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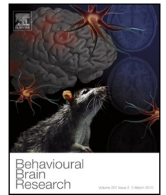

Research report

Involvement of cannabinoid receptors in the amygdala and prefrontal cortex of rats in fear learning, consolidation, retrieval and extinction

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HIGHLIGHTS

- Drug study on fear memory and cannabinoids in prefrontal cortex and amygdala.
- Cannabinoids reduce fear learning in amygdala and prefrontal cortex.
- Cannabinoid antagonists impair fear extinction in the prefrontal cortex.

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ABSTRACT

Cannabinoid receptors 1 (CB1R) have been shown to be a crucial part of the neuromodulatory endocannabinoid system which is involved in emotional learning and memory.

We here investigated in rats the role of CB1R in the basolateral amygdala (BLA) and medial prefrontal cortex (mPFC) in different phases of fear learning, memory and extinction. We used the fear potentiated startle paradigm to measure the effects of local microinfusion of the CB1R agonist WIN 55,212-2 (WIN) or the CB1R antagonist AM251 on acquisition, consolidation, retrieval and extinction of fear.

No effects on fear acquisition of WIN or AM251 were found in the BLA or mPFC. WIN impaired fear retrieval in the BLA and in mPFC. Also, WIN reduced fear consolidation in the BLA but not in the mPFC. AM251 decreased fear consolidation after mPFC infusion. Likewise, fear extinction was impaired by AM251 infused into the mPFC.

Our data indicate that fear memory consolidation and retrieval, as well as extinction are regulated differentially by amygdaloid and cortical CB1R.

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1. Introduction

Endocannabinoid signalling regulates transmitter release via cannabinoid receptors 1 (CB1R) that are located presynaptically on several transmitter systems in the brain [1–3]. CB1R inhibit transmitter release as heteroreceptors mainly on glutamatergic and GABAergic nerve terminals in the cerebellum and in the forebrain. Hence, CB1R and endocannabinoid signalling are in strategic positions to regulate a variety of cognitive functions, such as learning and memory. For example, enhancement of endocannabinoid signalling by the inhibition of its metabolizing enzyme modulates acquisition and extinction of spatial memory [4], the selective CB1R-antagonist AM251 has an amnestic effect in the inhibitory avoidance paradigm [5], and the synthetic CB1R agonist WIN 55,212-2 (WIN) impairs spatial memory retrieval [6]. In addition the genetic deletion of CB1R in mice impaired the extinction of conditioned fear [7]. Many recent studies support the important role of endocannabinoid signalling in mnemonic processes [8–14]. However, there are still some open questions concerning the involvement of CB1R with respect to different brain systems and different phases of learning and memory.

We here focus on fear conditioning and fear memory. Classical fear conditioning comprises different phases of learning and memory processes such as acquisition, consolidation, retrieval and extinction [15,16]. Recently, different paradigms of fear conditioning were used to study the role of endocannabinoid signalling in fear conditioning. But the differences of for example olfactory fear conditioning [12], auditory fear conditioning [11], contextual fear conditioning [10], visual fear conditioning [17] and trace fear conditioning [13] make direct comparisons between these studies difficult. Furthermore, different species (mice or rats), knockout and wildtype animals and various drugs in different doses were used in these studies. Therefore, we here investigated in a systematic approach in rats the role of CB1R in the basolateral complex of the amygdala (BLA) and the medial prefrontal cortex (mPFC) in different phases of fear learning. We used the fear-potentiated startle (FPS) paradigm and tested the effects of the CB1R agonist WIN and

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the antagonist AM251 on acquisition, consolidation, retrieval and extinction of fear after local microinfusion of the drugs.

2. Materials and methods

2.1. Subjects

A total of 265 adult male Wistar rats (Hannover strain, Harlan-Winkelmann, Borchen, Germany) weighing between 250 and 300 g were used. Animals were housed in groups of five in Macrolon cages (type IV) under standard conditions on a 12 h light-dark cycle with lights on at 7 o'clock. All experimental procedures were performed during the rats' light cycle. They received free access to tap water and were maintained on their experimental body weight by controlled feeding of 12 g rodent chow per rat per day.

The experiments were performed in accordance with the National Institutes of Health ethical guidelines for the care and use of laboratory animals for experiments and were approved by the local animal care committee and authorities.

2.2. Experimental procedures

Upon arrival, the rats were allowed to acclimatize to the vivarium for three days with access to food and water ad libitum. Afterwards, feeding was reduced to 12 g per animal per day and daily handling was conducted for one week. Then, stereotaxic surgeries were performed and guide cannulae were either implanted into the prelimbic part (PrL) of mPFC or into the BLA. Three weeks after arriving in the laboratory animals underwent an ASR matching procedure, which was done in order to individually identify the acoustic startle stimulus intensity for each animal that differed least from the mean value of the group. This matching procedure was done in order to reduce the variability of different baseline ASR reactivity. Three days later the fear conditioning training was commenced. At 24 h following the fear conditioning session, animals were returned to the same chambers and presented with the first FPS test. During the next three days the same FPS test was executed in a 24 h rhythm and again at 14 days following these tests. Three to four days later some of the animals were also tested in the elevated plus-maze. After completion of the experiments all laboratory animals were killed by an overdose of chloral hydrate (in accordance with the Animal Welfare Act).

2.3. Matching, fear conditioning and fear potentiated startle

For matching, fear conditioning and FPS measuring a Startle Response System (TSE Systems, Bad Homburg) was used. Startle-boxes consisted of two different sets of cages (Set I and II) with Plexiglas doors and floors resting on a piezo-sensitive platform mounted inside of a sound attenuated and ventilated chamber. Vibrations of the cages caused by the whole body acoustic startle response (ASR) were transduced into analogy signals and then digitized and stored by a computer using the TSE software. For fear conditioning an electrical stimuli were delivered through a floor grid (only Set I). All acoustic startle stimuli (AS) were presented as broadband noise at the respective sound levels. Pure sine wave tones of 4 kHz and a sound pressure level of 72 decibel (dB) were used as conditioned stimuli (CS). At the beginning of each session, animals were placed into the startle chambers for a 5-min acclimatization period without stimuli. All boxes were equipped with a fan that produced a broadband noise level of 60 dB (sound pressure level).

2.4. Matching

During the matching procedure animals were exposed to AS of four different intensities (90, 95, 100, 105 dB). Each stimulus was presented eight times for 30 ms. Altogether, a total of 32 stimuli were presented in pseudo randomized order. Interstimulus intervals (ITI) differed from 20 to 30 s and were also presented in pseudo randomized order.

2.5. Fear conditioning

Conditioning was performed by pairing a CS with a foot shock. During training a tone (4 kHz, 72 dB, 4000 ms) was presented eight times with a pseudo randomized delay between the presentations (90–180 s). After 3500 ms duration the CS was paired with the delivery of a 0.5 mA foot shock (500 ms) as an unconditioned stimulus (US).

2.6. FPS test

In order to evaluate FPS as an operational measure of fear the AS intensity from the matching was used was each rat individually. Following an acclimatization period of 5 min this individually defined AS was presented ten times (Block I). Then, 30 trials followed in pseudo randomized order consisting of three different types of trials. (1) Ten trials involved the presentation of the AS (Block II), (2) ten trials involved the presentation of the CS, for 4000 ms, and, at 3500 ms after its onset, the simultaneous presentation of the AS. (3) Ten trials involved the presentation of a neutral signal tone (10 kHz, 72 dB), for 4000 ms, and, at 3500 ms after its onset, the simultaneous presentation of the AS. Finally, ten AS alone presentations were presented (Block III). Interstimulus intervals differed from 20 to 30 s. The FPS was calculated as the percent difference between the ASR in the presence or in the absence of the CS (100% × [AS with CS − AS alone]/AS alone) according to previous studies [18–20].

2.7. Surgery

Rats were anesthetized with an intraperitoneal injection of chloral hydrate (360 mg/kg, Sigma–Aldrich, Steinheim, Germany) and placed in a stereotaxic device. Subsequent supplements of chloral hydrate were administered intraperitoneally if necessary. Incisions were made in the scalp to expose the skull, burr holes were drilled, and the dura overlying the BLA or mPFC was perforated. All rats were bilaterally implanted with stainless steel guide cannulae (21 gauge) aiming 1 mm above the intended injection site in either mPFC (PrL) or BLA. These cannulae were permanently secured to the skull with dental cement as well as bone screws and closed by removable mandrins before and between behavioural experiments. A post-surgery recovery period of 6–10 days was allowed.

Stereotactic coordinates based on Bregma according to the atlas of Paxinos and Watson [21] used for the final injection sites were for prelimbic mPFC: antero-posterior (AP) +2.7 mm, latero-medial (LM) ± 0.8 mm, dorso-ventral (DV) −3.7 mm; BLA: AP −3.1 mm, LM ±5.0 mm, DV −6.0 mm.

