (PFC); and ii). if the acute antipsychotic-like effects of CBD would last for 24-h. AMPH (5 mg/kg) and MK-801 (0.5 mg/kg) impaired PPI. CBD (30 and 60 mg/kg), similar to clozapine (5 mg/kg), attenuated AMPH- and MK801-induced PPI disruption. AMPH, but not MK-801, increased global DNA methylation in the ventral striatum, an effect prevented by CBD. CBD and clozapine increased, by themselves, DNA methylation in the prefrontal cortex. The acute effects of CBD (30 or 60 mg/kg) on the PPI impairment induced by AMPH or MK-801 was also detectable 24 h later. Altogether, the results show that CBD induces acute antipsychotic-like effects that last for 24-h. It also modulates DNA methylation in the ventral striatum, suggesting a new potential mechanism for its antipsychotic-like effects.

1. Introduction

Schizophrenia is a complex psychiatric disorder that affects about 1% of the population in the world. It involves genetic and epigenetic factors that cause neurochemical and development changes in the central nervous system (Liu et al., 2018a; Penades et al., 2020; Weiss and Feldon, 2001). The current antipsychotics drugs remain problematic, as it can induce significant side effects, commonly resulting in discontinuation of treatment (Kaar et al., 2019; Meltzer, 1999). This fact, together with the poor effectiveness against the negative and cognitive symptoms of schizophrenia, results in a constant search for new therapeutic approaches.

Preclinical and clinical studies suggest that the endocannabinoid system is implicated in the pathophysiology and treatment of schizophrenia (Leweke et al., 2018; Zamberletti et al., 2012). Cannabidiol (CBD) is the main non-psychotomimetic compound of the *Cannabis sativa* plant (Mechoulam and Shvo, 1963) with multiple pharmacological effects (Campos et al., 2012; Davies and Bhattacharyya, 2019), including antipsychotic properties (Iseger and Bossong, 2015; Osborne et al., 2017; Zuardi et al., 1995).

CBD shows antipsychotic effects in diverse animal models. For example, it reduces stereotyped and hyperlocomotion induced by psychotomimetic drugs (Moreira and Guimaraes, 2005; Zuardi et al., 1991). CBD also restores deficits in prepulse inhibition of the startle response

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(PPI). This behavioral change is proposed to reflect the impaired sensorimotor gating present in schizophrenia patients. PPI disruption can be induced by drugs that facilitate dopaminergic- or inhibit glutamatergic- neurotransmissions such as amphetamine (AMPH) or dizocilpine (MK-801), an *N*-methyl-D-aspartate (NMDA) receptor antagonist (Gomes et al., 2014; Long et al., 2006; Pedrazzi et al., 2015). In schizophrenia patients, CBD treatment was well tolerated and presented efficacy comparable to the antipsychotic amisulpride (Leweke et al., 2012; McGuire et al., 2018).

The exact molecular mechanisms involved in CBD-induced behavioral effects are not fully understood. CBD has a low affinity for cannabinoid receptors (Thomas et al., 1998), but it can act as a negative allosteric modulator on both CB1 and CB2 receptors (Laprairie et al., 2015; Martinez-Pinilla et al., 2017). Furthermore, it may also increase anandamide signaling by inhibiting its intracellular degradation catalyzed by the fatty acid amide hydrolase (FAAH) enzyme (Bisogno et al., 2001; Leweke et al., 2012; Watanabe et al., 1996).

CBD effects also involve non-cannabinoid mechanisms, such as facilitation of 5-HT1A-mediated neurotransmission (Mishima et al., 2005; Rodrigues da Silva et al., 2020; Russo et al., 2005; Sales et al., 2018; Sonego et al., 2016) and activation of transient receptor potential vanilloid type 1 receptors (TRPV1) (Bisogno et al., 2001; De Petrocellis et al., 2011). It also modulates glutamate and monoamine levels in the brain (Linge et al., 2016), among other mechanisms (for review see (Ibeas Bih et al., 2015)).

Endocannabinoid signaling and CBD have been associated with the modulation of epigenetic mechanisms such as DNA methylation (Pucci et al., 2013; Sales et al., 2020). DNA methylation consists in the addition of a methyl radical to the cytosine carbon 5 in dinucleotides cytosine-phosphate-guanine (CpGs), catalyzed by the DNA methyltransferases (DNMTs) enzymes. These enzymes are widely expressed in the brain (Moore et al., 2013). In vitro, CBD, and anandamide modulate DNA methylation (Paradisi et al., 2008; Pasquariello et al., 2009; Pucci et al., 2013). Anandamide, via the CB1 receptor, increases DNMTs activity and consequently DNA methylation levels in genes associated with skill cells (Paradisi et al., 2008). CBD prevents DNMT activity and global DNA methylation changes in the hippocampus and prefrontal cortex (PFC) of stressed animals (Sales et al., 2020), suggesting that this epigenetic mechanism could be related to its sustained antidepressant-like effects observed in rodent animal models (Sales et al., 2019).

DNA methylation changes have also been associated with schizophrenia (Liu et al., 2018b). The levels of S-adenosyl L-methionine, a methyl radical donor, are increased in the prefrontal cortex (PFC) of schizophrenia patients, which also overexpress DNMT1mRNA (Guidotti et al., 2007). Antipsychotic drugs, including clozapine, activate DNA demethylation in genes associated with GABAergic neurotransmission (Dong et al., 2008). The development of schizophrenia-like symptoms induced by the antimitotic agent methylazoxymethanol acetate (MAM) was prevented by peripubertal CBD treatment. This effect was accompanied by a reversal of MAM-induced changes in DNA methylation of the CB1 gene in the rat PFC (Stark et al., 2019). Furthermore, epigenetic modifications induced by drugs of abuse play an important role in their behavioral responses and the concomitant neuronal plasticity changes (McCowan et al., 2015). Psychostimulant drugs modulate DNA methylation of key genes involved in the pathogenesis of psychotic disorders in the PFC and nucleus accumbens (Mychasiuk et al., 2013).

