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Brief Report

# Short- and long-term effects of cannabinoids on the extinction of contextual fear memory in rats

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ABSTRACT

Facilitation of memory extinction by manipulation of the endocannabinoid (eCB) system has been recently studied in several paradigms. Our previous results pointed to facilitation of contextual fear memory extinction by a low dose of a cannabinoid agonist, with a suggestion of short-term effects. The aim of the present study was to further investigate the effects of cannabinoid drugs in the short- and long-term extinction of conditioned fear using an extended extinction protocol. Male Wistar rats were placed in a conditioning chamber and after 3 min received a footshock (1.5 mA, 1 s). On the next day, they received i.p. drug treatment (WIN55212-2 0.25 mg/kg, AM404 10 mg/kg, SR141716 A 1 mg/kg) and were re-exposed to the conditioning chamber for 30 min (extinction training). No-Extinction groups received the same drug treatment, but were exposed for 3 min to the conditioning chamber. A drug-free test of contextual memory (3 min) was performed 7 days later. The cannabinoid agonist WI55212-2 and the inhibitor of eCB metabolism/uptake AM404 facilitated short-term extinction. In addition, long-term effects induced by treatments with WIN55212 and AM404 were completely divergent to those of SR141716A treatment. The present results confirm and extend previous findings showing that the eCB system modulates short-term fear memory extinction with long-lasting consequences.

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The pioneer findings of Marsicano et al. (2002) on the importance of the endocannabinoid (eCB) system for extinction of aversive memories motivated several studies on this subject. Some of them approached the implications of the eCB system for the extinction of distinct kind of memories (Hill, Froese, Morrish, Sun, & Floresco, 2006; Holter et al., 2005; Parker, Burton, Sorge, Yakiwchuk, & Mechoulam, 2004; Varvel, Anum, & Lichtman, 2005; Varvel & Lichtman, 2002), thus reinforcing the preferential role of eCBs on the extinction of memories with aversive motivation (Niyuhire et al., 2007). Other groups applied psychopharmacology to facilitate fear memories extinction (Chhatwal, Davis, Maguschak, & Ressler, 2005; Pamplona, Prediger, Pandolfo, & Takahashi, 2006).

So far, the facilitation of extinction was observed after inhibition of eCB uptake/degradation in the fear-potentiated startle task (Chhatwal et al., 2005), after genetic deletion or pharmacological inhibition of the eCB-degrading enzyme fatty acid amide hydrolase (FAAH) in the water maze reversal task (Varvel, Wise, Niyuhire, Cravatt, & Lichtman, 2007) and after treatment with low doses of different cannabinoid agonists in the cocaine-induced conditioned place preference (Parker et al., 2004), in an attention set-shifting alternation task (Hill et al., 2006) and in contextual conditioned fear (Pamplona et al., 2006). To the best of our knowledge, only our results pointed to a facilitation of both short- and long-term extinction of conditioned fear by a low dose of a cannabinoid agonist. For this reason, and because of the recent contention that cannabinoid agonists-induced facilitation of fear extinction might be confounded by locomotor effects (Varvel et al., 2007), we aimed to further investigate the short- and long-term effects of eCB modulation on contextual fear extinction using an extended extinction protocol.

Male adult (3 months old) Wistar rats from the colony of the Universidade Federal de Santa Catarina (UFSC) were used. They were housed collectively in plastic cages (5–6 per cage), under controlled temperature (23 ± 2 °C) and a 12:12 h light/dark cycle (lights on at 7:00 a.m.), with rat chow and tap water available ad libitum. All procedures performed complied with the "Principles of laboratory animal care" from NIH.

WIN55212-2 (Tocris, USA), AM404 (Tocris, USA) and SR141716A (Sanofi-Aventis, France) were dissolved in saline with 10% DMSO plus 0.1% Tween80. The control solution consisted of the drugs' vehicle. Doses of AM404 (10 mg/kg, i.p.), WIN55212-2 (0.25 mg/kg, i.p.) and SR141716A (1 mg/kg, i.p.) were selected based on previous reports (Bortolato et al., 2006; Chhatwal et al., 2005; Pamplona et al., 2006). The drugs were injected i.p. (2 ml/kg) 20 min before the extinction session.

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The contextual fear conditioning and extinction were performed as described before (Pamplona et al., 2006), with minor modifications based on (Suzuki et al., 2004). For conditioning, rats ($n = 9$ per group) were placed in the conditioning chamber for 3 min and received a footshock (1.5 mA, 1 s), after which they remained for an additional min in the chamber before being returned to their home cages. Twenty-four hours after, they were injected with WIN55212-2, AM404, SR141716A or vehicle (control) and subjected to a 30-min non-reinforced exposure to the conditioning chamber (extinction training). No-Extinction groups received the same drug treatment, but were exposed for 3 min to the conditioning chamber. Seven days after, all animals were placed in the conditioning chamber for 3 min to test for contextual fear memory in a drug-free state. All groups were conditioned and tested in a fully counterbalanced experimental design. Freezing (immobility in a crouching position, except for breathing movements) was used as an index of contextual fear memory (Blanchard & Blanchard, 1969). The freezing time (s) was recorded offline by an experienced experimenter who was unaware of the treatment condition.

Statistical analyses of results were carried out using two-way ANOVA with treatment or 3-min bins/days (repeated measure) as independent factors. Following significant ANOVAs, differences between groups were evaluated by Duncan's post hoc test. Pearson's correlation test was used to test the correlation between freezing time expressed during the 30-min extinction session and the drug-free test. The accepted level of significance was $p < 0.05$. Statistical analyses were performed using the Statistica 6.0® software package (StatSoft, USA).