2.8. Microinjection

Bilateral injections of WIN, AM-251 or vehicle were carried out on hand-restrained rats with the injection cannulae (26 gauge) extending 1 mm beyond the tip of the guide cannula using micro-litre syringes (1 μl, SGE Analytical Science, Ringwood, Australia) attached to flexible polyethylene tubes. The rate of injection was 0.15 μl/min, and the injection cannulae were left in place for an additional 1 min to allow adequate absorption of WIN, AM-251 or vehicle by the surrounding tissue. According to the different learning and memory processes investigated the time interval between microinjection and testing differed.

2.9. Drugs

For intra-cerebral microinjection, a dose of 5 μg/0.3 μl WIN 55,212-2 (Sigma–Aldrich, Steinheim, Germany) or 1 μg/0.3 μl AM-251 was used and bilaterally infused into the region of interest. WIN and AM-251 were each dissolved in dimethyl sulfoxide (DMSO; Sigma–Aldrich, Steinheim, Germany) and Tween-80 and diluted in Ringer's solution for injection Braun (Braun AG, Melsungen, Germany) at a ratio of 10% DMSO, 3% Tween80 to 85% Ringer's solution for injection. A composite of the solvents of the same ratio served as control. Drugs were freshly prepared before being used.

2.10. Histology

After completion of the experiments the rats were deeply anaesthetized with 720 mg/kg chloral hydrate and transcardially perfused with 0.1 M phosphate-buffered formaldehyde (pH 7.4). Brains were removed from the skull, post-fixed in a sucrose-formalin solution (30 g Sucrose, Merck, Darmstadt, Germany; 20 ml of 37% formalin, Merck, Darmstadt) for two days. Afterwards, the sucrose–formalin solution was replaced by a 30% sucrose solution (in 0.2 M phosphate buffer) and the brains were again stored for two days. Subsequently, the brains were cut into 40-μm coronal sections on a cryostat microtome. To verify the appropriate location of tips of the infusion cannulae, the sections were Nissl-stained with thionin and analyzed using a light microscope (Axioscop, Zeiss, Göttingen). The atlas of Paxinos and Watson (1998) was used for the re-construction of the injections sites.

2.11. Statistical analysis

The descriptive statistics is based on means, and variance is indicated by the standard error of the mean (±SEM). All analyses were performed with the statistical software SigmaStat (version 2.03 for Windows). Results were analyzed using one-way or two-way analyses of variance (ANOVA). After significant ANOVAs, differences between groups were evaluated by post hoc Tukey's t test. A P-value <0.05 was considered to represent a significant effect.

3. Results

3.1. Histology

A total of 265 animals underwent stereotactic surgery. The target areas were missed in nine animals. Data from these rats were not included in the analysis. Three animals died during surgery. Fig. 1A–D shows the localization of the injection cannulae in the BLA. In 127 rats evaluation of thionine-stained brain sections (Fig. 2A and B) using light microscopy indicated that the target area

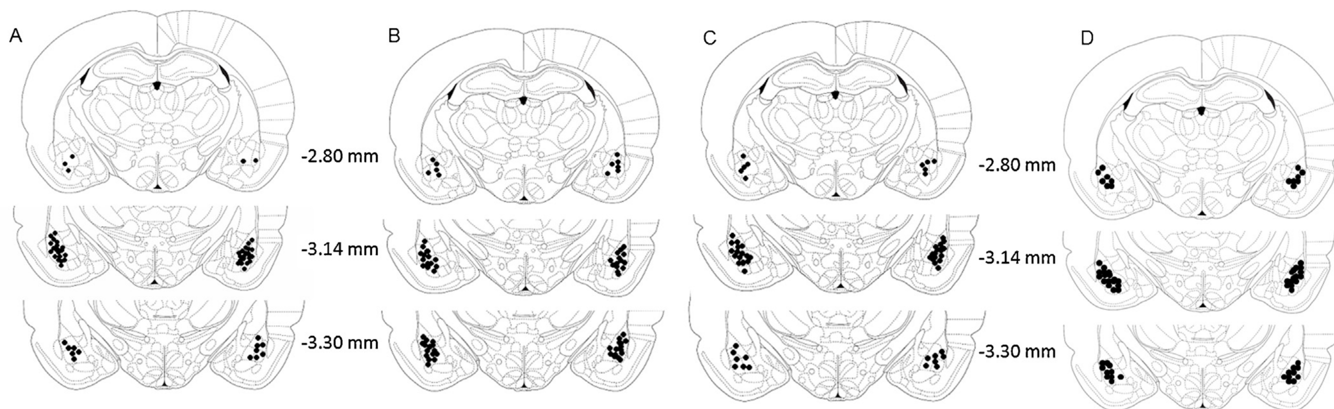

Fig. 1. Reconstruction of infusion sites into the BLA for the investigation of acquisition (A), consolidation (B), retrieval (C) and extinction (D) of fear depicted on schematic drawings of coronal brain sections (distance from bregma) according to the atlas of Paxinos and Watson (1998).

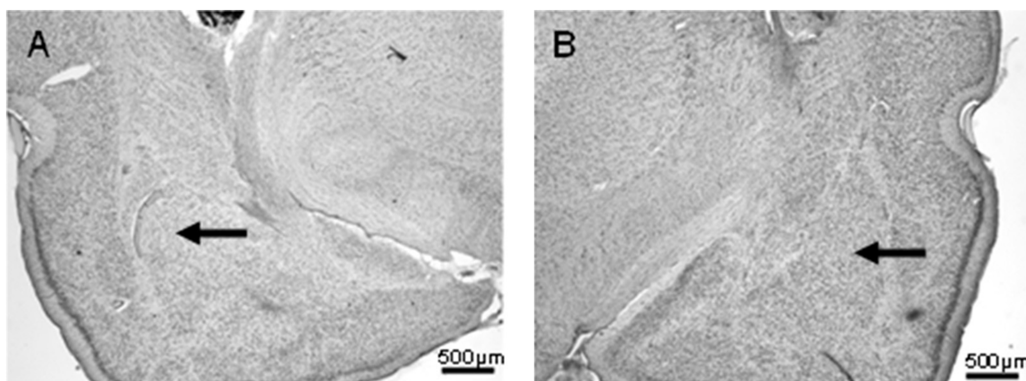

Fig. 2. Photomicrograph of brain sections showing representative infusion sites (arrows) within the BLA (A: left hemisphere, B: right hemisphere).

of the IC microinjection was accurately hit. Fig. 3A–D illustrates the localization of the injection cannulae in the mPFC (PrL). In 126 of the rats the Nissl-stained sections (Fig. 4A and B) revealed that the target area was hit.

3.2. FPS

3.2.1. Acquisition

In order to investigate drug effects on fear acquisition, the rats were bilaterally injected with either WIN ($n = 10$), AM251 ($n = 10$) or a vehicle (Control, $n = 10$) into the BLA 10–20 min prior to fear conditioning. There was no significant difference between treatments at any time point (24 h, 48 h, 72 h, 96 h and 14 d 96 h) in the percent FPS measured in animals exposed to the 4 kHz CS (Fig. 5, ANOVA: $p > 0.05$). Qualitatively similar results were found after presentation of the 10 kHz tone (data not shown). Table 1 lists the average ASR values determined during Block II of FPS-test in the absence of the CS.

Likewise, rats were bilaterally injected with either WIN ($n = 10$), AM251 ($n = 10$) or a control solution ($n = 10$) into the mPFC

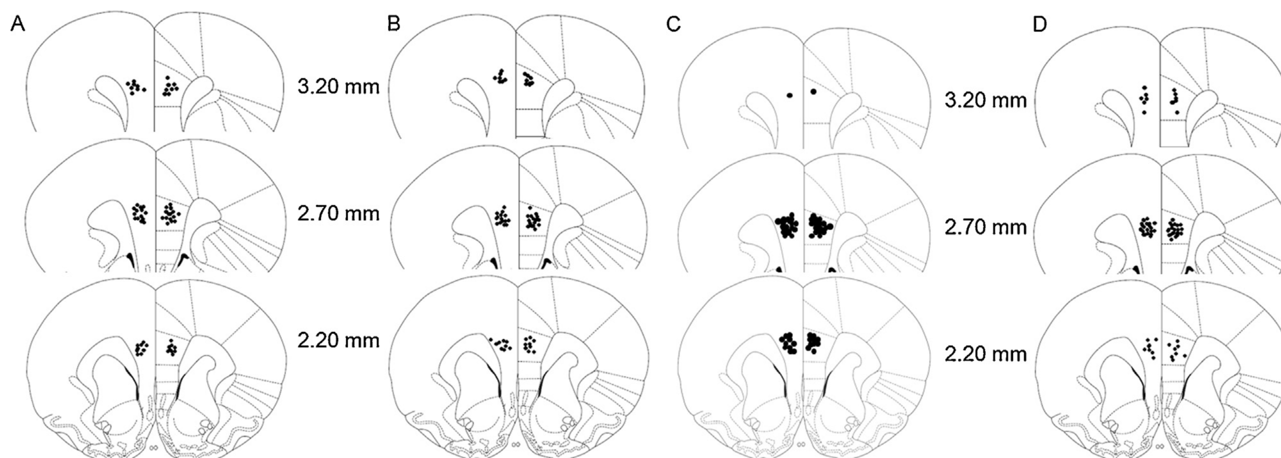

Fig. 3. Reconstruction of infusion sites into the mPFC (PrL) for the investigation of acquisition (A), consolidation (B), retrieval (C) and extinction (D) of fear depicted on schematic drawings of coronal brain sections (distance from bregma) according to the atlas of Paxinos and Watson (1998).

