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ORIGINAL INVESTIGATION

The cannabinoid receptor agonist WIN 55,212-2 facilitates the extinction of contextual fear memory and spatial memory in rats

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Abstract
Rationale Previous studies demonstrated that pharmacological blockade of CB1 cannabinoid receptors decreases the extinction of conditioned fear and spatial memory in rodents. However, the effects of CB1 cannabinoid receptor activation in this response remain unclear.
Objectives To evaluate the effects of the cannabinoid agonist WIN 55,212-2 (WIN) and the cannabinoid antagonist SR 147778 (SR) on the extinction of contextual fear memory in rats 24 h or 30 days after fear conditioning.
Methods For fear conditioning, rats were placed in the conditioning chamber for 3 min and received a 1-s electric foot shock (1.5 mA). Retrieval testing consisted of a 3-min exposure to the conditioning chamber and extinction training consisted of successive 9-min exposures at 24-h intervals. Rats were also evaluated in the open field and water maze reversal task.
Results The administration of SR (1.0 mg/kg, i.p.) and WIN (0.25 mg/kg, i.p.) before extinction training disrupted and facilitated, respectively, the extinction of 24 h contextual fear memory. These effects were not related to any disturbance in memory retrieval, unconditioned freezing expression, or locomotor activity. WIN (0.25 mg/kg, i.p.) also facilitated the extinction of 30-day-old contextual fear memory, while the prior administration of SR (0.2 mg/kg, i.p.) antagonized this response. The facilitative effect of WIN on memory extinction does not seem to be specific for contextual fear memory because it was also observed in the water maze reversal task.
Conclusions These results suggest cannabinoid receptor agonists as potential drugs to treat anxiety disorders related to the retrieval of aversive memories.

Keywords Fear conditioning · Spatial memory · Extinction · Cannabinoid · WIN 55,212-2 · SR 147778

Introduction

The endocannabinoid system has become a major focus in the search for novel therapies for many common mental disorders (Makriyannis et al. 2005) because an increasing amount of evidence suggests its important role in regulation of emotional states and cognitive processes (Terranova et al. 1996; Lichtman 2000; Marsicano et al. 2002; Takahashi et al. 2005). The physiological importance of the endocannabinoid system in emotional learning is supported by the dense expression of the CB1 cannabinoid receptors and the presence of endocannabinoids in brain regions known to be important for anxiety and aversive learning, including the amygdala and hippocampus (Herkenham et al. 1990; Di Marzo et al. 2000). Behavioral studies also provide compelling support for the involvement of the cannabinoid system in learning and memory processes. Cannabinoid agonists often induce cognitive impairments in rodents

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(Lichtman et al. 1995; Ferrari et al. 1999; Da Silva and Takahashi 2002; Varvel and Lichtman 2002; Pamplona and Takahashi 2006), whereas the antagonism of CB1 receptors generally enhances rodent performance in many memory tasks (Terranova et al. 1996; Reibaud et al. 1999; Lichtman 2000; Takahashi et al. 2005).

Special interest was shown in cannabinoid modulation of fear memories, as numerous similarities link the expression of fear and anxiety in humans suffering, such as phobias, posttraumatic stress disorder (PTSD), and other anxiety disorders, to the expression of conditioned fear in animals (Brewin and Holmes 2003). In fear conditioning paradigms, a conditioned stimulus (such as a context) is paired with an unconditioned stimulus (such as foot shock). When placed back in the context, the animal shows conditioned fear responses such as freezing. The duration of nonreinforced reexposures to the context is a crucial determinant of subsequent memory processing: brief reminders lead to reconsolidation, whereas longer reminders result in memory extinction, which tends to weaken the expression of the original memory (Suzuki et al. 2004). After this, a recent study at our laboratory demonstrated that the activation of CB1 cannabinoid receptors impairs the acquisition of contextual fear conditioning in rats with no effect on retrieval at all (Pamplona and Takahashi 2006). Furthermore, the endocannabinoids anandamide and 2-arachidonoylglycerol are released in the periaqueductal gray matter during stressful situations (Hohmann et al. 2005) and in the basolateral amygdala during the extinction of fear memories (Marsicano et al. 2002). Consequently, the genetic deletion of CB1 cannabinoid receptors results in a strong impairment of short-term and long-term extinction of conditioned fear, which was confirmed by the use of rimonabant, a selective CB1 cannabinoid receptor antagonist. The recent availability of SR 147778 (SR), a newly developed antagonist with high affinity and specificity for CB1 cannabinoid receptors (Rinaldi-Carmona et al. 2004), leads to the possibility of confirming and extending these previous findings observed with rimonabant (Rinaldi-Carmona et al. 1995). Moreover, in light of the fact that fear memories become increasingly resistant to extinction with age (Suzuki et al. 2004), it seems to be of interest to investigate whether the cannabinoid system may influence extinction of remote fear memories as well.

