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ORIGINAL INVESTIGATION

Effects of the fatty acid amide hydrolase inhibitor URB597 on coping behavior under challenging conditions in mice

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Abstract
Rationale Recent evidence suggests that in addition to controlling emotional behavior in general, endocannabinoid signaling is engaged in shaping behavioral responses to challenges. This important function of endocannabinoids is still poorly understood.
Objectives Here we investigated the impact of blockade of fatty acid amide hydrolase (FAAH), the degrading enzyme of anandamide on behavioral responses induced by challenges of different intensity.
Methods Mice treated with FAAH inhibitor URB597 were either manually restrained on their backs (back test) or received foot-shocks.
Results The behavior of mice showed bimodal distribution in the back test: they either predominantly showed escape attempts or equally distributed time between passivity and escape. URB597 increased escapes in animals with low escape scores. No effects were noticed in mice showing high escape scores, which is likely due to a ceiling effect. We hypothesized that stronger stressors would wash out individual differences in coping; therefore, we exposed mice to foot-shocks that decreased locomotion and increased freezing in all mice. URB597 ameliorated both responses. The re-exposure of mice to the shock cage 14 days later without delivering shocks or treatment was followed by reduced and fragmented sleep as shown by electrophysiological recordings. Surprisingly, sleep was more disturbed after the reminder than after shocks in rats receiving vehicle before foot-shocks. These reminder-induced disturbances were abolished by URB597 administered before shocks.
Conclusions These findings suggest that FAAH blockade has an important role in the selection of behavioral responses under challenging conditions and—judging from its long-term effects—that it influences the cognitive appraisal of the challenge.

Keywords Back test · Conditioned fear · Coping · FAAH · Inescapable foot shock · Mouse · URB597

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Introduction

Active coping is a therapeutic goal in a variety of physical and mental diseases (Cooke et al. 2007; Westerhuis et al. 2011). A growing body of evidence suggests that the endocannabinoid system—in addition to its effects on anxiety-like and depression-like behavior—affects the way animals cope with challenges. Early work with knockout animals already showed that the effects of cannabinoid type-1 receptor (CB1) gene disruption depend on environmental conditions in the elevated plus-maze (Haller et al. 2004). Particularly, the behavior of CB1 knock-out (CB1 KO) and wild-type mice was similar under low light—i.e., less aversive conditions—but CB1 gene disruption increased anxiety-like behavior under the more aversive high light condition. Thus, the anxiogenic-like effect of CB1 gene disruption was restricted to the more stressful unfamiliar environment, suggesting that disrupted endocannabinoid signaling exacerbates anxiety under challenging conditions.

Recent work with the fatty acid amide hydrolase (FAAH) inhibitor URB597—which increases the brain availability of the endocannabinoid anandamide—supports the notion that the effects of endocannabinoids depend on environmental conditions; particularly, behavioral effects become more prominent under conditions of environmental aversiveness. The first indication came from a study by Naidu et al.

(2007), who showed that the compound decreased depression- and anxiety-like behaviors under specific conditions only, more precisely under conditions that have been perceived as stressful for subjects. By studying the issue further, Haller et al. (2009) showed that FAAH inhibition by URB597 decreases anxiety by blunting the behavioral impact of aversive conditions; the aversiveness of the testing environment was evidenced by glucocorticoid measurements in this study. Similar findings were obtained with the monoacylglycerol lipase inhibitor JZL184 that enhances signaling by 2-arachidonoylglycerol and another endogenous CB1 agonist by inhibiting its hydrolyzing enzyme (Aliczki et al. 2012; Sciolino et al. 2011). These findings suggest that in contrast to CB1 gene disruption that exacerbates behavioral responses to challenging conditions, increased endocannabinoid signaling dampens the effects of such challenging conditions. In a recent study, McLaughlin et al. (2012) showed that URB597 promotes active coping in the forced swimming test, which also supports the notion that endocannabinoids are involved in coping with environmental challenges.

To further assess the role of endocannabinoid signaling in challenge responding, we studied here the effects of the FAAH inhibitor URB597 in the back test where subjects are manually restrained on their backs for 1 min, and escape attempts from this forced unnatural position are considered as active coping responses (Hawley et al. 2010; Lambert et al. 2006; Ruis et al. 2001). The compound increased escape attempts in subjects that showed low, but not in those showing high, escape scores at baseline. To control for this putative ceiling effect, a different set of mice was exposed to unavoidable foot-shocks that result in freezing, i.e., in passive challenge responding in all undrugged mice. We hypothesized that URB597 would diminish inactivity induced by shocks in all mice, i.e., it would promote active coping with the challenge. Based on earlier data on the relationship between active coping and resilience under adverse conditions (Koolhaas et al. 1999, 2007), we also hypothesized that URB597 would reduce the long-term impact of shocks on emotional responses triggered by contextual reminders. The reminder was administered to the same subjects 14 days later and performed without the administration of foot-shocks or pharmacological treatments. Emotional responses were evaluated by freezing responses shown during and sleep patterns shown after the contextual reminder.

