Involvement of the prelimbic cortex in the disruptive effect of cannabidiol on fear memory reconsolidation

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PURPOSE OF STUDY

A dysfunctional aversive memory processing contributes to the development of the posttraumatic stress disorder;

It is thought that aversive memories can be attenuated by targeting pharmacologically its reconsolidation;

Fear memory reconsolidation depends on prelimbic (PL) cortex activity;

We sought to investigate whether cannabidiol (CBD), a non-psychotomimetic component of *Cannabis sativa,* disrupts fear memory reconsolidation, and whether PL cortex cannabinoid type-1 (CB1) receptors contribute to this effect.

METHODS

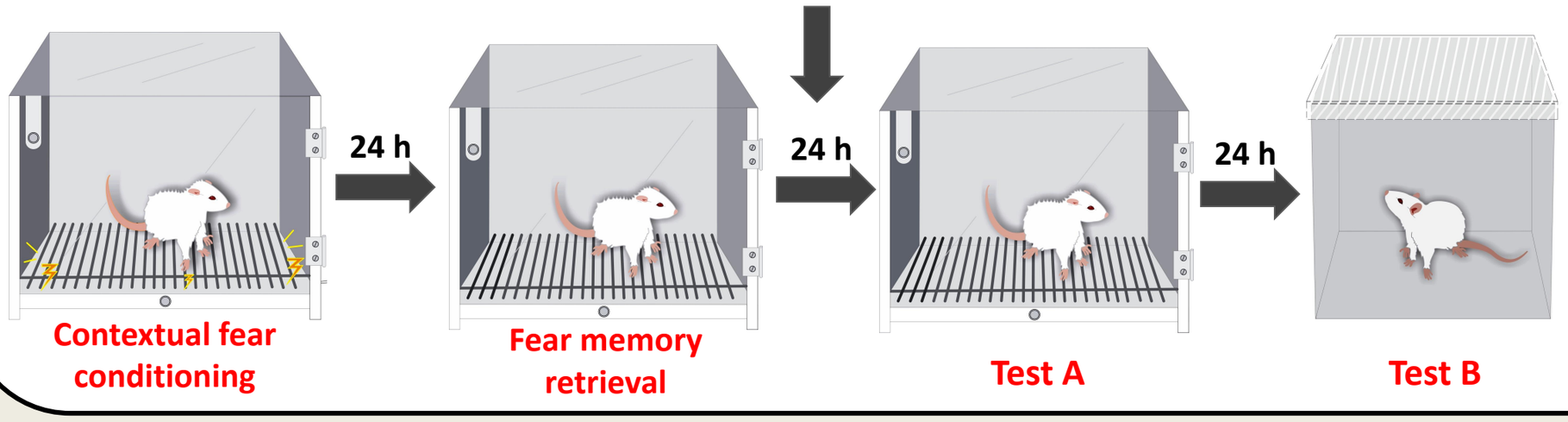
Male Wistar rats aged 3 months

Stereotaxic surgery targeting PL cortex