Together, these results suggest that changes in DNA methylation could be involved in the antipsychotic-like effects of the CBD. The present study aimed at investigating this possibility in mice. We assessed if CBD's acute behavioral effects in PPI impairments induced by amphetamine (AMPH) or the NMDA receptor antagonist MK-801 are associated with changes in global DNA methylation in the ventral striatum and PFC. We also verified if this single CBD treatment would produce sustained behavioral effects.

2. Material and methods

2.1. Animals

Male Swiss mice (from Ribeirão Preto Campus of the University of São Paulo, Brazil) weighing 25–30 g were used ($n = 176$). The animals were housed in groups of eight and maintained at a controlled light/dark cycle (12–12 h, lights on at 07:00 h) and temperature (23 ± 1 °C) conditions. Food and drinking water were available ad libitum. The experiments were performed in compliance with the US National Institutes of Health Guide for Care and Use of Laboratory Animals recommendations. The experimental protocol was approved by the local Ethical Committee (CEUA, n° 2015.1.143.58.6). A total of 147 animals were used in this study.

2.2. Drugs and treatments

Drugs were freshly prepared on the day of testing, and the doses were chosen according to previously published studies (Issy et al., 2014; Issy et al., 2009; Moreira and Guimaraes, 2005; Pedrazzi et al., 2015). D-amphetamine (AMPH, Sigma-Aldrich, USA) was dissolved in 0.9% sterile saline in a dose of 5 mg/kg. Cannabidiol (CBD, BSPG Laboratories, United Kingdom, 30 and 60 mg/kg) was dissolved in Tween 80 to a final concentration of 2% (*v/v*) and in 0.9% sterile saline. MK-801 (Sigma-Aldrich, USA, 0.5 mg/kg) was dissolved in 0.9% sterile saline. Clozapine (Leponex, Novartis, Brazil, 5 mg/kg.) was dissolved in sterile saline/acetic acid 0.5% (*v/v*). All of the solutions were administered intraperitoneally in a volume of 10 ml/kg.

2.3. Prepulse inhibition (PPI)

The sessions were conducted simultaneously in two identical standard operant conditioning chambers (startle response systems; Med Associates, Inc., USA). A continuous acoustic signal provided a background white noise level of 65 dB. The pulse (pulse alone) was a burst of the white noise of 105 dB with a rise/decay of 5 ms and duration of 20 ms, and the prepulse intensities were set at 80, 85, and 90 dB of pure tone, 7000-Hz frequency, and duration of 10 ms.

2.4. Platform calibration

The cages were calibrated daily before the tests to ensure equal sensitivity of both response platforms throughout the tests. The platform calibration was done by adjusting the gain on the load cell amplifier to 150 arbitrary units (AU) at a standard weight appropriated for mice (40 g). The limits of the load cell were − 2047 to +2047 AU.

2.5. Behavioral procedure

After a 5-min acclimatization period, in which mice received no stimuli except for the 65-dB background noise, they were presented with a series of 10 stimuli (pulse alone). The first 10 pulse-alone trials allow the within-session habituation to the startle stimulus and are not considered for statistical analysis of PPI percentage. The PPI test consisted of 64 trials pseudo-randomly divided into eight different categories presented with an inter-stimulus interval of 30 s: pulse alone (105 dB), prepulse alone (80, 85, or 90 dB), prepulse+pulse with 100-ms interval between prepulse and pulse, and null, where no stimulus was presented. Prepulse stimulus did not elicit an acoustic startle response. Mean acoustic startle response to pulse-alone (P) trials and each prepulse+pulse (PP + P) trial was calculated for each subject. These data were used in the statistical analysis to assess drug-induced changes in startle amplitude in PPI. The level of PPI was determined by expressing the prepulse+pulse startle amplitude as a percentage decrease from pulse-alone startle amplitude, according to the following formula: %PPI = [100-(((PP+ P)/P)*100)]. Using this formula, a 0% value denotes no

difference between the startle reflex response to the pulse alone and the prepulse plus pulse, indicating no PPI. A low percentage score indicates a PPI deficit.

2.6. Tissue collection

Immediately after the PPI test, the animals were deeply anesthetized with 5% chloral hydrate (10 ml/kg) and then decapitated. The brain structures (PFC and ventral striatum) were dissected, homogenized in lysis buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 2 mM EDTA pH 8.0, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitor (10% *v*/v; # P2714, Sigma-Aldrich) and stored at −80 °C until analysis.

2.7. DNA methylation analysis by enzyme-linked immunoassay (ELISA)

The DNA was extracted using the Wizard DNA Purification kit (#A1120, Promega Corporation, USA) according to the manufacturer's instructions. The purified DNA was digested with the enzyme Nuclease P1 (# P2640, Sigma-Aldrich, 2 U/μg DNA, 4 h at 65 °C in 20 mM acetate buffer pH 5.3) and the enzyme alkaline phosphatase (# N7640, Sigma-Aldrich, 0.3 U/μg DNA, 2 h at 65 °C in 20 mM Tris-HCl pH 7.5). The DNA was precipitated with ethanol and 5 M NaCl at −20 °C for at least 48 h. It was then centrifuged at 4 °C, 10000 *g* for 15 min. The pellet was resuspended in ultra-pure water. Then, the amount of total DNA was read by the SpectraMax 190 plate reader (version 6.2.1, Molecular Devices, Sunnyvale, CA; absorbance 280/260 nm), and the samples stored at −20 °C until the time of use. According to the manufacturer's instructions, purified methylated DNA was quantified by the DNA Methylation EIA kit (# 589324, Cayman Chemicals). The absorbance produced by the assay was measured by the SpectraMax 190 plate reader (version 6.2.1, Molecular Devices, Sunnyvale, CA, absorbance 280/260 nm). Several concentrations of 5-methyl-2'deoxycytidine were used to construct the standard curve. The results were expressed as methylated cytidine/total DNA (pg /ng).