Materials and methods

Subjects

Subjects were 2–3-month-old male CD1 mice (Charles River Laboratories; Hungary) weighing approximately 30 g. Food and water were available ad libitum; temperature and relative humidity were kept at 22±2 °C and 60±10 %, respectively. Mice were maintained in a normal light cycle of 12 h with lights on at 07:00 h. In contrast to rats that are highly social, individual housing is not stressful in the mouse, which is a solitary species (Arndt et al. 2009; Benton and Brain 1981; Capanna et al. 1984). Moreover, mice establish strong dominance hierarchies (Capanna et al. 1984; Poshivalov 1980), which would have constituted a confounding factor in this experimental design if the mice were housed in groups. Therefore, all mice were housed individually for 2 weeks before experimentation. Mice were experimentally naïve with no drug history prior to the experiment and used in one experiment only.

Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were reviewed and approved by the Animal Welfare Committee of our Institute.

Drugs and doses

URB597 (Sigma, Budapest, Hungary) was dissolved in 0.2 ml dimethylsulfoxide, which was diluted to a final volume with saline that contained 0.4 % methylcellulose, a biologically neutral solvent, and was injected intraperitoneally at a dose of 0.3 mg/kg and a volume of 10 ml/kg 40 min before testing. At this time point, 0.3 mg/kg URB597 has a maximal effect on brain anandamide, which is increased about threefold over basal levels (Kathuria et al. 2003; Piomelli et al. 2006).

Behavioral testing

All behavioral tests were conducted in the first 3 h of the light period in an experimental room at 400-lx light intensity. Behavior during testing was recorded with a Sony DCR-SR75 digital camcorder (Sony Electronics, San Diego, CA, USA). Later, behavioral recordings were analyzed with the H77 event recorder software (Jozsef Haller, Budapest, Hungary).

Back test The back test was developed in piglets (Ruis et al. 2001) and was later adapted to rats to evaluate coping with a challenging situation, e.g., a forced unnatural position (Hawley et al. 2010; Lambert et al. 2006). Subjects are manually restrained on their backs for 1 min, and escape attempts are considered indicative of active coping. This behavior shows a biphasic distribution: the behavior of subjects is either dominated by escape attempts or by more passive responses, e.g., resting. Consequently, subjects with values close to the group mean were less frequent than "extremes" (passive and active coping), i.e., overall averages describe behavior poorly in this test. Therefore, subjects are usually divided into active and passive copers, who are evaluated separately (Hawley et al. 2010; Lambert et al. 2006; Koolhaas et al. 1999, 2007; Ruis et al. 2001). The behavior of the two phenotypes is rather consistent over time. We employed a repeated-measures design

here to evaluate the impact of URB597 in conjunction with the pre-existing phenotype of mice (see "Experimental design").

As the test was not employed in mice so far, we performed a preliminary study in 18 mice to identify the behaviors shown in this test and to establish whether the test was repeatable without major changes in behavior. Mice were exposed to the test three times at 7-day intervals. We identified four distinct behaviors: *resting* (no movements except for breathing and small head movements), *escape attempts* (vigorous body and limb movements aimed at escaping restraint), *body twitches*, and *bites* directed towards the hand of the experimenter. Regarding bites, we note that the experimenter wore thick gloves over the latex examination gloves, which prevented pain and injuries from bites; therefore, the manual restraint was consistent and had no impact on the behavior of subjects. Twitches and bites were very short (< 1 s); consequently, mice divided test time between escape attempts and resting. Overall, time devoted to escape was longer than that devoted to resting. Similar to piglets and rats, individual differences were large and allowed the identification of distinct coping styles based on escape/resting ratios (see later discussion). The duration of resting and escape attempts was consistent over time (Spearman correlation coefficients for behavior shown in the three trials were between 0.627 and 0.697, while p values were between 0.005 and 0.001). As expected, a bimodal distribution of coping styles was seen in this preliminary study; only 17.6 % of subjects showed a mixed strategy; the rest were either more passive or more active. This preliminary study showed that the behavior of mice is well characterized by the duration of resting and escape attempts and that the repeated-measures approach is applicable.

Unavoidable foot-shocks and the contextual reminder Unavoidable foot-shocks are dramatic stressors that—in contrast to forcing mice on their backs—do not differentiate coping styles because all subjects show a robust inhibition of behavior throughout shock exposure. In addition, behavior is altered in the long run by a single exposure—a phenomenon known as conditioned fear—which per se precludes repeatability. Therefore, foot-shocks were administered once, and the effects of URB597 were evaluated in groups that were studied in parallel. The test was employed to study the impact of this compound on coping with a severe stressor and to evaluate the long-term consequences of both the shock per se and URB597 treatment administered before shocks.