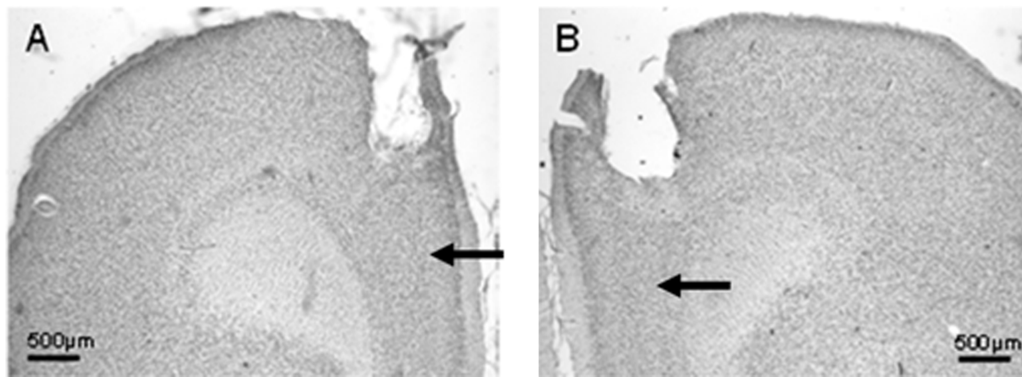

Fig. 4. Photomicrograph of brain sections showing representative infusion sites (arrows) within the mPFC (PrL; A: left hemisphere, B: right hemisphere).

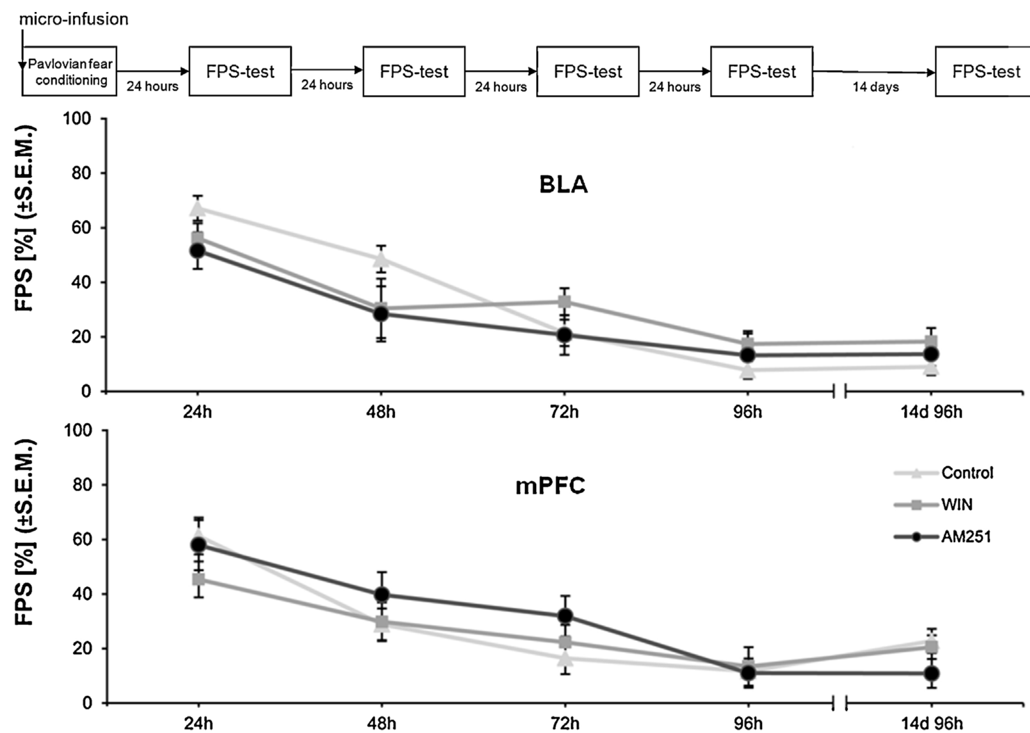

Fig. 5. Effects of bilateral intra BLA and intra mPFC (PrL) control- (0.3 μl), WIN- (5 μg/0.3 μl) and AM251-infusion (1 μg/0.3 μl) on percent FPS in fear acquisition. Data are means ± SEM (BLA and mPFC: control: $n = 10$, WIN: $n = 10$, AM251: $n = 10$). There was no significant difference between treatments at any time point (24 h, 48 h, 72 h, 96 h and 14 d 96 h) in the evaluation of percent FPS measured in animals exposed to the 4 kHz CS (ANOVA: $p > 0.05$).

10–20 min prior to fear conditioning. There was no significant difference between treatments at any time point (24 h, 48 h, 72 h, 96 h and 14 d 96 h) in percent FPS measured in rats exposed to the 4 kHz CS (Fig. 5, ANOVA: $p > 0.05$). Qualitatively similar results were found after presentation of the 10 kHz tone (data not shown). Table 2 lists the averaged ASR values determined during Block II of FPS-test in the absence of the CS.

3.2.2. Consolidation

In order to investigate fear consolidation, the rats were bilaterally injected with either WIN ($n = 11$), AM251 ($n = 11$) or vehicle ($n = 11$) into the BLA 30–45 min after fear conditioning. Twenty four hours after fear conditioning the animals that had received WIN injections exhibited reduced FPS following exposure to the 4 kHz CS compared to rats treated with vehicle (Fig. 6). Statistical

Table 1
ASR magnitude during FPS-test after intra-BLA infusion (control: $n = 10$, WIN: $n = 10$, AM251: $n = 10$) in fear acquisition. Data are means (±SEM) of 10 presentation of the AS in the absence of the CS (Block II).

Treatment	24 h		48 h		72 h		96 h		14 d 96 h	
	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.
Control	317	56.7	289	45.0	241	21.0	295	34.8	235	35.8
WIN	470	76.4	342	58.2	272	28.6	310	35.5	302	37.5
AM251	479	49.4	355	45.2	312	39.1	329	41.8	302	40.4

Table 2
ASR magnitude during FPS-test after intra-mPFC (PrL) infusion (control: $n = 10$, WIN: $n = 10$, AM251: $n = 10$) in fear acquisition. Data are means (±SEM) of ten presentations of the AS in absence of CS (Block II).

	24 h		48 h		72 h		96 h		14 d 96 h	
Treatment	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.
Control	514	43.6	445	46.2	360	32.1	316	33.1	341	34.8
WIN	591	82.5	537	87.2	402	46.5	373	53.5	416	56.3
AM251	552	63.2	317	34.1	291	26.4	280	32.5	320	33.3

