

Miquel Martin · Catherine Ledent · Marc Parmentier
Rafael Maldonado · Olga Valverde

Involvement of CB1 cannabinoid receptors in emotional behaviour

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Abstract *Rationale:* Endogenous and exogenous cannabinoids acting through the CB1 cannabinoid receptors are implicated in the control of a variety of behavioural and neuroendocrine functions, including emotional responses, and learning and memory processes. Recently, knockout mice deficient in the CB1 cannabinoid receptor have been generated, and these animals result in an excellent tool to evaluate the neurophysiology of the endogenous cannabinoid system. *Objectives:* To establish the role of the CB1 cannabinoid receptor in several emotional-related behavioural responses, including aggressiveness, anxiety, depression and learning models, using CB1 knockout mice. *Methods:* We evaluated the spontaneous responses of CB1 knockout mice and wild-type controls under different behavioural paradigms, including the light/dark box, the chronic unpredictable mild stress, the resident–intruder test and the active avoidance paradigm. *Results:* Our findings showed that CB1 knockout mice presented an increase in the aggressive response measured in the resident–intruder test and an anxiogenic-like response in the light/dark box. Furthermore, a higher sensitivity to exhibit depressive-like responses in the chronic unpredictable mild stress procedure was observed in CB1 knockout mice, suggesting an increased susceptibility to develop an anhedonic state in these animals. Finally, CB1 knockout mice showed a significant increase in the conditioned responses produced in the active avoidance model, suggesting an improvement of learning and memory processes. *Conclusions:* Taken together these findings demonstrate that endogenous cannabinoids through the activation of CB1 receptors are implicated in the control of emotional behaviour

and participate in the physiological processes of learning and memory.

Keywords Cannabinoid · CB1 receptor · Chronic unpredictable mild stress · Resident-intruder test · Active avoidance · Depression-like state

Introduction

The endocannabinoid system is involved in controlling several behavioural responses in the central nervous system (CNS), such as locomotor activity (Dewey 1986), nociception (Lichtman and Martin 1991a, 1991b; Calignano et al. 1998; Ledent et al. 1999), learning and memory (Ferrari et al. 1999; Reibaud et al. 1999; Böhme et al. 2000), as well as in the physiopathological processes leading to drug abuse (Tanda et al. 1997, 2000; Martin et al. 2000; Valjent and Maldonado, 2000). However, the implication of this endogenous system in regulating emotional responses is poorly understood. Physiological actions of endocannabinoids in the CNS are mediated by the activation of a specific cannabinoid receptor, the CB1 receptor (Matsuda et al. 1990). This receptor is widely distributed in the CNS (Herkenham et al. 1991; Tsou et al. 1997), being present in the limbic system and in the brain areas related to stress responses, such as the central amygdala and the paraventricular nucleus of the hypothalamus. In addition, endocannabinoids could activate the hypothalamic–pituitary–adrenal (HPA) axis (Weidenfel et al. 1994), the neuroendocrine system involved in the responses to emotional stress. Both the neuroanatomical localisation of the CB1 cannabinoid receptor and its physiological implication in the control of HPA axis support the idea that this receptor participates in regulating emotional responses (Devane et al. 1988, 1992). However, previous studies have reported unclear and contradictory results concerning on the role of CB1 cannabinoid receptors in anxiety.

In line with this, the acute administration of cannabinoids may cause anxiogenic responses in humans

M. Martin · R. Maldonado · O. Valverde (✉)
Laboratori de Neurofarmacologia,
Facultat de Ciències de la Salut i de la Vida,
Universitat Pompeu Fabra, C/ Doctor Aiguader 80,
08003 Barcelona, Spain
e-mail: olga.valverde@cexs.upf.es
Tel.: +34-93-5422830, Fax: +34-93-5422802

C. Ledent · M. Parmentier
IRIBHN, Université libre de Bruxelles, 1070 Brussels, Belgium

(Zuardi et al. 1982) even though they have been reported to be consumed in humans exposed to stressful situations (Williamson and Evans 2000). However, animal studies reveal that cannabinoids can induce both anxiolytic and anxiogenic-like responses, depending on the doses and the familiarity of the environment (Rodríguez de Fonseca et al. 1996). Thus, cannabinoids display a dose-dependent biphasic profile in rats when using standard anxiety models. Low doses of these compounds seem to produce anxiolytic-like responses, whereas higher doses result in anxiety-like reactions (Onaivi et al. 1990; Navarro et al. 1993; Rodríguez de Fonseca et al. 1996). Furthermore, blockade of the endogenous cannabinoid tone with SR 141716A, a specific CB1 cannabinoid receptor antagonist, induces anxiety-like responses in the elevated plus-maze and in the defensive withdrawal test in rats (Navarro et al. 1997).