Therefore, the main objective of the present study was to examine whether the administration of the cannabinoid agonist WIN 55,212-2 (WIN) could facilitate the extinction of recent and/or remote contextual fear memory in rats. Further, we investigated the role of the CB1 cannabinoid receptors in the extinction processes using the newly developed selective CB1 cannabinoid receptor antagonist SR. The water maze reversal task was also used to investigate whether the influence of the cannabinoid system on memory extinction would generalize to extinction of spatial memory in rats.

Materials and methods

Animals

Male adult Wistar rats (3 months old) bred and raised in the animal facility of the Department of Pharmacology of Universidade Federal de Santa Catarina (UFSC) were used. The animals were kept in collective plastic cages (five to six rats per cage) with food and water available ad libitum. They were maintained in a room under controlled temperature (23±2°C) and a 12:12-h light/dark cycle (lights on at 7:00 A.M.). Each behavioral test was conducted during the light phase of the cycle (between 8:00 A.M. and 5:00 P.M.) using independent experimental groups consisting of seven to ten animals per group. All the experimental procedures were performed according to the guidelines on animal care of the UFSC Ethics Committee on the Use of Animals, which follows the "principles of laboratory animal care" from NIH.

Drugs and treatment

WIN [*R*-(+)-(2,3-dihydro-5-methyl-3-[{4-morpholinyl}methyl] pyrol [1,2,3-de-]-1,4-benzoxazin-6-yl)(1-naphthalenyl) methanone mesylate] (Tocris, USA) and SR [5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-ethyl-*N*-(1-piperidinyl)-1*H*-pyrazole-3-carboxamide] (Sanofi-Aventis, France) were dissolved in 0.9% NaCl (saline) with 10% dimethylsulfoxide plus 0.1% Tween 80. The control solution consisted of a drug vehicle. All drug doses, selected according to previous literature (Lichtman et al. 1995; Chhatwal et al. 2005; Takahashi et al. 2005; Pamplona and Takahashi 2006), were administered intraperitoneally in a volume of 0.2 ml/100 g of body weight. WIN and SR were administered 30 and 20 min, respectively, before behavioral test, except in experiment 3 in which SR was administered 20 min before WIN.

Behavioral procedures

Fear conditioning

The conditioning chamber consisted of a modified shuttle box (Automatic Reflex Conditioner model 7531, Ugo Basile, Italy) made of gray opaque Plexiglas. One of the compartments (22×22×25 cm) of the chamber was used for tone and contextual fear conditioning. Contextual conditioning tests were conducted in the chamber and tone conditioning tests were conducted in a different context, consisting of a

transparent glass cage (30×30×30 cm). The experiments were carried out in a sound-attenuated room under low intensity light (10 lx) and a microvideo camera was mounted at the top of the chamber, allowing the experimenter to observe the rats on a monitor placed in an adjacent room. Tone and contextual fear conditioning were performed with modifications from a procedure previously described by Corodimas et al. (2000). For contextual fear conditioning, rats were placed in the conditioning chamber for 3 min and received a 1-s electric foot shock (1.5 mA), after which they were kept for an additional minute in the chamber before being returned to their home cages. For tone fear conditioning, the rats were placed in the conditioning chamber, and after 3 min a sound (1,000 Hz, 80 dB) was presented for 10 s that coterminated with a 1-s electric foot shock (1.5 mA). The rats were kept for an additional minute in the chamber before being returned to their home cages. Independent groups of animals were used in each experiment. Freezing, defined as a stereotyped crouching position with complete immobility of the animal, except for the movements necessary for breathing, was used as a memory index during the subsequent nonreinforced reexposures to the context or tone (Blanchard and Blanchard 1969; Fanselow 1980). Freezing time was recorded with stopwatches by an experienced observer who was blind to the conditions of the treatment. The same observer recorded freezing in all the experiments to avoid individual variabilities and obtain more reliable results.

Experiment 1: effects of cannabinoid receptor ligands on extinction of recent contextual fear memory Successive long exposures to the conditioning chamber were used to test the effects of cannabinoids on short-term (within-exposure) and long-term (between-exposure) extinction of conditioned fear. For this, 24 h after contextual fear conditioning, the animals were exposed to the conditioning chamber for 9 min and the freezing behavior was evaluated. This extinction procedure was executed three times at 24-h intervals to give an index of long-term extinction of conditioned freezing. Moreover, the percentage of freezing during the first extinction session was used to investigate any possible within-session effects of drug treatment (Quirk et al. 2000; Marsicano et al. 2002; Fernandez-Espejo 2003). The animals were treated with WIN (0.25, 1.25, or 2.50 mg/kg, i.p.), SR (0.2, 1.0, or 2.0 mg/kg, i.p.) or control solution before each extinction session.

