Psychopharmacology
DOI 10.1007/s00213-015-3939-5

ORIGINAL INVESTIGATION

Role of CB2 receptors in social and aggressive behavior in male mice

Marta Rodríguez-Arias[1,2] · Francisco Navarrete[2,3] · M. Carmen Blanco-Gandia[1,2] · M. Carmen Arenas[1,2] · María A. Aguilar[1,2] · Adrián Bartoll-Andrés[3] · Olga Valverde[2,4] · José Miñarro[1,2] · Jorge Manzanares[2,3]

Received: 20 October 2014 / Accepted: 14 April 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract
Rationale Male CB1KO mice exhibit stronger aggressive responses than wild-type mice.
Objective This study was designed to examine the role of cannabinoid CB2r in social and aggressive behavior.
Methods The social interaction test and resident–intruder paradigm were performed in mice lacking CB2r (CB2KO) and in wild-type (WT) littermates. The effects of the CB2r selective agonist JWH133 (1 and 2 mg/kg) on aggression were also evaluated in Oncins France 1 (OF1) mice. Gene expression analyses of monoamine oxidase-A (MAO-A), catechol-o-methyltransferase (COMT), 5-hydroxytryptamine transporter (5-HTT), and 5-HT_{1B} receptor ($5HT_{1B}$r) in the dorsal raphe nuclei (DR) and the amygdala (AMY) were carried out using real-time PCR.
Results Group-housed CB2KO mice exhibited higher levels of aggression in the social interaction test and displayed more aggression than resident WT mice. Isolation increased aggressive behavior in WT mice but did not affect CB2KO animals; however, the latter mice exhibited higher levels of social interaction with their WT counterparts. MAO-A and 5-HTT gene expression was significantly higher in grouped CB2KO mice. The expression of $5HT_{1B}$r, COMT, and MAO-A in the AMY was more pronounced in CB2KO mice than in WT counterparts. Acute administration of the CB2 agonist JWH133 significantly reduced the level of aggression in aggressive isolated OF1 mice, an effect that decreased after pretreatment with the CB2 receptor antagonist AM630.
Conclusion Our results suggest that CB2r is implicated in social interaction and aggressive behavior and deserves further consideration as a potential new target for the management of aggression.

Highlights
- Grouped CB2KO mice show higher levels of offensive aggression than WT mice in the social interaction test.
- CB2KO mice show higher levels of offensive aggression than WT mice in the resident intruder paradigm.
- COMT, MAO-A, 5-HTT and $5HT_{1B}$r mRNA levels differ in WT and CB2KO mice.
- Administration of JWH133 decreases the level of aggression in OF1 mice.
- Treatment with JWH133 normalizes alterations of MAO-A and COMT gene expression in aggressive isolated mice.

Electronic supplementary material The online version of this article (doi:10.1007/s00213-015-3939-5) contains supplementary material, which is available to authorized users.

✉ Marta Rodríguez-Arias
marta.rodriguez@uv.es

[1] Unidad de Investigación Psicobiología de las Drogodependencias, Departamento de Psicobiología, Facultad de Psicología, Universitat de València, Avda. Blasco Ibáñez, 21, 46010 Valencia, Spain

[2] Red Temática de Investigación Cooperativa en Salud (RETICS-Trastornos Adictivos), Instituto de Salud Carlos III, MICI NN and FEDER, Madrid, Spain

[3] Instituto de Neurociencias, Universidad Miguel Hernández-CSIC, Avda. Ramón y Cajal s/n, 03550 San Juan de Alicante, Alicante, Spain

[4] Neurobiology of Behavior Research Group (GReNeC), Department of Health and Experimental Sciences, University Pompeu Fabra, IMIM (Hospital del Mar), Barcelona Biomedical Research Park (PRBB), C/ Dr. Aiguader, 08003 Barcelona, Spain

Keywords CB2KO mice · Social encounters · Resident–intruder paradigm · Aggression · OF1 mice · JWH133 · Gene expression

Published online: 29 April 2015

Introduction

A relation between cannabinoids and aggression was established in the 1970s. Using the resident–intruder paradigm, Miczek (1978) demonstrated that delta 9-tetrahydrocannabinol (THC) induced a dose-dependent decrease in attack behavior in three different species of mammals. However, in contrast, other studies since then have highlighted how cannabis administration in stressful situations can cause or exacerbate aggression in rats. In line with this, chronic administration of cannabis sativa extract or THC after food, sleep, or drug deprivation was shown to elicit aggressive behavior in rodents (Carlini and Gonzales 1972; Carlini et al. 1976). In addition, shock-induced defensive aggression was found to increase the number of aggressive responses exhibited by rats treated with cannabis (Carder and Olson 1972). Mouse-killing behavior was induced in group-housed rats after chronic administration of daily doses of THC, while a single dose was enough to provoke this response in rats housed in isolation (Ueki et al. 1972). In the case of the endocannabinoid anandamide, its effects have been shown to depend on the dose employed; high doses reduce aggression in aggressive mice, while low doses increase aggressive responses in timid mice (Sulcova et al. 1998). A recent report has associated intrauterine exposure to cannabis with an increased risk of aggressive behavior and attention deficit at an age as early as 18 months in girls, but not in boys (El Marroun et al. 2012), whereas the CB1 receptor (CB1r) inverse agonist taranabant has been shown to produce irritability and anger/aggression when administered to obese and overweight patients (Proietto et al. 2010).

Most of the abovementioned studies have focused on the CB1r. Experiments with CB1KO mice have revealed that these animals present anxiogenic- and depressive-like phenotypes (for review see Valverde and Torrens 2012) and alterations in the regulation of social and aggressive behaviors. In one study, CB1KO mice exposed to the resident–intruder procedure exhibited stronger aggressive responses than wild-type mice, though these differences were not observed in subsequent encounters (Martin et al. 2002). Our group recently confirmed and extended these results, showing that CB1KO mice behave more aggressively than their wild-type (WT) counterparts in a social interaction test when confronted with an anosmic standard opponent. Moreover, isolation, which increases aggression in WT mice, did not have any effect on this behavior in CB1KO mice. Furthermore, pharmacological manipulation of CB1r with the agonist ACEA confirmed the critical role of this receptor in the control of aggression (Rodríguez-Arias et al. 2013). Interestingly, our results related highly aggressive behavior of grouped CB1KO mice with increased gene expression of catechol-o-methyltransferase (COMT) in median and dorsal raphe nuclei (MnR and DR, respectively) and the amygdala (AMY) and increased monoamine oxidase-A (MAO-A) gene expression in the AMY.

Little is known about the role of CB2 receptors (CB2r) in mediating aggressive behavior. CB2r have been detected under normal conditions in the brainstem of rats, mice, and ferrets (Van Sickle et al. 2005). Subsequent studies in rats have identified a wide distribution of CB2r in different brain areas, including the spinal nucleus, hippocampus, olfactory nucleus, cerebral cortex, amygdala, striatum, thalamus, and cerebellum (Gong et al. 2006; Onaivi 2006). Interestingly, the presence of CB2r in areas related to response to stress, anxiety, and depression, such as the hippocampus and amygdala, implicates them in the regulation of mood disorders (Onaivi et al. 2008). In addition, CB2r knockout mice (CB2−/−) display an increased vulnerability to stressful stimuli. Deletion of CB2r reduces motor activity in the open field test, but enhances the response to acute cocaine administration and produces mood-related alterations, prepulse inhibition deficit, and cognitive impairment (Ortega-Álvaro et al. 2011). On the other hand, transgenic mice overexpressing CB2r (CB2xP) have been shown to have an endophenotype that is resistant to acute and chronic depressive-like behaviors (García-Gutiérrez et al. 2010). Several studies have suggested that CB2r also play a key role in the regulation of anxiety (García-Gutiérrez and Manzanares 2011), producing opposite effects depending on the schedule of pharmacological manipulation (García-Gutiérrez et al. 2012).

The aim of the present study was to examine the role of CB2r in social and aggressive behavior in mice lacking CB2r (CB2KO) housed in groups and in isolation using the social interaction test and resident–intruder paradigm. Anxiety profile and spontaneous motor activity were also evaluated in group-housed WT and CB2KO mice. The behavioral effects of a CB2r agonist (JWH133) on social activity and aggression were also evaluated in isolated highly aggressive Oncins France 1 (OF1) mice. In addition, real-time PCR experiments were performed to analyze the expression of COMT and MAO-A in the amygdala, the 5-HT transporter (5-HTT) in the DR, and the $5HT_{1B}$ receptor ($5HT_{1B}$r) in the AMY of CB2KO mice (and their corresponding WT animals) and saline- or JWH133-treated OF1 mice.