The short-term effects of the manipulation of the eCB system on the extinction of contextual fear memory are illustrated on Fig. 1A. Two-way ANOVA (treatment, repeated measure) for the extinction training revealed effects for treatment [$F(3,32) = 4.68$, $p < 0.01$], repeated measure [$F(9,288) = 37.55$, $p < 0.0001$] and for the interaction between these factors [$F(27,288) = 2.45$, $p < 0.001$]. Further comparison indicated no difference between the experimental groups at the start of the extinction session ($p > 0.05$), but it developed within the session. WIN55212-2-treated group showed less freezing in the interval of 6–18 min of the session compared to the control group ($p < 0.05$) and AM404-treated group showed less freezing from 12 to 21 min compared to the control group ($p < 0.05$). SR141716A-treated group presented higher freezing level in the last 3 min compared to the control group ($p < 0.05$). The long-term effects of the manipulation of the eCB system on the extinction of contextual fear memory were analyzed by comparison of the first 3 min of the extinction session with the 3-min drug-free test performed 7 days later. The results are illustrated on Fig. 1B. Two-way ANOVA (treatment, repeated measure) revealed effect for treatment [$F(3,32) = 10.78$, $p < 0.0001$], repeated measure [$F(1,32) = 35.79$, $p < 0.0001$] and for the interaction between these factors [$F(3,32) = 3.96$, $p < 0.05$]. Post hoc analysis indicated that the groups treated with WIN55212-2 and AM404 expressed less freezing compared to the control group in the drug-free test ($p < 0.05$). On the other hand, the SR141716A-treated group showed more freezing compared to the control group ($p < 0.05$). The results of the No-Extinction controls are illustrated on Fig. 1C. Two-way ANOVA (treatment, repeated measure)

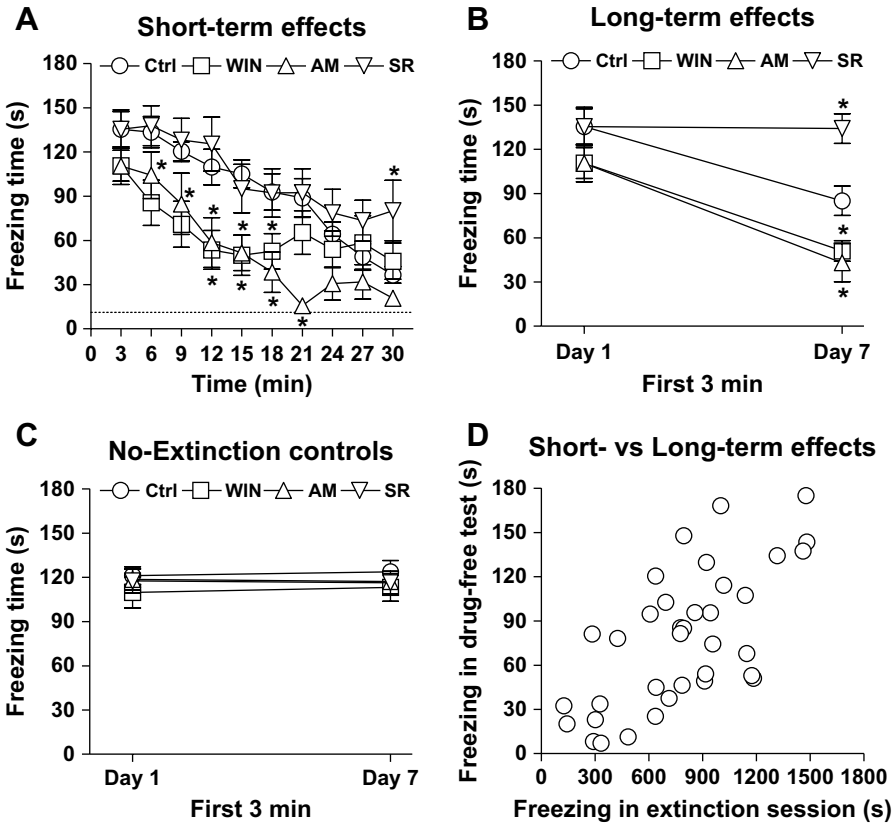


**Fig. 1.** Effects of the cannabinoid agonist WIN55212-2 (WIN), the inhibitor of endocannabinoid uptake/metabolism AM404 (AM) and the antagonist of $CB_1$ cannabinoid receptors SR141716A (SR) on the extinction of contextual fear memory in rats. The animals ($n = 9$ per group) were conditioned and 24 h after treated i.p. with WIN55212-2 (0.25 mg/kg), AM404 (10 mg/kg) or SR141716A (1 mg/kg) and re-exposed to the conditioning chamber for a single extinction session of 30 min (A). Seven days after the extinction session, the animals were placed in the conditioning chamber for 3 min to test for contextual fear memory in a drug-free state (B). No-Extinction controls received the same drug treatment, but were exposed for 3 min to the conditioning chamber (Day1) and re-exposed 7 days later in a drug-free state (C). Correlation between freezing time expressed during extinction session (Day 1) and drug-free test (Day 7) for Extinction groups (D). Data are represented as mean ± SEM. The dotted line in 1A represents pre-conditioning freezing time (mean of all groups). $^{*}p < 0.05$ compared to the control group (Duncan's post hoc test).

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