Shocks were delivered and contextual reminders were administered in a separate, quiet experimental room. On the first day, mice were introduced into the Plexiglas box (30×30×30 cm). Shocks were administered via the grid floor of the box. Two shock trains of 1 s were administered per minute for 5 min (i.e., each mouse received ten shocks). Each shock train (100 V, 3 mA) was 1 s in length and consisted of 0.01-s shocks separated by 0.02-s-long breaks. The box was cleaned with ethanol between each subject.

Behavior was recorded by a video camera placed 1.5 m above the Plexiglas box. For behavioral scoring, the box where mice received shocks was covered by a 10×10-cm grid on the video screen. Locomotion was evaluated by counting the lines crossed by animals by all four paws. Runs prompted by shocks per se and locomotion between shocks was counted separately. Exploration directed in the air or the walls (upward search) and those directed below the metallic grid (downward search) were counted separately as well. Freezing (complete immobility, no movements of the snout) was also scored.

Surgery and EEG/EMG recordings

We instrumented the subjects with EEG and EMG electrodes as described previously (Kantor et al. 2009). Briefly, under anesthesia with ketamine–xylazine (100 and 10 mg/kg ip), stainless steel screw electrodes were implanted epidurally over the left frontal cortex (1.5 mm lateral and 1 mm anterior to Bregma) and left parietal cortex (1.5 mm lateral and 1.0 mm anterior to Lambda) for fronto-parietal EEG recordings. The ground electrode was placed over the cerebellum. EMG signals were acquired by a pair of stainless steel spring wires (Plastics One Inc., Roanoke, VA, USA) inserted into the neck extensor muscles.

The recording cable was attached to a low torque commutator, fixed above the cages that allowed free movement. The EEG/EMG signals were acquired using MultiAmp amplifiers (Supertech Ltd., Pecs, Hungary), analog band pass-filtered at 0.53–80 Hz, and digitized at 256 Hz using a sleep scoring system (SleepSign, Kissei Comtec, Matsumoto, Japan). The signals were then digitally filtered (EEG, 0.5–60 Hz; EMG, 5–60 Hz) and semiautomatically scored in 10-s epochs. K.S. and S.K. visually inspected all scoring and made corrections when appropriate. To examine the changes in sleep architecture of shock-trained mice, sleep amount was recorded and calculated every 30 min in the first 210 min that followed the shock or the contextual reminder. Sleep amount was expressed as % of these 30-min-long periods. The number of awakenings was also counted and was expressed as the number of awakenings per minute of sleep. This variable was referred to as sleep fragmentation.

Experimental design

In experiment 1, we studied the effects of URB597 on coping styles in the back test. Mice were tested three times at 7-day intervals after treatments that were administered 40 min prior to testing (N=40). The first trial preceded by vehicle injections was considered habituation to treatment and testing procedure. The coping style of mice was established based on behavior shown during the second trial. All mice received vehicle before this trial. Overall, the duration of escape

attempts was larger than the duration of resting, confirming that the stressor was mild and did not induce a general inhibition of behavior. Mice were categorized as passive copers if the escape/resting ratio was smaller than 1.5. In these mice, the duration of escape attempts was larger than the duration of resting by 33 % or less. Mice were assigned to the active coping group if the escape/resting ratio was larger than 3. In these mice, the duration of escape attempts was larger than the duration of resting by 66 % or more. Mice showing intermediate scores were considered mixed copers. Mice were treated either with URB597 or with saline before the third trial. Assignment was based on behavior shown in the second trial to ensure similar shares of coping styles within treatment groups.

In experiment 2, we studied the behavioral and emotional responses elicited by unavoidable foot-shocks and a contextual reminder administered 14 days later. Mice were instrumented with EEG and EMG electrodes as described in "Surgery and EEG/EMG recordings" and were allowed 1 week to recover. They were habituated to the cables for 4–5 days before the experiments. On the day when shocks were administered, mice were connected to the recording cables at 7 AM. Baseline values for sleep patterns were obtained between 8 and 9 AM. At 20 min later, mice were treated with vehicle or URB597, and after another 40 min they were disconnected from the recording cables and were transferred to a quiet room (separated from the housing room) where foot-shocks were delivered. Behavior shown during shock exposure was video-recorded and analyzed later by an experimenter blind to treatments. Recordings were restarted in the home-cage immediately after shock delivery and lasted 210 min.