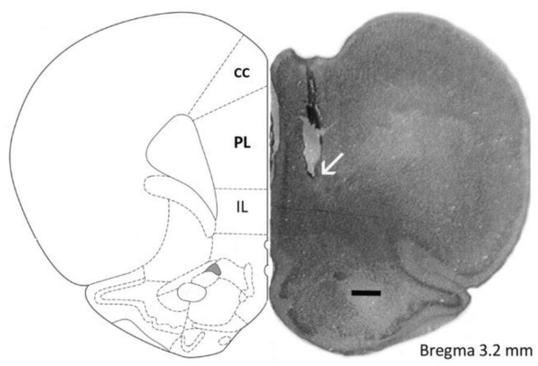
Previous familiarization to Context A

Bilateral drug administration

Zif268 labelling



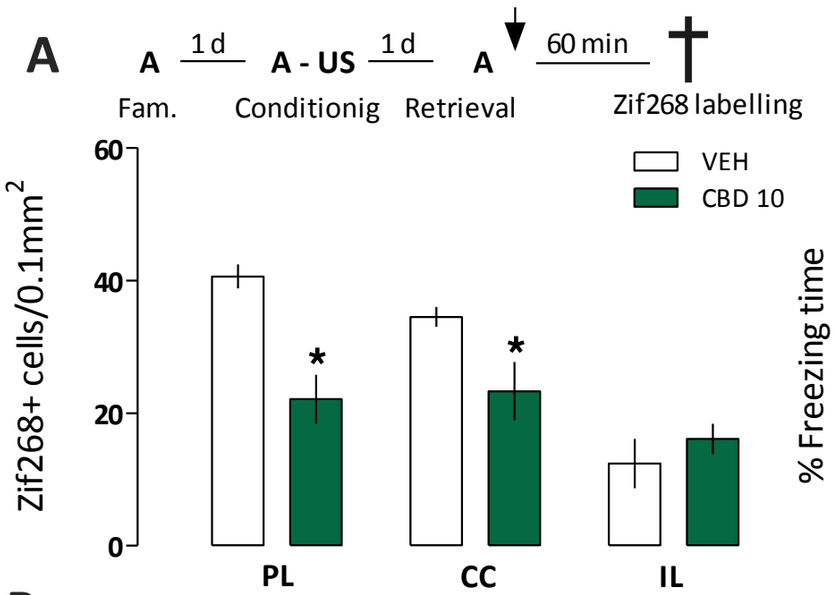
HISTOLOGIC ANALYSIS



Photomicrography of representative infusion sites placement in prelimbic cortex (PL; arrow).

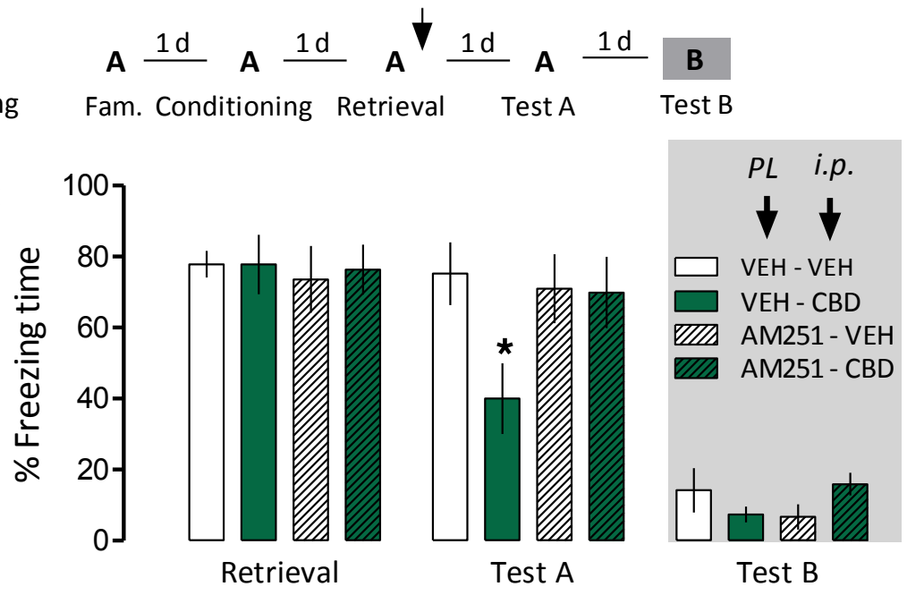
RESULTS

Experiment 1: Effects of CBD (i.p.) on Zif268/Egr1 expression along the medial prefrontal cortex

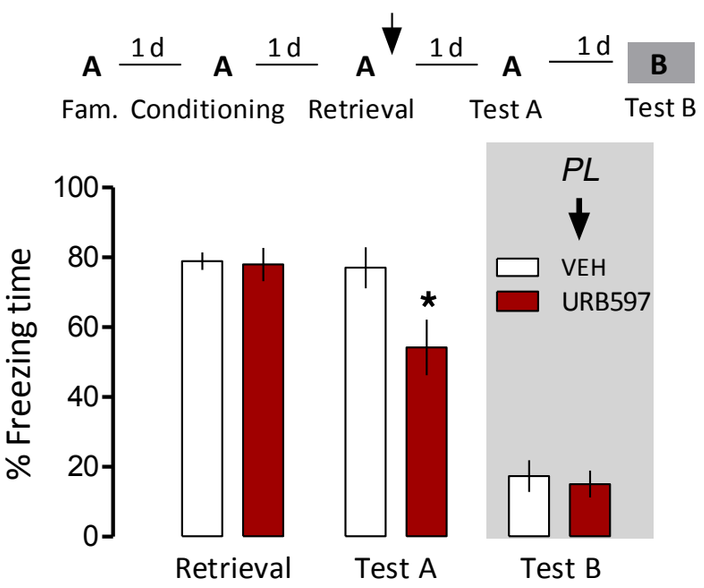


Panel A: Mean ± S.E.M. of Zif268/Egr1 positive cells. **P*<0.05 relative to respective controls (One-way ANOVA followed by Newman-Keuls test).
Panel B: Photomicrography of Zif268 expression along the medial prefrontal cortex.
VEH = vehicle; CBD 10 mg/kg
PL: prelimbic cortex**; CC:** cingulate cortex**; IL:** infralimbic cortex.