3. Experimental design

Experiment 1: Acute behavioral effects induced by a single injection of cannabidiol or clozapine in mice treated with amphetamine or MK-801 and submitted to the PPI

Mice received i.p. injections of clozapine (5 mg/kg), CBD (30 and 60 mg/kg), or respective vehicle (10 ml/kg) followed, 20 min later, by an injection of AMPH (5 mg/kg), MK-801 (0.5 mg/kg) or saline (10 ml/kg). Twenty minutes later, the animals were submitted to the PPI test (Fig. 1A and B).

Experiment 2: DNA methylation changes induced by cannabidiol or clozapine in the PFC and ventral striatum of mice treated with amphetamine or MK-801 and submitted to the PPI

Mice received i.p. injections of clozapine (5 mg/kg), CBD (30 and 60 mg/kg), or respective vehicle (10 ml/kg) followed, 20 min later, by an injection of AMPH (5 mg/kg), MK-801 (0.5 mg/kg) or saline (10 ml/kg).

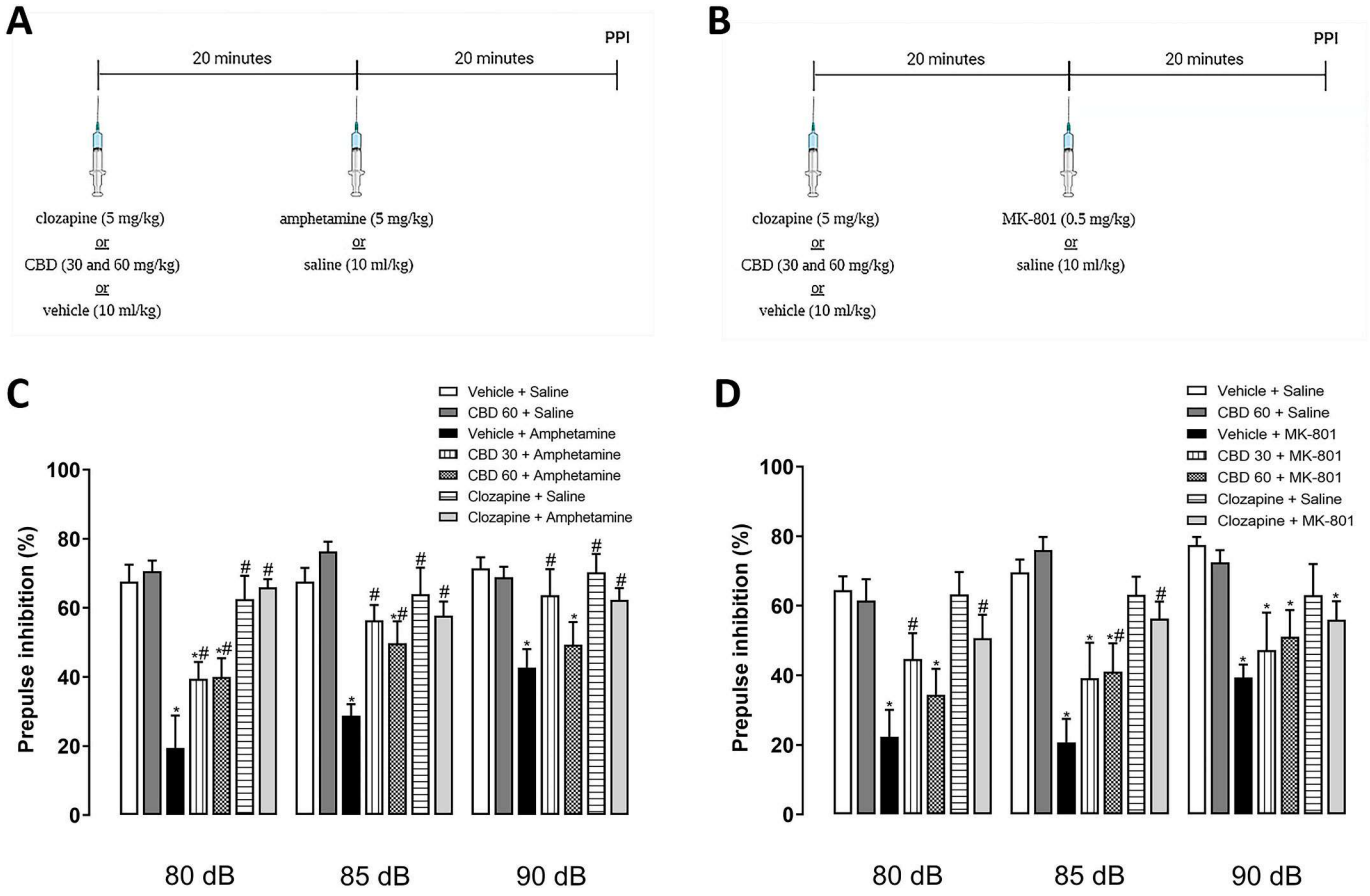

Fig. 1. Behavioral effects induced by pretreatment of cannabidiol (CBD) or clozapine in mice treated with amphetamine (AMPH) or MK-801 and submitted to the prepulse inhibition of startle reflex (PPI). (A) Experimental protocol of animals treated with AMPH or (B) MK-801. (C) CBD (30 and 60 mg/kg) attenuated the AMPH-induced disruption at all intensities analyzed ($n = 7$–8 mice/ group). D) CBD (30 mg/kg) was able to attenuate the disruption at 80 dB intensity, while the dose of 60 mg/kg attenuated this effect at intensity of 85 dB. Clozapine attenuated MK-801- induced disruption in both intensities 80 and 85 dB ($n = 7$–8 mice/group). The data are presented as mean ± SEM. Duncan post hoc test. * $P < 0.05$ compared to the vehicle + saline group. #$P < 0.05$ compared to the vehicle + AMPH or MK-801 group. Figure designed using imagens from BioRender.com.

Twenty minutes later, the animals were submitted to the PPI test. Immediately after the test, they were anesthetized, decapitated, and the brain regions (ventral striatum and PFC) were dissected for molecular analysis (Fig. 2A and D).

Experiment 3: Sustained behavioral effects induced by a single injection of cannabidiol in mice treated with amphetamine or MK-801 and submitted to the PPI

Mice received i.p. injections of clozapine (5 mg/kg), CBD (30 and 60 mg/kg) or respective vehicle (10 ml/kg) followed, 24 h later, by an injection of AMPH (5 mg/kg), MK-801 (0.5 mg/kg) or saline (10 ml/kg). Twenty minutes later, the animals were submitted to the PPI test (Fig. 3A and B).

4. Statistical analysis

