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ORIGINAL INVESTIGATION

# Cannabidiol effects in the prepulse inhibition disruption induced by amphetamine

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**Abstract**
*Rationale* The information processing appears to be deficient in schizophrenia. Prepulse inhibition (PPI), which measures the inhibition of a motor response by a weak sensory event, is considered particularly useful to understand the biology of information processing in schizophrenia patients. Drugs that facilitate dopaminergic neurotransmission such as amphetamine induce PPI disruption in human and rodents. Clinical and neurobiological findings suggest that the endocannabinoid system and cannabinoids may be implicated in the pathophysiology and treatment of schizophrenia. Cannabidiol (CBD), a non-psychotomimetic constituent of the *Cannabis sativa* plant, has also been reported to have potential as an antipsychotic.
*Objective* Our aim was to investigate if CBD pretreatment was able to prevent PPI disruption induced by amphetamine. Since one possible mechanism of CBD action is the facilitation of endocannabinoid-mediated neurotransmission through anandamide, we tested the effects of an anandamide hydrolysis inhibitor (URB597) in the amphetamine-induced PPI disruption.
*Methods* Male *Swiss* mice were treated with CBD systemic or intra-accumbens, or URB597 (systemic) prior to amphetamine and were exposed to PPI test.
*Results* Amphetamine (10 mg/kg) disrupted PPI while CBD (15–60 mg/kg) or URB597 (0.1–1 mg/kg) administered alone had no effect. Pretreatment with CBD attenuated the amphetamine-disruptive effects on PPI test after systemic or intra-accumbens administration. Similar effects were also found with the inhibitor of anandamide hydrolysis.
*Conclusion* These results corroborate findings indicating that CBD induces antipsychotic-like effects. In addition, they pointed to the nucleus accumbens as a possible site of these effects. The increase of anandamide availability may be enrolled in the CBD effects.

**Keywords** Cannabidiol · Prepulse inhibition · Amphetamine · Nucleus accumbens · Schizophrenia · Antipsychotic

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## Introduction

Schizophrenia is a multifactorial psychiatric disorder that affects about 1 % of the population in the world and involves genetic factors, neurochemical and development changes in the nervous system (Weiss and Feldon 2001; Lau et al. 2003). The main pathophysiological hypothesis of schizophrenia is based, at least in part, on drug responses. Dopamine receptor antagonism is the main pharmacological property shared by antipsychotic drugs, and psychotomimetic effects may be induced by drugs that increase dopaminergic neurotransmission, such as apomorphine and amphetamine, or by antagonists of the glutamate receptor subtype N-methyl-D-

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aspartate (NMDA), such as phencyclidine (Moore et al. 1999; Bird et al. 2001; Lipska and Weinberger 2005).

Prepulse inhibition (PPI) of startle response is characterized by the reduction of an acoustic startle reflex to an intense acoustic stimulus (pulse) when immediately preceded by a lower-intensity stimulus (prepulse; Swerdlow et al. 2001). PPI is a translational model particularly useful to comprehend the information processing in schizophrenia patients. PPI appears to be present in all mammals, including rats and humans (Swerdlow et al. 1994, 2000) and is disrupted in schizophrenia (Braff and Geyer 1990; Braff et al. 1978, 1992, 1999; Weike et al. 2000). This impairment can be experimentally reproduced by acute or chronic treatment with dopamine agonists, such as apomorphine and quinpirole, drugs that enhance dopaminergic neurotransmission, such as amphetamine and cocaine, or by glutamate NMDA receptor antagonists (Swerdlow et al. 1986; Peng et al. 1990; Mansbach et al. 1988). On the other hand, antipsychotic drugs such as haloperidol, clozapine, and risperidone prevent PPI disruption (Geyer et al. 2001).

Since antipsychotics can induce side effects such as pharmacological parkinsonism, acute dystonia, tardive dyskinesia, weight gain, diabetes, and sedation (Kane 2001; Meltzer 1999), it is important to search for new therapeutic approaches. In this way, preclinical and clinical studies have suggested that cannabidiol (CBD) could be an effective antipsychotic (for review, Campos et al. 2012; Zuardi et al. 2012).