Recently, knockout mice deficient in the CB1 cannabinoid receptor have been generated (Ledent et al. 1999). These mice represent a useful tool for clarifying the previous controversial findings on the involvement of CB1 receptors in emotional behaviour. For this purpose, we have evaluated the emotional responses of CB1 knockout mice in different behavioural models of aggressiveness, anxiety and depressive-like responses. Anxiety-like responses have been evaluated using the light/dark box (Filliol et al. 2000), a model of anxiety in which mice are exposed to a conflict represented by the novelty and aversive characteristics of the lit compartment of the box. The chronic unpredictable mild stress model (CMS) has been proposed to be a valid animal model of depression (Willner 1990, 1997). This procedure simulates anhedonia, a loss of responsiveness to pleasant events, which is a core symptom of depression and the defining feature of melancholia. CMS also causes a generalised decrease in reward sensitivity, and all these deficits have been reported to be reversed by chronic administration of tricyclic antidepressants (Willner 1990, 1997). Aggressive behaviour was also studied in these knockout mice using the resident-intruder test (König et al. 1996). Under this paradigm, we compare the attack behaviour of an intruder animal when it was placed in the resident's home cage. Finally, the responses of CB1 knockout mice were evaluated in the active avoidance procedure (Sansone 1975). Both anxiety and learning and memory processes play an important role in the performance of animals under this latter paradigm.

Materials and methods

Animals

Male CB1 knockout mice and wild-type littermates were used in all the experiments. The generation of mice lacking CB1 receptors was described previously (Ledent et al. 1999). In order to homogenise the genetic background of the mice, the first generation of heterozygotes were bred for 15 generations on a CD1 (Charles River, France) background, with selection for the mutant CB1 gene at each generation. All animals used in a given experiment originated from the same breeding series and were matched for age and weight.

Mice were housed in a temperature-controlled room ($21\pm 1^\circ\text{C}$) with a 12-h/12-h light/dark cycle. Lights were on between 0800 hours and 2000 hours in all experiments except the CMS procedure, where the light/dark cycle was reversed.

Food and water were available ad libitum except as described below for the CMS. All animal procedures met the guidelines of the European Communities Directive 86/609/EEC regulating animal research and were approved by the local ethics committee. All experiments were performed under blinded conditions. The same observer was in charge of the performance of the complete set of experiments.

Chronic unpredictable mild stress procedure

Two weeks prior to beginning the CMS procedure, the animals ($n=30$ for each genotype) were single housed in a reversal light/dark cycle room. Five days after single housing, mice were habituated to intake sucrose solution 2% (Monleon et al. 1995). This solution was substituted for 24 h in the place of drinking water. After this first period of habituation to sucrose solution, we measured baseline sucrose consumption for each mouse for 2 weeks (three measures per week), presenting the solution in small receptacles (23 mm diameter top \times 17 mm bottom \times 33 mm height; 12-ml capacity). On the days of sucrose consumption measurements, food, water and bedding were removed at 0800 hours. Three hours later, the receptacles containing sucrose solution (2%) were fixed in the corner of the house cage, allowing the mice to have access to the sucrose solution. Mice had access to sucrose solution for a period of 1 h, after that the receptacles were removed, and the food, water and bedding were replaced. Sucrose intake was evaluated as the difference in the weights of the receptacles before and after the session. Animals were weighed 3 days per week, just prior each sucrose intake measurement.

After obtaining a stable baseline of sucrose consumption, the mice of each genotype were separated into two different groups ($n=15$ per genotype), matched by the weight and the mean of sucrose solution intake. One group was subjected throughout the experiment to CMS for the next 5 weeks and the other group was under similar experimental conditions without CMS exposure. As previously reported (Monleon et al. 1995; Valverde et al. 1997), the regime of stress per week consisted of: (1) three 3-h periods of food and water deprivation with bedding removal, immediately prior to the sucrose test; (2) two 17-h periods in a soiled cage (150 ml water in 100 g sawdust bedding); (3) one or two periods per week (7 h and 17 h) of 45° cage tilt; (4) one or two periods per week of increased cage floor temperature to 32°C ; and (5) one period per week of changed light/dark cycle. Animals were exposed to a total of seven stress sessions per week.

During the stress regime, sucrose intake was measured three times per week (on Monday, Wednesday and Friday). The non-stressed control groups were also deprived of food and water (with bedding removal) for 3 h preceding each sucrose intake test.