Materials and methods

Subjects

A total of 55 male cannabinoid receptor CB2KO and 59 male WT mice, all 42 days old, were employed in the first experiment. Male CB2KO mice on a C57BL/6J congenic background (kindly provided by Nancy E. Buckley, Cal State Polytechnic Univ., Pomona, CA, USA) were crossed with outbreed CD1 (Charles River, France) background (Buckley et al. 2000) for eight generations. CB2KO homozygote mice and their corresponding WT littermates—both derived from

heterozygous parents—were used. For the second study, we employed 112 OF1 male mice (Charles River, Barcelona, Spain), also of an age of 42 days. OF1 mice were employed as they exhibit aggressive behavior from the age of 6 weeks, a behavior that increases with age. Isolation for 21 days has been shown to enhance this behavior (Rodríguez-Arias et al. 1998). Eighty-eight per cent of the isolated OF1 mice showed aggressive behavior, while this was the case among only 10 % of their group-housed counterparts. All animals were housed under standard laboratory conditions: constant temperature (21±1 °C), a reversed light schedule (white lights off; 0730 to 1930 hours), and food and water available ad lib, except during behavioral testing. For the first experiment, half of the experimental animals were housed individually for 21 days in transparent, plastic cages (24×13.5×13 cm), while the other half was housed in groups of four in plastic cages (25×25×14.5 cm) during the same period. For the second experiment, 62 mice were housed in isolation under the same experimental conditions. The remaining mice were housed in groups of four to be used later as standard opponents or saline-treated, group-housed mice. All procedures were conducted in compliance with the guidelines of the European Council Directive 2010/63/UE regulating animal research and were approved by the local ethical committees.

Drug treatment and experimental design

Two sets of mice were employed in the first experiment. The first set performed the elevated plus maze (EPM), the open field test, and the social interaction test (in that order), with a week's interval between each test. Subsequently, their brains were removed for PCR analysis. The second set of mice performed the resident–intruder test. Two sets of mice were also used in the second experiment. OF1 mice performed the social interaction test and WT and CB2KO mice performed the resident–intruder paradigm after being treated, in all cases, with the CB2 cannabinoid agonist JWH133. In this second experiment, mice were injected intraperitoneally (i.p.) with doses of 1, 2, and 4 mg/kg of the CB2 cannabinoid agonist JWH133 in a volume of 0.01 ml/g (Tocris Bioscience, Bristol, UK). The CB2 antagonist AM630 (Biogen, Madrid, Spain) was also administered at a dose of 3 mg/kg. The control group was injected with a mixture of DMSO, Tween 80, and distilled water (1:1:8 proportion), which was also used for dissolving the drugs. The doses were chosen based on previous reports (García-Gutiérrez et al. 2012).

Social encounters

This test consisted of confronting an experimental animal with a standard opponent in a neutral cage (61×30.5×36 cm) for 10 min following a 1-min adaptation period. Standard opponents were rendered temporarily anosmic by intranasal lavage with a 4 % zinc sulfate solution 1 day before testing (Smoothy et al. 1986). This kind of mouse induces an attack reaction in its opponent but does not outwardly provoke or defend itself, since it cannot perceive a pheromone that is present in the urine of the experimental animals and functions as a cue for eliciting aggressive behavior in mice with a normal sense of smell (Brain et al. 1981). A more detailed description of the behaviors evaluated can be found in Rodríguez-Arias et al. (1998) and in the supplementary material.

Resident–intruder procedure

CB2KO and WT mice underwent four 5-min episodes of the resident–intruder procedure, which evaluates aggressive behavior in rodents (Miczek and O'Donnell 1978). Resident mice were housed individually for 10 days prior to the experimental procedure. Intruder animals of a similar age and weight were housed in cages in groups of five. Each session consisted of placing an intruder mouse in the resident's home cage for a period of 5 min. Animals received two training sessions on day 1 and two test sessions on day 2. Threat and attack behaviors were ethologically analyzed.

Spontaneous motor activity

Spontaneous locomotor activity was automatically measured by an actimeter (CIBERTEC S.A., Spain) consisting of eight cages (33×15×13 cm), each with eight infrared lights located in a frame around the cage. In this apparatus, beams are positioned on the horizontal axis 2-cm apart, at a height just above the bottom of the cage (body level of mice). The different frames are separated from each other by a distance of 4 cm and, since they are opaque, prevent animals from seeing conspecifics. Spontaneous motor activity was recorded for 1 h, without previous adaptation to the actimeter.

Elevated plus maze

Animals performed the EPM in accordance with previously described protocols (Rodríguez-Arias et al. 2011). The experimental room was illuminated with a dim red light (40 lx at 1 m above floor level). The measurements recorded during the test period were frequency of entries and time and percentage of time spent in each section of the apparatus (open arms, closed arms, central platform). An arm was considered to have been visited when the animal placed all four paws on it.

Real-time PCR analyses

Gene expression studies were carried out in selected brain regions from untreated CB2KO and WT group-housed mice. In the second experiment, the main targets involved in the regulation of aggressive and social behavior were studied in

OF1-treated mice. Mice were killed and their brains removed and frozen over dry ice. Brain sections were manipulated as previously described (Navarrete et al. 2012). Total RNA was isolated from brain tissue micropunches using TRIzol reagent (Invitrogen, Madrid, Spain) and was subsequently retrotranscribed to complementary DNA. Quantitative analysis of the expression of the genes MAO-A (Mm00558004_m1), COMT (Mm00514377_m1), 5-HTT (Mm00439391_m1), and $5HT_{1B}r$ (Mm00439377_s1)—relatively abundant in all cases—was performed with the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). All reagents were obtained from Applied Biosystems, and manufacturer protocols were followed. 18S ribosomal RNA (rRNA) was employed as the reference gene and was detected using TaqMan ribosomal RNA control reagents. All primer–probe combinations were optimized and validated for relative quantification of gene expression. In brief, the data for each target gene were normalized to the endogenous reference gene, and the fold change in target gene messenger RNA (mRNA) abundance was determined using the $2^{-\Delta\Delta Ct}$ method (Schmittgen et al. 2000). This quantification method involves comparing the Ct values of samples with a control or calibrator, such as a non-treated sample or RNA from normal tissue. The Ct values of both the calibrator and samples are normalized to an appropriate endogenous housekeeping gene (18S rRNA). Intact CB2KO (n=8) and WT (8) animals were used to study receptor gene expression under baseline conditions. Grouped (n=10) and isolated (n=30) OF1 treated animals were sacrificed and their brains removed 2 h and 30 min after injection in order to study receptor gene expression changes under pharmacological treatment conditions.

Statistical analyses

Non-parametric Kruskal–Wallis tests were used to assess the variance of the behavioral measures in different treatment groups according to the times allocated to threat, attack, latency of threat and attack, unit of threat and attack, and number of attacks. Subsequently, appropriate paired comparisons were carried out using Mann–Whitney U tests to compare behaviors following the different treatments. Data for other behaviors evaluated by the social interaction test were analyzed using a two-way ANOVA with two between variables: housing (with two levels, grouped or isolation) and genetics (with two levels, wild-type and CB2KO). One-way ANOVA was employed to evaluate the effects of AM630 on the antiaggression effects of JWH. Data from the resident–intruder test were analyzed using two-way ANOVA with one between variable—genetics—and one within variable—encounters (with four levels). To study the effect of JWH on CB2KO mice, we employed an ANOVA with two between variables—genetics (with two levels, WT and KO) and treatment (with two levels, JWH and saline). Spontaneous motor activity was analyzed with a mixed ANOVA with the between variable genetics and a within variable—time (with six levels). Data from the EPM were analyzed with a one-way ANOVA with the between variable genetics.

Student's t test analyses were carried out for gene expression comparison between CB2KO and WT mice and between group-housed and isolated OF1 mice. One-way ANOVA with one between variable was employed to analyze data for OF1 JWH133-treated mice: treatment (with four levels: grouped, isolated+VEH, isolated+JWH133-1, and isolated+JWH133-2). The Bonferroni test was employed for post hoc comparisons.