Experiment 2: Antagonism of CB1 receptors in PL cortex abolished the CBD disrupting effects on fear memory reconsolidation



Experiment 3: Inhibition of FAAH in the PL cortex impaired fear memory reconsolidation



**P*<0.05 relative to respective controls (repeated-measures ANOVA followed by Newman-Keuls test; values are expressed as mean + S.E.M).
VEH = vehicle; CBD 10 mg/kg; AM251 = 50 pmol/0.2 µL/side; URB597 = 30 pmol/0.2µL/side.

CONCLUSIONS

Cannabidiol attenuates the expression of Zif268/Egr1, the product of an immediate early gene associated with memory reconsolidation, in prelimbic and anterior cingulate cortices;

Activation of cannabinoid type-1 receptor located in prelimbic cortex contributes to the disrupting effects of cannabidiol on fear memory reconsolidation;

Indirectly increasing the endocannabinoid transmission, via inhibition of anandamide degradation, into the prelimbic cortex also disrupts fear memory reconsolidation.

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We have no conflict of interest

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Purpose of the study: A dysfunctional aversive memory processing contributes to the development of the posttraumatic stress disorder [1]. The maintenance and/or strength of this memory can be attenuated by targeting pharmacologically its reconsolidation, which is regulated by brain regions such as the prelimbic (PL) cortex [2]. The major non-psychotomimetic component of the *Cannabis sativa* plant, cannabidiol, (CBD) is able to disrupt fear memory reconsolidation in laboratory animals through cannabinoid type-1 (CB1) receptor-mediated signaling [3]. The objective of the present study was to investigate whether CB1 receptors in the PL cortex are involved in CBD-induced reconsolidation disruption.

Methods: Male Wistar rats were contextually-fear conditioned in Context A. On the next day, the retrieval session was conducted in order to induce memory labilization. It consisted of a short exposure of the animals to the same context. All treatments were performed immediately after the retrieval session. In experiment 1, the subjects received systemic injections of vehicle (VEH) or CBD (10mg/kg). After 1 hour, they were submitted to transcardiac perfusion for posterior evaluation of Zif268/Egr-1 protein expression along the medial prefrontal cortex (PL, cingulate and infralimbic), by immunohistochemistry. Results were expressed as the number of Zif268/Egr-1 positive cells/0.1mm^2. In experiment 2, rats bilaterally implanted with guide cannulas received an infusion of VEH or the CB1 receptor antagonist AM251 (50pmol/0.2µL) into PL and, immediately after, received systemic injections of VEH or CBD (10mg/kg). In experiment 3, rats received VEH or URB597 (30pmol/0.2µL), an inhibitor of the enzyme that metabolizes anandamide, into the PL. To assess reconsolidation impairment, the animals were re-exposed to Context A one day later (Test A). Freezing behavior was assessed during retrieval session and Test A as an index of fear memory.

Results: VEH-treated rats displayed significantly more Zif268/Egr-1 expression in PL (40.6±1.8) and cingulate (34.5±1.5) than in infralimbic (12.4±3.7) cortex following contextual fear memory retrieval. Systemic CBD administration prevented such differences (PL: 22.1±3.7; cingulate: 23.3±4.4; infralimbic: 16.1±2.3). In experiment 2, despite all groups having presented similar freezing levels during retrieval (VEH/VEH: 77.9±3.8%; VEH/CBD: 77.8±8.4%; AM251/VEH: 73.6±9.4%; AM251/CBD: 76.3±7%), CBD-treated animals showed less freezing behavior during Test A (VEH/VEH: 75.2±8.8%; VEH/CBD: 43±8%). CB1 antagonism in the PL, however, prevented the CBD effect (AM251/VEH: 74.3±8%; AM251/CBD: 70.0±8%). In experiment 3, all groups presented similar freezing levels during the retrieval session (VEH: 78.9±2.5%; URB597: 78±4.7%). During Test A, URB597-treated group presented less freezing than the control group (VEH: 77.0±5.8%; URB597: 54.2±7.9%).

Conclusions: The present work gives further support for the involvement of the PL in fear memory reconsolidation. It also indicates that the PL is a potential site for CBD disruptive effects on this process. In addition, the results suggest the indirect potentiation of the endocannabinoid system in this area is a possible mechanism to impair fear memory reconsolidation.

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Keywords

Memory and cognitive disorders

Immediate early genes

Neuropharmacology