Light/dark box procedure

Mice housed five per cage were individually exposed for 5 min to a box consisting of a small compartment (15 \times 20 \times 25 cm) with black walls and black floor dimly lit (5 lux) connected by a 4-cm-long tunnel to a large compartment (30 \times 20 \times 25 cm) with white walls and floor, under intense illumination (500 lux). Lines were drawn on the floor of both compartments to allow measurement of locomotor activity by counting the number of squares (5 \times 5 cm) crossed. Floor lines separated the lit compartment into three equal zones, from the tunnel to the opposite wall, designated as proximal (zone 1), median (zone 2) and distal (zone 3). Each animal was placed in the dark compartment facing the tunnel at the beginning of the observation session. The time spent in, the number of the entries in each compartment and the number of visits into each zone of the lit compartment were recorded (Filliol et al. 2000). The number of squares crossed per unit of time in both lit and dark compartments was also evaluated as an index of locomotor activity.

Resident–intruder procedure

This procedure evaluates aggressive behaviour in rodents (König et al. 1996). Resident mice had been housed individually for 10 weeks prior to the beginning of the experimental procedure. Intruder animals of a similar age and weight were housed five per cage. Each session consisted of putting together resident and intruder mice for a period of 4 min. Then, we measured the attack frequency and duration based on the presence of an attack or menace (beating tail) by the resident for each 10-s period. Animals received two training sessions in the morning and two test sessions in the afternoon.

Active avoidance procedure

Active avoidance procedure is a model in which anxiety, learning and memory play an important role in the performance of the animals. In this test, mice were trained to avoid an aversive stimulus associated with the presentation of a conditioned stimulus (CS) in a two-way shuttle box apparatus. The apparatus consists of a box with two compartments (20×10 cm) connected by a 3×3-cm door. A light (10 W) switched on in the compartment in which the mouse was placed was used as a CS. The CS preceded by 5 s the onset of the unconditioned stimulus (US) and overlapped it for 25 s. Using this procedure, the light was presented in the compartment for 30 s (5 s alone and 25 s together with the US). At the end of the 30-s period, both CS and US were automatically turned off. The US was an electric shock (0.2 mA) continuously applied to the grid of the floor. A conditioned response was recorded when the animal avoided the US by changing from the compartment where the animal received the CS into the opposite compartment within the 5 s after the onset of the CS. If animals failed to avoid the shock, they could escape it by crossing during the US (25 s). Between each trial session, there was an intertrial interval of 30 s.

Ten wild-type and twelve knockout mice, housed five per cage, were subjected to five daily 100-trial active avoidance sessions. Each day the mice were placed in the shuttle box 10 min before the start of each session to allow them to explore the box and to habituate to the apparatus.

The initial reactivity to the electric foot-shock exposure was evaluated in a preliminary experiment in a separate groups of wild-type and knockout mice. Thus, ten mice of each genotype were exposed to exactly the same procedure described above, but only for 1 day, and the primary behavioural responses to the electric foot shock were evaluated. Locomotor activity was evaluated during the period of habituation to the apparatus 10 min before electric foot-shock exposure by measuring the number of squares crossed and the number of rearings. The following responses were measured during the period of electric foot-shock exposures: number of rearings, squares crossed (5×6 cm), jumpings and vocalisations.

Analysis of the data

The CMS data were analysed using two-way analyses of variance (ANOVAs) for each week of stress procedure and for each genotype. The factors of variation were exposure to chronic stress (between subjects) and day (between subjects). Subsequent one-way ANOVAs were calculated for treatment effects for each testing day and for each genotype. Active avoidance and the resident–intruder tests data were analysed using two-way ANOVA with genotype (between subjects) and day (for active avoidance test) or sessions (for intruder test; within subjects) as factors of variation. In the case of the active avoidance, subsequent one-way ANOVAs were calculated to compare results between genotypes for each day and changes observed in each genotype through the 5 days of the experiment. In the case of the preliminary experiment to evaluate the basal reactivity of animals in the active avoidance model, the Student's *t*-test was used to compare behavioural data obtained between wild-type and knockout mice. In the case of the intruder test, subsequent one-way ANOVAs were computed to compare re-

sults between genotypes for each session. Data from the light/dark box were analysed using the Student's *t*-test. The level of significance was set at $P < 0.05$ for all the experiments.

Results

CB1 knockout mice exhibit an anxiogenic-like response in the light/dark box

Several changes observed in the behavioural performance of CB1 knockout mice in the light/dark box suggest that these mice present an anxiogenic-like response. A significant decrease in the number of entries in the lit compartment was observed for CB1 knockout mice ($F_{1,37}=3.627$, $P < 0.01$), whereas no difference in this variable was observed in the dark compartment ($F_{1,37}=0.519$, NS; Fig. 1B). CB1 knockout mice exhibited an increase in the time spent in the dark compartment of the box ($F_{1,37}=5.125$, $P < 0.01$; Fig. 1C), whereas the opposite effect was found in the lit compartment; thus, a decrease in the time spent in the light compartment was observed for the CB1 knockout mice ($F_{1,37}=4.426$, $P < 0.01$; Fig. 1C). No differences between genotypes were obtained in the latency to visit the lit compartment the first time (Fig. 1C; $F_{1,37}=1.688$, NS) or in the time spent in the tunnel that connected both compartments ($F_{1,37}=0.411$, NS; data not shown). The activity index of wild-type and knockout mice was similar in the dark compartment. In the lit compartment, locomotor activity of knockout mice was decreased when compared with locomotion in the wild-type group ($F_{1,37}=6.548$, $P < 0.05$; Fig. 1A).