CBD is the main non-psychotomimetic compound of *Cannabis sativa* (Mechoulam and Shvo 1963). CBD presents multitude pharmacological actions (Izzo et al. 2009; Campos et al. 2012). It has low affinity for cannabinoid receptors (Petited et al. 1998; Thomas et al. 1998) but can act as an antagonist or inverse agonist of these receptors (Thomas et al. 1998; Pertwee et al. 2008). CBD may also decrease anandamide hydrolysis and re-uptake, facilitating endocannabinoid-mediated neurotransmission (Bisogno et al. 2001). CBD effects also involve non-cannabinoid mechanisms, facilitating 5-HT1A-mediated neurotransmission in vitro (Russo et al., 2005) and *in vivo* (Mishima et al. 2005), and activating transient receptor potential vanilloid type 1 receptors (Bisogno et al. 2001).

Previous studies have shown that CBD blocks PPI impairment induced by MK-801, an NMDA receptor antagonist (Long et al. 2006). However, CBD effects on PPI impairment induced by amphetamine are still unknown. Moreover, the brain sites of CBD effects in this model have not been investigated. Therefore, the objective of the present paper was to investigate whether CBD systemically or injected into the nucleus accumbens would attenuate the PPI impairment induced by amphetamine. Since the facilitation of endocannabinoid-mediated neurotransmission through inhibition of fatty acid amidohydrolase (FAAH), the enzyme responsible for the metabolism of the endocannabinoid anandamide, is suggested as a possible mechanism of action of CBD, we compare CBD effects with those induced by the FAAH inhibitor URB597. The atypical antipsychotic clozapine was used as a positive control.

## Material and methods

### Animals

The experiments were performed in compliance with the recommendations of SBNeC (Brazilian Society of Neuroscience and Behavior), which are based on the US National Institutes of Health Guide for Care and Use of Laboratory Animals. The experimental protocol was approved by the local Ethical committee. Male Swiss mice (University of São Paulo, Campus Ribeirão Preto, Brazil) weighing 25–30 g were used ($n$=88). The animals were housed in groups of eight and maintained at controlled conditions of light/dark cycle (12–12 h, lights on at 07:00 h) and temperature (23±1 °C). Food and drinking water were available ad libitum.

### Drugs

Drugs were freshly prepared on the day of testing, and the doses were chosen according to previously published studies (Moreira and Guimarães 2005, Issy et al. 2009, 2014; Gomes et al. 2011). D-amphetamine (SIGMA) was dissolved in 0.9 % sterile saline. CBD (THC Pharm, Germany) and URB597 (Calbiochem, USA) were dissolved in Tween 80 to a final concentration of 2 % (*v*/*v*) and in 0.9 % sterile saline. Clozapine (Leponex, Novartis) 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. For intra-cerebral injections, CBD was diluted in grape seed oil (Campos and Guimarães 2008).

### PPI

The operant sessions were conducted simultaneously in two identical standard operant conditioning chambers (startle response systems; Med Associates, Inc., USA; for details see Issy et al. 2014). A continuous acoustic signal provided a background white noise level of 65 dB. The pulse (pulse alone) was a burst of white noise of 105 dB with a rise/decay of 5 ms and duration of 20 ms, and the prepulse intensities were set to 80, 85, and 90 dB of pure tone, 7000-Hz frequency, and duration of 10 ms.

### 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.

### 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 the percentage of PPI. 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 and 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−[100×(PP+P/P)]. Using this formula, a 0 % value denotes no difference between the startle reflex response to the pulse alone and to the prepulse *plus* pulse and thus indicates no PPI. A low percentage score indicates a PPI deficit.

**Pretest session** Using the same protocol, mice were subjected to a PPI pretest session to select those animals with PPI response superior to 0 %. This procedure was not used to assign animals to groups with comparable PPI or startle magnitude. No animal was excluded in the present study.

### Systemic treatment

Four days after the pretest session, the mice were re-subjected to the PPI test to analyze the effects of the pharmacological treatment. Mice were treated with an intraperitoneal injection of vehicle (saline plus Tween 80; 10 ml/kg; $n$=8) or CBD (15, 30, 60 mg/kg; $n$=8/10 per group) or URB597 (0.1, 0.3, 1 mg/kg; $n$=8 per group) or clozapine (5 mg/kg; $n$=8) and, 30 min after, received an intraperitoneal injection of saline or amphetamine (10 mg/kg; $n$=8). The PPI test was performed 30 min after the second treatment.

