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THC inhibits the expression of ethanol-induced locomotor sensitization in mice

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ABSTRACT

The motivational circuit activated by ethanol leads to behavioral changes that recruit the endocannabinoid system (ECS). Case reports and observational studies suggest that the use of *Cannabis* sp. mitigates problematic ethanol consumption in humans. Here, we verified the effects of the two main phytocannabinoid compounds of *Cannabis* sp., cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC), in the expression of ethanol-induced locomotor sensitization in mice. Male adult DBA/2 mice were exposed to locomotor sensitization by daily intraperitoneal injections of ethanol (2.5 g/kg) for 12 days; control groups received saline. After the acquisition phase, animals were treated with cannabinoids: CBD (2.5 mg/kg); THC (2.5 mg/kg); CBD + THC (1:1 ratio), or vehicle for 4 days with no access to ethanol during this period. One day after the last cannabinoid injection, all animals were challenged with ethanol (2.0 g/kg) to evaluate the expression of the locomotor sensitization. Mice treated with THC alone or THC + CBD showed reduced expression of locomotor sensitization, compared to the vehicle control group. No effects were observed with CBD treatment alone. Our findings showing that phytocannabinoid treatment prevents the expression of behavioral sensitization in mice provide insight into the potential therapeutic use of phytocannabinoids in alcohol-related problems.

Introduction

The endocannabinoid system (ECS) plays an important regulatory role in several neurotransmission systems (Basavarajappa, 2007; Mechoulam & Parker, 2013), including those involved in motivational circuitry (Friemel, Zimmer, & Schneider, 2014; Maldonado, Valverde, & Berrendero, 2006; Parolaro, Vigano, Realini, & Rubino, 2007). The ECS has been linked with drug-associated behavior and neuronal plasticity related to the motivational process (Maldonado et al., 2006; Parsons & Hurd, 2015; Prud'homme, Cata, & Jutras-Aswad, 2015). For example, chronic exposure to ethanol has been associated with changes in endocannabinoid signaling (Basavarajappa, 2007; Wang, Liu, Harvey-White, Zimmer, & Kunos, 2003), and blockade of endocannabinoid receptors has been shown to reduce ethanol intake (Arnone et al., 1997; Cippitelli et al., 2005; Colombo et al., 2007; Gallate & McGregor, 1999; Maccioni, Colombo, & Carai, 2010; Wang et al., 2003).

Animal models of locomotor sensitization have been used to assess motivational salience provoked by recurrent exposure to drugs of abuse, such as ethanol (Abrahao et al., 2013; Coelhoso et al., 2013; Robinson & Berridge, 2008; Steketee & Kalivas, 2011). Repeated administration of ethanol induces progressive and persistent increase of locomotor activity, even after prolonged periods of withdrawal (Boehm, Goldfarb, Serio, Moore, & Linsenbardt, 2008; Coelhoso et al., 2013; Steketee & Kalivas, 2011). Sensitization to ethanol increases dopamine release and neuronal plasticity in the striatum (Abrahao et al., 2013; Oleson & Cheer, 2012), a region

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known to regulate motivational behaviors (Koob & Volkow, 2016). Evidence suggests that ethanol-induced plasticity is modulated by the ECS (Hungund, Szakall, Adam, Basavarajappa, & Vadasz, 2003; Lovinger, 2010; Maldonado et al., 2006), but whether exogenous manipulation of the ECS can affect the behavioral expression of ethanol-induced sensitization remained unknown until now. To address this question, we used two of the main phytocannabinoid compounds found in *Cannabis sativa*: delta-9-tetrahydrocannabinol (THC), which acts as a direct agonist of cannabinoid receptors, and cannabidiol (CBD), which indirectly increases the levels of endocannabinoids (Devinsky et al., 2014; Mechoulam & Parker, 2013).

Methods

A total of 84 animals were submitted to this protocol (40 received ethanol and 44 received saline). DBA/2 mice were originally acquired from The Jackson Laboratory. The animals were bred and raised in the Instituto de Farmacologia e Biologia Molecular (INFAR) – Universidade Federal de São Paulo, Brazil. In this study, the related strain was designated as DBA/2 to indicate that the animals used were born after three generations of breeding from the initial matrix (DBA/2J) received from The Jackson Laboratory. Male adult mice were separated into groups of 8 subjects and kept in home cages (40 × 34 × 17 cm) in a light/dark cycle (12/12 h, lights on at 7:00 AM), with free access to food and water. The study was carried out in strict accordance with the recommendations established by the National Institute of Health (Publications No. 8023, revised 1978) for the care and use of laboratory animals.

Ethanol 15% (Synth®) was diluted in saline (NaCl 0.9% in water solution), and was administered in doses of 2.0 g/kg and 2.5 g/kg, intraperitoneally (i.p.). CBD and THC, originally obtained from the National Institute of Health of the United States, were kindly provided by Prof. Elisaldo Carlini (Preventive Medicine Department/ Universidade Federal de São Paulo – UNIFESP). A single dose of 2.5 mg/kg of CBD and THC was tested. This dose was selected according to previous studies demonstrating no deleterious effects on locomotor activity (El-Alfy et al., 2010; Long et al., 2010; Tai et al., 2015). DMSO 8% (Sigma–Aldrich®), Tween 20 1% (Biorad®), and saline were used in the cannabinoids dilution.