Experiment 2: effects of cannabinoid receptor ligands on retrieval of contextual fear memory Contrasting with the extinction procedure, a single short exposure to the conditioning chamber was used to test the effect of cannabinoids on retrieval of conditioned fear with minimal interference of within-session extinction (McKay et al. 2002). For this, 24 h after contextual fear conditioning, the animals were exposed for 3 min to the conditioning chamber and the freezing behavior was evaluated (Sorg et al. 2004). The animals were treated with WIN (0.25, 1.25, or 2.50 mg/kg, i.p.), SR (0.2, 1.0, or 2.0 mg/kg, i.p.), or control solution before being reexposed to the conditioning chamber.

Experiment 3: effects of the cannabinoid agonist WIN on extinction of remote contextual fear memory Thirty days after being simultaneously subjected to tone and contextual fear conditioning, the animals were exposed to the conditioning chamber for 9 min for freezing evaluation. Because aversive memories become increasingly resistant to disruption with age (Suzuki et al. 2004), this extinction procedure was executed five times at 24-h intervals.To investigate whether the effects of WIN on extinction of contextual fear memory in rats were related to the activation of CB1 cannabinoid receptors, the animals were treated with SR (0.2 mg/kg, i.p.) or control solution (i.p.), and 20 min later they were injected with WIN (0.25 mg/kg, i.p.) or control solution (i.p.) 30 min before each extinction session. Also, to investigate whether the WIN effects were selective to the memory that was extinguished, 24 and 48 h after the end of the extinction protocol (fifth day), the rats were tested in a drug-free state for retrieval of the tone and contextual fear conditioning, respectively. For retrieval of tone fear conditioning, they were placed in a different context (transparent acrylic cage, 30×30×30 cm) and three 1-min sound presentations were made with 1-min intervals. Twenty-four hours after, the rats were exposed to the conditioning chamber for 3 min for retrieval of the contextual fear conditioning. Freezing behavior was evaluated during each test.

Unconditioned freezing behavior

Experiment 4: effects of cannabinoid receptor ligands on the expression of unconditioned freezing behavior Rats were placed in the conditioning chamber for 3 min and after this period they received a 1-s electric foot shock (1.5 mA), after which they were kept for one additional minute in the chamber before being returned to their home cages. Twenty-four hours after, they were treated with WIN (0.25, 1.25, or 2.50 mg/kg, i.p.), SR (0.2, 1.0, or 2.0 mg/kg, i.p.), or control solution and exposed for 3 min to a new context (transparent glass cage, 30×30×30 cm) for evaluation of unconditioned freezing behavior.

Open field

The open field apparatus was made of white painted wood with a white 100×100 cm floor (divided into 25 squares of 20×20 cm) and 40-cm-high white walls.

Experiment 5: effects of cannabinoid receptor ligands on locomotor activity Rats were injected with WIN (0.25, 1.25, or 2.50 mg/kg, i.p.), SR (0.2, 1.0, or 2.0 mg/kg, i.p.), or control solution and placed in the center of the open field for 3 min of free exploration. The number of squares crossed was registered and used as an index of locomotor activity.

Water maze reversal task

To test whether the effects of the activation and blockade of CB1 cannabinoid receptors on extinction of contextual fear memory could be generalized to another hippocampus-dependent task with different sensory, motivational, and performance demands, the rats were tested in the water maze reversal task previously described by Varvel and Lichtman (2002). The water maze consisted of a circular swimming pool made of black painted fiberglass (inside diameter 1.70 m and 0.8 m high, filled to a depth of 0.6 m with water maintained at 25°C). The target platform (10×10 cm) was made of transparent Plexiglas and was submerged 1–1.5 cm beneath the surface of the water. Starting points for the animals were marked on the outside of the maze as north (N), south (S), east (E), and west (W). The platform was located in the center of the northeast quadrant at a point 35 cm from the wall of the maze. Four distant visual cues (55×55 cm) were placed on the walls of the experimental room to allow spatial orientation by the animals.

Experiment 6: effects of cannabinoid receptor ligands on extinction of spatial memory in rats Rats were assigned to two training sessions separated by an interval of 24 h, each of which consisted of six consecutive trials with the platform remaining in the fixed position. The animals were left in one of the aforementioned starting points facing the wall of the maze and were allowed to swim freely to the platform. If an animal did not find the platform during a period of 60 s, it was gently guided to the platform's location and allowed to remain for 10 s on it before being removed from the water maze for 20 s and subsequently placed at the next starting point. Twenty-four hours after the second training session, rats received WIN (0.25 mg/kg, i.p.), SR (1.0 mg/kg, i.p.), or control solution (i.p.) and were subjected to a reversal task in which the platform was moved to the opposite side of the tank (center of the southwest quadrant). The starting points and the intertrial intervals were identical to those of the training sessions. The time the animals spent reaching the platform (escape latency) was used as the learning/memory index in both the training sessions and the reversal task.