### Stereotaxic surgery and central treatment

Seven days before the experiment, mice were anesthetized with an association of ketamine (70 mg/kg) and xilazine (30 mg/kg, 1.5 ml/kg, intraperitoneally) and fixed in a stereotaxic frame (Stoelting, Wood Dale, IL, USA). After scalp anesthesia with 2 % lidocaine, the skull was surgically exposed and stainless steel guide cannulae were implanted unilaterally into the nucleus accumbens [ântero-posterior=+1.4 mm from bregma; lateral=+1.2 mm from the medial suture; vertical=−4.mm from the skull with a lateral inclination of 0° (Franklin and Paxinos 2008)]. The cannulae were fixed to the skull with dental cement and a metal screw. An obturator inside the guide cannulae prevented obstruction. The animals received antibiotic (Amoxicillin-Union Chemicals) during the first 7 days post-surgery. One week after stereotaxic surgery, the animals received an intra-accumbens microinjection of CBD (60 nmol; 0.2 μl) or vehicle (0.2 μl grape seed oil), followed immediately by a systemic injection of amphetamine 10 mg/kg. Ten minutes later, the animals were submitted to the PPI test. An infusion pump (Scientific, USA) microsyringe (Hamilton; 10 μl) and a polyethylene catheter (P10) connected to a 30-gauge dental needle were used for the microinjections. CBD or vehicle was microinjected for 5 min, and the needle was kept in the cannula for 1 additional minute to avoid reflux.

### Location of the cannulae in the nucleus accumbens

Mice were deeply anesthetized with a lethal dose of ketamine and xilazine association intraperitoneally, and transcardially perfused with phosphate-buffered saline (PBS) followed by 4 % paraformaldehyde (PFA 4 %) in 0.1 M phosphate buffer (PB). Then, the brains were removed and post-fixed in the same fixative solution (PFA 4 %) for 2 h, and after they were immersed in 30 % sucrose in 0.1 M PB for cryoprotection during 48 h. The brains were frozen at −40 °C on dry ice and isopentane, and 25-μm-thick serial coronal sections were obtained using a cryostat microtome at −20 °C.

### Statistical analysis

The PPI results were analyzed by mixed-design analysis of variance (ANOVA) with treatments (treatment 1, vehicle or CBD; treatment 2, saline or amphetamine) as the main independent factors, and prepulse intensity (80, 85, and 90 dB) as the repeated factor. Specifically, data from the FAAH inhibitor URB597 were analyzed by repeated measures with treatment as between-subject factors and prepulse intensity as a within-subject factor. Startle results were analyzed by one-way ANOVA. In all cases, Duncan test was used for multiple comparisons. The significance level was set at $P<0.05$. All of the data are expressed as the mean±SEM.

## Results

### CBD pretreatment attenuates amphetamine-disruptive effects in PPI: systemic administration

Mixed-design ANOVA indicated significant effects of prepulse intensity ($F2,134=17.23$, $P<0.001$), treatment 1 ($F3,67=2.7$, $P=0.05$), and treatment 2 ($F1,67=73.61$, $P<0.001$). There was also an interaction between prepulse intensity and treatment 2 ($F2,134=7.71$, $P=0.003$). Two-way ANOVA analyses conducted at each prepulse intensity showed significant effects of treatment 1 at 80 and 85 dB (80 dB, $F3,67=3.07$, $P=0.03$; 85 dB, $F3,67=2.65$, $P=0.05$) and of treatment 2 at all prepulse intensities (80 dB, $F1,67=52.44$, $P<0.001$; 85 dB, $F1,67=85.12$, $P<0.001$; 90 dB, $F1,67=50.90$, $P<0.001$). Additionally, a tendency for interaction between treatment 1 versus treatment 2 was observed at 80 dB ($F3,67=4.09$, $P=0.07$). Post hoc analysis indicated that animals treated with vehicle+amphetamine showed a significant impairment of PPI compared to control at all prepulse intensities (vehicle+saline; Duncan, $P<0.05$). At 80 and 85 dB, however, PPI impairment induced by amphetamine was attenuated by CBD (30 and 60 mg/kg; Fig. 1a). Moreover, CBD, by itself, did not induce any change (Duncan, $P>0.05$). There was no significant change in the acoustic startle response (Fig. 1b).

Clozapine was used as a positive control. Pretreatment with clozapine prevented the disruptive effect of amphetamine at prepulse intensities of 80 and 90 dB (Duncan, $P<0.05$; Fig. 1c). Clozapine treatment significantly increased the startle reflex amplitude (one-way ANOVA, Duncan, $P<0.05$; Fig. 1d).