When we measured the percentage of visits for each zone of the lit compartment, the CB1 knockout mice showed a significant increase in number of visits to the proximal zone of the lit compartment (Fig. 1D) close to the tunnel ($F_{1,37}=3.151$, $P < 0.01$) and a significant decrease in number to the distal zone ($F_{1,37}=3.052$, $P < 0.01$) compared with wild-type animals. No differences between genotypes were observed in the medial zone ($F_{1,37}=0.592$, NS).

CB1 knockout mice exhibited an increased anhedonia during the CMS procedure

During the 2-week period of habituation, a similar stable baseline of sucrose intake was obtained in CB1 knockout mice and the wild-type group. The values of sucrose intake solution at the start of the stress procedure were 1.59 ml for the CB1 knockout group and 1.80 ml for the wild-type group.

After this period, we started the stress procedure and quantified sucrose intake as a measure of the anhedonic state of the animals. Three different measures of sucrose intake were performed each week of the stress procedure (Fig. 2).

During the first 3 weeks of CMS procedure, two-way ANOVA revealed an effect of the day in wild-type and

Fig. 1A–D Anxiogenic-like responses exhibited by CB1 knockout mice in the light/dark box. CB1 knockout mice are represented by the *open columns*, and their control wild-type littermates by the *filled columns* ($n=15$ for each genotype). Different variables were evaluated in this model for 5 min. **A** Index of activity (squares crossed per unit of time); **B** number of entries in each compartment; **C** Time (s) spent in each compartment and the latency (s) to visit the lit area the first time; **D** percentage of visits for each zone of the lit compartment. Results are expressed as mean±SEM. *Open asterisks* – $P<0.05$ (*), $P<0.01$ (**) – represent genotype comparisons (unpaired two-tailed Student's *t*-test). *Filled asterisks* – $P<0.01$ (***) – represent dark versus lit compartment measured in the same genotype (paired two-tailed Student's *t*-test)

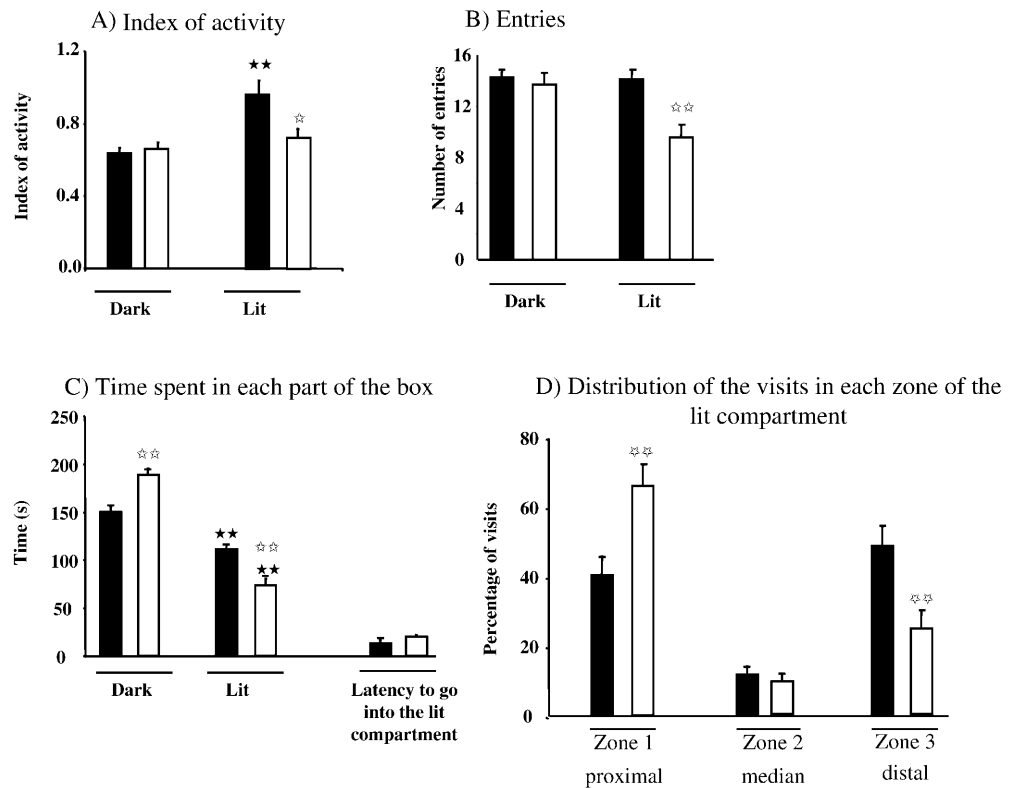


Table 1 Initial reactivity of CB1 knockout and wild-type mice exposed to the electric foot-shock under the same conditions as in the active avoidance procedure. Results are expressed as

mean±SEM. No significant differences were observed between genotypes in any of the behavioural responses (Student's *t*-test)