Results

First experiment

Social behaviors

Kruskal–Wallis analysis showed a significant effect with respect to the time spent in *threat* (χ^2(df=3, p=0.001)=17.301) and *attack* (χ^2=(df=3, p=0.01)=12.825) (Fig. 1a), *unit of threat* (χ^2(df=3, p=0.001)=21.260) and attack (χ^2(df=3, p=0.001)=17.127) (Fig. 1b), and *number of attacks* (χ^2(df=3, p=0.01)=13.608) (Fig. 1c). The Mann–Whitney U test revealed that group-housed CB2KO mice spent more time in threat and attack, engaged in longer episodes of threat and attack, and performed a higher number of attacks than group-housed WT mice (p<0.05 in all cases). These behaviors were also more pronounced in isolated WT mice than in those housed in groups (p<0.001 in all cases).

Kruskal–Wallis analysis also showed a significant effect of the time needed to exhibit the first threat (*latency to threat*, Fig. 1d) (χ^2(df=3, p=0.02)=9.716). The Mann–Whitney U test showed that grouped CB2KO mice engaged in threat earlier than group-housed WT animals (p<0.01), while no differences were observed among isolated mice. Isolated WT mice engaged in threat behavior quicker than grouped WT mice (p<0.01), with no differences observed among CB2KO mice.

The time spent engaged in *social investigation* (Table 1) showed an effect of the variable housing [F(1,44)=32.479; p<0.001] and the interaction housing×genetics [F(1,44)=4.114; p<0.05]. Isolation increased this parameter in both types of mice (p<0.01), although CB2KO mice spent more time in social investigation than WT mice (p<0.05). The unit of social investigation revealed an effect of the variable housing [F(1,44)=15.450; p<0.001], since isolation increased the mean time spent by both types of mice in each social encounter (p<0.01).

Non-social investigation (Table 1) showed an effect of the variable housing [F(1,44)=36.406; p<0.001], with group-

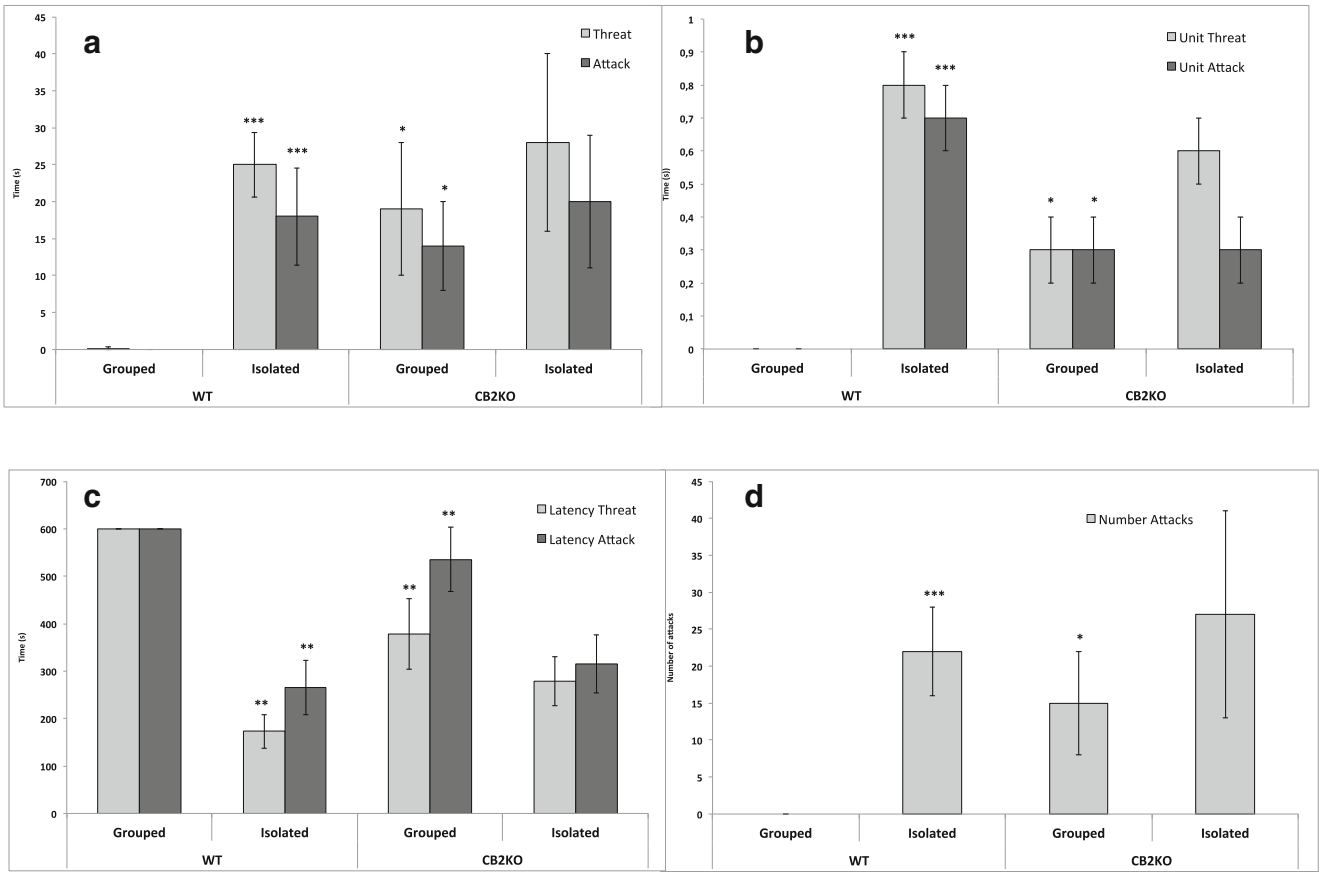

Fig. 1 Means of accumulated times (in seconds, with ±SEM) spent in threat and attack (**a**), unit of threat and attack (**b**), latency of threat and attack (**c**), and number of attacks (**d**) exhibited by adult CB2KO and WT (group-housed or isolated) during the social interaction test (grouped WT $n=12$, grouped CB2KO $n=12$, isolated WT $n=12$, and isolated CB2KO $n=10$). Differences with respect to grouped WT mice $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$

housed mice spending more time engaged in this behavior than animals housed in isolation ($p<0.001$).

Resident–intruder paradigm

The data of the time spent in threat during the resident–intruder encounters are presented in Fig. 2 (see Table 1 of the supplementary material for data of the time spent in attack and the number of attacks). The variable genetics showed a significant effect for time spent in threat [$F(1,30)=94.758$; $p<0.001$] and attack [$F(1,30)=27.840$; $p<0.001$], and number of attacks [$F(1,30)=31.771$; $p<0.001$]. CB2KO mice spent more time in threat and attack and performed a higher number of attacks than WT animals in all of the four encounters. For the time spent engaged in threat, the interaction genetic×encounters also showed a significant effect [$F(3,90)=3.062$; $p<0.01$], with CB2KO mice exhibiting higher levels of threat in the third and fourth encounter than in the first ($p<0.01$).

Table 1 Means of accumulated times (in seconds, with ±SEM) allocated to different categories of behavior by CB2KO and WT adult mice (group-housed or isolated) during the social interaction test

	Grouped		Isolated	
	WT	CB2KO	WT	CB2KO
Non-social exploration	488±14	444±15	395±16***	365±12***
Social investigation	41±5	32±6	84±17**	123±16**+
Unit of social investigation	1.06±0.1	1.13±0.2	1.7±0.2**	1.8±0.2**
Latency to social investigation	23±7	21±4	11±4	14±5

WT grouped $n=12$, CB2KO grouped $n=12$, WT isolated $n=12$, and CB2KO isolated $n=10$. Differences with respect to their corresponding WT group $+p<0.05$; differences with respect to grouped animals $^{**}p<0.01$, $^{***}p<0.001$; differences with respect isolated WT group $+p<0.05$

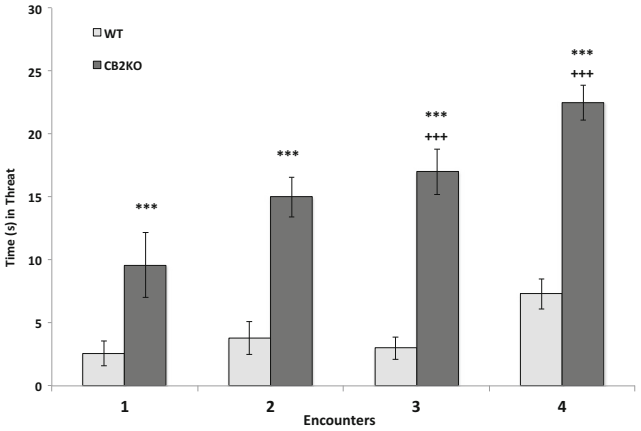

Fig. 2 Means of accumulated times (in seconds, with ±SEM) of time spent in threat by CB2KO and WT adult mice during the resident–intruder test. Animals underwent two training sessions on day 1 and two test sessions on day 2 (WT n=17; CB2KO n=15). Differences with respect to WT mice ***p<0.001

Spontaneous motor activity

The ANOVA of the mice's motor activity over 1 h (WT 1053±48 and CB2KO 1685±48 photocell cuts/h) revealed an effect of the interaction genetics×time [F(1, 30)=12.921; p<0.001], with CB2KO mice exhibiting more activity than WT in all the measures recorded (p<0.001 in all cases).