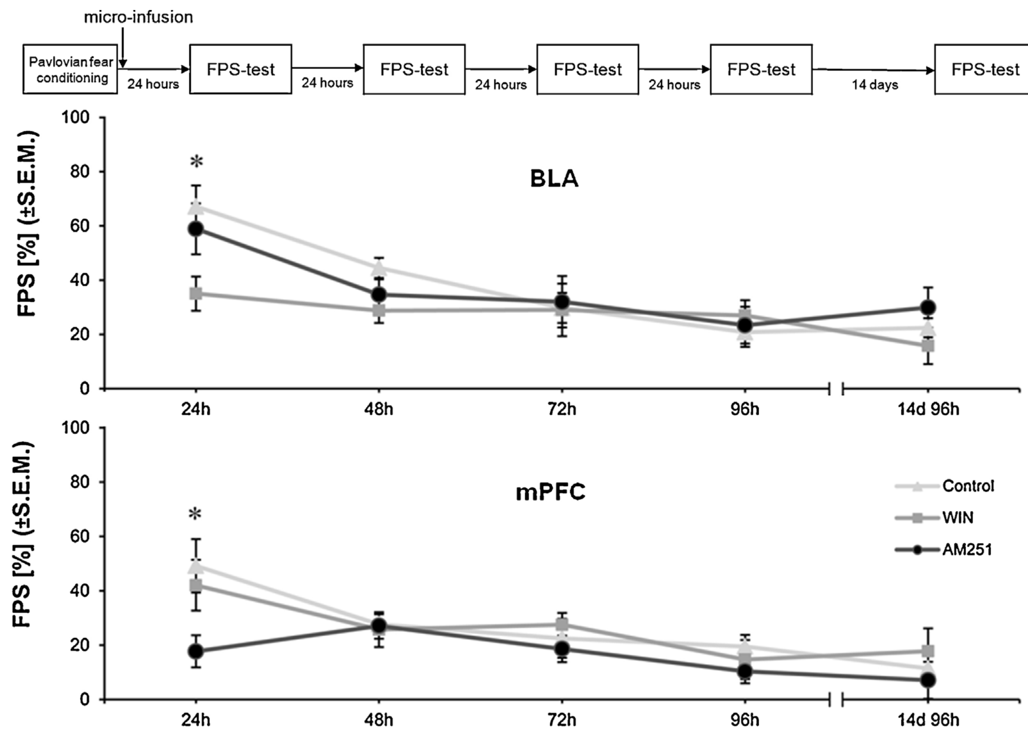

Fig. 6. Effects of bilateral intra BLA and intra mPFC (PrL) control- (0.3 μl), WIN- (5 μg/0.3 μl) and AM251-infusion (1 μg/0.3 μl) on percent FPS in fear memory consolidation. Data are means ± SEM (BLA: control: $n = 11$, WIN: $n = 11$, AM251: $n = 11$; mPFC: control: $n = 10$, WIN: $n = 11$, AM251: $n = 10$). 24 h after fear conditioning WIN intra BLA and AM251 intra mPFC reduced FPS significantly (* $p > 0.05$).

analysis using one-way ANOVA revealed a significant difference among groups ($F_{2,30} = 4.459$; $p = 0.020$), and post hoc analysis with Tukey's *t*-test indicated a significant difference between the control group and the group treated with WIN ($p = 0.020$). There was no significant difference between treatments at time points (48 h, 72 h, 96 h and 14 d 96 h) in the evaluation of percent FPS measured in rats exposed to the 4 kHz CS (ANOVA: $p > 0.05$, Fig. 6). Qualitatively similar results were found after presentation of the 10 kHz tone (data not shown). Table 3 lists the average ASR values determined during Block II of FPS-test in absence of CS.

Likewise, rats were bilaterally injected with either WIN ($n = 11$), AM251 ($n = 10$) or vehicle ($n = 10$) into the mPFC 30–45 min after fear conditioning. Twenty four hours after fear conditioning the animals that had received AM251 injections exhibited reduced FPS following exposure to the 4 kHz CS compared to animals treated with control solution (Fig. 6). Statistical analysis showed a significant difference among groups ($F_{2,28} = 3.575$; $p = 0.041$), and post hoc Tukey's *t*-tests indicated a significant difference between controls group and rats treated with AM251 ($p = 0.043$). There was no significant difference between treatments at time points (48 h, 72 h, 96 h and 14 d 96 h) in the evaluation of percent FPS measured in rats exposed to the 4 kHz CS (ANOVA: $p > 0.05$, Fig. 6). Qualitatively similar results were found after presentation of the 10 kHz tone (data not shown). Table 4 lists the average ASR values determined during Block II of FPS-test in the absence of the CS.

3.2.3. *Retrieval*

For the investigation of fear memory retrieval, the rats were bilaterally injected with either WIN ($n = 11$), AM251 ($n = 10$) or vehicle (Control, $n = 10$) into the BLA 10–20 min prior the first measurement of FPS (24 h after fear conditioning). The rats that had received WIN injections exhibited reduced FPS upon exposure to

Table 3
ASR magnitude during FPS-test after intra-BLA infusion (control: $n = 11$, WIN: $n = 11$, AM251: $n = 11$) in fear memory consolidation. Data are means (±SEM) of ten presentations of the AS in the absence of the CS (Block II).

	24 h		48 h		72 h		96 h		14 d 96 h	
Treatment	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.
Control	557	70.3	367	36.5	388	51.8	367	47.9	430	59.4
WIN	523	53.1	364	42.2	376	34.6	347	41.1	428	63.4
AM251	554	69.9	336	52.4	299	40.5	266	25.8	288	23.8

Table 4
ASR magnitude during FPS-test after intra-mPFC (PrL) infusion (control: $n = 10$, WIN: $n = 11$, AM251: $n = 10$) in fear memory consolidation. Data are means (±SEM) of 10 presentations of the AS in the absence of the CS (Block II).

	24 h		48 h		72 h		96 h		14 d 96 h	
Treatment	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.
Control	423	29.3	329	24.0	346	38.5	346	42.8	354	32.3
WIN	714	83.8	445	58.2	359	45.3	342	43.2	355	41.7
AM251	742	99.6	486	68.2	472	65.8	374	55.3	423	58.1

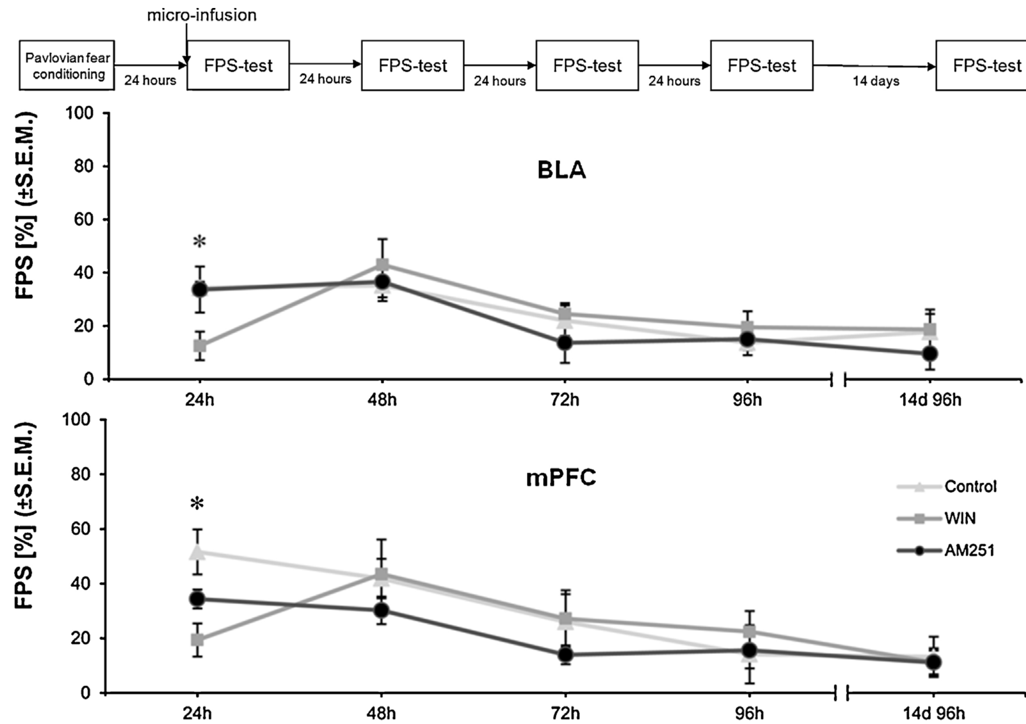

Fig. 7. Effects of bilateral intra BLA and intra mPFC (PrL) control- (0.3 μl), WIN- (5 μg/0.3 μl) and AM251-infusion (1 μg/0.3 μl) on percent FPS in fear memory retrieval. Data are means ± SEM (BLA and mPFC: control: $n = 10$, WIN: $n = 11$, AM251: $n = 10$). 24 h after fear conditioning WIN intra BLA and intra mPFC reduced FPS significantly (*$p > 0.05$).

the 4 kHz CS compared to rats treated with vehicle (Fig. 7). Statistical analysis indicated a significant difference among groups ($F_{2,28} = 4.414$; $p = 0.022$), and post hoc Tukey's *t*-tests revealed a significant difference between the control and the WIN group ($p = 0.039$). There was no significant difference between treatments at time points (48 h, 72 h, 96 h and 14 d 96 h) in percent FPS measured in animals exposed to the 4 kHz CS (ANOVA: $p > 0.05$, Fig. 7). Qualitatively similar results were found after presentation of the 10 kHz tone (data not shown). Table 5 lists the average ASR values in the absence of the CS.

Likewise, rats were bilaterally injected with either WIN ($n = 11$), AM251 ($n = 10$) or vehicle (Control, $n = 10$) into the mPFC 10–20 min before the first measurement of FPS (24 h after fear conditioning). The animals that had received WIN showed reduced FPS after exposure to the 4 kHz CS compared to rats treated with vehicle (Fig. 7). Statistical analysis showed a significant difference among groups ($F_{2,28} = 6.813$; $p = 0.004$), and post hoc Tukey's *t*-tests indicated a significant difference between the control and the WIN group ($p = 0.003$). There was no significant difference between treatments at time points (48 h, 72 h, 96 h and 14 d 96 h) in percent FPS measured in rats exposed to the 4 kHz CS (ANOVA: $p > 0.05$, Fig. 7). Qualitatively similar results were found after presentation of the 10 kHz tone (data not shown). Table 6 lists the average ASR values determined during Block II of FPS-test in the absence of the CS.