The percentage of PPI was analyzed by repeated-measures analysis of variance (ANOVA) with the treatment as the independent factor and the prepulse intensity (80, 85, and 90 dB) as the repeated measure. The acoustic startle response and DNA methylation were analyzed by one-way ANOVA. In the case of significant effects detected in ANOVAs, the Duncan test was used for multiple comparisons. The level of significance was set at $P < 0.05$. Statistical analyses were performed using SPSS software (Statistical Package for Social Sciences) version 22.0. The graphs were built with the aid of the program GraphPadPrism 7.0. In all the graphs, the bars represent the mean +/− standard error of the means.

5. Results

5.1. CBD pretreatment acutely attenuates amphetamine- and MK-801-disruptive effects in PPI

- Protocol amphetamine

Acute treatment with AMPH significantly impaired PPI at all prepulse intensities analyzed (treatment factor: F6,48 = 13.64, $P < 0.001$, Duncan, $P < 0.05$, Fig. 1C). There was a significant prepulse intensity effect (F2,96 = 8.517, P < 0.001) and an interaction between treatment versus intensity (F12,96 = 2.37, $P = 0.01$). CBD 30 and 60 mg/kg, similar to clozapine, decreased amphetamine effect at all prepulse intensities (Duncan, $p < 0.05$). No treatment changed the acoustic startle response in this assay (F6,48 = 2.169, $P = 0.062$; Duncan, $P < 0.05$, Table 1).

- Protocol MK-801

Acute treatment with MK-801 significantly impaired PPI at all prepulse intensities analyzed (treatment factor: F6,45 = 8.56, $P < 0.001$, Duncan, $P < 0.05$, Fig. 1D). There was a significant prepulse intensity effect (F2,90 = 10.54, $P < 0.001$) and a trend for treatment versus intensity interaction (F12,90 = 1.70, $P = 0.078$). CBD 30 and 60 mg/kg decreased MK-801 effect at 80 dB and 85 dB, respectively (Duncan, $P < 0.05$). The vehicle + MK-801 treatment showed a significant difference in relation to the clozapine + MK-801 treatment in the acoustic startle response (F6,42 = 2.91, $P = 0.016$; Duncan, $P < 0.05$, Table 2).

5.2. CBD pretreatment prevents the global DNA methylation changes induced by amphetamine in the ventral striatum

- Protocol amphetamine

Acute treatment with AMPH increased global DNA methylation in the ventral striatum. Pretreatment with both doses of CBD, similarly to the clozapine, prevented this increase (F6,41 = 4.90, $P < 0.001$; Duncan, $P < 0.05$, Fig. 2B). AMPH did not alter the global DNA methylation in the PFC (Fig. 2B) whereas clozapine + saline treatment significantly increased global DNA methylation in this structure (F6,43 = 3.43, $P =$