Data analysis

The statistical comparison of results was carried out using one-way ANOVA with treatment as the independent factor or two-way ANOVA with treatment and trials (repeated measure) as independent factors. After significant ANOVAs, differences between groups were evaluated by post hoc Duncan's test. The accepted level of significance for the tests was $p \leq 0.05$. All statistical analyses were performed using the Statistica® 6.0 software package (StatSoft, USA).

Results

Experiment 1: effects of cannabinoid receptor ligands on extinction of recent contextual fear memory The effects of SR (0.2, 1.0, or 2.0 mg/kg, i.p.) on extinction of contextual fear memory evaluated 24 h after fear conditioning are given in Fig. 1a. Two-way ANOVA revealed a significant effect for treatment [$F(3,26)=5.18$, $p<0.01$] and trials [$F(2,52)=11.67$, $p<0.0001$], but no treatment × trial interaction. Post hoc comparisons indicated that the extinction protocol of 3 days significantly decreased the freezing time across successive reexposures of the control group to the conditioning chamber ($p \leq 0.05$, second and third trials compared to the first). The intermediate dose of SR (1.0 mg/kg, i.p.) disrupted the extinction of contextual fear memory as indicated by an increased freezing time compared to the control group ($p \leq 0.05$).

The effects of WIN (0.25, 1.25, or 2.50 mg/kg, i.p.) on extinction of contextual fear memory, evaluated 24 h after fear conditioning, are given in Fig. 1b. Two-way ANOVA revealed a significant effects for treatment [$F(3,29)=6.84$, $p<0.001$] and trials [$F(2,58)=17.31$, $p<0.00001$], but no treatment × trial interaction. Post hoc comparisons indicated that the control group presented a partial extinction of contextual fear conditioning after three reexposures to the conditioning chamber ($p \leq 0.05$, third compared to the first exposure). The administration of WIN promoted a dose-dependent effect on the extinction process. The group treated with the lowest dose of WIN (0.25 mg/kg, i.p.) exhibited a decreased freezing time during the first 9-min exposure compared to the control group ($p \leq 0.05$) and it underwent partial extinction on the third trial ($p \leq 0.05$, compared to the first), suggesting a facilitative effect of this dose in the extinction of contextual fear conditioning. In contrast, the higher dose of WIN (2.50 mg/kg, i.p.) disrupted the extinction of conditioned fear as evidenced by the lack of reduction in the freezing time across the trials and an increased freezing time compared to the group treated with the lowest dose of WIN (0.25 mg/kg, i.p.). The intermediate dose of WIN (1.25 mg/kg, i.p.) exhibited a