Prepulse trials did not elicit startle response in all prepulse intensities (mean±SEM of all treatments of CBD group (vehicle+saline; CBD 60 mg/kg+saline; vehicle+amphetamine; CBD 15 mg/kg+amphetamine; CBD 30 mg/kg+amphetamine; CBD 60 mg/kg+amphetamine; 80 dB (54±2.17); 85 dB (58±1.73); 80 dB (65±1.75)).

### Location of the cannulae in the nucleus accumbens

The cannula sites in the nucleus accumbens core or shell of animals included in data analyses can be seen in Fig. 2. Animals with the injection sites outside the nucleus accumbens core or shell were used to test CBD effects as a negative control. In these animals, the CBD microinjection did not modify amphetamine-induced PPI disruption (Duncan, $P>0.05$; vehicle+amphetamine, 80 dB (13±23.4); 85 dB (11±23.3); 90 dB (27±8.5), $n=4$; CBD+amphetamine, 80 dB (22.25±5.6); 85 dB (18±16.7); 90 dB (36.25±5.4), $n=4$).

### CBD administration into accumbens attenuates amphetamine-disruptive effects in PPI

Mixed-design ANOVA indicated significant effect of prepulse intensity ($F2,50=3.24$, $P=0.05$). There was also an interaction between prepulse intensity and treatment 2 ($F1,25=27.17$, $P<0.001$). Two-way ANOVA analyses conducted at each prepulse intensity showed significant effects of treatment 1 at 90 dB ($F1,25=6.12$, $P=0.02$), and of treatment 2 at all prepulse intensities (80 dB, $F1,25=9.35$, $P=0.005$; 85 dB, $F1,25=29.36$, $P<0.001$; 90 dB, $F1,25=28.35$, $P<0.001$). Additionally, there is a significantly interaction between treatment 1 versus treatment 2 at 90 dB ($F1,25=6.38$, $P=0.018$). Post hoc analysis indicated that animals treated with vehicle+amphetamine showed a significant impairment of PPI compared to control at all prepulse intensities (vehicle+saline; Duncan, $P<0.05$). At 90 dB, however, PPI impairment induced by amphetamine was attenuated by CBD (60 nmol); Fig. 3a). Moreover, CBD, by itself, did not induce any change (Duncan, $P>0.05$).

The startle amplitude was significantly decreased by amphetamine (Fig. 3b). A reduction in the startle amplitude would account for a "floor" effect in the prepulse+pulse trials and should not reflect a real reduction in the PPI, as statement by Swerdlow and coauthors (2000). Therefore, in order to determine whether the decrease of PPI percent by amphetamine was merely a reflection of the decreased startle amplitude rather than a true decrease in PPI response, a subgroup analysis was performed on the mice matched for startle amplitudes, following similar protocol presented by Flood and coworkers (2010). The matching was performed by selecting mice in each group of amphetamine treatment to yield subgroups with similar means for startle amplitude. The median was used to select amphetamine subgroups exhibiting the highest or the lowest startle amplitudes. We do not found a significant difference between groups (highest startle, 48±2.97, $n=8$; lowest startle, 27±10.27, $n=8$). Although amphetamine decreased the startle response, intra-accumbens administration of CBD did not change this response.

Prepulse trials did not elicit startle response in all prepulse intensities (mean±SEM of all treatments; 80 dB (42±5.85); 85 dB (50±3.74); 80 dB (53±3.92)).

### URB597 pretreatment attenuates amphetamine-disruptive effects in PPI: systemic administration

The repeated measures ANOVA revealed a significant main effect of treatment [$F5,40=8.899$, $P<0.001$], of the prepulse intensity [$F2,80=5.713$, $P=0.005$], and interaction between prepulse intensity and treatment [$F10,80=2.009$, $P=0.043$]. Post hoc analysis indicated that animals treated with vehicle+amphetamine showed a significant impairment of PPI