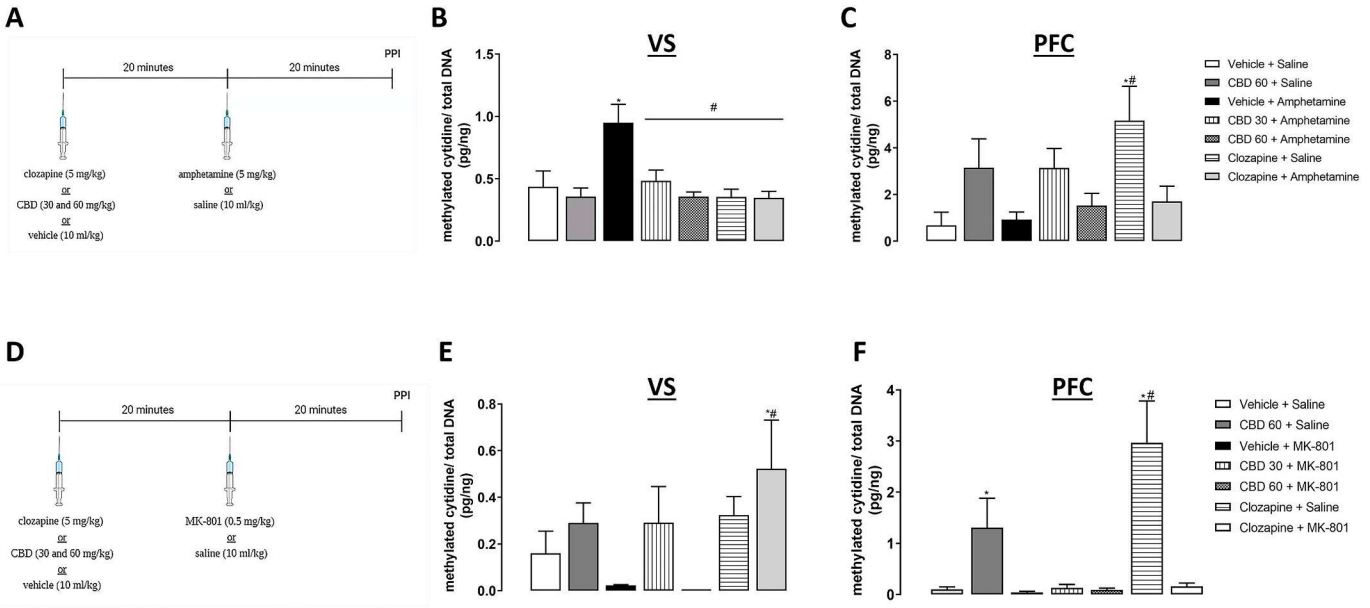

Fig. 2. Effects induced by pretreatment of cannabidiol (CBD) or clozapine in global DNA methylation of mice treated with amphetamine (AMPH) or MK-801 and submitted to the prepulse inhibition of startle reflex (PPI). (A) Experimental protocol of animals treated with AMPH or (D) MK-801. B) acute amphetamine treatment promoted increased global methylation in the ventral striatum (VS). The interaction between the two doses of CBD and AMPH promoted a significant decrease in AMPH-induced global DNA methylation, similar to that observed in the clozapine + AMPH interaction (n = 7–8 mice/group). (C) acute treatment with AMPH did not promote changes in global DNA methylation in the prefrontal cortex (PFC). The clozapine + saline treatment promoted a significant increase in global DNA methylation in this brain structure (n = 7–8 mice/group). (E) acute treatment with MK-801 promoted changes in global methylation in the VS, but not in (F) PFC. (E) The clozapine + MK-801 treatment promoted a significant increase in global DNA methylation in the VS (n = 5–6 mice/group) and (F) CBD (60 mg/kg) or clozapine + saline treatments promoted a significant increase in global DNA methylation in the PFC (n = 5–6 mice/group). The data are presented as mean ± SEM., Duncan post hoc test.* $P < 0.05$ compared to the vehicle + saline group. #$P < 0.05$ compared to the vehicle + AMPH or MK-801group. Figure designed using imagens from BioRender.com.