	Locomotor activity during habituation		During electric foot shock		
	Rearing	Squares crossed	Squares crossed	Jumping	Vocalisation
Wild-type	21.6±5.7	118.8±15.5	594.5±32.0	33.3±11.4	10.0±3.9
Knockout	30.2±9.2	105.0±18.0	547.1±31.3	54.4±12.9	6.0±3.9

knockout mice, without any effects of stress or an interaction between these two factors in any group of animals. Thus, for wild-type mice, an effect of day was observed in week 1 ($F_{2,56}=6.337$, $P<0.01$), week 2 ($F_{2,56}=12.778$, $P<0.001$) and week 3 ($F_{2,56}=14.526$, $P<0.001$). Similarly, for the CB1 knockout group, a day effect was also observed in week 1 ($F_{2,56}=13.249$, $P<0.001$), week 2 ($F_{2,56}=5.639$, $P<0.01$) and week 3 ($F_{2,56}=5.835$, $P<0.01$).

In week 4, two-way ANOVA calculated for wild-type mice did not show any effects. However, in the case of the CB1 knockout mice, a two-way ANOVA showed an effect of day ($F_{2,54}=4.483$, $P<0.05$) and of stress procedure ($F_{1,27}=5.786$, $P<0.05$) and a significant interaction between these two factors ($F_{2,54}=3.774$, $P<0.05$). One-way ANOVA revealed an effect of stress in the knockout group on the last 2 days of exposure to the CMS procedure (day 11, $F_{1,28}=8.569$, $P<0.01$; day 12, $F_{1,28}=6.518$, $P<0.05$; Fig. 2B).

Finally, on the fifth week of stress, two-way ANOVA in the group of wild-type mice revealed an effect of day

($F_{2,52}=3.665$, $P<0.05$) and an effect of stress ($F_{1,26}=5.832$, $P<0.05$), without interaction between these two factors. In the case of the CB1 knockout mice, we observed an effect of stress ($F_{1,27}=6.402$, $P<0.05$), without an effect of day and without an interaction between these two factors. The lack of significant interaction between the factors day and stress was due to the fact that the consumption of sucrose solution was stabilised on the fifth week in both stressed and non-stressed animals. Therefore, only a main significant effect of stress was revealed in both wild-type and knockout groups during this week (Fig. 2).

CB1 knockout mice showed an enhanced performance during the active avoidance test

In a preliminary experiment, we have evaluated the initial behavioural reactivity to foot-shock exposure in wild-type and knockout mice (Table 1). No significant