Elevated plus maze

The EPM data are presented in Table 2. The ANOVA for the time spent in the closed arms of the maze [F(1, 28)=9.138; p<0.01] and in the central area [F(1, 28)=15.092; p<0.001], revealed an effect of genetics, as the CB2KO mice spent more time in the closed arms but less time in the central area than their WT counterparts.

MAO-A, COMT, 5-HTT, and 5HT$_{1B}$r gene expression in CB2KO versus WT mice

Real-time PCR analyses revealed that expression of the MAO-A gene was enhanced in the AMY [Student t test: t=−2.767, df=14, p=0.01] and the DR [Student t test: t=−2.781, df=14, p<0.01] of CB2KO mice. On the other hand, the expression of the COMT gene was significantly higher in the AMY [Student t test: t=−2.142, df=14, p<0.05], while a decrease was detected in the DR [Student t test: t=4.215, df=14, p<0.01]. In CB2KO animals, 5HT$_{1B}$r and 5-HTT gene expression was upregulated in the AMY [Student t test: t=−7.811, df=14, p<0.001] and DR [Student t test: t=−3.829, df=14, p<0.001], respectively (Fig. 3a, b).

Table 2 Means of accumulated times (in seconds, with ±SEM) in the elevated plus maze (WT n=13; CB2KO n=17)

	WT	CB2KO
Time OA	32±6	36±6
% time OA	11±2	12±2
Time central	160±6	123±6***
Time CA	107±7	141±6***
Open entries	7±1	5±1
% open entries	24±5	22±3
Closed entries	18±1	16±1
Total entries	25±1	22±2

Differences with respect to WT mice ***p<0.001

Second experiment

Social behaviors

Kruskal–Wallis analysis showed a significant effect of administration of JWH in isolated OF1 mice with respect to the time spent in threat (χ^2(df=4, p=0.001)=25.340) and attack (χ^2(df=4, p=0.001)=21.947) (Fig. 4a), the latency to threat (χ^2(df=4, p=0.001)=19.336) or attack (χ^2(df=4, p=0.01)=16.210) (Fig. 4b), the unit of threat (χ^2(df=4, p=0.001)=26.345) and attack (χ^2(df=4, p=0.001)=23.983) (Fig. 4c), and the number of attacks (χ^2(df=4, p=0.001)=19.317) (Fig. 4d) (see Table 2 of the supplementary material for the Threat data). The Mann–Whitney U test revealed that isolated mice spent more time engaged in aggression and initiated this behavior sooner (p<0.001 in all cases). JWH133 significantly decreased aggression at all the doses tested (threat: p<0.001 for JWH-4 and p<0.01 for JWH-1 and 2; attack: p<0.001 for JWH-4, p<0.01 for JWH-2, and p<0.05 for JWH-1; latency of threat: p<0.05 in all cases; latency of attack: p<0.05 for JWH-2 and p<0.01 for JWH-4; unit of threat and attack: p<0.001 in all cases; number of attacks: p<0.01 in all cases). To rule out whether or not this effect was due to motor impairment, the motor effects of 2 and 4 mg/kg of this drug were tested. No differences were observed (data not shown).

In order to test the specificity of the actions of JWH, the effect of the CB2 antagonist AM630 was analyzed in isolated OF1 mice treated with 2 or 4 mg/kg and subsequently undergoing the social interaction test (see Table 3). The ANOVA showed an effect of AM630 on time engaged in attack [F(3, 35)=10.410; p<0.001] and number of attacks [F(3,35)=13.322; p<0.001]. The groups treated with AM630 plus JWH spent significantly more time engaged in attack and performed a higher number of attacks than those treated only with 2 (p<0.001) or 4 mg/kg (p<0.05) of JWH.

In addition, JWH (2 mg/kg) was tested in WT and CB2KO mice in the resident–intruder paradigm. The variable genetics showed a significant effect for threat, attack, and number of attacks. In all cases, CB2KO mice exhibited higher levels of

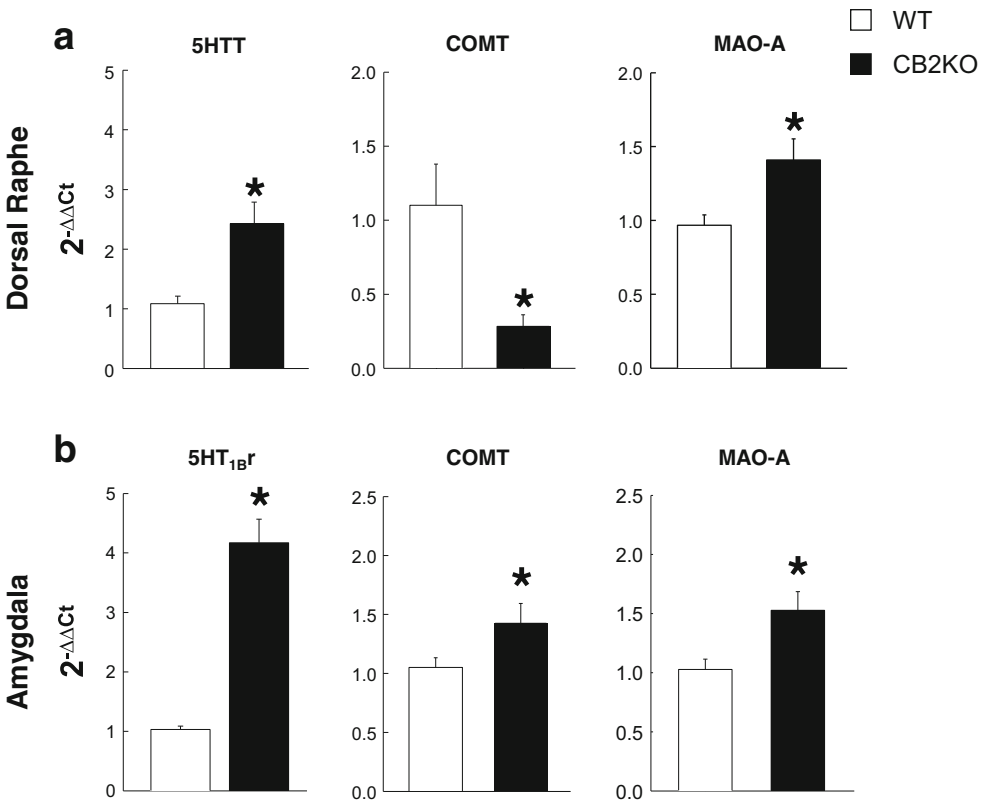

Fig. 3 MAO-A, COMT, 5-HTT, and $5HT_{1B}r$ relative gene expression in the DR and AMY of CB2KO and WT mice according to real-time PCR (n=8 in all the groups). *Columns* represent the means and *vertical lines* the ±SEM of relative gene expression ($2^{-\Delta\Delta Ct}$ method) in the DR (**a**) and AMY (**b**). *Asterisks* indicate values of CB2KO mice significantly different ($p<0.05$) from those of WT mice

aggression than WT mice ($p<0.001$, $p<0.01$, and $p<0.05$, respectively). ANOVA did not reveal any effect of this CB2 agonist (see Table 4).