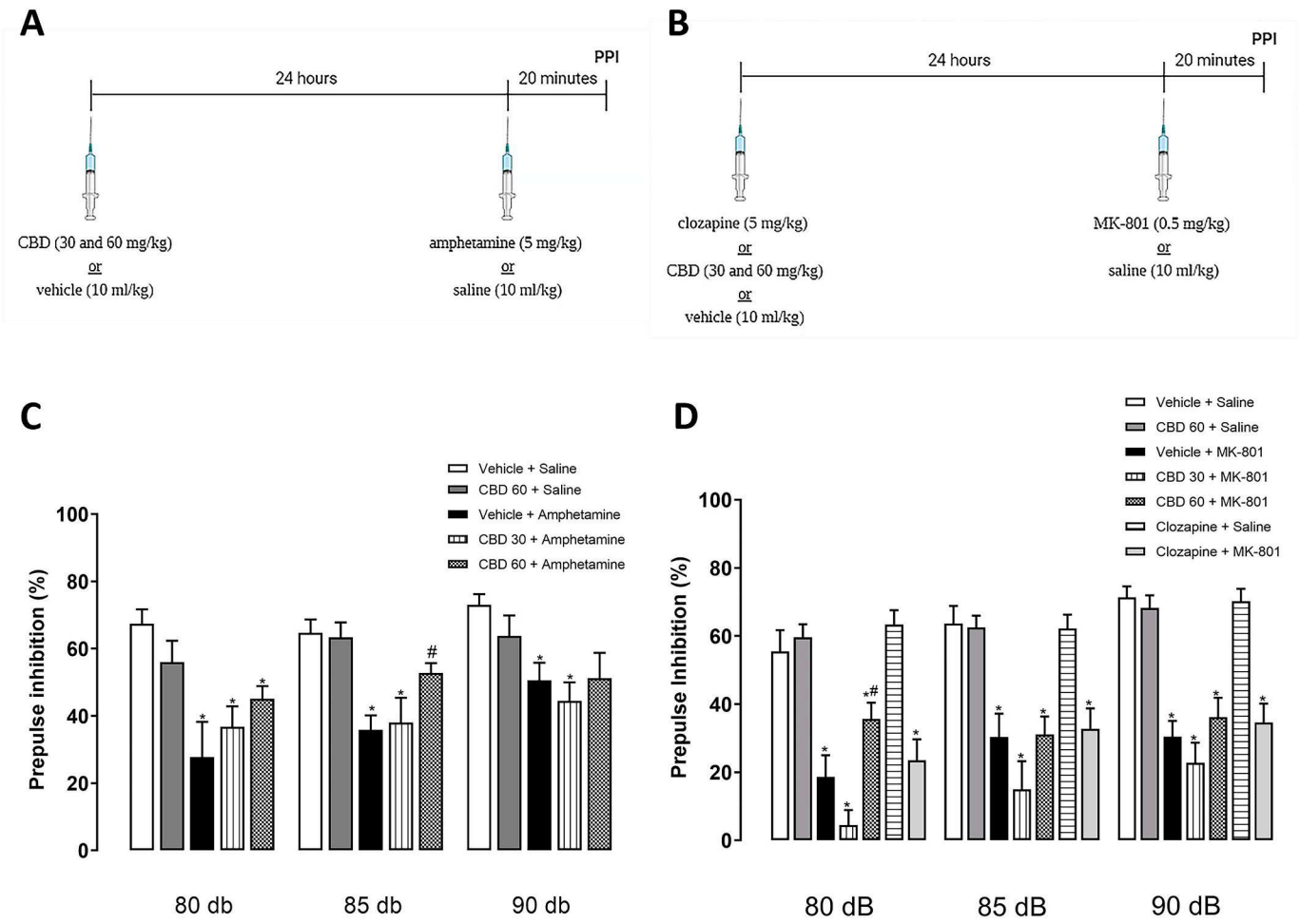

Fig. 3. Sustained behavioral effects induced by pretreatment of cannabidiol (CBD) in mice treated with amphetamine (AMPH) or MK-801 and submitted to the prepulse inhibition of startle reflex (PPI). (A) Experimental protocol of animals treated with AMPH or (B) MK-801. (C) CBD (60 mg/kg) resulted in sustained effect attenuating the AMPH-induced disruption at intensity of 85 dB (n = 7–8 mice/group). (D) CBD (60 mg/kg) modestly resulted in sustained effect attenuating the MK-801-induced disruption at intensity of 80 dB (n = 7–8 mice/group). The data are presented as mean ± SEM. Duncan post hoc test. * $P < 0.05$ compared to the vehicle + saline group. #$P < 0.05$ compared to the vehicle + AMPH or MK-801 group. Figure designed using imagens from BioRender.com.

Table 1
Starle response amplitude of mice treated with CBD 20 min before PPI.

Treatment with saline or amphetamine preceded by vehicle, CBD or clozapine	
Treatment	Startle response (A.U.)
Vehicle + Saline	532.2 ± 81.36
CBD60 + Saline	530.8 ± 72.41
Vehicle + Amphetamine	749.1 ± 78.52
CBD30 + Amphetamine	740.2 ± 49.64
CBD60 + Amphetamine	669.9 ± 76.08
Clozapine + Saline	493.1 ± 94.14
Clozapine + Amphetamine	556.1 ± 49.45

Table 2
Starle response amplitude of mice treated with CBD 20 min before PPI.

Treatment with saline or MK-801 preceded by vehicle, CBD or clozapine	
Treatment	Startle response (A.U.)
Vehicle + Saline	665.2 ± 93.79
CBD60 + Saline	566.0 ± 65.06
Vehicle + MK-801	870.9 ± 131.4
CBD30 + MK-801	596.8 ± 80.96
CBD60 + MK-801	901.7 ± 135.1
Clozapine + Saline	614.0 ± 69.36
Clozapine + MK-801	437.6 ± 45.82 #

#$P < 0.05$ compared to the vehicle + MK-801 group.

0.007; Duncan, P < 0.05, Fig. 2C).

- Protocol MK-801

MK-801 did not change global DNA methylation in the ventral striatum (Fig. 2E). CBD (30 mg/kg) and clozapine increased global DNA methylation in this structure (F6,33 = 2.583, $P = 0.036$; Duncan, P < 0.05, Fig. 2E). In the PFC, no alteration was observed after MK-801 treatment while CBD (60 mg/kg) or clozapine + vehicle increased global DNA methylation in this structure (F6,32 = 7.183, P < 0.001; Duncan, P < 0.05, Fig. 2F).

5.3. Acute CBD pretreatment results in sustained (24-h) attenuation of amphetamine- and MK-801-disruptive effects in PPI

- Protocol amphetamine

Amphetamine disrupted PPI at all prepulse intensities (treatment factor: F4,33 = 7.09, $p < 0.001$; Duncan, P < 0.05, Fig. 3C). There was also significant intensity effect (F2,66 = 8.657, P < 0.001) but no interaction of treatment versus intensity interaction. The PPI disruption was prevented by CBD 60 mg/kg at the 85 dB prepulse intensity (Duncan, P < 0.05, Fig. 3C). No treatment changed the acoustic startle

responses in this assay ($F4,33 = 0.399$, $P = 0.807$; $P < 0.05$, Table 3).

- Protocol MK-801

MK-801 disrupted PPI at all prepulse intensities (treatment factor: $F6,51 = 29.307$, $P < 0.001$, respectively; Duncan, $P < 0.05$, Figs. 3D). There was also significant intensity effects ($F2,102 = 10.80$, $P < 0.001$), but no interaction of treatment versus intensity interaction.

The PPI disruption was prevented by CBD 60 mg/kg at the 80 dB prepulse intensity (Duncan, $P < 0.05$, Fig. 3D). No treatment changed the acoustic startle responses in this assay ($F6,52 = 1.377$, $P = 0.241$; $P < 0.05$, Table 4).

6. Discussion

The main finding in this study is that a single administration of CBD results in rapid and sustained antipsychotic-like effects in the PPI. The acute attenuation of the PPI deficits induced by AMPH or MK-801 were similar to those observed with clozapine. Moreover, they were associated with changes in DNA methylation in areas related to schizophrenia.