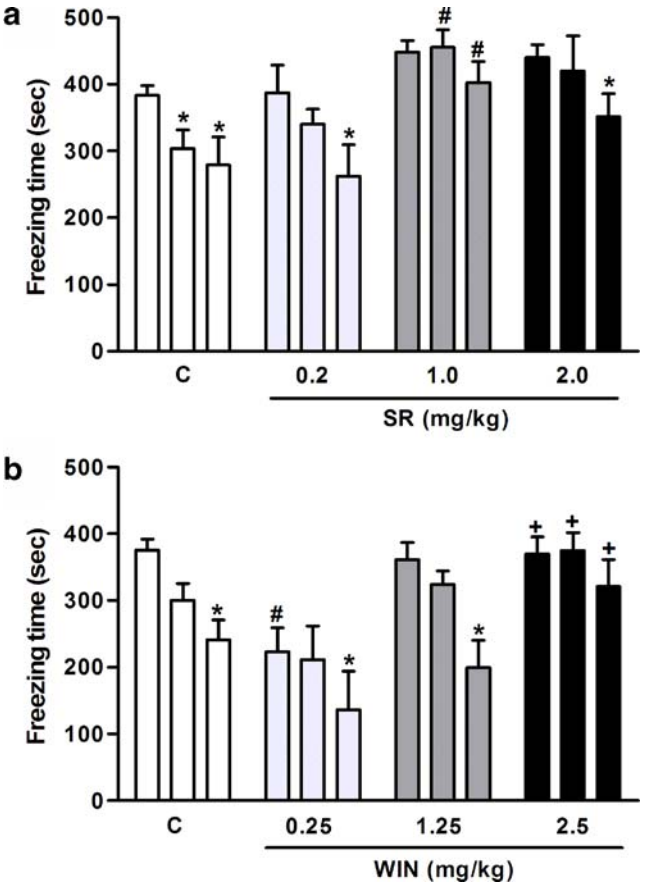

Fig. 1 Effects of the selective CB1 cannabinoid receptor antagonist SR (0.2, 1.0 or 2.0 mg/kg, i.p.) and cannabinoid agonist WIN (0.25, 1.25, or 2.50 mg/kg, i.p.) on the extinction of recent contextual fear memory in rats. Data are expressed as mean±SEM of the time spent freezing expressed by SR-treated rats (**a**) and WIN-treated rats (**b**) during three 9-min exposures to the conditioning chamber with 24-h intervals (each *bar* represents the data of one session). *Asterisk*: $p \leq 0.05$ compared to the first session of the corresponding group. *Number sign*: $p \leq 0.05$ compared to the control group during the corresponding session. *Plus sign*: $p \leq 0.05$ compared to the group treated with the lowest dose of WIN (0.25 mg/kg, i.p.) during the corresponding session (Duncan's post hoc test). (Control n=8, SR 0.2 n=7, SR 1.0 n=8, and SR 2.0 n=7) (Control n=9, WIN 0.25 n=7, WIN 1.25 n=7, and WIN 2.5 n=10)

profile of extinction similar to that of the control group. As reduction of freezing time in the group treated with WIN (0.25 mg/kg, i.p.) might suggest that WIN affected the retrieval of memory and not its extinction, the results of the first extinction session (9 min) were reanalyzed in 3-min bins. Further analysis of freezing levels showed no significant difference during the first 3-min bin [$F(3,29)$= 2.57, p=0.07], but a marked treatment effect was noted in the second [$F(3,29)$=8.1, p=0.0004] and third [$F(3, 29)$= 6.06, p=0.002] 3-min bins. Post hoc comparisons revealed that WIN (0.25 mg/kg, i.p.) did not influence memory retrieval (first 3 min), but facilitated short-term extinction, reducing the freezing time in the second and third 3-min bins compared to the control group ($p \leq 0.05$ for both). This result was confirmed in experiment 2.

Experiment 2: effects of cannabinoid receptor ligands on retrieval of contextual fear memory The effects of SR (0.2, 1.0, or 2.0 mg/kg, i.p.) and WIN (0.25, 1.25, 2.5 mg/kg, i.p.) on the retrieval of contextual fear memory are given in subpanels a and b in Fig. 2, respectively. One-way ANOVA of the results of each experiment revealed a nonsignificant effect for treatment with SR [$F(3,32)$=0.38, p=0.77] or WIN [$F(3,28)$=1.56, p=0.22].

Experiment 3: effects of the cannabinoid agonist WIN on extinction of remote contextual fear memory The effects of WIN (0.25 mg/kg, i.p.) on extinction of 30-day-old contextual fear memory in rats are given in Fig. 3. Two-way ANOVA revealed a significant effect for treatment [$F(2,29)$=13.62, p<0.0001] and trials [$F(4,116)$=18.02, p<0.00001], but no treatment × trial interaction. Post hoc comparisons indicated that the administration of WIN (0.25 mg/kg, i.p.) significantly decreased the freezing time compared to the control group ($p \leq 0.05$), suggesting a facilitative effect of WIN on the extinction of remote contextual fear memory. Moreover, a per se ineffective dose of SR (0.2 mg/kg, i.p.) antagonized the effect of WIN (0.25 mg/kg, i.p.) ($p \leq 0.05$), suggesting that it was related to the activation of the CB1 cannabinoid receptors.

As illustrated in Fig. 3b, to investigate whether the WIN effects were selective toward the memory that was extinguished, 24 and 48 h after the end of the extinction protocol (fifth day), the rats were tested in a drug-free state for retrieval of the tone and context fear conditioning. One-way ANOVA revealed no significant treatment effect on the freezing time during tone presentation [$F(2,29)$=0.71, p=0.50], demonstrating that the tone-shock association was unaffected by the extinction of contextual fear memory (Fig. 3b). However, one-way ANOVA revealed significant treatment effect on the freezing time during reexposure to the context [$F(2,29)$=4.48, p<0.005]. Indeed, 48 h after the end of the fifth extinction session, the control group continued to express pronounced freezing behavior when reexposed to the conditioning chamber, whereas the time spent freezing by drug-free rats previously given WIN was significantly shortened ($p \leq 0.05$) (Fig. 3b). This latter effect was antagonized by SR (0.2 mg/kg, i.p.), emphasizing the involvement of CB1 cannabinoid receptors on the facilitative effects of WIN on extinction of remote contextual fear memory (Fig. 3b).