3.2.4. Extinction

In order to investigate fear memory extinction, the rats were bilaterally injected with either WIN ($n = 11$), AM251 ($n = 11$) or vehicle (Control, $n = 11$) into the BLA 10–20 min prior to both the second (48 h after fear conditioning), third (72 h after fear conditioning) and fourth (96 h after fear conditioning) measurement of FPS. There was no significant difference between treatments at any time point (24 h, 48 h, 72 h, 96 h and 14 d 96 h) in the evaluation of percent FPS measured in rats exposed to the 4 kHz CS (Fig. 8, ANOVA: $p > 0.05$).

Table 5
ASR magnitude during FPS-test after intra-BLA infusion (control: $n = 10$, WIN: $n = 11$, AM251: $n = 10$) in fear memory retrieval. Data are means (±SEM) of ten trials after the presentation of the AS in the absence of the CS (Block II).

	24 h		48 h		72 h		96 h		14 d 96 h	
Treatment	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.
Control	521	75.2	421	76.7	370	57.3	347	42.2	562	89.6
WIN	631	90.4	421	56.2	336	57.7	331	37.9	515	94.0
AM251	529	52.7	364	33.9	343	27.6	331	34.5	444	47.5

Table 6
ASR magnitude during FPS-test after intra-mPFC (PrL) infusion (control: $n = 10$, WIN: $n = 11$, AM251: $n = 10$) in fear memory retrieval. Data are means (±SEM) of ten trials after the presentation of the AS in the absence of the CS (Block II).

	24 h		48 h		72 h		96 h		14 d 96 h	
Treatment	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.
Control	629	47.0	533	45.9	466	61.1	405	56.0	459	69.0
WIN	781	57.8	466	55.1	475	42.4	346	30.9	399	48.6
AM251	506	46.2	395	55.9	317	42.1	256	36.7	394	68.9

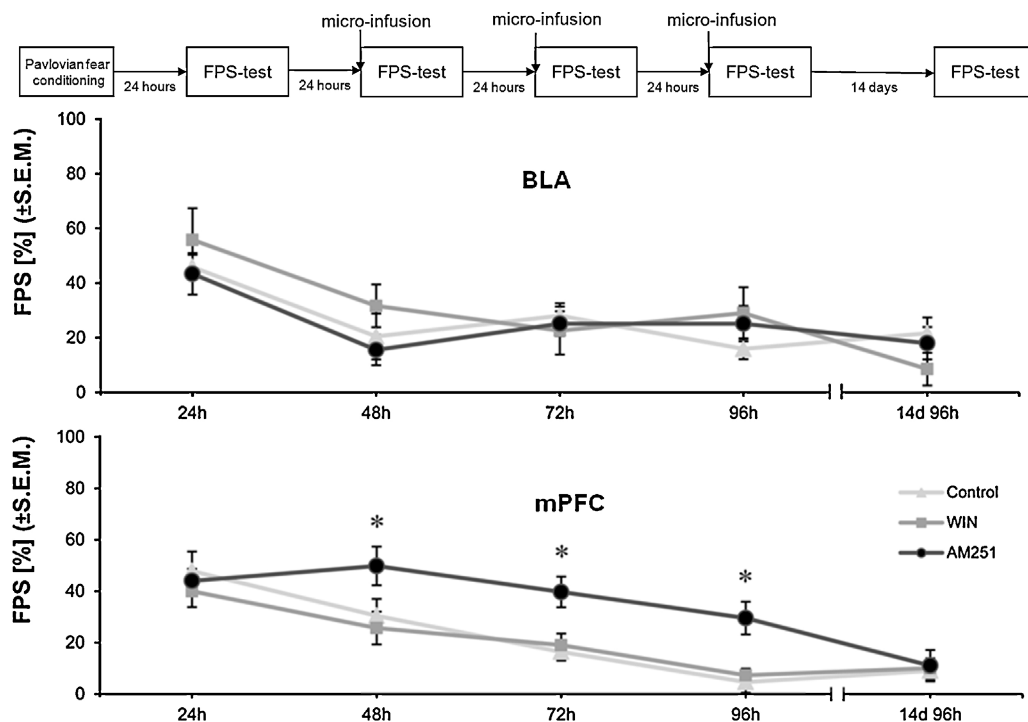

Fig. 8. Effects of bilateral intra BLA and intra mPFC (PrL) control- (0.3 μl), WIN- (5 μg/0.3 μl) and AM251-infusion (1 μg/0.3 μl) on fear extinction. Data are means ± SEM (BLA and mPFC: control: $n = 11$, WIN: $n = 11$, AM251: $n = 11$). In the BLA groups there were no significant treatment effects at any time point (24 h, 48 h, 72 h, 96 h and 14 d 96 h) in the evaluation of percent FPS measured in rats exposed to the 4 kHz CS (ANOVA: $p > 0.05$). At 48, 72 and 96 h after fear conditioning intra mPFC AM251 significantly reduced extinction (*$p > 0.05$).

Qualitatively similar results were found after presentation of the 10 kHz tone (data not shown). Table 7 lists the averaged ASR values determined during Block II of FPS-test in the absence of the CS.

Likewise, rats were bilaterally injected with either WIN ($n = 11$), AM251 ($n = 11$) or vehicle (Control, $n = 11$) into the mPCF 10–20 min before the second (48 h after fear conditioning), third (72 h after fear conditioning) and fourth (96 h after fear conditioning) measurement of FPS. After exposure to the 4 kHz CS, rats that had received AM251 exhibited increased FPS on the second day of measurement, the third day of measurement and the fourth day of measurement compared to animals that had received vehicle (Fig. 8). Statistical analysis using ANOVA and post hoc Tukey's t-tests indicated significant differences between groups on the second ($F_{2,31} = 3.476$; $p = 0.043$), third ($F_{2,31} = 7.134$; $p = 0.003$), and fourth day ($F_{2,31} = 8.933$; $p < 0.001$). The first measurement of FPS took place 24 h after fear conditioning. In this test no significant difference in the magnitude of FPS among experimental groups was found (ANOVA: $p > 0.05$). Eighteen days after treatment no significant treatment effect on FPS was seen (Fig. 8; ANOVA: $p > 0.05$). Table 8 lists the averaged ASR values determined during Block II of FPS-test in the absence of the CS.

4. Discussion

Cannabinoid 1 receptors (CB1R) in the amygdala and the prefrontal cortex play important roles in associative learning [12,22–25]. The results of the present fear-conditioning study support this notion and suggest that the various phases of learning and memory in the rat can be influenced differentially in the mPFC and BLA by the synthetic CB1R ligands WIN and AM251.

Table 7
ASR magnitude during FPS-test after intra-BLA infusion (control: $n = 11$, WIN: $n = 11$, AM251: $n = 11$) in extinction trials. Data are means (±SEM) of 10 presentations of the AS in the absence of the CS (Block II).

	24 h		48 h		72 h		96 h		14 d 96 h	
Treatment	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.
Control	479	64.7	413	49.7	366	27.6	346	23.8	345	38.6
WIN	594	67.6	415	39.2	455	35.6	430	38.4	546	66.6
AM251	612	67.6	454	57.5	458	55.6	399	56.0	443	47.6

Table 8
ASR magnitude during FPS-test after intra-mPFC (PrL) infusion (control: $n = 11$, WIN: $n = 11$, AM251: $n = 11$) in extinction trials. Data are means (±SEM) of ten presentations of the AS in the absence of the CS (Block II).

	24 h		48 h		72 h		96 h		14 d 96 h	
Treatment	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.	ASR	±S.E.M.
Control	669	77.4	456	53.4	381	39.7	411	38.6	472	67.6
WIN	621	75.6	515	83.6	527	61.2	589	65.1	448	64.5
AM251	697	53.2	444	33.8	469	57.9	419	48.4	472	80.3

4.1. Acquisition

Fear conditioning was not affected by intracerebral microinjection of WIN or AM251 into the BLA or mPFC. Other studies on the influence of CB1R ligands on the acquisition of fear have yielded slightly different results. For example, systemic application of AM251 in normal mice improved acquisition [13], but impaired acquisition in CB1R knock-out mice with contextual fear conditioning [8], whereas in rats, acquisition was impeded by the CB1R antagonist in a dose-dependent manner. Other studies with CB1R knockout mice [7,26] are consistent with our results, in which fear acquisition was not dependent on CB1R. Modulation of transmitter release via CB1R ligands has already been demonstrated for the lateral nucleus of the amygdala [27] as well as the PFC [28–30]. It is possible that acquisition cannot be sufficiently disrupted in the BLA as a result of compensation by the central nucleus of the amygdala [31,32].