MAO-A, COMT, 5-HTT, and $5HT_{1B}r$ gene expression in group-housed versus isolated vehicle-treated OF1 mice

The effect of isolation on MAO-A, COMT, 5-HTT, and $5HT_{1B}$ gene expression was evaluated by means of real-time PCR in group-housed and isolated vehicle-treated OF1 animals. MAO-A mRNA levels were significantly lower in the AMY of isolated OF1 mice [Student t test: t=2.957, df=18, $p<0.010$], though no differences were observed in the DR [Student t test: t=−0.534, df=18, $p<0.601$]. On the other hand, COMT gene expression in the DR was significantly reduced by isolation [Student t test: t=3.083, df=18, $p<0.01$], while no differences were observed in the AMY [Student t test: t=−0.303, df=18, $p<0.766$]. Finally, there was a significant increase of 5-HTT [Student t test: t=−3.219, df=18, $p<0.01$] and $5HT_{1B}r$ [Student t test: t=−2.703, df=18, $p<0.01$] in the DR and AMY, respectively, of isolated OF1 mice (Fig. 4a and b).

MAO-A, COMT, 5-HTT, and $5HT_{1B}r$ gene expression in isolated OF1 mice treated with the CB2r agonist JWH133

Real-time PCR experiments were carried out to evaluate the effect of JWH133 administration on isolated OF1 animals. MAO-A gene expression in the DR [$F(2,29)$=5.024; $p<0.01$] and AMY [$F(2,29)$=10.157; $p<0.001$] of isolated OF1 mice was significantly increased by treatment with this CB2r agonist. On the other hand, this treatment induced an increase of COMT gene expression in the AMY of isolated OF1 animals [$F(2,29)$=15.479; $p<0.001$], whereas there was a trend toward an increase in the DR, without reaching statistical significance [$F(2,29)$=2.944; $p<0.074$]. The CB2r agonist did not alter 5-HTT mRNA levels in the DR of isolated OF1 mice [$F(2,29)$=0.181; $p<0.836$] or significantly reduce $5HT_{1B}r$ gene expression in the AMY of the same animals [$F(2,29)$=2.001; $p<0.159$] (Figs. 5a, b and 6).

Discussion and conclusions

The present results demonstrate that CB2rs play a significant role in the regulation of aggressive behavior: (1) in the social interaction test, CB2KO mice are more aggressive than their WT counterparts when housed in groups; (2) in the resident–intruder paradigm, CB2KO are more aggressive that WT animals; (3) an acute dose of the CB2r agonist JWH133 decreases aggressive behavior in aggressive isolated OF mice; and (4) significant changes in MAO-A, 5-HTT, and $5\text{-}HT_{1B}$ gene expression occur in the DR and AMY of CB2KO versus WT mice and in aggressive OF1 mice treated with a vehicle versus JWH133.

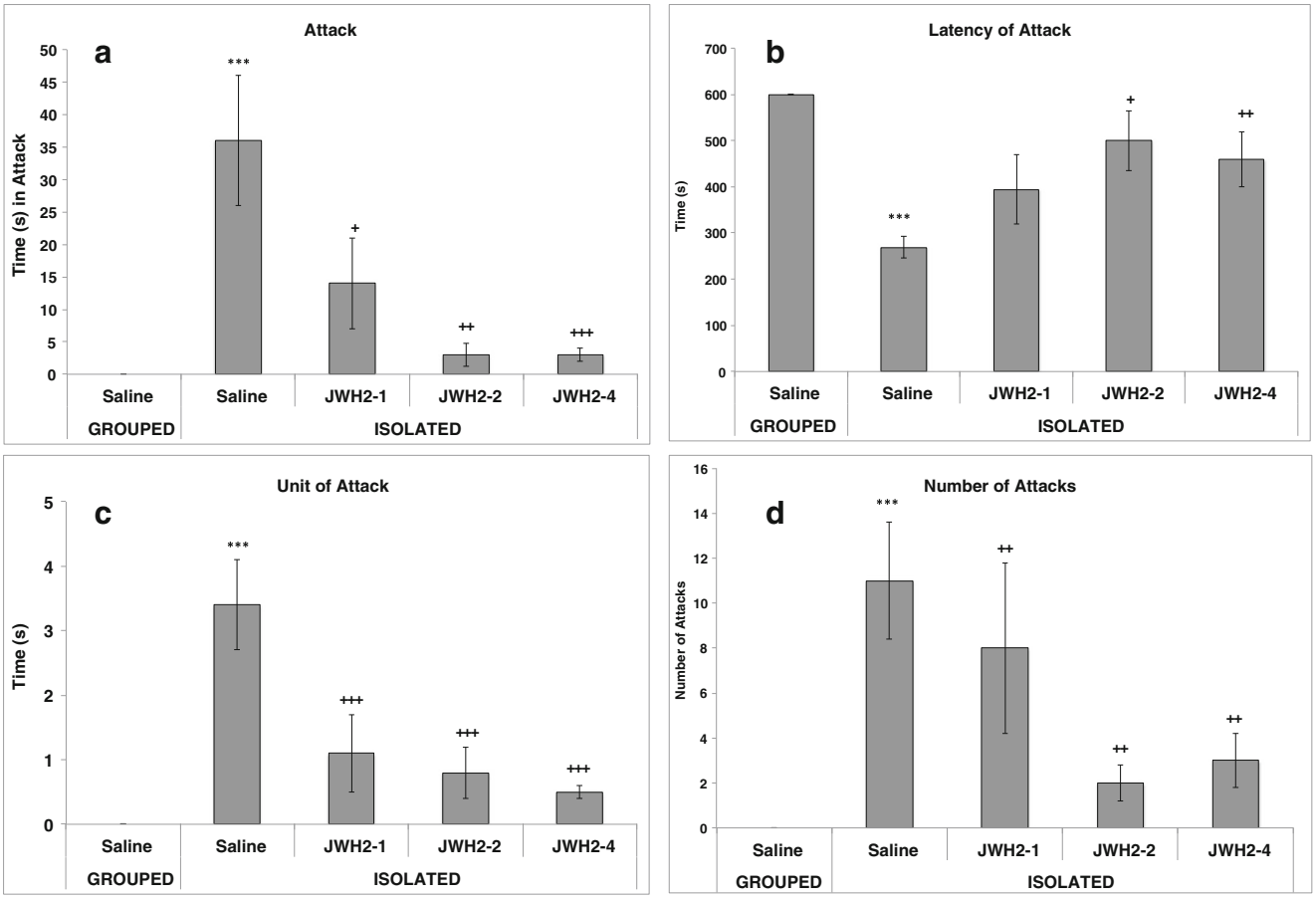

Fig. 4 Means of accumulated times (in seconds, with ±SEM) of attack (**a**), unit of attack (**b**), latency of attack (**c**), and number of attacks (**d**) exhibited by group-housed or isolated OF1 mice treated with vehicle or JWH133 (1, 2, and 4 mg/kg) during the social interaction test (grouped $n=10$, isolated $n=8$, JWH-1 $n=11$, JWH-2 $n=9$, JWH-4 $n=10$). Differences with respect to saline-treated grouped mice $^{***}p<0.001$; differences with respect to saline-treated isolated mice $^{+}p<0.05$; $^{++}p<0.01$; $^{+++}p<0.001$

In the social interaction test, an experimental mouse is confronted with a standard opponent that does not provoke aggression; in this way, aggressive behavior is always expressed by the experimental animal. When housed in groups, our CB2KO mice displayed higher levels of aggression during the social interaction test than their WT counterparts (which lacked this kind of behavior). The former mice

Table 3 Means of accumulated times (in seconds, with ±SEM) allocated to different categories of behavior during the social interaction test by isolated OF1 mice treated with JWH133 (2 and 4 mg/kg) plus AM630 (3 mg/kg)

	Sal		AM630-3	
	JWH-2	JWH-4	JWH-2	JWH-4
Threat	17±2	6±1	13±1	10±1
Latency of threat	213±40	290±65	143±28	178±32
Unit of threat	0.9±0.3	0.6±0.3	0.6±0.01	0.6±0.01
Attack	4±1.8	3±1	12±1***	8±1*
Latency of attack	296±54	459±51	261±37	331±33
Unit of attack	0.8±0.3	0.5±0.1	0.6±0.01	0.5±0.1
Number of attacks	1.9±0.8	3.7±1.2	19±1.8***	12±3*