The contextual reminder was administered 14 days after shock exposure. As during the shock day, mice were connected to the recording cables at 7 AM, and baseline sleep patterns were established between 8 and 9 AM. Mice were transferred to the shock-associated Plexiglas boxes at 10 AM. Neither pharmacological treatment nor shocks were administered. Behavior shown in the shock-associated environment was video-recorded and was later analyzed by an experimenter blind to the treatments. Note that treatments were administered 14 days earlier during shock exposure. Freezing—a typical conditioned fear-related behavior—was scored. EEG recordings were resumed in the home-cage immediately after the contextual reminder and lasted 210 min.

Statistical analyses

The back test Correlations were estimated by linear regression (predictor variable: behavior during trial 2; dependent variable: behavior during trial 3). Behavioral differences were evaluated by three-factor ANOVA (factor 1: treatment; factor 2: coping style; repeated-measures factor 3: trial). As the interaction between these three factors was significant, separate two-factor ANOVAs were run for the treatment groups (factor 1: coping; factor 2: trial).

Foot-shocks and reminder Behaviors typical to shock exposure (e.g., "shock-runs" and behaviors shown between shock presentations) were evaluated by two-factor ANOVA (factor 1: treatment; repeated-measures factor 2: time, i.e., the sequential number of shocks or pauses between shocks). Sleep patterns were compared by two-factor ANOVA. Factor 1 was the experimental condition that had four levels: (1) vehicle-treated before shock, measured after shock; (2) vehicle-treated before shock, measured after reminder; (3) URB597-treated before shock, measured after shock; and (4) URB597-treated before shock, measured after reminder. Post-shock and post-reminder measurements were made in the same animals. Repeated-measures factor 2 was the time elapsed from shock or reminder.

Where the main effect was significant, post-hoc Fisher's LSD tests were performed for pair-wise comparisons. Significance level was set at $p<0.05$. P values underwent Bonferoni correction for multiple comparisons (Holm's procedure).

Results

The effects of URB597 on escape attempts in the back test

As the duration of resting and escape attempts was mutually exclusive (the other two behaviors being very short; see earlier discussion), only data on escape attempts were shown here. URB597 did not affect the duration of escape attempts overall. However, the correlation between behaviors shown in trials 2 and 3 showed interesting differences between the treatment groups. The correlation between the two trials was significant in mice treated with vehicle before both trials ($R=0.479$; $p=0.03$) (Fig. 1a). These findings replicated those obtained in the preliminary study (see "Materials and methods"). There was no correlation between behaviors shown in the two trials when trial 3 was preceded by URB597 injections ($R=0.128$; $p=0.59$) (Fig. 1b). Particularly, mice showing low escape scores after vehicle spent considerably more time with escape attempts after URB597. The most active animals showed escape attempts for most of the time; as such, the duration of this behavior was likely unable to show further increases due to a ceiling effect. To control for the impact of baseline behavior, data were reanalyzed by taking coping styles into account. These were delimited as described in "Materials and methods". Coping styles showed a bimodal distribution as expected based on earlier findings. Only 26.3 % of subjects showed a mixed strategy; the rest adopted either more passive (39.5 %) or more active coping (34.2 %). The behavior of mice was determined by an interaction between treatment (factor 1), coping (factor 2), and trial (repeated-measures factor 3) (Wilk's lambda=0.174;

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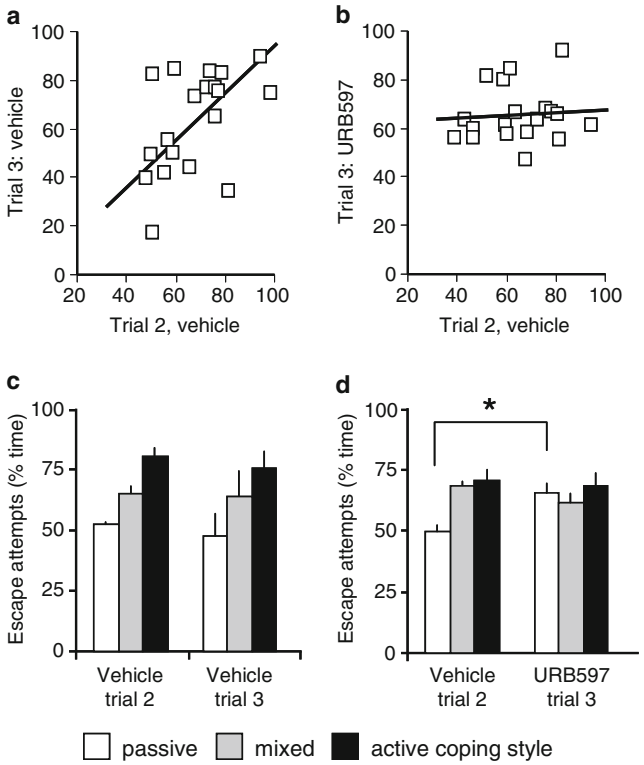