The mPFC has close anatomical [33,34] and functional [12,35,36] connections with the amygdala. In the context of fear learning and memory, the connections between the infralimbic (IL) and the PrL mPFC and the BLA are particularly important [37,38]. There is physiological evidence that these parts of the mPFC regulate fear learning in the BLA [35,39] probably involving CB1R [12]. Using olfactory fear-conditioning it was shown that CB1R in the BLA affect the neuronal activity and fear acquisition [25,40]. Our data and those of studies using CB1R knock-out mice [7] do not support this finding. This discrepancy is probably due to methodological differences between these studies. The advantage of our present study is that we used the same paradigm and methodological approach to test the possible role of CB1R in the BLA in fear acquisition, consolidation, retrieval and extinction.

4.2. Consolidation

Within a temporal window of up to 6 h after conditioning, it is possible to inhibit processes of memory consolidation [41–43]. Therefore, in the present study the local microinjections were done 30–45 min after fear conditioning. Twenty four hours after conditioning, there was a reduction in FPS as a result of WIN-infusions into the BLA, whereas in the mPFC, the CB1R antagonist AM251 induced a reduction of fear.

Compared to the control treatment, AM251 had no measurable effect on FPS in the BLA. This result is supported by a study in mice, in which no effect of AM251 on consolidation of fear conditioning with discrete stimuli was observed [13] and by a study in rats with olfactory fear conditioning [40]. As suggested by Pistis and colleagues [44], modulation of consolidation within the BLA may also take place via a cannabinoid receptor other than CB1R. These receptors may be activated by WIN and hence lead to a decrease in synaptic transmission. A selective CB1R antagonist such as AM251 would not bind to such a receptor and as result would have no effect on consolidation. In another study in rats using bilateral infusion of WIN and AM251 into the BLA, it was shown that CB1R ligands in this area of the brain are probably involved in the regulation of memory consolidation [45]. Our results support this finding. In contrast, Tan and colleagues [40] found no effect of intra-BLA WIN microinfusion on consolidation of olfactory fear memory.

Our data suggest no effect of WIN in the mPFC on memory consolidation. This is unexpected, since a reduction by WIN of glutamatergic and GABAergic transmission has been found in the mPFC in rats [46,47]. On the other hand, we found an effect of the CB1R antagonist AM251 in the mPFC on fear memory consolidation. Since Marsicano and colleagues showed no effect of systemic administration of a CB1R antagonist on consolidation of auditory fear conditioning in either mice or CB1R knockout mice [7], our findings have to be interpreted carefully. It has already been demonstrated that in the hippocampus, cannabinoids can affect memory consolidation. However, the results are inconsistent. In some cases, consolidation of memory [5,48] and LTP [49] in the hippocampus was impaired with AM251. In another paper, the same effect was seen with the agonist WIN [50,51]. It was also shown that a CB1R agonist can suppress protein synthesis for synaptic plasticity in the hippocampus [52]. An effect of the cannabinoid system on the amygdala-prefrontal cortical pathway has already been shown for mPFC [12,26]. Microinjection of the CB1R antagonist AM251 into the mPFC blocked emotional learning [26]. LTP (electrodes were placed in the BLA) was suppressed by AM251on the pathway between the BLA and the mPFC [26]. However, in these studies the treatment with CB1R ligands took place prior to an olfactory fear conditioning and, therefore, no direct conclusions can be drawn regarding consolidation.

Modulation of consolidation can be brought about by the cannabinoid system both in the mPFC as well as in the amygdala. Neither WIN nor AM251 had an effect on acquisition, so it is likely that the consolidation processes react more sensitively to the neuromodulatory effects of CB1R. The apparent contrasting effect on consolidation of fear conditioning from CB1R agonist and antagonist in the BLA and the mPFC suggests reciprocal interactions of both areas of the brain during the formation of emotional memory [35,39,53].

4.3. Retrieval

In this study, infusion of WIN both in the BLA and in the mPFC shortly before retrieval reduced FPS compared to the control treatment. This effect of WIN was no longer detectable after an additional 24 h (48 h FPS test). Percent FPS at this point was similar to the control group suggesting that successful fear conditioning took place.

Previous studies on spatial memory retrieval have already demonstrated an important role of CB1R since memory recall was impaired by CB1R agonists [54,55]. However, experiments on fear expression have yielded inconsistent results. In one study, fear expression in mice was improved by systemic administration of AM251 [13], whereas in another, no effect was found [11] and intra-BLA AM251 microinfusion in rats had no effect on the expression of olfactory fear conditioning [40]. Marsicano and colleagues (2002) have demonstrated that endocannabinoids appear to be involved in the retrieval of fear response. They found elevated levels of endocannabinoid in the BLA (but not in the mPFC) of mice during the presentation of the sound 24 h after auditory fear conditioning. In

the present study, any disruption of the physiological balance of the endocannabinoid system within the BLA may have reduced fear retrieval after WIN treatment.

The results obtained in the present study for the mPFC are in contrast to those of Laviolette and Grace [12], who found a reduced fear response following olfactory fear conditioning after intracerebral injection of AM251 into the mPFC, although there was no effect of WIN. However, a reduction in fear has been shown in studies with temporary inactivation of the mPFC [56,57]. In addition, electrophysiological studies have shown an influence of the mPFC on fear expression [35,58,59]. The BLA pyramidal neurons can be stimulated by direct mPFC efferents [58] or may be inhibited by GABAergic interneurons in the lateral nucleus receiving efferents from a subpopulation of neurons in the mPFC [35]. It is thought that this inhibitory mPFC-BLA pathway functions primarily in extinction [60].

4.4. Extinction

If the CS is repeatedly presented without the US following fear conditioning, a reduced conditioned response (CR) is observed. The loss of the CR is termed extinction. Extinction is a learning process also characterized by acquisition, consolidation and retrieval [61,62]. In an attempt to influence extinction, microinjections of CB1R ligands into the BLA and the mPFC were performed 48, 72 and 96 h after fear conditioning, immediately before measuring FPS. Thus, each time the direct effect on retrieval of extinction was tested, as well as the indirect effect on acquisition of extinction by measuring FPS on the next day (72 and 96 h after fear conditioning).

Microinjection of WIN or AM251 into the BLA had no effect on extinction of the FPS compared to the control treatment. There is evidence suggesting that extracellular signal-regulated kinases activity in the BLA can be modulated by cannabinoids during extinction [63]. Behavioural studies of BLA-lesioned rats have shown that other structures within the amygdala may compensate for BLA dysfunction and hence enable extinction [64,65]. Although blocking of extinction in the inhibitory avoidance paradigm can be achieved by AM251 in the BLA, WIN had no effect [66]. Clearly, the different experimental designs contribute to the variation of the results.

Retrieval of extinction was not modulated by AM251 or WIN in the BLA. This result suggests that the processes of retrieval of extinction are mediated by other mechanisms and/or structures compared to retrieval of fear conditioning, since this is influenced by WIN in the BLA.

Extinction was inhibited by microinjection of AM251 into the mPFC. This was observed on each of the three test days on which the microinjection was given shortly before recall of the extinction. Lesion studies have already shown that the mPFC plays an important role in extinction [61,62]. Studies with an NMDA receptor antagonist [67] and a MAPK inhibitor [68] as well as lesion studies [69,70] have shown that the IL is probably responsible for both the consolidation and the expression of extinction. In contrast, this attributes no essential significance for the acquisition of extinction to the mPFC [61,71]. The results of the present study likewise suggest that the mPFC is involved in the expression of extinction. It is not exactly clear which molecular processes are activated during extinction and how much they are influenced by AM251. However, over the course of the three treatment days, the FPS of rats decreased in a parallel to the FPS values of control animals. It can therefore be assumed that despite treatment, an extinction occurred that possibly consisted of habituation-like processes that can be influenced by CB1R ligands [72,73]. WIN microinjections into the mPFC performed in the present study have shown no effect at any time point compared to a control treatment. It is noteworthy that in our study as well, the inhibitory effect of WIN on FPS of retrieval of fear conditioning was not observed following microinjection into the mPFC.

Lin and colleagues [74] showed contrasting results in a study in which rats were locally injected with AM251 into the IL and extinction was prevented. In addition, WIN promoted extinction. However, the microinjections always took place prior to an unpaired presentation of the CS, while the test was carried out only 24 h later and not directly afterwards as it was done in the present study. Thus, the Lin et al. study primarily showed the acquisition of extinction. As a result, the effect of the different areas of the mPFC can only be compared indirectly.

Systemic studies of mice and rats have shown that administration of CB1R antagonists decreases extinction [7,13,16,26,75] and WIN apparently had no effect [16]. This finding is consistent with the results of the present study. However, there was also evidence that acquisition of extinction can be delayed by CB1R antagonists [7,26]. Extinction can be prevented by systemic administration of a CB1R antagonist in the context of fear conditioning, whereas WIN facilitates extinction [9]. Similarly, various studies with CB1R knockout mice have found a negative effect on extinction [7,26,63,72].