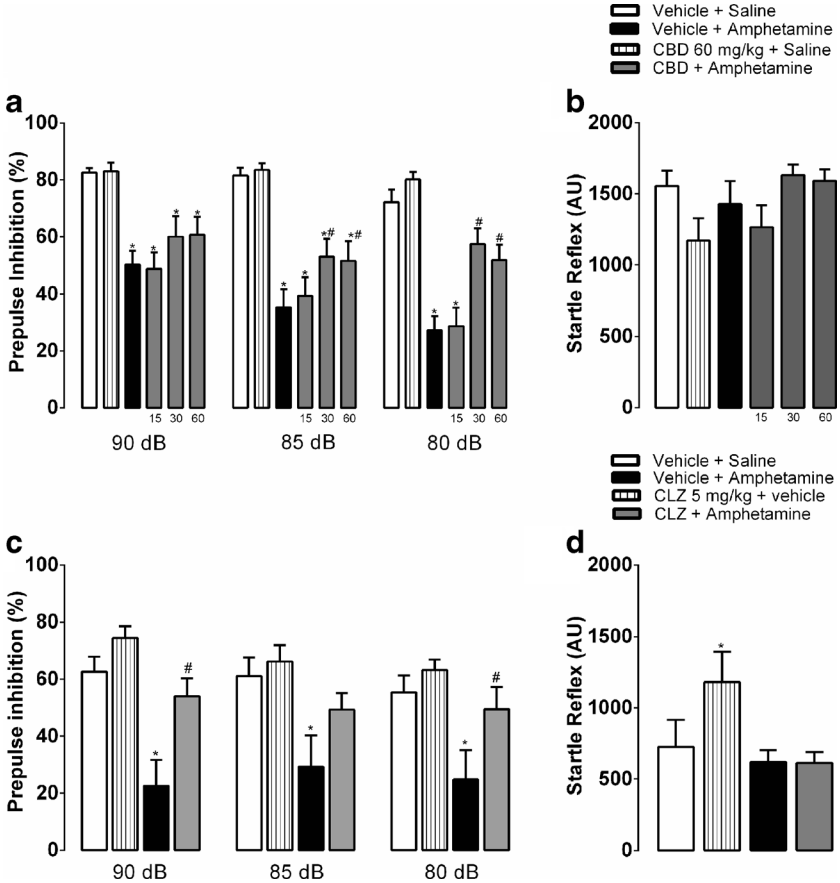


**Fig. 1** **a** Effects of CBD pretreatment in the amphetamine-induced PPI disruption. The disruptive effect of amphetamine treatment (10 mg/kg) in the PPI was attenuated by CBD pretreatment (30 and 60 mg/kg; i.p.) in all prepulse intensities. The effects of CBD were tested at all doses (15, 30, and 60 mg/kg), despite only the major one is showed. **b** Startle response amplitude (pulse-only trials, arbitrary units, AU) was assessed in mice treated with saline or amphetamine preceded by vehicle or CBD. Startle amplitude was not modified. **c** Clozapine pretreatment effects on amphetamine-induced PPI disruption. The disruptive effect of amphetamine treatment (10 mg/kg) in the PPI test was attenuated by clozapine pretreatment (5 mg/kg; i.p.). **d** Startle response amplitude was assessed in mice treated with saline or amphetamine preceded by vehicle or clozapine. Clozapine treatment significantly increased the startle reflex amplitude. Duncan's post hoc test $^{*}P<0.05$ compared to vehicle+saline group. $^{\#}P<0.05$ compared to saline+amphetamine group. Data are expressed as mean±SEM. $n$=8–10 per treatment (**a**, **b**); $n$=8 per treatment (**c**, **d**)

compared to control at all prepulse intensities (vehicle+saline; Duncan, $P<0.05$). At all prepulse intensities, PPI impairment induced by amphetamine was attenuated by URB597 dose-dependently (0.3 mg/kg at all prepulse intensities, and 1 mg/kg at 90 dB, Fig. 4a). There was no significant change in the acoustic startle response (Duncan, $P>0.05$, Fig. 4b).

## Discussion

This study shows that CBD attenuates the PPI disruption induced by amphetamine. Moreover, our data suggests that CBD infusion into the nucleus accumbens seems to be sufficient for the attenuation of PPI disruption. However, these results do not exclude the potential role of other structures in the CBD effects. In addition, we found that the FAAH inhibitor, similar to CBD, attenuated the amphetamine-induced PPI deficit. These results allow the suggestion that CBD action mechanism may be related to the facilitation of endocannabinoid-mediated neurotransmission through anandamide increase. Further studies are required to describe CBD mechanisms of action in the PPI modulation.

The present results agree with previous reports of CBD effects in predictive models for antipsychotic effects based on the dopamine hypothesis of schizophrenia. For example, CBD and haloperidol attenuated apomorphine-induced stereotypy in rats, but only the latter produced catalepsy, a model associated with extrapyramidal side effects induced by typical antipsychotics (Carlsson 1998; Howes and Kapur 2009; Zuardi et al. 1991). Additionally, Moreira and Guimarães (2005) observed that CBD decreased amphetamine-induced hyperlocomotion in mice. These effects were observed at the dose of 60 mg/kg, the same dose that attenuated the PPI disruption induced by amphetamine in our study.

