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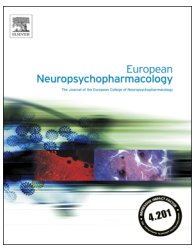

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Δ^9-Tetrahydrocannabinol alone and combined with cannabidiol mitigate fear memory through reconsolidation disruption

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Abstract
Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the major constituents of the *Cannabis sativa* plant, which is frequently consumed by subjects exposed to life-threatening situations to relief their symptomatology. It is still unknown, however, whether THC could also affect the maintenance of an aversive memory formed at that time when taken separately and/or in conjunction with CBD. The present study sought to investigate this matter at a preclinical level. We report that THC (0.3–10 mg/kg, i.p.) was able to disrupt the reconsolidation of a contextual fear memory, resulting in reduced conditioned freezing expression for over 22 days. This effect was dependent on activation of cannabinoid type-1 receptors located in prelimbic subregion of the medial prefrontal cortex and on memory retrieval/reactivation. Since CBD may counteract the negative psychotropic effects induced by THC and has been shown to be a reconsolidation blocker, we then investigated and demonstrated that associating sub-effective doses of these two compounds was equally effective in attenuating fear memory maintenance in an additive fashion and in a dose ratio of 10 to 1, which contrasts with that commonly found in *C. sativa* recreational samples. Of note, neither THC alone nor CBD plus THC interfered with anxiety-related behaviors and locomotor activity, as assessed in the elevated plus-maze test, at a time point coinciding with that used to evaluate their effects on memory reconsolidation. Altogether, present findings suggest a potential therapeutic value of using THC and/or CBD to

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mitigate a dysfunctional aversive memory through reconsolidation disruption in post-traumatic stress disorder patients.

1. Introduction

Cannabis has commonly been the most widely used illicit substance worldwide (Volkow et al., 2014). It's already described effects on brain function are primarily attributed to Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) (Pertwee, 2008; Izzo et al., 2009), which directly or indirectly potentiate the endocannabinoid system through cannabinoid type-1 (CB1) receptor signaling mechanisms (Mechoulam and Lichtman, 2003; Pertwee, 2012). Interestingly, accumulating evidence has indicated that whereas appropriate doses of CBD are able to counteract THC-induced negative psychotropic effects, such as psychotic-like behaviors (Bhattacharyya et al., 2009), the impairing effects of these phytocannabinoids on learning and memory processing may be similar (Klein et al., 2011; Morgan et al., 2012; Niesink and van Laar, 2013).

Considering the augmented *Cannabis* use by post-traumatic stress disorder (PTSD) patients (Kessler et al., 1995; Cornelius et al., 2010), which may present changes in endocannabinoid system functioning (Hauer et al., 2013; Neumeister et al., 2013), this conduct could represent an empirical self-medication attempt to affect the maintenance of a dysfunctional aversive learning, which is believed to underlie this psychiatric condition (Parsons and Ressler, 2013; Trezza and Campolongo, 2013). Recent preclinical findings from our research group have supported this hypothesis, as CBD was able to mitigate aberrant and enduring fear memories by disrupting their reconsolidation process (Stern et al., 2012; Gazarini et al., in 2015). However, it is still unknown whether THC could also alleviate, in a long-lasting manner, the outcome of an aversive memory through reconsolidation disruption when taken either separately or in conjunction with CBD. If so, it could encourage further investigation of their efficacy in PTSD patients, whose dysfunctional memories are usually resilient to therapeutic approaches targeting their permanent attenuation (Cain et al., 2012; Debiec, 2012; Pitman et al., 2012).

The first objective of the present study was to investigate whether systemic administration of THC could disrupt the reconsolidation of a contextual fear memory in rats. Its second objective was to investigate how and where in the brain THC could induce this effect. Thereafter, since CBD is less potent than THC in mitigating a fear memory through reconsolidation disruption, we hypothesized that the association of sub-effective doses of these two compounds could be as effective as THC alone when administrated in a high CBD-low THC dose ratio, which contrasts with that found commonly in *Cannabis* products (Zamengo et al., 2014).

2. Experimental procedures

2.1. Animals

Experiments were performed in male Wistar rats (bred and raised by the animal house of the Federal University of Santa Catarina, Florianopolis, Brazil) aged 14–16 weeks and kept grouped on a 12 h light/dark cycle, with lights switched on at 7:00 AM and food and water ad libitum. All procedures were approved by the Institutional Ethical Committee for the care and use of laboratory animals of our University in compliance with Brazilian legislation.

2.2. Drugs

THC (THC-Pharma, Germany; 0.1–10 mg/kg i.p.) was dried and resuspended in 5% of DMSO (Sigma, USA) and dissolved in a PBS solution containing 0.1% of bovine serum albumin (Sigma, USA). CBD (THC-Pharma, Germany; 1.0 and 3.0 mg/kg) and AM251 (Tocris, USA; 1.0 mg/kg i.p. and 50 pmol bilaterally into the prelimbic cortex) were dissolved in NaCl 0.9% containing 5% of Tween 80® (Vetec, Brazil). The choice of CBD and AM251 doses was based on pilot experiments and/or previously published studies (Stern et al., 2012; Gazarini et al., in 2015).

2.3. Stereotaxic surgery and drug infusion

Each animal was anesthetized using 1.0 ml/kg of a solution containing xylazine (10 mg/ml; Carlier, Brazil) and ketamine (100 mg/ml; Sespo, Brazil), in association with local anesthesia (3.0% lidocaine with 1:50000 norepinephrine; Dentsply, Brazil), and then positioned in a stereotaxic frame. Two stainless steel guide cannulas were implanted bilaterally aiming at the prelimbic cortex and fixed to the skull with acrylic resin and two stainless steel screws. The cannula tips were 1.5 mm above the site of drug infusion. A stylet was introduced inside each guide cannula to reduce possible occlusion. For post-surgery analgesia, each animal received subcutaneous flunixin meglumine (Schering-Plough, Brazil; 2.5 mg/kg).

Ten days after the stereotaxic surgery, each animal received a bilateral infusion with dental needles introduced through the guide cannulas until their tips were 1.5 mm below the end of the cannula. Using two microsyringes connected to an infusion pump, a 0.2 μl/hemisphere of either vehicle or AM251 was injected during 1 min. A polyethylene catheter was interposed between the upper end of the dental needles and the microsyringes. To monitor drug flow, the displacement of an air bubble inside the polyethylene catheter was used. To prevent backflow, the needles were removed 30 s after the end of injections.

After the conclusion of experiment 4B, rats were intraperitoneally anesthetized and injected through the cannulas with 0.2 μl/side of Evans Blue, to mark the sites where drugs were previously infused, and then transcardially perfused with 0.9% of NaCl followed by 10% of formalin solution. Each rat brain was removed and immersed in a 10% formalin solution. Slices (50 μm thick) were obtained in a cryostat (Leica, Germany), mounted on glass microscope slides, and stained with Giemsa to localize anatomically the Evans Blue marks in diagrams from Paxinos and Watson's (2009) rat brain atlas. Their location in prelimbic cortex ranged from 3.7 to 3.0 mm anterior to Bregma. Figure 4C shows a schematic drawing of infusion sites placement into the prelimbic cortex. Animals receiving bilateral vehicle or AM251 infusion outside this medial prefrontal cortex subregion were excluded from the analysis.