Experiment 4: effects of cannabinoid receptor ligands on the expression of unconditioned freezing behavior The effects of WIN (0.25, 1.25, or 2.5 mg/kg, i.p.) or SR (0.2, 1.0, or 2.0 mg/kg, i.p.) on the expression of unconditioned

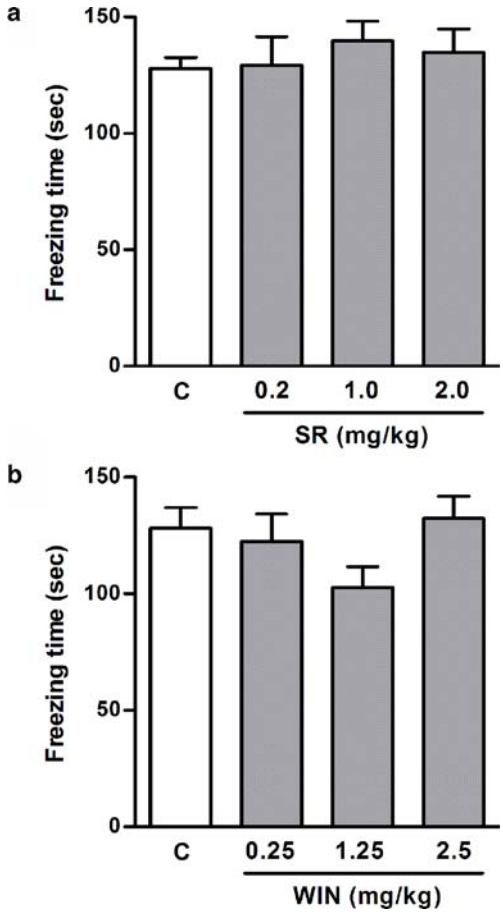

Fig. 2 Effects of the selective CB1 cannabinoid receptor antagonist SR (0.2, 1.0, or 2.0 mg/kg, i.p.) and cannabinoid agonist WIN (0.25, 1.25, or 2.50 mg/kg, i.p.) on the retrieval of recent contextual fear memory in rats. Data are expressed as mean±SEM of the time spent freezing expressed by SR-treated rats (**a**) and WIN-treated rats (**b**) during a 3-min exposure to the conditioning chamber. (Control n=9, SR 0.2 n=8, SR 1.0 n=10, and SR 2.0 n=9) (Control n=10, WIN 0.25 n=7, WIN 1.25 n=7, and WIN 2.5 n=8)

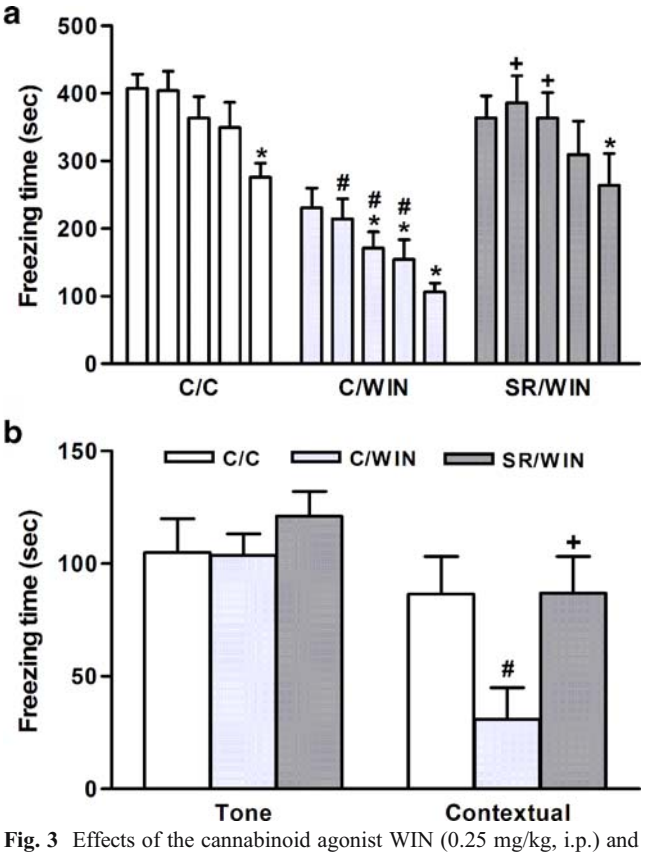

Fig. 3 Effects of the cannabinoid agonist WIN (0.25 mg/kg, i.p.) and pretreatment with the selective CB1 cannabinoid receptor antagonist SR (0.2 mg/kg, i.p.) on extinction of remote contextual fear memory in rats. The animals received one injection of SR or control solution (**c**) followed by one injection of WIN or control solution before each extinction session. **a** Mean±SEM of the time spent freezing expressed by the animals during five 9-min exposures to the conditioning chamber with 24-h intervals (each *bar* represents the data of one session). **b** (*Left*) Mean±SEM of the time spent freezing during a 3-min drug-free tone presentation, 24 h after the extinction of contextual fear conditioning; (*right*) mean±SEM of the time spent freezing during a 3-min drug-free exposure to the conditioning chamber, 48 h after the extinction of contextual fear conditioning. *Asterisk*: $p \leq 0.05$ compared to the first session of the corresponding group. *Number sign*: $p \leq 0.05$ compared to the C/C group during the corresponding session. *Plus sign*: $p \leq 0.05$ compared to the C/WIN group during the corresponding session (Duncan's post hoc test). (C/C n=9, C/WIN n=12, and SR/WIN n=11)

freezing behavior in rats are summarized in Table 1. One-way ANOVA revealed no significant effect for treatment on the time of unconditioned freezing [$F(6,52)=1.02$, $p=0.42$].