Our finding that AM251 infusion into the mPFC impaired fear extinction is consistent with a new concept of fear relief proposed by Riebe and colleagues [76]. According to that extinction of auditory-cued fear memories is regulated by the endocannabinoid system.

Overall, CB1R have a modulating effect on the extinction of conditioned fear. Hence, we conclude that CB1R-mediated processes in the mPFC play an important role in the extinction of the fear response.

4.5. FPS in response to 10 kHz pure tones

These experiments were conducted in order to determine whether there is a generalization of fear. In all experiments, a significant FPS was observed in response to the unconditioned 10 kHz tone. However, on average this effect was weaker compared to the FPS that was elicited by the 4 kHz CS. Likewise, the treatments led to qualitatively similar effects, although FPS values were generally lower and the magnitude of the effect was not always the same. We were not able to precisely determine whether this result is attributable to a pure generalization process, because we performed fear conditioning in the test chambers, yielding a considerable degree of context conditioning [77,78].

Previous studies have already shown that endocannabinoid signalling especially in the mPFC is important for the regulation of a variety of anxiety- and stress-related behaviours. For example the endocannabinoid analogue methanandamide, or THC has anxiolytic effects in the elevated-plus maze at certain doses in the mPFC [79,80]. Interestingly, at high doses CB1R agonists may have anxiogenic effects, possibly via activation of *transient receptor potential vanilloid type 1 channels* [81]. Obviously, CB1R in the mPFC play a more general role in the neuronal processing of adverse events, such as stress responses [82].

In summary, the present microinfusion study investigated the effects of the CB1R agonist WIN and the CB1R antagonist AM251 on conditioned fear in rats. The FPS paradigm was used because it allows the distinction of drug effects on individual phases of learning, memory and extinction. We here show an involvement of the endocannabinoid system in the mPFC and BLA on consolidation and retrieval of FPS, supporting the contention of a cannabinoid modulation of the connections between BLA and mPFC. In addition to the results of CB1R knockout studies, it is particularly noteworthy that consolidation as well as extinction was influenced by cannabinoid receptor ligands.

References

[1] Elphick MR, Egertova M. The neurobiology and evolution of cannabinoid signalling. Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences 2001;356:381–408.
[2] Piomelli D. The molecular logic of endocannabinoid signalling. Nature Reviews Neuroscience 2003;4:873–84.
[3] Howlett AC, Breivogel CS, Childers SR, et al. Cannabinoid physiology and pharmacology: 30 years of progress. Neuropharmacology 2004;47(Suppl. 1):345–58.
[4] Varvel SA, Wise LE, Niyuhire F, et al. Inhibition of fatty-acid amide hydrolase accelerates acquisition and extinction rates in a spatial memory task. Neuropsychopharmacology 2007;32:1032–41.
[5] de Oliveira AL, de Oliveira LF, Camboim C, et al. Amnestic effect of intrahippocampal AM251, a CB1-selective blocker, in the inhibitory avoidance, but not in the open field habituation task, in rats. Neurobiology of Learning and Memory 2005;83:119–24.
[6] Wegener N, Kuhnert S, Thuns A, et al. Effects of acute systemic and intracerebral stimulation of cannabinoid receptors on sensorimotor gating, locomotion and spatial memory in rats. Psychopharmacology 2008;198:375–85.
[7] Marsicano G, Wotjak CT, Azad SC, et al. The endogenous cannabinoid system controls extinction of aversive memories. Nature 2002;418:530–4.
[8] Mikics E, Dombi T, Barsvari B, et al. The effects of cannabinoids on contextual conditioned fear in CB1 knockout and CD1 mice. Behavioural Pharmacology 2006;17:223–30.
[9] Pamplona FA, Prediger RD, Pandolfo P, Takahashi RN. The cannabinoid receptor agonist WIN 55,212-2 facilitates the extinction of contextual fear memory and spatial memory in rats. Psychopharmacology 2006;188:641–9.
[10] Pamplona FA, Takahashi RN. WIN 55212-2 impairs contextual fear conditioning through the activation of CB1 cannabinoid receptors. Neuroscience Letters 2006;397:88–92.
[11] Arenos JD, Musty RE, Bucci DJ. Blockade of cannabinoid CB1 receptors alters contextual learning and memory. European Journal of Pharmacology 2006;539:177–83.
[12] Laviolette SR, Grace AA. Cannabinoids potentiate emotional learning plasticity in neurons of the medial prefrontal cortex through basolateral amygdala inputs. Journal of Neuroscience 2006;26:6458–68.
[13] Reich C, Mohammadi M, Alger B. Endocannabinoid modulation of fear responses: learning and state-dependent performance effects. Journal of Psychopharmacology 2008;22:769–77.
[14] McLaughlin RJ, Cannabinoids Gobbi G, emotionality. A neuroanatomical perspective. Neuroscience 2012;204:134–44.
[15] Abel T, Lattal KM. Molecular mechanisms of memory acquisition, consolidation and retrieval. Current Opinion in Neurobiology 2001;11:180–7.
[16] Nader K. Memory traces unbound. Trends in Neurosciences 2003;26:65–72.
[17] Chhatwal JP, Davis M, Maguschak KA, Ressler KJ. Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. Neuropsychopharmacology 2005;30:516–24.
[18] Fendt M, Fanselow MS. The neuroanatomical and neurochemical basis of conditioned fear. Neuroscience and Biobehavioral Reviews 1999;23:743–60.
[19] Davis M, Falls WA, Campeau S, Kim M. Fear-potentiated startle: a neural and pharmacological analysis. Behavioural Brain Research 1993;58:175–98.
[20] Koch M. The neurobiology of startle. Progress in Neurobiology 1999;59:107–28.
[21] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. San Diego: Academic Press; 1998.
[22] Roche M, O'Connor E, Diskin C, Finn DP. The effect of CB(1) receptor antagonism in the right basolateral amygdala on conditioned fear and associated analgesia in rats. European Journal of Neuroscience 2007;26:2643–53.
[23] Azad SC, Monory K, Marsicano G, et al. Circuitry for associative plasticity in the amygdala involves endocannabinoid signaling. Journal of Neuroscience 2004;24:9953–61.
[24] Kim MJ, Loucks RA, Palmer AL, Brown AC, Solomon KM, Marchante AN, et al. The structural and functional connectivity of the amygdala: from normal emotion to pathological anxiety. Behavioural Brain Research 2011;223: 403–10.
[25] Tan H, Lauzon NM, Bishop SF, et al. Integrated cannabinoid CB1 receptor transmission within the amygdala-prefrontal cortical pathway modulates neuronal plasticity and emotional memory encoding. Cerebral Cortex 2010;20:1486–96.
[26] Varvel SA, Anum EA, Lichtman AH. Disruption of CB(1) receptor signaling impairs extinction of spatial memory in mice. Psychopharmacology 2005;179:863–72.
[27] Azad SC, Eder M, Marsicano G, et al. Activation of the cannabinoid receptor type 1 decreases glutamatergic and GABAergic synaptic transmission in the lateral amygdala of the mouse. Learning and Memory 2003;10:116–28.
[28] Lafourcade M, Elezgarai I, Mato S, et al. Molecular components and functions of the endocannabinoid system in mouse prefrontal cortex 1. PLoS ONE 2007;2:e709.
[29] Oropeza VC, Mackie K, Van Bockstaele EJ. Cannabinoid receptors are localized to noradrenergic axon terminals in the rat frontal cortex. Brain Research 2007;1127:36–44.
[30] Tzavara ET, Davis RJ, Perry KW, et al. The CB1 receptor antagonist SR141716A selectively increases monoaminergic neurotransmission in the medial prefrontal cortex: implications for therapeutic actions. British Journal of Pharmacology 2003;138:544–53.
[31] Wilensky AE, Schafe GE, Kristensen MP, LeDoux JE. Rethinking the fear circuit: the central nucleus of the amygdala is required for the acquisition, consolidation, and expression of Pavlovian fear conditioning. Journal of Neuroscience 2006;26:12387–96.
[32] Maren S. Pavlovian fear conditioning as a behavioral assay for hippocampus and amygdala function: cautions and caveats. European Journal of Neuroscience 2008;28:1661–6.
[33] McDonald AJ. Cortical pathways to the mammalian amygdala. Progress in Neurobiology 1998;55:257–332.
[34] Vertes RP. Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. Neuroscience 2006;142:1–20.
[35] Rosenkranz JA, Moore H, Grace AA. The prefrontal cortex regulates lateral amygdala neuronal plasticity and responses to previously conditioned stimuli. Journal of Neuroscience 2003;23:11054–64.
[36] Rosenkranz JA, Grace AA. Cellular mechanisms of infralimbic and prelimbic prefrontal cortical inhibition and dopaminergic modulation of basolateral amygdala neurons in vivo. Journal of Neuroscience 2002;22:324–37.
[37] Pape HC, Pare D. Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. Physiological Reviews 2010;90:419–63.
[38] Sotres-Bayon F, Quirk GJ. Prefrontal control of fear: more than just extinction. Current Opinion in Neurobiology 2010;20:231–5.
[39] Laviolette SR, Lipski WJ, Grace AA. A subpopulation of neurons in the medial prefrontal cortex encodes emotional learning with burst and frequency codes through a dopamine D4 receptor-dependent basolateral amygdala input. Journal of Neuroscience 2005;25:6066–75.
[40] Tan H, Lauzon NM, Bishop SF, et al. Cannabinoid transmission in the basolateral amygdala modulates fear memory formation via functional inputs to the prelimbic cortex. Journal of Neuroscience 2011;31:5300–12.
[41] Davis HP, Squire LR. Protein synthesis and memory: a review. Psychological Bulletin 1984;96:518–59.
[42] Nader K, Schafe GE, Le Doux JE. Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature 2000;406:722–6.
[43] Schafe GE, LeDoux JE. Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. Journal of Neuroscience 2000;20:RC96.
[44] Pistis M, Perra S, Pillolla G, et al. Cannabinoids modulate neuronal firing in the rat basolateral amygdala: evidence for CB1- and non-CB1-mediated actions. Neuropharmacology 2004;46:115–25.
[45] Campolongo P, Roozendaal B, Trezza V, et al. Endocannabinoids in the rat basolateral amygdala enhance memory consolidation and enable glucocorticoid modulation of memory. Proceedings of the National Academy of Sciences of the United States of America 2009;106:4888–93.
[46] Ferraro L, Tomasini MC, Cassano T, et al. Cannabinoid receptor agonist WIN 55,212-2 inhibits rat cortical dialysate gamma-aminobutyric acid levels. Journal of Neuroscience Research 2001;66:298–302.
[47] Ferraro L, Tomasini MC, Gessa GL, et al. The cannabinoid receptor agonist WIN 55,212-2 regulates glutamate transmission in rat cerebral cortex: an in vivo and in vitro study. Cerebral Cortex 2001;11:728–33.
[48] de Oliveira AL, Genro BP, Diehl F, Quillfeldt JA. Differential role of the hippocampal endocannabinoid system in the memory consolidation and retrieval mechanisms. Neurobiology of Learning and Memory 2008;90:1–9.
[49] de Oliveira AL, Genro BP, Vaz BR, et al. AM251, a selective antagonist of the CB1 receptor, inhibits the induction of long-term potentiation and induces retrograde amnesia in rats. Brain Research 2006;1075:60–7.
[50] Clarke JR, Rossato JI, Monteiro S, et al. Posttraining activation of CB1 cannabinoid receptors in the CA1 region of the dorsal hippocampus impairs object recognition long-term memory. Neurobiology of Learning and Memory 2008;90:374–81.
[51] Yim TT, Hong NS, Ejaredar M, et al. Post-training CB1 cannabinoid receptor agonist activation disrupts long-term consolidation of spatial memories in the hippocampus. Neuroscience 2008;151:929–36.
[52] Mackowiak M, Chocyk A, Dudys D, Wedzony K. Activation of CB1 cannabinoid receptors impairs memory consolidation and hippocampal polysialylated neural cell adhesion molecule expression in contextual fear conditioning. Neuroscience 2009;158:1708–16.
[53] Milad MR, Vidal-Gonzalez I, Quirk GJ. Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner. Behavioral Neuroscience 2004;118:389–94.
[54] Niyuhire F, Varvel SA, Martin BR, Lichtman AH. Exposure to marijuana smoke impairs memory retrieval in mice. Journal of Pharmacology and Experimental Therapeutics 2007;322:1067–75.
[55] Egashira N, Mishima K, Iwasaki K, Fujiwara M. Intracerebral microinjections of delta 9-tetrahydrocannabinol: search for the impairment of spatial memory in the eight-arm radial maze in rats. Brain Research 2002;952: 239–45.
[56] Blum S, Hebert AE, Dash PK. A role for the prefrontal cortex in recall of recent and remote memories. Neuroreport 2006;17:341–4.
[57] Sierra-Mercado Jr D, Corcoran KA, Lebron-Milad K, Quirk GJ. Inactivation of the ventromedial prefrontal cortex reduces expression of conditioned fear and impairs subsequent recall of extinction. European Journal of Neuroscience 2006;24:1751–8.
[58] Likhtik E, Pelletier JG, Paz R, Pare D. Prefrontal control of the amygdala. Journal of Neuroscience 2005;25:7429–37.
[59] Vidal-Gonzalez I, Vidal-Gonzalez B, Rauch SL, Quirk GJ. Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. Learning and Memory 2006;13:728–33.