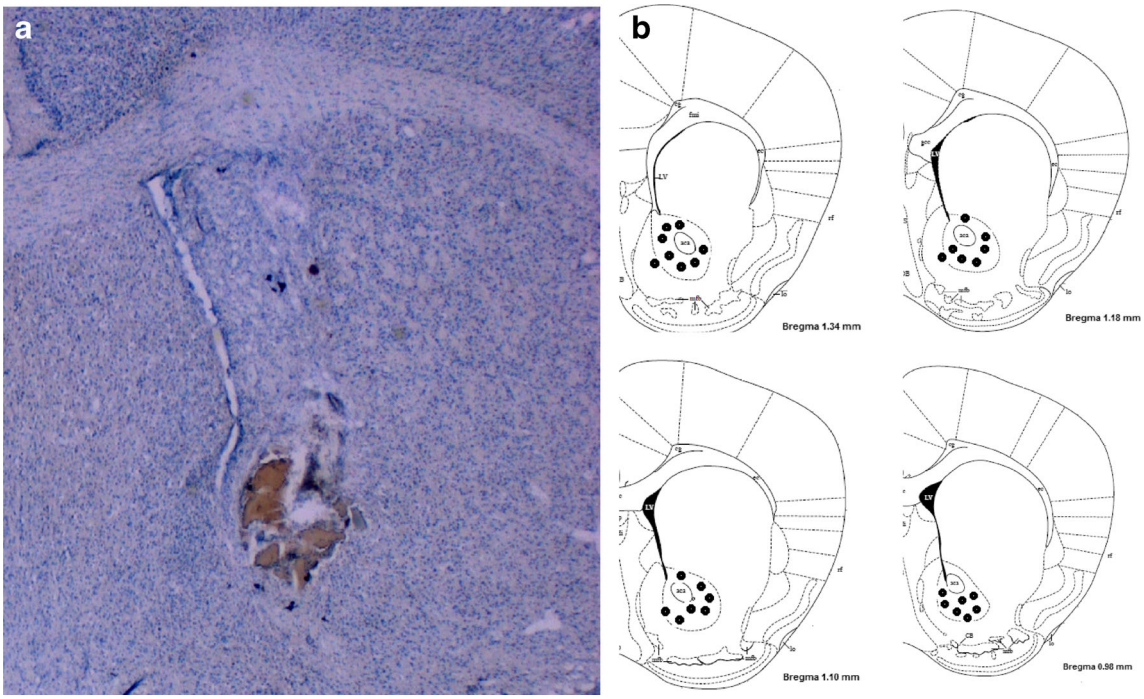


**Fig. 2** Representative photomicrograph (**a**) and figure of the injection site location in the nucleus accumbens (**b**). The cannula sites were located in plates 1.34 to 0.98 mm from bregma according to the Franklin and Paxinos (2008) mouse brain atlas

CBD was also able to attenuate the hyperlocomotion induced by ketamine, extending its antipsychotic-like effects to schizophrenia models based on the glutamatergic system (Moreira and Guimarães 2005). In the same study, similar to clozapine, CBD did not induce catalepsy. These results corroborate the hypothesis that CBD may act as an atypical antipsychotic. In agreement with this possibility, Long and colleagues (2006) found that CBD (5 mg/kg), similar to clozapine (4 mg/kg), reversed the PPI impairment induced by MK-801 (1 mg/kg). Since it also improved the reduced social interaction caused by this treatment, it was suggested that CBD could attenuate both the positive and negative symptoms of schizophrenia (Long et al. 2006; Gururajan et al. 2011).

Together, these preclinical results indicate that CBD possesses antipsychotic properties. This was recently confirmed in spontaneously hypertensive rats (SHRs). These animals exhibit behavioral changes such as a basal disruption in PPI that have been associated with schizophrenia, since they are reversed by clozapine (Levin et al. 2011). Similar to antipsychotic drugs, CBD also increases the PPI response in these