JWH-2 $n=9$, JWH-4 $n=10$, JWH-2+AM630 $n=10$, and JWH-4+AM630 $n=10$. Differences with respect to JWH-2- or JWH-4-treated mice $^{*}p<0.05$; $^{***}p<0.001$

Table 4 Means of accumulated times (in seconds, with ±SEM) allocated to different categories of behavior during the resident–intruder paradigm by WT and CB2KO mice treated with saline or JWH133 (2 mg/kg)

	Sal		JWH-2	
	WT	CB2KO	WT	CB2KO
Threat	5±1	15±2.4***	5±1.8	10±2.7***
Latency of threat	68±21	56±29	51±25	37±25
Unit of threat	0.5±0.1	0.7±0.1	0.5±0.1	0.5±0.1
Attack	12±2.3	20±5.3**	6±2.1	20±6.2**
Latency of attack	81±21	113±38	56±25	70±28
Unit of attack	0.7±0.1	0.7±0.1	0.5±0.1	0.7±0.1
Number of attacks	15±2.5	25±7*	8±3	20±6*

WT+Sal $n=9$, WT+JWH $n=9$, CB2KO+Sal $n=9$, CB2KO+JWH $n=9$. Differences with respect to WT mice $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$

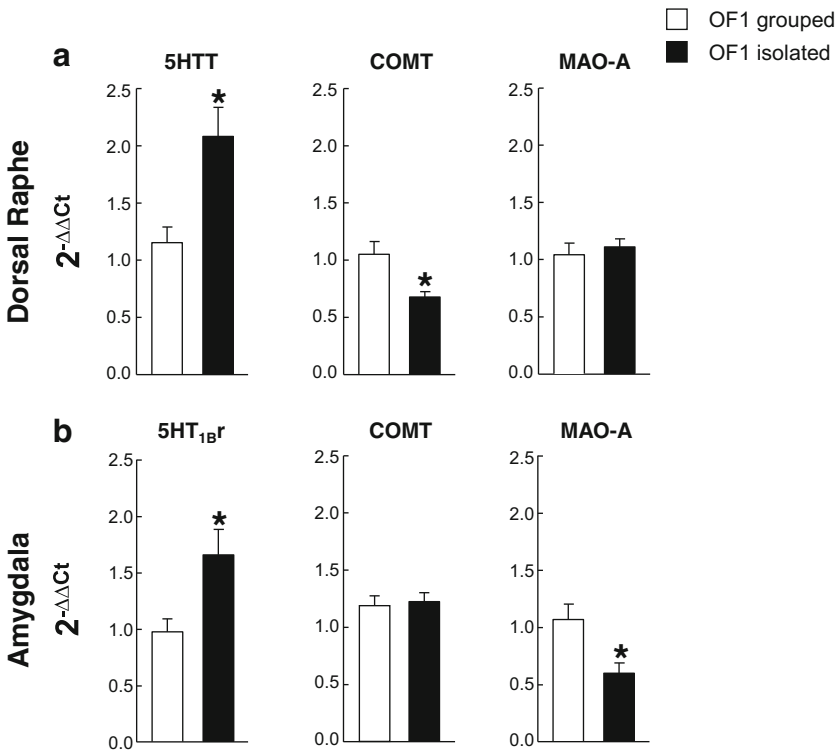

Fig. 5 Real-time PCR analysis of MAO-A, COMT, 5-HTT, and $5HT_{1B}r$ relative gene expression in the DR and AMY of group-housed and isolated saline-treated OF1 mice (n=10 in all the groups). *Columns* represent the means and *vertical lines* the ±SEM of relative gene expression ($2^{-\Delta\Delta Ct}$ method) in the DR (**a**) and AMY (**b**). *Asterisks* indicate values of isolated OF1 mice significantly different (p<0.05) from those of grouped OF1 mice

threatened and attacked their standard opponents in the absence of specific provocation. Similar results were obtained in the resident–intruder paradigm. Resident CB2KO mice threatened and attacked intruders more often than resident WT animals in all the staged encounters. Moreover, the former mice spent significantly more time engaged in threatening behavior (and showed a non-significant increment in attack) in all four encounters, an increase that was not observed in

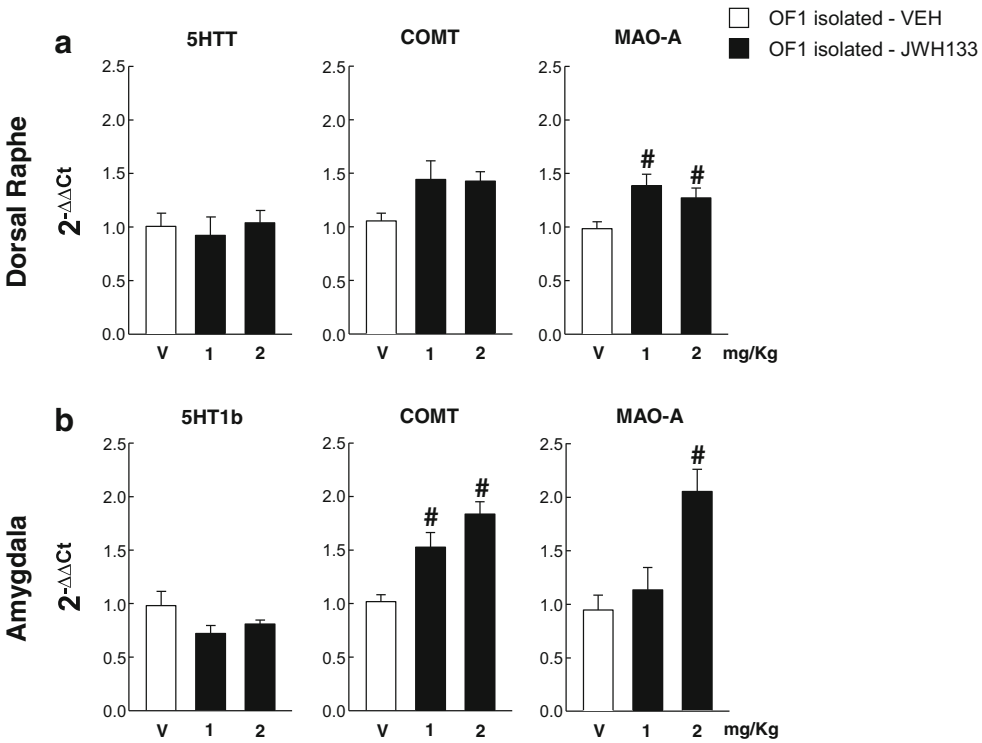

Fig. 6 Real-time PCR analysis of MAO-A, COMT, 5-HTT, and $5HT_{1B}r$ relative gene expression in the DR and AMY of isolated and JWH133-treated OF1 mice (n=10 in all the groups). *Columns* represent the means and *vertical lines* the ±SEM of relative gene expression ($2^{-\Delta\Delta Ct}$ method) in the DR (**a**) and AMY (**b**). *Octothorpes* indicate values of isolated JWH133-treated OF1 mice significantly different (p<0.05) from those of isolated vehicle-treated OF1 mice

WT mice. It should be taken into consideration that the higher levels of aggression observed in CB2KO mice in these two paradigms could have been indirectly influenced by the animals' increased motor activity. However, CB2KO mice did not show a different anxiety profile when they performed the EPM under low stress conditions.

On the other hand, long-term social isolation is also known to induce offensive and aggressive behavior in mice (Malick 1979; Valzelli 1985). In the present study, WT mice behaved aggressively after being isolated for 3 weeks, whereas no changes were observed in the aggressive behavior of CB2KO mice housed in the same conditions. The lack of an increase in aggression in CB2KO mice could have been due, among other reasons, to a ceiling effect with respect to the aggression displayed by group-housed KO mice or a lack of an effect of isolation on these mice. These results are in line with the behavior observed in CB1KO animals in a previous study in our laboratory (Rodriguez-Arias et al. 2013). Despite their higher level of aggression, group-housed CB2KO mice engaged in similar non-aggressive social interactions as WT mice. Following isolation, an increase in these social contacts was observed in both types of mice, though more so in CB2KO mice. These results contrast with previous reports concerning CB1KO mice, among which social contacts did not increase after isolation, and which showed avoidance and flee behaviors in accordance with the high levels of anxiety associated with these animals (Haller et al. 2002; Maccarrone et al. 2002; Martin et al. 2002; Urigüen et al. 2004). Such behaviors were absent in our CB2KO mice, though anxiogenic-like responses have been reported in some previous studies (Ortega-Alvaro et al. 2011). Therefore, we can affirm that group-housed CB2KO mice present a highly aggressive behavioral profile that is not modified by isolation, although this procedure did increase social interaction.