Experiment 5: effects of cannabinoid receptor ligands on locomotor activity The effects of WIN (0.25, 1.25, or 2.5 mg/kg, i.p.) or SR (0.2, 1.0, or 2.0 mg/kg, i.p.) on the locomotor activity of rats in the open field test are summarized in Table 1. One-way ANOVA revealed no significant effect for treatment on the number of squares crossed [$F(6,49)=1.81$, $p=0.12$].

Experiment 6: effects of cannabinoid receptor ligands on extinction of spatial memory in rats The effects of WIN (0.25 mg/kg, i.p.) or SR (1.0 mg/kg, i.p.) on rats subjected to the water maze reversal task are illustrated in Fig. 4. Two-way ANOVA revealed a significant effect of trials on escape latency during the two training sessions [day 1 $F(5,105)=24.63$, $p<0.00001$; day 2 $F(5,105)=9.45$, $p<0.00001$] with no difference between groups (Fig. 4a). Two-way ANOVA for the data of the reversal task revealed a significant effect for trials [$F(5,105)=17.16$, $p<0.00001$] and treatment × trial interaction [$F(10,105)=2.61$, $p<0.005$]. Post hoc comparisons indicated that WIN-treated (0.25 mg/kg, i.p.) animals showed decreased escape latencies in the first trial of the water maze reversal task, whereas SR-treated (1.0 mg/kg, i.p.) animals showed increased escape latencies in the second trial of the water maze reversal task compared to the control group ($p \leq 0.05$) (Fig. 4b).

Table 1 Effects of WIN (0.25, 1.25, or 2.5 mg/kg, i.p.) and SR (0.2, 1.0, or 2.0 mg/kg, i.p.) on unconditioned freezing and open field behavior

Treatment (mg/kg)	Unconditioned freezing (s)	Number of samples	No. of squares crossed	Number of samples
Control	31.9±5.9	11	63±6	13
SR 0.2	36.7±10.4	8	70±4	7
SR 1.0	41.1±8.4	8	69±4	7
SR 2.0	25.5±5.0	8	74±9	7
WIN 0.25	35.6±8.1	8	58±3	7
WIN 1.25	18.0±7.1	8	59±4	7
WIN 2.5	23.7±10.3	8	50±6	8

Discussion

The present findings confirm and extend those of previous studies demonstrating that the disruption of CB1 cannabinoid receptor signaling decreases the extinction of conditioned fear in rodents. More importantly, our results suggest that the extinction of contextual fear memory in rats may be facilitated by the cannabinoid agonist WIN, and that this response was antagonized by the new selective CB1 cannabinoid receptor antagonist SR. Furthermore, the present facilitative effects of WIN on memory extinction in rats cannot be attributed to alterations in memory retrieval or sensorimotor deficits and does not seem to be specific for conditioned fear memory because it was also observed for spatial memory.

In the present study, we present evidence that the administration of the new selective CB1 cannabinoid receptor antagonist SR (1.0–2.0 mg/kg, i.p.) disrupts the extinction of contextual fear memory in rats evaluated 24 h after fear conditioning. Our findings are in accordance with those of recent studies showing that CB1 knockout mice and mice and rats treated with the selective CB1 cannabinoid receptor antagonist rimonabant exhibit a pronounced deficit in the extinction of conditioned fear (Marsicano et al. 2002; Suzuki et al. 2004; Chhatwal et al. 2005). Furthermore, the present results demonstrate that a low dose of the cannabinoid agonist WIN (0.25 mg/kg, i.p.) may facilitate the extinction of conditioned fear in rats. This last finding extends to fear memory the previous results of Parker et al. (2004), showing that low doses of Δ^9–tetrahydrocannabinol and cannabidiol promote extinction of conditioned place preference in rats. It is interesting to note that we failed to show any enhancement of memory extinction using higher doses of WIN (1.25–2.5 mg/kg, i.p). Accordingly, WIN (5.0 mg/kg, i.p.) did not facilitate the extinction of fear-potentiated startle (Chhatwal et al. 2005). A potential discrepancy in the present study is the notion that rats treated with WIN (0.25 mg/kg, i.p) and showing reduced freezing during the first extinction session might have experienced some kind of impairment in fear memory retrieval. However, in keeping with the present results and previous reports (Lichtman 2000; Da Silva and Takahashi 2002; Marsicano et al. 2002; Varvel and Lichtman 2002; Chhatwal et al. 2005; Varvel et al. 2005; Pamplona and Takahashi 2006), neither WIN nor SR modified the performance in memory retrieval tasks, suggesting that the present effects of pharmacological manipulations of the cannabinoid system are specific for memory extinction. It could also be speculated that the present results may reflect some combination of sensorimotor deficits induced by drug treatment, rather than the facilitation of memory extinction. However, freezing behavior can hardly account for the present results because neither SR nor WIN altered the number of squares crossed in the open field test or the amount of unconditioned freezing expressed by rats.