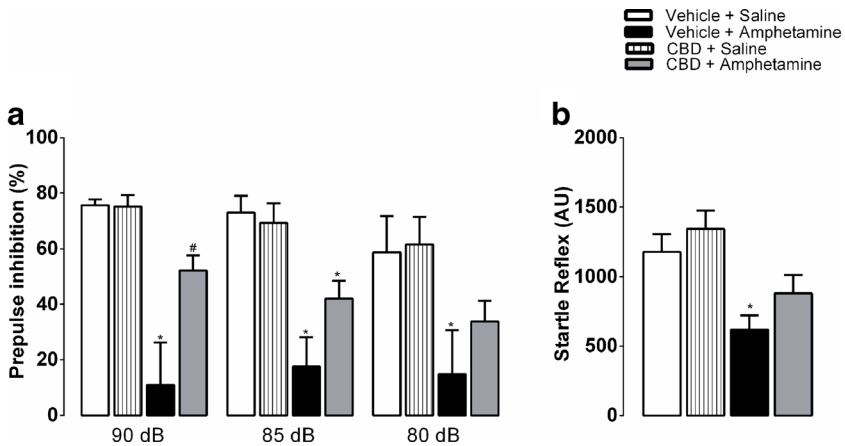


**Fig. 3** Effects of CBD microinjection into nucleus accumbens in the amphetamine-induced PPI disruption. **a** CBD (60 nmol) intra-accumbens microinjection attenuated the disruptive effect of amphetamine (10 mg/kg) systemic treatment in the PPI response. **b** Startle response amplitude (pulse-only trials) was assessed in mice systemically treated with saline or amphetamine preceded by intra-accumbens microinjection of vehicle or CBD. Amphetamine treatment significantly decreased the startle reflex amplitude. Duncan's post hoc test $^{*}P<0.05$ compared to vehicle+saline group. $^{\#}P<0.05$ compared to saline+amphetamine group. Data are expressed as mean±SEM; $n=7$–9 per treatment

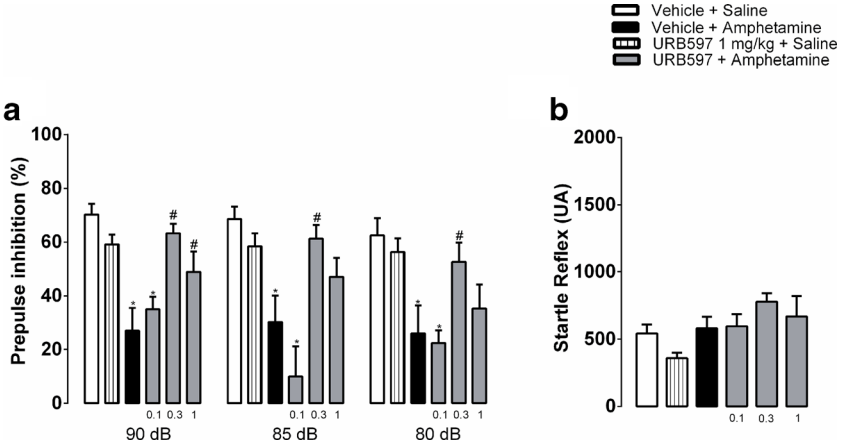


**Fig. 4** **a** Effects of URB597 pretreatment in the amphetamine-induced PPI disruption. The disruptive effect of amphetamine treatment (10 mg/kg) on PPI response was attenuated by URB597 pretreatment (0.3 and 1 mg/kg; i.p.). Only the highest dose of URB597 was tested as control. **b** Startle response amplitude (pulse-only trials) was assessed in mice treated with saline or amphetamine preceded by vehicle or URB597. Duncan's post hoc test $^{*}P<0.05$ compared to vehicle+saline group. $^{\#}P<0.05$ compared to saline+amphetamine group. Data are expressed as mean±SEM; $n=8$–10 per treatment

animals (Levin et al. 2014). In contrast to our results, these authors also found that anandamide uptake inhibitor did not modify PPI response in SHR strain. This pharmacological approach was not tested in the amphetamine-induced PPI disruption.

The antipsychotic properties of CBD are also confirmed by clinical studies. For example, it prevented the psychotomimetic effects of high doses of delta-9-tetrahydrocannabinol (Zuardi et al. 1982; Englund et al. 2013). Also, the symptom improvement induced by CBD in schizophrenic patients was initially detected in open studies with small samples (for review see Zuardi et al. 2012). It was recently confirmed in a double-blind study that CBD was as effective as amisulpride but did not induce common antipsychotic undesirable effects such as extrapyramidal symptoms, prolactin increase, and weight gain (Leweke et al. 2012).

