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Short communication

Intra-accumbal blockade of endocannabinoid CB1 receptors impairs learning but not retention of conditioned relief

Jorge R. Bergado Acosta [a], Miriam Schneider [b], Markus Fendt [a,c,*]

[a] Institute for Pharmacology and Toxicology, Otto-von-Guericke University Magdeburg, Germany
[b] Department of Psychology, Heidelberg University, Germany
[c] Center of Behavioral Brain Sciences, Otto-von-Guericke University Magdeburg, Germany

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ABSTRACT

Humans and animals are able to associate an environmental cue with the feeling of relief from an aversive event, a phenomenon called relief learning. Relief from an aversive event is rewarding and a relief-associated cue later induces an attenuation of the startle magnitude or approach behavior. Previous studies demonstrated that the nucleus accumbens is essential for relief learning. Here, we asked whether accumbal cannabinoid type 1 (CB1) receptors are involved in relief learning. In rats, we injected the CB1 receptor antagonist/inverse agonist SR141716A (rimonabant) directly into the nucleus accumbens at different time points during a relief learning experiment. SR141716A injections immediately before the conditioning inhibited relief learning. However, SR141716A injected immediately before the retention test was not effective when conditioning was without treatment. These findings indicate that accumbal CB1 receptors play an important role in the plasticity processes underlying relief learning.

1. Introduction

A basic motivation of animals and humans is to avoid potentially threatening events since this can have critical impact on the well-being or survival of the organism (Lang, Davis, & Öhman, 2000; LeDoux, 2012). While experiencing a threatening event is highly aversive, the relief from such an incident is rewarding (Leknes, Lee, Berna, Andersson, & Tracey, 2011; Seymour et al., 2005). Notably, animals and humans can associate this rewarding feeling with environmental cues, a phenomenon entitled 'Relief Learning' (Denny, 1971; Gerber et al., 2014). In experimental paradigms of relief learning, a conditioned relief stimulus induces behavioral changes such as approach behavior or attenuation of the startle response (Andreatta et al., 2012; Navratilova et al., 2012; Yarali et al., 2008). These behavioral changes are usually observed in the presence of appetitive stimuli (e.g., Conzelmann et al., 2009; Friederich et al., 2006; Lang, Bradley, & Cuthbert, 1990; Schmid, Koch, & Schnitzler, 1995; Schneider & Spanagel, 2008). A series of studies in humans and rodents demonstrated that the nucleus accumbens (NAC), a central part of the brain reward system (e.g., Ikemoto, 2007), is crucial for relief learning in mammals (Andreatta et al., 2012; Bergado Acosta, Kahl, Kogias, Uzuneser, & Fendt, 2017; Bruning, Breitfeld, Kahl, Bergado-Acosta, & Fendt, 2016; Kahl & Fendt, 2016; Leknes et al., 2011; Mohammadi, Bergado Acosta, & Fendt, 2014; Mohammadi & Fendt, 2015; Navratilova et al., 2012).

At the level of the NAC, the endocannabinoid (eCB) system, and in particular cannabinoid type 1 (CB1) receptors, has been reported to act as a fundamental mediator of the encoding of reward and incentive cues by allowing neural synchrony and rhythmicity patterns to emerge during reinforcement processes (Hernandez & Cheer, 2012). The eCB system is an evolutionarily ancient and widely distributed neuromodulatory system (Elphick, 2012) which is critically involved in the regulation and modulation of a plethora of neurophysiological processes, such as motor control, emotional homeostasis, memory storage, or reward processing (Kano, Ohno-Shosaku, Hashimotodani, Uchigashima, & Watanabe, 2009; Moreira & Lutz, 2008).

The present study was performed to address the hypothesis whether accumbal CB1 receptors are involved in relief learning. In experiment 1, we submitted animals to different conditioning procedures to demonstrate that the startle attenuation that is observed in relief conditioning experiments is due to the associative status of the conditioned relief stimulus. In two further experiments, we injected the CB1 receptor antagonist/inverse agonist SR141716A (rimonabant) directly into the NAC of rats. In experiment 2, we evaluated the role of accumbal CB1 receptors on the acquisition of conditioned relief, i.e. injections were performed

* Corresponding author at: Institute for Pharmacology and Toxicology, Otto-von-Guericke University Magdeburg, Leipziger Strasse 44, 39120 Magdeburg, Germany.
E-mail address: markus.fendt@med.ovgu.de (M. Fendt).

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immediately before relief learning and the retention test on learned relief was performed without injections. In experiment 3, the role of accumbal CB1 receptors on the expression of conditioned relief was evaluated, i.e. rats were submitted to relief learning without any treatment and SR141716A injections were performed immediately before the retention test on learned relief.

2. Methods and materials

2.1. Subjects

Male Sprague-Dawley rats (250–350 g) were used for the present experiment. They were kept in groups of 4–6 animals per cage under a light:dark cycle of 12 h:12 h (lights on 6:00 am) and had free access to water and food. All experiments and surgeries were done during the light phase. The experiments were performed in accordance with international guidelines for the use of animals in experiments (2010/63/EU) and were approved by the local ethical committee (Landesverwaltungsamt Sachsen-Anhalt, Az. 42502-2-1309 UniMD).