Fig. 1 The impact of URB597 on behavior shown in the back test. Note that the first trial served to habituate mice to injections and procedure. **a** Correlation between behaviors shown during the second and third trial, when both were preceded by vehicle treatment; **b** correlation between behaviors in mice which received URB597 before the third trial; **c** coping style-dependent duration of escape attempts in mice treated with vehicle two times; **d** coping style-dependent duration of escape attempts in mice treated with URB597 before the third trial. *Asterisk* significant effect of URB597 on the duration of escape attempts in passive copers ($p=0.02$)

$F_{interaction}(4, 66)=22.97$; $p<0.0001$). In a second analysis (permitted by the significant interaction between the three factors), we investigated behavioral changes within treatment groups. In subjects treated with vehicle before both trials, behavior was affected by coping styles but not by the trial ($F_{coping}(2, 32)=9.41$, $p=0.0006$; $F_{trial}(1, 32)=0.75$, $p=0.39$; $F_{interaction}(2, 32)=0.66$, $p=0.52$). The duration of escape attempts was similar in the two trials for all three coping styles ($p>0.5$) (Fig. 1c). In contrast, in mice treated with URB597 before trial 3, behavior was defined by an interaction between coping and trial ($F_{interaction}$ $(2, 32)=8.63$, $p=0.001$). Particularly, mice that adopted a passive style in the vehicle trial shifted towards a more active style after URB597 ($p=0.02$) (Fig. 1d). No similar changes were seen in mice adopting mixed or active styles in trial 2. In addition, the passive/active difference evident after vehicle ($p=0.0001$) disappeared after URB597 ($p=0.9$).

Taken together, these findings suggest that URB597 promoted an active coping style in mice that showed passive coping at baseline. One can hypothesize that a similar change was prevented in the other two coping groups by a ceiling effect.

Behavioral responses to unavoidable foot-shocks

In experiment 2, each shock elicited runs of about 50 cm (five line crossings) that were not affected by URB597 treatment ($F_{treatment}$ $(1, 12)=0.15$; $p<0.2$) (Fig. 2a). This suggests that pain sensitivity was not affected by URB597. However, FAAH inhibition significantly affected the behavior observed between shock presentations. Locomotion shown during the 30-s breaks that separated shocks was gradually reduced from about 10 cm to almost nil in vehicle-treated mice, but not in mice treated with the FAAH inhibitor. There were two waves of locomotion bouts in URB597-treated mice, resulting in an overall increase in locomotion ($F_{treatment}$ $(1, 12)=7.07$; $p<0.03$) (Fig. 2b). In parallel with this change, freezing—a fear-like behavior in this test—was reduced by FAAH inhibition ($F_{treatment}$ $(1, 12)=5.32$; $p<0.05$) (Fig. 2c). Interestingly, the direction of exploration was also changed. Particularly, URB597-treated mice increased the exploration of the space between and beneath the metallic grid by which shocks were delivered ($F_{treatment}$ $(1, 12)=4.77$; $p<0.05$) (Fig. 2d). Exploration directed towards the air ("upward search") was not affected (data not shown).

Taken together, these findings show that electric shocks markedly reduced behavioral activity, and URB597 ameliorated this behavioral inhibition.

Sleep in response to shocks and reminder

Sleep amount and sleep fragmentation were presented in Tables 1 and 2, respectively. The most interesting differences occurred between the shock day and the reminder. Figure 3 was compiled to clearly show these differences. Noteworthy, statistics was done on the raw data. Figure 3 was presented for clarity only.

Sleep amount was affected by an interaction between the condition (treatment administered before shocks and the situation, i.e., shock or reminder) and the time elapsed from exposure to shocks or reminder ($F_{condition*time}$ $(21, 168)=1.98$; $p<0.01$). Surprisingly, the impact of shocks on sleep amount was not particularly strong. During the 30 min that followed shock exposure, 20–30 % of time was already spent sleeping, and pre-shock levels of 80 % were resumed within about 90 min. This was true for both vehicle- and URB597-treated mice (Table 1). In contrast, sleep was considerably more reduced in control mice after the contextual reminder, although no shocks were delivered this time (Table 1 and Fig. 3a). At the end of the investigation period, control mice slept significantly more after the reminder than after the shock, a possible rebound effect. No similar changes were noticed in the URB597 group, where sleep amount was similar after shocks and reminder (Table 1; Fig. 3b).

Shock exposure resulted in fragmented sleep that was affected by an interaction between condition and time after shock or reminder ($F_{condition*time}$ $(21, 112)=2.03$; $p=0.0097$).