The effects of the cannabinoid system on the extinction of remote aversive memories in rats were also investigated.

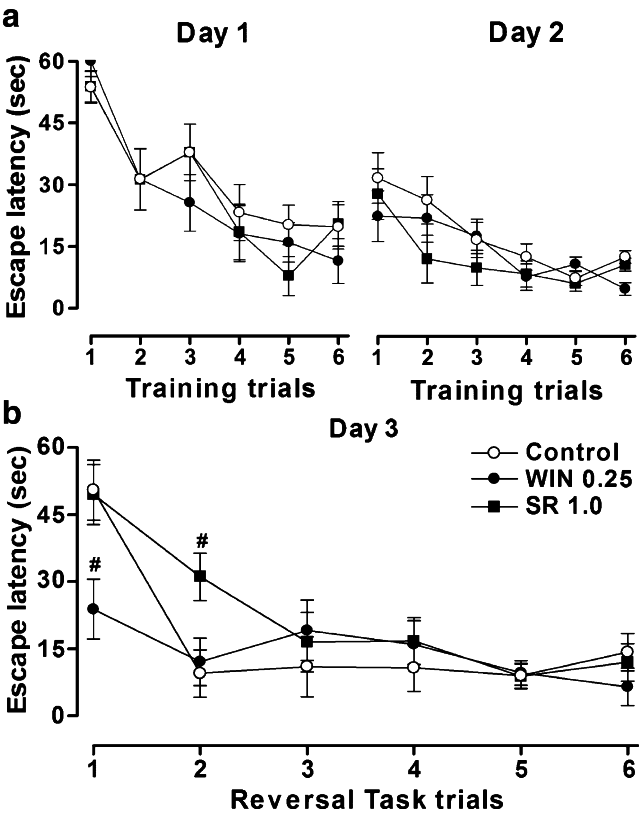

Fig. 4 Effects of the selective CB1 cannabinoid receptor antagonist SR (1.0 mg/kg, i.p.) and cannabinoid agonist WIN (0.25 mg/kg, i.p.) on the performance of rats in the water maze reversal task. **a** The animals were trained to find a submerged platform in a fixed position during six trials on two consecutive days. **b** One day later, they received drug treatment and were tested in the reversal task in which the platform location was changed to the opposite quadrant of the water maze. Each *point* represents the mean±SEM of the escape latency (s) to reach the platform location. *Number sign*: $p \leq 0.05$ compared to the control group during the corresponding trial (Duncan's post hoc test). (Control n=8, WIN n=8, and SR n=8)

As previously reported by Suzuki et al. (2004), the age of a specific memory is strongly determinant of the ease of its disruption. Corroborating a previous study (Suzuki et al. 2004), the remote contextual fear memory (30 days) was harder to extinguish than a recent one (24 h) because it required a protocol of five extinction sessions to exhibit a partial extinction. Nevertheless, the cannabinoid agonist WIN (0.25 mg/kg, i.p.) also facilitated the extinction of remote aversive memories through the activation of CB1 cannabinoid receptors. Furthermore, the effect of WIN was selective for the memories, which were extinguished and had long-lasting consequences, which clearly emphasizes the long-term facilitative effects of WIN on extinction of conditioned fear.

In addition, our findings also suggest that the endocannabinoid system modulates the extinction of spatial memory in rats evaluated in the water maze because the administration of SR (1.0 mg/kg, i.p.) and WIN (0.25 mg/kg, i.p.) transiently disrupted and improved, respectively, the performance of rats in the water maze reversal task. It must be conceded that the Wistar rats employed have poor visual capabilities, which may partially compromise these results. Nevertheless, our results are in accordance with those of earlier studies that demonstrate deficits in the extinction of previously learned spatial information in mice as a consequence of CB1 cannabinoid receptor deletion or blockade (Varvel and Lichtman 2002; Varvel et al. 2005).

In conclusion, the present results reinforce those of previous studies demonstrating that the disruption of CB1 cannabinoid receptor signaling impairs the extinction of both conditioned fear and spatial memory in rodents. More importantly, our results suggest that the extinction of contextual fear memory and spatial memory in rats may be facilitated by the cannabinoid agonist WIN with long-lasting effects. Because it was demonstrated that a drug that facilitates extinction of conditioned fear in laboratory animals may also be utilized with success in humans (Walker et al. 2002; Ressler et al. 2004), pharmacotherapies directed at the endocannabinoid system may represent a viable approach to the treatment of a variety of psychiatric disorders related to the retrieval of fear memories, including panic, phobias, and PTSD.

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