Our data are consistent with previous studies showing that CBD attenuates AMPH and MK-801 effects in the PPI test (Long et al., 2006; Zuardi et al., 1995; Gomes et al., 2014; Pedrazzi et al., 2015). Extending these results, however, we found that a single administration of CBD promotes a sustained (24 h) antipsychotic-like effect in the AMPH model. A similar, but more modest, result occurred in animals that received MK-801.

Recently, long-lasting CBD behavioral effects have been described in animal models of depression (Sales et al., 2019). In this study, CBD's antidepressant action was associated with increased expression of synaptic proteins, brain-derived neurotrophic factor (BDNF) levels, and dendritic spine density, through rapid activation of the BDNF-TrkB-mTOR signaling in the PFC. This latter pathway is involved in several neuronal functions impaired in depression and restored by antidepressant drugs, including synaptic plasticity (Autry and Monteggia, 2012; Lee, 2015; Park and Poo, 2013; Saxton and Sabatini, 2017; Switon et al., 2017).

Alterations in synaptic plasticity have also been involved in the neurobiology of schizophrenia (Forero et al., 2016; Hall et al., 2015; Kurian et al., 2011; Le-Niculescu et al., 2007). Genetic and epigenetic modifications, such as DNA methylation, are proposed as possible mechanisms to explain these synaptic changes and the pathophysiology of schizophrenia (Dong et al., 2016; Ovenden et al., 2018; Pries et al., 2017; Snijders et al., 2018). DNA methylation in promoter regions, an epigenetic mechanism catalyzed by DNA methyltransferase enzymes (DNMTs), is associated with the downregulation of specific gene expression (Snijders et al., 2018). Moreover, the antipsychotic effects of clozapine could involve DNA methylation changes (Kinoshita et al., 2017; Melas et al., 2012), leading to the normalization of proteins levels altered in schizophrenia, including BDNF and DNMTs (Dong et al., 2015a; Dong et al., 2019; Dong et al., 2008; Dong et al., 2015b). Analysis of genome-wide DNA methylation identified alterations in several candidate genes that have previously been associated with schizophrenia and antipsychotic drugs. These genes regulate dopaminergic, GABAergic, and glutamatergic signaling, among others (Abdolmaleky et al., 2005; Abdolmaleky et al., 2014; Abdolmaleky et al., 2011; Ayalew et al., 2012; Grayson and Guidotti, 2013; Kinoshita et al., 2017; Le-Niculescu et al., 2007; Mill et al., 2008; Nishioka et al., 2013; Shi et al., 2017; Tang et al., 2014).

PPI deficits induced by prenatal stress in mice showed a significant association with increased DNMTs expression and DNA methylation in GABAergic genes (reelin and GAD67) promoters. These effects were accompanied by reduced levels of proteins regulated by these genes in the frontal cortex. The administration of clozapine corrected the behavioral and molecular alterations (Matrisciano et al., 2013). Moreover, DNMT1 KO mice showed an improved PPI performance compared to controls (Morris et al., 2016). Corroborating these results, our study showed that AMPH treatment causes a rapid global DNA methylation change in the ventral striatum. This brain area contains the nucleus accumbens, one of the convergence centers of different neurotransmitter systems that regulate PPI. It receives dense dopaminergic innervation from the ventral tegmental area (Koch, 1999).

There is an increased dopamine influx in the nucleus accumbens of rats showing PPI impairment after AMPH (Zhang et al., 2000). Also, lesions that increase dopaminergic receptor sensitivity, or direct application of drugs that facilitate dopaminergic neurotransmission into this brain area, impair PPI (Swerdlow et al., 1990; Swerdlow et al., 1986; Swerdlow et al., 1992; Wan et al., 1994). These effects are reversed by dopaminergic antagonists (Swerdlow et al., 1994; Wan et al., 1994). CBD microinjection into the nucleus accumbens, similarly to what had been observed after systemic administration, attenuates AMPH-induced PPI disruption in mice (Pedrazzi et al., 2015). The AMPH-disruptive effects on PPI were accompanied by a rapid increase in the DNA methylation levels in the ventral striatum. Previous evidence suggests that AMPH alters transcription factors that interact with genomic DNA (McCowan et al., 2015). Even after two weeks of the withdrawal from AMPH administered for 14 days, rats present increased global DNA methylation in brain structures involved in schizophrenia, such as the nucleus accumbens (Mychasiuk et al., 2013). Also, the offspring of rats submitted to methamphetamine treatment showed hypermethylation of DNA in promoter regions of genes involved in learning, memory, and synaptic plasticity (Itzhak et al., 2015). Like clozapine, CBD prevented the DNA methylation changes induced by AMPH in the ventral striatum.

To date, very few studies have investigated the effects of MK-801 on DNA methylation, particularly in animal models of schizophrenia. Glutamate upregulates the expression of DNMTs in astrocytes and increases total DNA methylation (Zhao et al., 2019). The glutamate-induced changes were blocked by pretreatment with MK-801, suggesting that DNMTs-mediated DNA methylation may be necessary for glutamate-related effects (Zhao et al., 2019). Although MK-801 promoted a robust PPI disruption, it did not change global DNA methylation in the ventral striatum. This finding might indicate that these two drugs act on at least partially distinct neural substrates to impair PPI. Corroborating this possibility, (De Leonibus et al., 2002) showed that systemic treatment with AMPH or MK-801 induced a distinct c-FOS mRNA expression pattern in rats (). An in vitro *study* suggested that DNA methylation regulation in mature neurons is associated with excitatory neuronal activity and calcium signaling dependent on the activation of

Table 3
Starle response amplitude of mice treated with CBD 24 h before PPI.