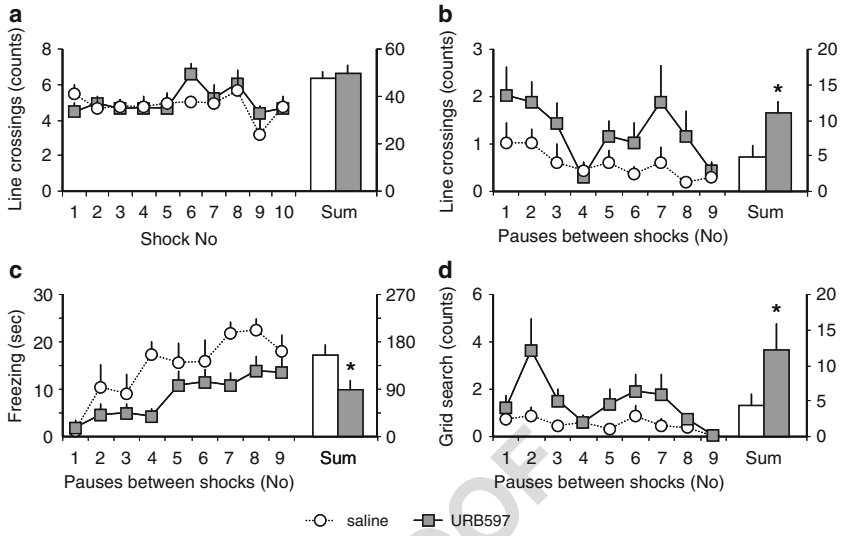

Fig. 2 Behavioral responses to unavoidable foot-shocks in vehicle and URB597-treated mice. **a** "Shock-runs" (rapid locomotion directly elicited by shocks); **b** locomotion between shock presentations (during the 30-s-long brakes that separated shocks); **c** freezing; **d** the exploration of the space between and beneath the metallic grid by which shocks were delivered. *Asterisk* significant effect of URB597 ($p<0.05$ at least)

The period of fragmented sleep covered the whole post-shock period albeit its magnitude was gradually reduced (Table 2). Surprisingly, the contextual reminder administered 14 days later increased sleep fragmentation immediately following the reminder as compared to the same period that followed shock exposure (Table 2; Fig. 3c). In contrast, mice submitted to shocks concurrently with FAAH inhibition did not show this increment in sleep fragmentation when exposed to the reminder. Moreover, sleep fragmentation after the reminder was ameliorated in this group as compared to the shock condition (Table 2; Fig. 3d).

Taken together, the findings suggest that the URB597-induced increase in active coping with shocks had a long-term emotional impact as shown by the sleep patterns.

Freezing during the contextual reminder and correlations

Mice readily showed freezing when re-exposed to the cage where they received electric shocks earlier (Fig. 3e). This behavior was marginally affected by URB597 treatments received before shock application, i.e., 14 days earlier (F (1, 11)=3.37; $0.1>p>0.05$). The marginally significant difference and the distribution of individual freezing score suggest that freezing may have been decreased in some mice, but this was not a general phenomenon. The correlation between freezing scores shown during and sleep variables shown immediately after shocks and reminder (first 30 min after the events) was not significant (*sleep amount*: $R=0.257$; $p=0.18$; *sleep fragmentation*: $R=-0.014$; $p=0.95$). In contrast, sleep amount and sleep fragmentation showed a strong negative correlation ($R=-0.782$; $p=0.00005$). This suggests that behavior shown during exposure is not significantly correlated with sleep patterns that follow exposure. Thus, the immediate response to the stressful event and its subsequent emotional impact are independent measures.

Discussion

Main findings

Overall, the behavior of mice was dominated by escape attempts in the back test, i.e., by an active coping strategy. However, large inter-individual variation was noticed, which allowed the identification of coping styles. As expected, these strategies were stable over time. Foot-shocks dramatically reduced behavioral activity in all undrugged mice, indicating

Table 1 Sleep amount after shocks and reminder in mice treated with vehicle and URB597 before shocks

Group	Period	BL	0–30	30–60	60–90	90–120	120–150	150–180	180–210
Vehicle	Shock	73.47±5.51	31.67±6.72	59.74±4.56	72.40±5.72	72.30±7.68	76.03±4.65	82.14±7.30	77.71±4.88
	Reminder	77.90±7.11	2.47±1.56	38.57±9.10	60.39±6.78	76.03±5.56	85.80±3.74	91.20±3.45	92.54±1.97
URB597	Shock	76.14±3.49	20.71±9.02	57.39±2.71	70.71±3.91	69.69±5.33	79.86±5.16	72.53±6.95	81.03±4.52
	Reminder	83.97±4.57	13.66±7.83	36.17±10.98	67.14±11.59	79.11±5.96	86.26±6.08	84.13±6.70	74.34±8.73