The brain sites of the antipsychotic-like effect of CBD had not yet been investigated but could involve the nucleus accumbens, an area proposed to mediate the effects of antipsychotic drugs on the positive symptoms of schizophrenia (for review, see Seeman et al. 2002). In a previous study, CBD, at doses similar to those used in the present work, increased neuronal activation measured by cFos expression in the nucleus accumbens (Guimarães et al. 2004). In addition, even if other regions such as the prefrontal cortex could also be involved (Csernasnky et al. 1993; Swerdlow et al. 2001), several pieces of evidence indicate that the nucleus accumbens is an essential structure in PPI modulation (Swerdlow et al. 2001).

The nucleus accumbens receives a dense dopaminergic innervation from the midbrain ventral tegmental area and contains several neurotransmitters that regulate PPI (Koch et al. 1999). Facilitation of dopamine-mediated neurotransmission in this structure impairs PPI (Swerdlow et al. 1992, 2001; Wan et al. 1994) whereas dopamine receptor antagonists reverse PPI deficits induced by dopamine direct or indirect agonists (Swerdlow et al. 1990, 1994; Wan et al. 1994). A similar effect was found after nucleus accumbens dopamine depletion after the use of the neurotoxin 6-OHDA (Zhang et al. 2000). Our results corroborate the proposed central role of the nucleus accumbens in the control of sensory-motor filter and suggest that CBD action in this brain region is important for its ability to decrease the effects of amphetamine on PPI.

The pharmacological mechanisms involved in the antipsychotic effects of CBD remain unclear. CBD has a lower affinity for CB1 and CB2 receptors but can indirectly increase endocannabinoid tonus by decreasing anandamide reuptake/metabolism (Bisogno et al. 2001). In fact, it has been suggested that CBD antipsychotic profile may be related to its ability to increase the availability of anandamide (Schubart et al. 2013). In a clinical study conducted by Giuffrida and coworkers (2004), schizophrenic patients were compared to healthy controls, and it was observed that the first group had higher concentrations of anandamide in cerebrospinal fluid, and this concentrations were negatively correlated with psychotic symptoms. This data may suggest that the increase of anandamide involves a negative feedback response in order to counteract the psychotic symptoms, and the D2 receptor hiperactivation.

In a complementary study conducted by Leweke and coworks (2012), a significant association between anandamide levels and the improvement of psychotic symptoms was found. These authors also found that the same CBD concentration that inhibits, in vitro, anandamide metabolism does not interact with receptors commonly associated with schizophrenia such as dopamine, GABA, serotonin, and glutamate. Moreover, facilitation of CB1-mediated neurotransmission by CBD also increases adult hippocampal neurogenesis (Campos et al. 2013), a mechanism that could be involved in cognitive

deficits observed in schizophrenic patients (Zuardi et al. 2011). CBD and anandamide can also activate TRPV1 channels, which could result in facilitation of glutamate presynaptic release (Zygmunt et al. 1999). This mechanism has been related to CBD blockade of PPI disruption induced by MK-801, a NMDA receptor antagonist (Long et al. 2006). Corroborating the hypothesis that anandamide has a role in the CBD antipsychotic-like effects, we provide the first demonstration that URB597, a known potent and selective inhibitor of the enzyme which catalyzes the hydrolysis of anandamida (Piomelli et al. 2006), has similar effects to CBD in the PPI test. The same action mechanism was found to attenuate the hyperactivity induced by dopamine D2/D3 agonism (Luque-Rojas et al. 2013). In addition, in rats, the properties of the anandamide transport inhibitor N-(4-hydroxyphenyl)-arachidonamide (AM404) counteracting behavioral responses associated with activation of dopamine D2 family receptors were demonstrated (Beltrano et al. 2000). The property of the endocannabinoid system as a relevant negative modulator of both D1 and D2 receptor–mediated behaviors was also demonstrated by Martín and coworkers (2008).

Furthermore, CBD also possesses several other mechanisms such as facilitation of 5HT1A receptor–mediated neurotransmission (an effect shared by the atypical antipsychotic aripiprazol) or anti-inflammatory/neuroprotective properties (for review see Campos et al. 2012) that could help to explain its antipsychotic effects. Further investigation is required.

In conclusion, the present study found that CBD attenuates PPI disruption induced by amphetamine. Moreover, we showed that this effect could be mediated by CBD direct action in the nucleus accumbens. Together, the results support the hypothesis that CBD might be a useful antipsychotic drug.

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**Conflicts of interest** There are no conflicts of interest.

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