Mice were handled daily for 1 week before experimental procedures, in order to reduce stress. Locomotor activity was recorded by a video camera located in the top of the open field that used as the sensitization apparatus. The distance traveled by the mice was measured in centimeters by Ethovision® software (Amsterdam, The Netherlands). All experiments were carried out during the afternoon. The apparatus used as sensitization context was made in wood boxes painted with acrylic white paint (22.5 × 23 cm × 35 cm). Animals were habituated to the experimental room for 30 min before the start of the experiment. The behavioral test was performed immediately after the injection. Brightness in the experimental room was 100 lux. The sensitization apparatus was cleaned with ethanol (70%) between each animal, to remove possible odor cues.

Locomotor sensitization was carried out as proposed by Stephen Boehm II and colleagues (2008) (Fig. 1). The baseline activity was registered on the first day of the experiment, when the animals received an i.p. injection of saline and were immediately placed in the sensitization context for 15 min. On the second day, animals received the first ethanol injection (2.0 g/kg, i.p.) and were promptly placed in the sensitization context for 15 min. This procedure allowed us to record the acute locomotor effects of ethanol administration. From the 2nd to the 11th day of the experiment, mice received saline or ethanol (2.5 g/kg) (i.p.) and were returned directly to their home cages. On the 12th day, the acquisition of locomotor sensitization was registered after ethanol (2 g/kg, i.p.) administration and a sequential 15 min of exposure to the sensitization context.

After the acquisition phase, both groups (saline and ethanol) were randomized into four groups (N = 10–12 per group), described below:

- Saline: Vehicle (Saline_VEH) – animals received saline during the acquisition phase and vehicle for 4 days as treatment; Cannabidiol (Saline_CBD) – animals received saline during the acquisition phase and 2.5 mg/kg CBD for 4 days as treatment; Tetrahydrocannabinol (Saline_THC) – animals received saline during the acquisition phase and 2.5 mg/kg THC for 4 days as treatment; Tetrahydrocannabinol + Cannabidiol (Saline_THC + CBD) – animals received saline during the acquisition phase and a mixture of 2.5 mg/kg of THC and CBD (1:1) for 4 days as treatment.
- Ethanol: Vehicle (Ethanol_VEH) – animals received ethanol during the acquisition phase and vehicle for 4 days as treatment; Cannabidiol (Ethanol_CBD) – animals received ethanol during the acquisition phase and 2.5 mg/kg CBD for 4 days as treatment; Tetrahydrocannabinol (Ethanol_THC) – animals received ethanol during the acquisition phase and 2.5 mg/kg THC for 4 days as treatment; Tetrahydrocannabinol + Cannabidiol (Ethanol_THC + CBD) – animals received ethanol during the acquisition phase and a mixture of 2.5 mg/kg of THC and CBD (1:1) for 4 days as treatment.

The VEH group received 5% DMSO, 1% Tween 20 in saline 0.9%. THC was administered, combined with CBD at a ratio of 1:1, at a dose of 2.5 mg/kg. Treatments were performed daily during 4 days after the acquisition phase.

One day after the phytocannabinoids treatment, all experimental groups, including saline control groups, were challenged with ethanol. Animals received ethanol injections (2.0 g/kg, i.p.) and were immediately placed in the sensitization context for 15 min, in order to measure the expression of locomotor sensitization.

All groups had been previously analyzed using the Shapiro–Wilk test and showed normal distribution in this evaluation. Analysis of variance tests (ANOVA) were conducted for the experimental analysis. Two-way ANOVA with repeated measures was performed to evaluate the baseline and acquisition of locomotor sensitization. In this test, baseline, 1st, and 12th acquisitions were considered the factor of time (repeated measures), the saline or ethanol was considered factor 1 (sensitization), and the vehicle or phytocannabinoid injection (vehicle, CBD, THC, THC + CBD) was considered factor 2 (treatment). For the expression phase, two-way ANOVA was performed considering two factors (factor 1: saline or ethanol during acquisition phase, and factor 2: vehicle or phytocannabinoid). After all experimental analyses, the Newman–Keuls *post hoc* test was used to verify the specific differences between the groups. The level of significance adopted was $p < 0.05$. All statistical analyses were made using the software Statistica 12®.

Results

In the baseline day, as expected, no differences were detected by the two-way ANOVA with repeated measures, since no interaction between the factors 'sensitization' and 'treatment' were found ($F_{(3,76)} = 1.1697$, $p = 0.32691$). The result demonstrates that there was no difference in basal locomotor prior to the ethanol treatment.

During the acquisition phase, all ethanol-treated animals presented increased locomotion when comparing day 1 and day 12 (factor time: $F_{(2,152)} = 167.56$, $p < 0.0001$; factor sensitization: $F_{(1,76)} = 278.24$, $p < 0.0001$; interaction: $F_{(6,152)} = 2.9396$, $p = 0.0096$). The Newman–Keuls *post hoc* test detected differences in the locomotor activity between day 1 and day 12 in all ethanol-