Figures in column headings indicate the starting and ending points of the periods analyzed (min). For ANOVA analysis, see text; for pairwise comparisons, see Fig. 3a, b

BL baseline

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t2.1 **Table 2** The impact of URB597 administered before shocks on the number of awakenings per minute of sleep following shock exposure and the contextual reminder

t2.2 Group	Period	BL	0–30	30–60	60–90	90–120	120–150	150–180	180–210
t2.3 Vehicle	Shock	0.20±0.02	1.40±0.39	1.27±0.24	0.99±0.19	0.71±0.23	0.60±0.19	0.41±0.12	0.44±0.08
t2.4	Reminder	0.12±0.02	3.75±1.18	1.56±0.32	1.00±0.25	0.59±0.12	0.36±0.09	0.24±0.07	0.19±0.04
t2.5 URB597	Shock	0.18±0.01	2.30±0.54	1.20±0.11	0.89±0.10	0.82±0.13	0.62±0.06	0.64±0.11	0.52±0.07
t2.6	Reminder	0.11±0.03	2.28±1.12	1.06±0.20	0.89±0.34	0.36±0.13	0.22±0.05	0.22±0.05	0.21±0.04

Boundaries in column headings indicate the starting and ending points of the periods analyzed; values represent minutes elapsed from the shock or the reminder. For ANOVA analysis, see text; for pairwise comparisons, see Fig. 3c, d

BL baseline

that they adopted a passive coping strategy when faced with this strong challenge. URB597 promoted active challenge responding in both tests. In the back test, this effect was restricted to mice characterized by a passive coping style. During foot-shocks, URB597 considerably ameliorated shock-induced behavioral passivity in all mice. Interestingly, behavioral activation was paralleled by increased grid search. Albeit findings related to the direction of exploration are difficult to interpret, an increased grid search suggests either a search for escape routes or an attempt to identify the source of the pain. As such, this behavior may reflect a more problem-oriented behavior. In line with earlier findings (Jha et al. 2005; Sanford et al. 2003), both shock exposure and the reminder were followed by a period of reduced and fragmented sleep. Surprisingly, larger sleep disturbances were noticed after the reminder than after shocks. URB597 administered before shocks had minor effects on shock-induced sleep deficits but abolished the increment of such deficits after the reminder.

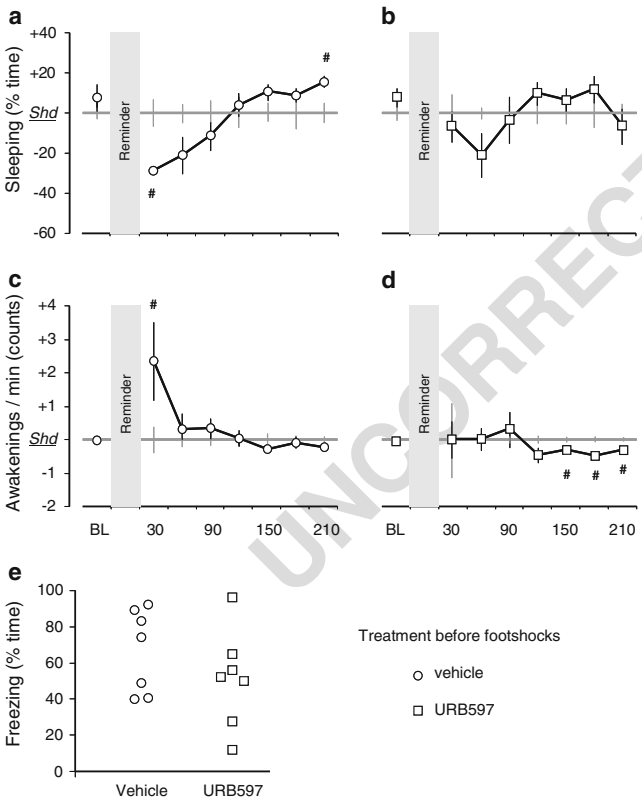

Fig. 3 The effects of URB597 on sleep following shocks and contextual reminders. Data show differences between the shock day and the reminder. The compound was administered before shocks only. For raw data, see Tables 1 and 2. **a** Sleep amount in mice treated with vehicle before shocks; **b** sleep amount in mice treated with URB597 before shocks; **c** fragmented sleep (awakenings/minute of sleep) in mice treated with vehicle before shocks; **d** fragmented sleep (awakenings/minute of sleep) in mice treated with URB597 before shocks; **e** the duration of freezing during the reminder (individual values were shown). *Thick horizontal gray lines* indicate the reference values of the shock day; *thin vertical gray lines* indicate the standard error of reference values. *BL* baseline, *number symbol* significant difference between shock and reminder ($p > 0.05$)

Taken together, these findings show that URB597 promoted active coping when mice were exposed to challenging situations; moreover, active coping shown during challenge exposure reduced the long-term emotional impact of the challenge.