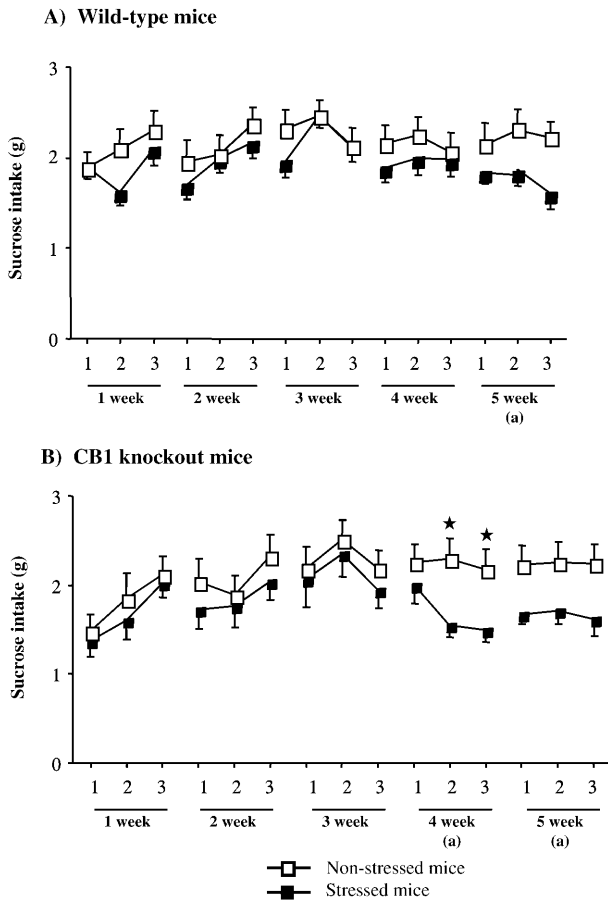


Fig. 2A, B CB1 knockout mice exhibited a hypersensitivity to chronic unpredictable mild stress model (CMS) procedure. *White squares* represent control mice ($n=15$) and *black squares* stressed animals ($n=15$). **A** Sucrose solution intake in wild-type animals. **B** Sucrose solution intake in CB1 knockout mice. The *horizontal axis* represents testing days of sucrose intake during the stress procedure, and the *vertical axis* represents the consumption of sucrose solution measured in grams. Results are expressed as mean \pm SEM. *Filled asterisk* $P<0.05$ (*) – represents treatment comparisons (one-way analysis of variance). ^a Indicates a significant main effect of stress ($P<0.05$) in the corresponding week

differences between wild-type and knockout mice were observed in the behavioural responses evaluated during the exposure to the electric foot shock (number of rearings, squares crossed, jumpings and vocalisations), indicating a similar basal reactivity to this experimental procedure. Besides, locomotor activity (number of rearings and squares crossed) was also similar in wild-type and knockout mice during the 10-min habituation prior to the exposure to the electric foot shocks.

Two-way ANOVA revealed a significant effect of day ($F_{4,80}=123.153$, $P<0.01$), genotype ($F_{1,20}=4.365$, $P<0.05$) and interaction between both factors ($F_{4,80}=2.649$, $P<0.05$). A progressive temporal increase in the performance in the active avoidance paradigm was observed in both wild-type and CB1 knockout mice. Indeed, subsequent one-way ANOVAs revealed a significant increase in

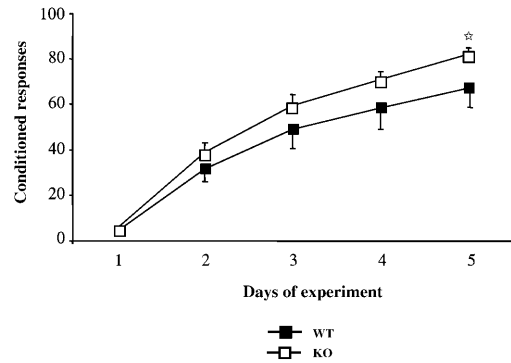


Fig. 3 CB1 knockout mice presented an enhancement in learning/memory evaluated during the active avoidance test (100-trial avoidance sessions per day for 5 days). CB1 knockout mice are represented by *white squares* and control wild-type littermates by *black squares* ($n=10-12$ per genotype). Values on the *vertical axis* represent the number of conditioned responses. The *horizontal axis* represents the consecutive days of the experiment. Results are expressed as mean \pm SEM. *Open asterisk* $P<0.05$ (*) – represents genotype comparison (one-way analysis of variance)

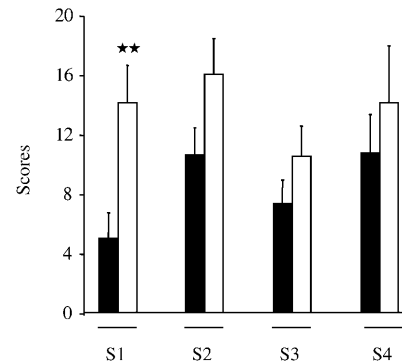


Fig. 4 CB1 knockout mice showed an increase in aggressive behaviour during the resident-intruder test. *Open columns* represent CB1 knockout mice ($n=10$). *Filled columns* represent CB1 wild-type animals ($n=10$). *Vertical axis* represents the score calculated as the presence of attack or menace (beating tail) by the resident for each 10-s period for a total time of 4 min (score from 0 to 48). *Black asterisks* $P<0.01$ (**) – represent genotype comparison (one-way analysis of variance)

the number of conditioned changes through the days in both wild-type ($F_{4,36}=30.038$, $P<0.01$) and CB1 knockout ($F_{4,44}=129.062$, $P<0.01$) mice. Genotype comparisons revealed a significant increase in the performance of the CB1 knockout mice on day 5 ($F_{1,20}=5.714$, $P<0.05$; Fig. 3).

CB1 knockout mice showed an increased aggressive behaviour during the resident-intruder test

Resident CB1 knockout mice were significantly more aggressive towards intruders than wild-type animals (Fig. 4). The enhanced aggressive behaviour was shown by an increase in the score calculated for the CB1 knockout mice when they were exposed to an intruder during the first session ($F_{1,17}=2.901$, $P<0.01$). However, the dif-

ferences between genotypes did not reach significance in sessions two, three and four (Fig. 4).