Treatment with saline or amphetamine preceded by vehicle or CBD	
Treatment	Startle response (A.U.)
Vehicle + Saline	602.1 ± 108.6
CBD60 + Saline	714.0 ± 96.57
Vehicle + Amphetamine	630.4 ± 74.76
CBD30 + Amphetamine	662.1 ± 54.48
CBD60 + Amphetamine	726.4 ± 77.03

Table 4
Starle response amplitude of mice treated with CBD 24 h before PPI.

Treatment with saline or MK-801 preceded by vehicle, CBD or clozapine	
Treatment	Startle response (A.U.)
Vehicle + Saline	1120 ± 199.8
CBD60 + Saline	1224 ± 115.0
Vehicle + MK-801	1491 ± 187.1
CBD30 + MK-801	1301 ± 164.6
CBD60 + MK-801	966.4 ± 142.8
Clozapine + Saline	1075 ± 149.9
Clozapine + MK-801	1447 ± 169.3

NMDA receptors (Nelson et al., 2008), which might help to explain the lack of effect of this drug in DNA methylation in our study. AMPH, by facilitating dopamine-mediated neurotransmission, would increase global DNA methylation by interfering with distinct intracellular cascades than those engaged by NMDA-receptor activation.

The lack of AMPH effect in the PFC, another brain region involved in the etiology of schizophrenia (Fromer et al., 2016), contrasts with the increased global DNA methylation observed in the PFC of rodents after AMPH withdrawal (Mychasiuk et al., 2013) or repeated methamphetamine treatment (Gonzalez et al., 2018). The reasons for this difference are unclear but could indicate a different role of D2 and D1 receptors in this effect after acute administration, since it is known that there is a large expression of D2 receptors in the striatum, particularly in the nucleus accumbens and a wide expression of D1 receptors in the prefrontal cortex (Missale et al., 1998; Zhang et al., 2000).

CBD and clozapine, however, increased DNA methylation in the PFC compared to the vehicle. The similar changes induced by these two drugs are compatible with the proposal that they might produce similar benefits in cognitive and negative symptoms associated with schizophrenia (Gomes et al., 2014).

Previous studies have already shown that CBD can modulate DNA methylation and gene expression. For example, CBD reverses schizophrenia-like effects and transcriptional changes induced by repeated administration of ketamine, another NMDA receptor antagonist, in genes associated with synaptogenesis and neuronal plasticity in the PFC of rats (Kozela et al., 2020). Pucci et al. (2013) found that CBD can control human skin cell proliferation and differentiation by increasing global DNA methylation and DNMT levels (Pucci et al., 2013). CBD also modulated DNMT activity and DNA methylation levels in the PFC and hippocampus of mice, an effect associated with its antidepressant-like effects (Sales et al., 2020). Moreover, the schizophrenia-like phenotype induced by prenatal exposure of rats to the antimitotic agent methylazoxymethanol acetate (MAM) is accompanied by reduced DNA methylation and increased transcriptional regulation of CB1 receptors. CBD treatment prevented the schizophrenia-like deficits and CB1 changes in the PFC at adulthood, possibly by increasing DNA methylation levels (Stark et al., 2019).

In summary, this study showed that CBD induces an acute antipsychotic-like effect that lasts for 24 h. It also acutely alters global DNA methylation in the ventral striatum and prefrontal cortex. Future studies are needed to investigate which genes are being affected by these methylation changes and if these alterations are responsible for the sustained (24-h) CBD effect.

Authors contributions

J.F.C.P. performed experiments, analyzed the data and wrote the paper. A.J.S helped to perform the molecular assays and the writing of the paper. F.S.G.; S.R.L.J.; J.A.S.C. and E.D.B. designed the experiments, supervised the project, and contributed to the writing the paper. All authors discussed the results and commented on the manuscript at all stages.

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Declaration of Competing Interest

F.S.G., and J.A.C. are coinventors of the patent “Fluorinated CBD compounds, compositions and uses thereof. Pub. No.: WO/2014/108899. International Application No.: PCT/IL2014/050023,” Def. US number Reg. 62193296; July 29, 2015; INPI on August 19, 2015 (BR1120150164927; Mechoulam R, Zuardi A.W., Kapczinski F, Hallak J.E.C, Guimarães F.S., Crippa J.A.S., Breuer A). The University of São Paulo has licensed this patent to Phytecs Pharm (USP Resolution No. 15.1.130002.1.1). The University of São Paulo has an agreement with Prati-Donaduzzi Pharm to “develop a pharmaceutical product containing synthetic CBD and prove its safety and therapeutic efficacy in the treatment of epilepsy, schizophrenia, Parkinson's disease, and anxiety disorders.” J.A.C ans F.S.G. are coinventors of the patent “Cannabinoid-containing oral pharmaceutical composition, method for preparing and using same”, INPI on September 16, 2016 (BR 112018005423-2). J.A.C. is a member of the International Advisory Board of the Australian Centre for Cannabinoid Clinical and Research Excellence (ACRE) – National Health and Medical Research Council (NHMRC). J.A.C. have received travel support to scientific meetings and personal consultation fees from BSPG-Pharm. J.A.C. is recipient of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) productivity fellowships (1 A). J. A.C. received a grant from the University Global Partnership Network (UGPN)— Global priorities in cannabinoid research excellence program. J.A.S.C. is a member of the International Advisory Board of the Australian Centre for Cannabinoid Clinical and Research Excellence (ACRE - National Health and Medical Research Council, NHMRC).

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