Earlier studies suggested that the impact of URB597 on behavioral responses triggered by environmental adversity is mediated by the CB1 receptor (Haller et al. 2009). Nevertheless, certain effects of endocannabinoids are mediated by receptors other than the CB1, e.g., by the vanilloid receptor type-1 and the poorly known cannabinoid receptor type-3 and receptors (Almeida-Santos et al. 2013; Hajos et al. 2001; Haller et al. 2002; Marinelli et al. 2007; Ryberg et al. 2007). In addition, URB597 blocks the degradation of several lipid mediators, e.g., anandamide, oleoylethanolamide, and palmitoylethanolamide (Fegley et al. 2005; Piomelli et al. 2006), and the latter two may exert their effects via peroxisome proliferator-activated receptor alpha (Mazzola et al. 2009). Therefore, the mechanisms by which URB597 affects stress coping need further studies.

Comparison with earlier findings

The role of endocannabinoids in stress coping was recently substantiated by several studies, e.g., it was shown that striatal anandamide participates in the emotional arousal resulting from a non-familiar social encounter (Trezza et al. 2012;

Gururajan et al. 2012) and is particularly important for coping responses to novel social contexts in terms of the time spent with social investigation (Marco et al. 2011). It was also shown that FAAH inhibition promotes active responses (e.g., swimming) and inhibits passive responses (e.g., immobility) in the forced swimming test (McLaughlin et al. 2012; Realini et al. 2011). Cognitive flexibility—an important characteristic of coping styles (Koolhaas et al. 1999, 2007)—was markedly inhibited by URB597 in another study (Sokolic et al. 2011). Direct effects on cannabinoid receptors—e.g., Δ^9-tetrahydrocannabinol treatments—also facilitated a switch from passive to active behavior in fear conditioning (Metna-Laurent et al. 2012).

Taken together, these findings substantiate the view that the endocannabinoid anandamide has an important role in coping with challenges. Particularly, anandamide signaling appears to promote active coping. This conclusion was supported here by studies involving two tests that pose challenges of different intensities. A coping strategy-dependent effect was noticed in the back test, which did not seem to be particularly challenging for mice. A more general effect was seen during foot-shocks, a very strong stressor that washed out differences between baseline coping styles.

We believe that the most interesting finding of this study is that active coping with a dramatic stressor has long-term consequences for the emotional impact of contextual reminders. It is highly unlikely that the pharmacological effects of a single URB597 treatment lasted 14 days (Piomelli et al. 2006). Nevertheless, active coping with a critical situation—prompted here by URB597—may have affected "subjective" feelings associated with that particular event, which may have ameliorated emotional responses elicited by its re-experiencing.

Theoretical implications

Although data point to the involvement of endocannabinoid signaling in the control of emotional behavior, the pharmacological evidence seems rather inconsistent (see, for reviews, Viveros et al. 2005; Witkin et al. 2005; Zanettini et al. 2011). One possible reason for such discrepancies is the role of cannabinoid signaling in coping with environmental adversity as shown here and in a number of recent publications (Haller et al. 2009, 2013; Marco et al. 2011; McLaughlin et al. 2012; Metna-Laurent et al. 2012; Naidu et al. 2007; Realini et al. 2011; Sciolino et al. 2011). Taken together, these publications suggest that endocannabinoid signaling decreases the emotional impact of aversive conditions and promotes active coping with adversities. In addition to anxiety- and depression-like behaviors, the effects of endocannabinoid signaling on learning may also be affected by this mechanism as the learning-related effects of endocannabinoid signaling also depend on the aversiveness of testing conditions (Abush and Akirav 2010; Campolongo et al. 2012, 2013). Taken together, these findings suggest that studying the effects of cannabinoids in conjunction with environmental conditions is a promising approach and may lead to a better understanding of the roles played by endocannabinoids in behavior. On a more general level, these findings—if supported by subsequent studies—may open new therapeutic windows for agents that increase endocannabinoid signaling. Active coping is a therapeutic goal in a variety of physical and mental diseases (Cooke et al. 2007; Westerhuis et al. 2011). Potentially, such goals may be achieved by enhancing anandamide signaling.

Conclusions

In behavioral terms, URB597 promoted active coping with two stressful situations, namely, a forced unnatural position and foot-shocks. The URB597-induced increase in active coping reduced the long-term emotional impact of contextual reminders. Taken together, these findings suggest that endocannabinoid signaling has an important role in the selection of behavioral responses under challenging conditions and—judging from its long-term effects—that it influences the cognitive appraisal of the challenge. These findings warrant further studies into the role of endocannabinoids in coping with challenges.

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