Discussion

We investigated the role played by the CB1 cannabinoid receptor in the control of a range of emotional responses using knockout mice lacking these CB1 receptors in different behavioural models and demonstrated that the endogenous cannabinoid system modulates emotional behaviour through the CB1 cannabinoid receptors. Thus, CB1 knockout mice exhibited an increase in basal level of anxiety-like responses during the light/dark test, an enhanced development of the anhedonic state during the CMS, an increase in the aggressive behaviour when exposed to the resident-intruder procedure, and a higher level of performance under the active avoidance paradigm.

The neuroanatomical localisation of CB1 cannabinoid receptors is consistent with the changes observed in this study in several emotional-like responses in CB1 knockout mice. Thus, CB1 cannabinoid receptors are located in the limbic system and other brain areas related to stress responses, such as the hypothalamus (Herkenham et al. 1991; Tsou et al. 1997). Furthermore, several previous pharmacological studies agree well with the present findings. Thus, acute administration of the selective CB1 receptor antagonist SR 141716A induced anxiety-like responses in the elevated plus maze and the defensive withdrawal tests (Navarro et al. 1997). However, previous studies have shown that CB1 agonists are able to display both anxiogenic- and anxiolytic-like responses, depending on the dose and the context of study (Rodríguez de Fonseca et al. 1991; Devane et al. 1992). Recently, it has been reported that high doses (25–100 µg) of the CB1 cannabinoid receptor agonist, delta 9-tetrahydrocannabinol (THC), given via the intracerebroventricular (i.c.v.) route, increased plasma adrenocorticotrophin and corticosterone hormone concentrations. This effect was blocked by naloxone (s.c.) and SR 141716A (i.c.v.). However, higher doses of this CB1 antagonist – SR 141716A (i.c.v.) – also increased adrenocorticotrophin and corticosterone secretion. In this respect, it has been recently suggested that a physiological role of the endogenous cannabinoids is tonically to inhibit the release of these hormones through the stimulation of the CB1 receptors (Manzanares et al. 1999a). Taking into account the direct relationship between these hormones and the endogenous response to stress, this hypothesis is in agreement with our present findings, showing an increase in the basal level of anxiety in mice lacking CB1 cannabinoid receptors.

CMS has been proposed as a valid animal model of depression. In this sense, CMS has been proposed as a model with construct validity because anhedonia is a core symptom of major depressive disorders (review in Willner 1990, 1997). We demonstrated an enhanced sensitivity of CB1 knockout mice in developing a depres-

sive-like state, supporting the notion that the endogenous cannabinoid system regulates the maintenance of mood. In this sense, two-way ANOVA revealed a main effect of the stress procedure only in the fifth week for the wild-type group. In the case of the knockout mice, the main effect of stress was revealed in the fourth week and it was maintained during the fifth week. Previous studies have evaluated the effects of the cannabinoids on the biogenic amine system. Thus, THC inhibited the uptake of norepinephrine and serotonin into synaptosomes generated from the hypothalamus, and this compound was also able to inhibit the uptake of dopamine into synaptosomes from the striatum (Banerjee et al. 1975; Hershkowitz and Szechtman 1979). Furthermore, THC decreased electrically induced ganglionic transmission, and doses below threshold for blocking transmission potentiated the inhibitory action of exogenous catecholamines (Goldstein et al. 1977). These authors proposed that the facilitation of the effects of norepinephrine in this model could be important for a hypothetical antidepressant effect of cannabinoids (Goldstein et al. 1977). In agreement with these early studies, it has been recently demonstrated using *in vivo* microdialysis that THC administration results in a dose-dependent increase of extracellular levels of dopamine in the striatum (Tanda et al. 1997; Malone and Taylor 1999). Besides, the *in vivo* release of dopamine induced by THC was dependent on the serotonin transmission, since it was facilitated by the administration of fluoxetine (Malone and Taylor 1999).

Indeed, in this study both increases and blockade of THC-induced striatal-dopamine release were observed with fluoxetine administration, depending on the route of fluoxetine administration. In addition, cannabinoids have been claimed to be useful in clinical practice to relieve depression associated with chronic pain (Williamson and Evans 2000). Taken together, these findings indicate that cannabinoids alter the homeostatic control of different neurochemical mechanisms in a complex way, which can explain the enhanced susceptibility of CB1 knockout mice to the CMS procedure to induce a state of anhedonia related to depression-like behaviour. We previously reported that these CB1 knockout mice exhibited a reduction in the conditioned response to acute stress after exposure to a strong stimulus involving opioid mechanism, like the electric foot shock in the conditioned suppression of motility test (Valverde et al. 2000a). In this model, animals react to the adverse situations by motor immobility, which is suppressed by antidepressants (Kameyama and Nagasaka 1982). The apparent discrepancies obtained in these two models of depression could be due to the differences in the temporal scheduling and the experimental procedure used to produce the depressive-like state (acute vs chronic stress regime; strong vs mild stress stimuli; predictable vs unpredictable stress).