A neural circuit involving several brain regions—including the AMY—has been implicated in the regulation of emotions. Functional or structural alterations of one or more of these regions, or of the interconnections between them, may increase susceptibility to aggression and violence (Davidson 2000). Serotonin (5-HT) has been consistently implicated in the neurobiological mechanisms that mediate aggressive behavior. Indeed, numerous studies have related impulsive, hostile, and violent behavior with a deficiency in 5-HT (for review see Takahashi et al. 2012). A recent meta-analysis concluded that the results in question, though heterogeneous, confirmed a small, inverse correlation between 5-HT function and aggression, anger, and hostility (Duke et al. 2013). 5-HT in the mammalian central nervous system is derived mainly from the DR and MnR, and inhibition of the metabolism of monoamines increases the availability of 5-HT in the brain. We observed increased MAO-A gene expression in the DR and AMY of CB2KO mice, which suggested enhanced 5-HT metabolization. Expression of the COMT gene in the AMY was also more pronounced in these mice, while it was decreased in the DR. In contrast, 5-HTT gene expression in the DR was enhanced in CB2KO mice. Therefore, it could be hypothesized that the availability of 5-HT in each structure is lower due to increase of MAO-A gene expression in the AMY or of 5-HTT gene expression in the DR. These results are in line with previous findings in CB1KO mice (Rodríguez-Arias et al. 2013). Indeed, there are concordant reports regarding the relation between COMT levels and aggression. Two recent meta-analyses have linked COMT gene polymorphism to aggression, pointing to an association between the homozygous genotype of lower COMT activity polymorphism and violence (Bhakta et al. 2012; Singh et al. 2012). However, an even more recent report has failed to confirm these findings (Soyka et al. 2015). On the other hand, Ginsberg et al. (2011) reported a significant upregulation of COMT expression in several brain regions of aggressive resident mice. We have to bear in mind that, although real-time PCR is a highly sensitive technique capable of detecting small mRNA level changes, it is not possible to directly correlate these alterations with an increase in protein content. Intracellular metabolic factors such as posttranscriptional phenomena could account for differences between gene transcription and final protein expression (Maier et al. 2009).

Moreover, the possibility that the highly aggressive phenotype of CB2KO mice was associated with a developmental loss of CB1r cannot be ruled out. However, previous studies have shown that CB1r gene expression in the spinal cord of CB2KO mice does not differ to that in WT mice (La Porta et al. 2013, Fig. 5a), which makes differences in the brain of the two strains unlikely. In addition, real-time PCR studies in CB2KO mice has revealed alterations in the gene expression of targets related with the serotonergic system that differ from those observed as a consequence of CB1r deletion (Rodríguez-Arias et al. 2013). Indeed, although we observed higher levels of aggression in CB1KO and CB2KO mice, COMT gene expression was increased in the MnR, DR, and AMY of CB1KO mice, but only in the AMY of CB2KO mice, whereas it was decreased in the DR of the latter animals.

Most previous studies have found a correlation between depleted MAO-A function and increased aggression (Scott et al. 2008; Stetler et al. 2014). However, inhibition of MAO-A activity reduces the oxidative metabolism of monoamines, which presumably increases the availability of 5-HT and other monoamines in the brain. MAO-A inhibitors can be used as antiaggression drugs, though they are known to also alter other behaviors (for review see Takahashi et al. 2011). In line with this, a recent study has detected increased MAO-A gene expression in rats stressed during adolescence and exhibiting heightened aggressive behavior in adulthood (Marquez et al. 2013). Similarly to the results obtained in CB1KO mice, we have observed an increase of MAO-A gene expression in the AMY of CB2KO mice; this may have

reduced 5-HT levels, which underlay the elevated expression of $5HT_{1B}$r observed in these mice. In most studies, local activation of $5HT_{1B}$r in projection regions has been found to reduce aggressive behavior in various procedures and species (see Takahashi et al. 2012).

The CB2r agonist JWH133 has been shown to significantly decrease aggression in aggressive mice after a 3-week isolation procedure (Rodríguez-Arias et al. 1998, 2013). In the present study, JWH133 reduced the time spent by isolated mice in aggressive behaviors and the mean time employed in threat or attack (unit of threat and attack), delayed latency to threat at all of the doses administered, and decreased the number of attacks. This effect was not due to motor impairment and seemed to be mediated by CB2 receptors, as antagonism of this receptor with AM630 abolished the antiaggression effect. In addition, JWH133 did not induce any effect in CB2KO mice in the resident–intruder test. These results suggest that activation of CB2rs regulates aggressive response-modulating serotoninergic function in different brain areas. Several studies using in vivo microdialysis to evaluate the effect of social isolation rearing have found little or no changes in basal tissue levels or synaptic overflow in the serotonin system under such circumstances, which indicates normal tonic release. However, they have observed marked decreases in response to drugs and stressors in the cortex and hippocampus (for review see Marsden et al. 2011). In general, isolation affects serotoninergic more than dopaminergic neurotransmission. For example, isolation rearing has been shown to deplete 5-HT content and increase 3,4-dihydroxyphenylacetic acid (DOPAC) levels and turnover of 5-HT and DA in the hippocampus (Brenes and Fornaguera 2009).

In the present study, isolated OF1 mice displayed increased 5-HTT gene expression in the DR, which may have been related with an enhanced reuptake of 5-HT and a concomitant reduction in COMT gene expression. On the other hand, isolation increased $5HT_{1B}$r and decreased MAO-A gene expression. While the increase in 5-HTT in the DR was maintained in isolated mice treated with JWH133, there was a decrease in the $5HT_{1B}$ receptor in the AMY of the same animals. Treatment of isolated mice with JWH133 increased MAO-A in the DR and AMY and COMT in the AMY, which may have been related with the lower serotonin levels detected in comparison with saline-treated isolated and group-housed mice. Thus, isolated JWH133-treated mice would be expected to have lower serotonin levels than saline-treated isolated or grouped mice. It is important to point out that increases in MAO-A gene expression in the AMY and DR were observed in both CB2KO and OF1 mice treated with JWH133, although a completely different behavioral outcome was observed between the two groups. This discrepancy highlights the differences between a KO model, in which CB2 signaling was absent throughout the period of brain development, and studying the impact of stimulating a receptor in a situation of stress by isolation.

Aggressive behavior is defined in the DSM-IV R and the new DSM-V as a symptom of numerous psychiatric disorders, including schizophrenia, anxiety disorder, impulse control disorder, oppositional-defiant conduct, posttraumatic and borderline personality disorders, or antisocial personality disorder (Boles and Miotto 2003; Raine 2002; Rydén et al. 2009; Volavka 2013). Our findings support an important role for CB2rs in social interaction and aggressive behavior. Pharmacological manipulation of this receptor deserves further investigation as a potential target in the management of aggression-related psychiatric disorders.

Acknowledgments This work was supported by the following research grants: Ministerio de Ciencia e Innovación (SAF2011-23420 awarded to Jorge Manzanares; SAF2010-15793 awarded to Olga Valverde); Ministerio de Economía y Competitividad, Dirección General de Investigación (PSI2011-24762 awarded to PI Jose Miñarro); Generalidad Valenciana, Consejería de Educación (PROMETEO/2009/072 awarded to PI Jose Miñarro); Generalitat de Catalunya (2009SGR684 awarded to Olga Valverde); Instituto de Salud "Carlos III" (FIS); Redes Telemáticas de Investigación Cooperativa en Salud (RETICS); Red de Trastornos Adictivos (RTA); fondos FEDER (RD06/0001/1004 and RD12/0028/0019 awarded to PI Jorge Manzanares, RD06/001/0016 and RD12/0028/0005 awarded to PI Jose Miñarro, and RD06/001/1001 and RD12/0028/0024 awarded to PI Olga Valverde).