[60] Ehrlich I, Humeau Y, Grenier F, et al. Amygdala inhibitory circuits and the control of fear memory. Neuron 2009;62:757–71.
[61] Quirk GJ, Mueller D. Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology 2008;33:56–72.
[62] Myers KM, Davis M. Mechanisms of fear extinction. Molecular Psychiatry 2007;12:120–50.
[63] Cannich A, Wotjak CT, Kamprath K, et al. CB1 cannabinoid receptors modulate kinase and phosphatase activity during extinction of conditioned fear in mice. Learning and Memory 2004;11:625–32.
[64] Anglada-Figueroa D, Quirk GJ. Lesions of the basal amygdala block expression of conditioned fear but not extinction. Journal of Neuroscience 2005;25:9680–5.
[65] Sotres-Bayon F, Bush DE, LeDoux JE. Emotional perseveration: an update on prefrontal-amygdala interactions in fear extinction. Learning and Memory 2004;11:525–35.
[66] Ganon-Elazar E, Akirav I. Cannabinoid receptor activation in the basolateral amygdala blocks the effects of stress on the conditioning and extinction of inhibitory avoidance. Journal of Neuroscience 2009;29:11078–88.
[67] Burgos-Robles A, Vidal-Gonzalez I, Santini E, Quirk GJ. Consolidation of fear extinction requires NMDA receptor-dependent bursting in the ventromedial prefrontal cortex. Neuron 2007;53:871–80.
[68] Hugues S, Deschaux O, Garcia R. Postextinction infusion of a mitogen-activated protein kinase inhibitor into the medial prefrontal cortex impairs memory of the extinction of conditioned fear. Learning and Memory 2004;11:540–3.
[69] Laurent V, Westbrook RF. Inactivation of the infralimbic but not the prelimbic cortex impairs consolidation and retrieval of fear extinction. Learning and Memory 2009;16:520–9.
[70] Quirk GJ, Russo GK, Barron JL, Lebron K. The role of ventromedial prefrontal cortex in the recovery of extinguished fear. Journal of Neuroscience 2000;20:6225–31.
[71] Herry C, Ferraguti F, Singewald N, et al. Neuronal circuits of fear extinction. European Journal of Neuroscience 2010;31:599–612.
[72] Kamprath K, Marsicano G, Tang J, et al. Cannabinoid CB1 receptor mediates fear extinction via habituation-like processes. Journal of Neuroscience 2006;26:6677–86.
[73] Kamprath K, Wotjak CT. Nonassociative learning processes determine expression and extinction of conditioned fear in mice. Learning and Memory 2004;11:770–86.
[74] Lin HC, Mao SC, Chen PS, Gean PW. Chronic cannabinoid administration in vivo compromises extinction of fear memory. Learning and Memory 2008;15:876–84.
[75] Suzuki A, Josselyn SA, Frankland PW, et al. Memory reconsolidation and extinction have distinct temporal and biochemical signatures. Journal of Neuroscience 2004;24:4787–95.
[76] Riebe CJ, Pamplona F, Kamprath K, Wotjak CT. Fear relief-toward a new conceptual frame work and what endocannabinoids gotta do with it. Neuroscience 2012;204:159–85.
[77] Pearce JM. A model for stimulus generalization in Pavlovian conditioning. Psychological Review 1987;94:61–73.
[78] Watson JB, Rayner R. Conditioned emotional reactions. 1920. American Psychologist 2000;55:313–7.
[79] Rubino T, Guidali C, Vigano D, et al. CB1 receptor stimulation in specific brain areas differently modulate anxiety-related behavior. Neuropharmacology 2008;54:151–60.
[80] Rubino T, Realini N, Castiglioni C, et al. Role in anxiety behavior of the endocannabinoid system in the prefrontal cortex. Cerebral Cortex 2008;18:1292–301.
[81] Fogaca MV, Aquiar DC, Moreira FA, Guimaraes FS. The endocannabinoid and endovanilloid systems interact in the rat prelimbic medial prefrontal cortex to control anxiety-like behavior. Neuropharmacology 2012;63:202–10.
[82] Hill M, McLaughlin RJ, Pan B, et al. Recruitment of prefrontal cortical endocannabinoid signaling by glucocorticoids contributes to termination of the stress response. Journal of Neuroscience 2011;31:10506–15.