Cannabinoid administration has been previously reported to modify aggressive behaviour in rodents. Thus, low doses of THC and other cannabinoid derivatives reduced aggressiveness (Cherek et al. 1980); whereas higher doses increased this behaviour (Alves et al. 1973;

Bac et al. 1998). In the present study, the lack of the CB1 cannabinoid receptor clearly increased aggressive behaviour measured during the resident–intruder test, but exclusively during the first session of the paradigm. Interestingly, THC-induced muricidal behaviour has been reported to become enhanced after repeated sessions (Bac et al. 1998). However, CB1 knockout mice showed a similar aggressiveness during the four sessions of the paradigm. The enhanced basal levels of anxiety observed in the CB1 knockout mice can also participate in the response observed and, thus, facilitate the increase in aggressive behaviour during the first session of the test. Muricidal mechanisms are of central origin, and a variety of neurotransmitters, including serotonin, histamine, dopamine and enkephalins, have been implicated in this response (Vergnes and Kempf 1982; König et al. 1996). Decreases in serotonin seem to play a major role in the induction of muricide (Vergnes and Kempf 1982), and a possible modification in the activity of this monoaminergic system in CB1 knockout mice might explain their increased aggressiveness. Important relationships between cannabinoids and endogenous enkephalins have been previously reported (Manzanares et al. 1999b; Ledent et al. 1999; Valverde et al. 2000b). In this sense, preproenkephalin knockout mice also exhibited an increase in the aggressive behaviour in the resident–intruder model (König et al. 1996).

CB1 knockout mice exhibited enhanced performance in the active avoidance test. Thus, the number of the conditioned changes to avoid electric foot shocks was higher in the knockout mice. This model has been proposed as useful for evaluating learning and memory, although an increase in the anxiety state of the mice can impair performance under this paradigm (Sansone 1975). However, the increase in the anxiety levels does not seem to have caused the responses observed in the active avoidance model. Indeed, decreases in stress and administration of anxiolytic compounds, such as the benzodiazepines, increase the performance under this paradigm (Sansone 1975). Taking into account the role of endogenous cannabinoids in nociception (Ledent et al. 1999; Valverde et al. 2000a), another possibility could be that the initial responsiveness to the electric foot shock in CB1 knockout mice was greater than in wild-type mice. We have therefore evaluated the primary behavioural responses to the foot-shock exposure in wild-type and knockout animals. The initial responsiveness of both genotypes was similar in all the behavioural responses evaluated, suggesting that the results observed in knockout mice during this test were not due to an enhanced reaction of the initial foot-shock stimulus. This is consistent with previous studies showing that the nociceptive threshold was not changed in the CB1 knockout mice (Ledent et al. 1999; Valverde et al. 2000a). However, these CB1 knockout mice exhibited a selective decrease of opioid-mediated, stress-induced analgesia without modification of the stress-induced analgesia mediated by non-opioid mechanisms (Valverde et al. 2000a).

The role of the cannabinoids in memory processes has been suggested by the disruption of short-term recall as well as the perceptual and disorienting effects observed in humans after THC administration (Miller and Branconner 1983; Chait and Pierri 1992). In rodents, endogenous cannabinoids have been reported to prevent the induction of the long-term potentiation in the hippocampus (Stella et al. 1997) and to impair memory in a delayed non-match-to-position task, an effect attenuated by SR 141716A (Mallet and Beninger 1998). In addition, stimulation of the CB1 cannabinoid receptors induced a short-term memory deficit due to information not being correctly encoded or retrieved (Schacter and Wagner 1999). Our present findings agree with previous studies in showing an enhancement of memory in a two-trial, object-recognition test (Reibaud et al. 1999) and a facilitation of the long-term potentiation in the hippocampus in the same line of CB1 knockout mice (Böhme et al. 2000). Different hypotheses have been postulated to explain the intrinsic mechanism involved in cannabinoid-induced memory impairment. In this sense, THC through the activation of the CB1 cannabinoid receptors reduced hippocampal extracellular acetylcholine concentration, which may be crucial for memory/learning processes (Gessa et al. 1997). Memory impairment and reduction of the extracellular acetylcholine concentration produced by cannabinoids have been reported to be produced by the concomitant activation of both CB1 cannabinoid and D2 dopamine receptors, the latter most likely being activated by endogenous dopamine released following THC administration (Nava et al. 2000). Further studies are necessary to elucidate the exact mechanisms involved in the impairment of learning/memory processes in mice deficient in CB1 cannabinoid receptors.

In summary, the present findings demonstrate that endogenous cannabinoids acting via the CB1 cannabinoid receptors regulate a variety of emotional responses, including anxiety, the control of mood, aggressive behaviour and higher integrative processes such as memory and learning. The involvement of the endogenous cannabinoid system in the control of these important behavioural responses provides new perspectives for the promising therapeutic potential of cannabinoid compounds.

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