2.2. Apparatus

A startle system with eight chambers (SR-LAB, San Diego Instruments, USA) was used. Each chamber was equipped with a loudspeaker (50 dB SPL background noise), a light cue (5 s, ca. 1000 lx) and a transparent animal enclosure (9 cm × 16 cm). As startle stimuli noise burst with a duration of 40 ms and an intensity of 96 dB SPL were used. As aversive stimuli, scrambled electric stimuli (0.5 s, 0.4 mA) were administered via a floor grid. The delivery of the startle, light and electric stimuli was controlled by the SR-LAB software. The responses to the startle stimuli and to the electric stimuli were measured by piezoelectric motion sensors underneath of the animal enclosure and further analyzed by the SR-LAB software. The mean sensor output in the time window 10–30 ms after startle stimulus onset was used as the startle magnitude, whereas the mean output during the whole stimulus period was used to quantify the response to the electric stimulus.

2.3. Surgery

For experiment 2 and 3, guide cannulas were implanted for intracranial injections. The animals were anesthetized with an isoflurane/oxygen mixture (2.0–2.5%) and fixed into a stereotaxic apparatus. The skull was exposed and stainless steel guide cannulas (custom-made; diameter: 0.7 mm, length: 8.0 mm) were bilaterally implanted aiming at NAC: 1.2 mm rostral, ±1.5 mm lateral, and 7.4 mm ventral to the bregma (Paxinos & Watson, 2014). Cannulas were fixed with dental cement and anchoring screws.

2.4. Behavioral procedures

Experiment 1: To evaluate potential unconditioned effects of the light CS, forty rats were placed into the startle boxes. Following 5 min of acclimation time, 10 startle stimuli were presented with an inter-trial interval of 30 s to habituate the animals. Subsequently, 20 further startle stimuli were presented, 10 of them without the light CS (startle alone trials) and 10 of them upon presentation of the light CS (CS-startle trials). The order of the trials with and without light CS was pseudo-randomized. The mean startle magnitudes on startle alone trials and on CS-startle trials, as well as the difference, were calculated.

The following day, rats were assigned to four groups with varying conditioning protocols: The group "CS only" was exposed to 15 presentations of the 5 s-light CS, i.e. no electric stimuli were delivered. The group "ISI 0" received 15 electric stimuli, directly followed by a 5 s-light CS (without any inter-stimulus interval). The group "ISI 3" was submitted to our established relief conditioning protocol, i.e. 15 presentations of an electric stimulus followed by the 5 s-light that was presented 3 s after the onset of the US. The last group "random" received randomized US and CS presentation, i.e. US and CS could also coincide.

On the third day, the test of the first day was repeated.

Experiment 2: Five days after implantation of the guide cannulas, a startle baseline test was performed. Rats were placed into the animal enclosure and after 5 min of acclimation, 10 startle stimuli were presented with an inter-trial interval of 30 s.

One day later, twenty-three rats received injections of either 0.3 μl vehicle (0.9% saline, 8.3% Tween 80, 1.6% ethanol) or 0.9 μg/0.3 μl SR141716A (dissolved in vehicle) solution. The dose and the vehicle for SR141716A were chosen based on previous publications (Malinen & Hyytia, 2008; Manzanares, Corchero, & Fuentes, 1999). Immediately after the injections, relief conditioning was performed as described above (experiment 1, group "ISI 3"). During this phase, locomotor response to foot shocks was also measured.

The following day, a retention test on conditioned relief was performed without any injections. The retention test was identical to the test used in experiment 1.

Experiment 3: As in experiment 2, first a baseline test was performed for twelve rats. The next day, rats were relief conditioned without treatment. Immediately before the retention test one day later, either 0.3 μl vehicle or 0.9 μg/0.3 μl SR141716A solution was injected into the NAC. A further day later, the animals were re-conditioned. For the second retention test, a cross-over design was used, i.e. animals that received vehicle before the first retention test now received SR141716A and vice versa.

2.5. Histology

The rats of experiments 2 and 3 were sacrificed after the behavioral experiments. The brain was removed, sectioned and Nissl-stained to verify injection sites into the NAC. Only animals with bilateral injections into the NAC shell and core regions were included into final analyses. The injection sites of these animals are shown in Fig. 1.

2.6. Statistical analysis

Statistical analyses were performed with Prism 6.0 (GraphPad Software Inc., La Jolla, CA, USA). Data were normally distributed (D'Agostino & Pearson omnibus normality test) and two-way ANOVAs were performed using trial type (startle alone, CS-startle), conditioning protocol (experiment 1) and treatment (experiment 2 + 3) as factors. The statistical threshold was set to $P < 0.05$.

3. Results

3.1. Experiment 1: associative character of the relief CS

Fig. 2A depicts the startle magnitudes in the two different startle trial types, i.e. in the absence (startle alone) or presence (CS-startle) of the light stimulus, before the animals were submitted to different conditioning protocols. Light stimulus did not affect the startle magnitude (t-test: $t = 0.42$, $P = 0.67$).

This was different in the startle tests which were performed after the rats have been submitted to different conditioning protocols. An ANOVA revealed a significant interaction of conditioning protocol and trial type ($F_{(3,44)} = 2.86$, $P = 0.048$; factor protocol: $F_{(3,44)} = 0.59$, $P = 0.63$; factor trial type: $F_{(1,44)} = 10.04$, $P = 0.0005$).