References

Bhakta SG, Zhang JP, Malhotra AK (2012) The COMT Met158 allele and violence in schizophrenia: a meta-analysis. Schizophr Res 140: 192–197. doi:10.1016/j.schres.2012.06.026

Boles SM, Miotto K (2003) Substance abuse and violence—a review of the literature. Aggress Violent Behav 8:155–174. doi:10.1016/S1359-1789(01)00057-X

Brain PF, Benton D, Childs G, Parmigiani S (1981) The effect of the type of opponent in test of murine aggression. Behav Process 6:319–327. doi:10.1016/0376-6357(81)90049-8

Brenes JC, Fornaguera J (2009) The effect of chronic fluoxetine on social isolation-induced changes on sucrose consumption, immobility behavior, and on serotonin and dopamine function in hippocampus and ventral striatum. Behav Brain Res 198:199–205. doi:10.1016/j.bbr.2008.10.036

Buckley NE, McCoy KL, Mezey E, Bonner T, Zimmer A, Felder CC, Glass M, Zimmer A (2000) Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB(2) receptor. Eur J Pharmacol 396:141–149. doi:10.1016/S0014-2999(00)00211-9

Carder B, Olson J (1972) Marihuana and shock induced aggression in rats. Physiol Behav 8:599–602. doi:10.1016/0031-9384(72)90081-9

Carlini EA, Gonzales C (1972) Aggressive behavior induced by marihuana compounds and by amphetamine in rats previously made dependent on morphine. Experimentia 28:542–544

Carlini EA, Lindsey CJ, Mustry RE, Monti JM (1976) Marihuana aggressiveness in REM-sleep-deprived rats: neurochemical and neurophysiological correlates. In: Braude MC, Szara S (eds) The pharmacology of marihuana. Raven, New York, pp 515–530

Davidson RJ (2000) Dysfunction in the neural circuitry of emotion regulation—a possible prelude to violence. Science 289:591–594. doi:10.1126/science.289.5479.591

Duke AA, Bègue L, Bell R, Eisenlohr-Moul T (2013) Revisiting the serotonin-aggression relation in humans: a meta-analysis. Psychol Bull 139:1148–1172. doi:10.1037/a0031544

El Marroun H, Hudziak JJ, Tiemeier H, Creemers H, Steegers EA, Jaddoe VW, Hofman A, Verhulst FC, van den Brink W, Huizink AC (2012) Intrauterine cannabis exposure leads to more aggressive behavior and attention problems in 18-month-old girls. Drug Alcohol Depend 118:470–474. doi:10.1016/j.drugalcdep.2011.03.004

García-Gutiérrez MS, Manzanares J (2011) Overexpression of CB2 cannabinoid receptors decreased vulnerability to anxiety and impaired anxiolytic action of alprazolam in mice. J Psychopharmacol 25:111–120. doi:10.1177/0269881110379507

García-Gutiérrez MS, Pérez-Ortiz JM, Gutiérrez-Adán A, Manzanares J (2010) Depression-resistant endophenotype in mice overexpressing cannabinoid CB(2) receptors. Br J Pharmacol 160:1773–1784. doi:10.1111/j.1476-5381.2010.00819.x

García-Gutiérrez MS, García-Bueno B, Zoppi S, Leza JC, Manzanares J (2012) Chronic blockade of cannabinoid CB2 receptors induces anxiolytic-like actions associated with alterations in GABA(A) receptors. Br J Pharmacol 165:951–964. doi:10.1111/j.1476-5381.2011.01625.x

Ginsberg SD, Che S, Hashim A, Zavadil J, Cancro R, Lee SH, Petkova E, Sershen HW, Volavka J (2011) Differential regulation of catechol-O-methyltransferase expression in a mouse model of aggression. Brain Struct Funct 216:347–356. doi:10.1007/s00429-011-0315-z

Gong JP, Onaivi ES, Ishiguro H, Liu QR, Tagliaferro PA, Brusco A, Uhl GR (2006) Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. Brain Res 1071:10–23. doi:10.1016/j.brainres.2005.11.035

Haller J, Bakos N, Szirmay M, Ledent C, Freund TF (2002) The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. Eur J Neurosci 16:1395–1398. doi:10.1046/j.1460-9568.2002.02192.x

La Porta C, Bura SA, Aracil-Fernández A, Manzanares J, Maldonado R (2013) Role of CB1 and CB2 cannabinoid receptors in the development of joint pain induced by monosodium iodoacetate. Pain 154:160–174. doi:10.1016/j.pain.2012.10.009

Maccarrone M, Valverde O, Barbaccia ML, Castañé A, Maldonado R, Ledent C, Parmentier M, Finazzi-Agrò A (2002) Age-related changes of anandamide metabolism in CB1 cannabinoid receptor knockout mice: correlation with behaviour. Eur J Neurosci 15:1178–1186. doi:10.1046/j.1460-9568.2002.01957.x

Maier T, Guell M, Serrano L (2009) Correlation of mRNA and protein in complex biological samples. FEBS Lett 583(24):3966–3973. doi:10.1016/j.febslet.2009.10.036

Malick JB (1979) The pharmacology of isolation-induced aggressive behavior in mice. Curr Rev Psychopharmacol 5:1–27

Márquez C, Poirier GL, Cordero MI, Larsen MH, Groner A, Marquis J, Magistretti PJ, Trono D, Sandi C (2013) Peripuberty stress leads to abnormal aggression, altered amygdala and orbitofrontal reactivity and increased prefrontal MAOA gene expression. Transl Psychiatry 3, e216. doi:10.1038/tp.2012.144

Marsden CA, King MV, Fone KC (2011) Influence of social isolation in the rat on serotonergic function and memory—relevance to models of schizophrenia and the role of 5-HT(6) receptors. Neuropharmacology 61:400–407. doi:10.1016/j.neuropharm.2011.03.003

Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O (2002) Involvement of CB1 cannabinoid receptors in emotional behaviour. Psychopharmacology 159:379–387. doi:10.1007/s00213-001-0946-5

Miczek KA (1978) Delta 9-tetrahydrocannabinol: antiaggressive effects in mice, rats, and squirrel monkeys. Science 199(4336):1459–1461

Miczek KA, O'Donnell JM (1978) Intruder-evoked aggression in isolated and nonisolated mice: effects of psychomotor stimulants and L-dopa. Psychopharmacology 57:47–55

Navarrete F, Perez-Ortiz JM, Manzanares J (2012) Cannabinoid CB2 receptor-mediated regulation of impulsive-like behaviour in DBA/2 mice. Br J Pharmacol 165:260–273. doi:10.1111/j.1476-5381.2011.01542.x

Onaivi ES (2006) Neuropsychobiological evidence for the functional presence and expression of cannabinoid CB2 receptors in the brain. Neuropsychobiology 54:231–246. doi:10.1159/000100778

Onaivi ES, Ishiguro H, Gong JP, Patel S, Meozzi PA, Myers L, Perchuk A, Mora Z, Tagliaferro PA, Gardner E, Brusco A, Akinshola BE, Hope B, Lujilde J, Inada T, Iwasaki S, Macharia D, Teasenfitz L, Arinami T, Uhl GR (2008) Brain neuronal CB2 cannabinoid receptors in drug abuse and depression: from mice to human subjects. PLoS ONE 3, e1640. doi:10.1196/annals.1432.036

Ortega-Alvaro A, Aracil-Fernández A, García-Gutiérrez MS, Navarrete F, Manzanares J (2011) Deletion of CB2 cannabinoid receptor induces schizophrenia-related behaviors in mice. Neuropsychopharmacology 36:1489–1504. doi:10.1038/npp.2011.34

Proietto J, Rissanen A, Harp JB, Erondu N, Yu Q, Suryawanshi S, Jones ME, Johnson-Levonas AO, Heymsfield SB, Kaufman KD, Amatruda JM (2010) A clinical trial assessing the safety and efficacy of the CB1R inverse agonist taranabant in obese and overweight patients: low-dose study. Int J Obes 34:1243–1254. doi:10.1038/ijo.2010.38

Raine A (2002) Biosocial studies of antisocial and violent behavior in children and adults: a review. J Abnorm Child Psychol 30:311–326

Rodríguez-Arias M, Miñarro J, Aguilar MA, Pinazo J, Simón VM (1998) Effects of risperidone and SCH 23390 on isolation-induced aggression in male mice. Eur Neuropsychopharmacol 8:95–103. doi:10.1016/S0924-977X(97)00051-5

Rodríguez-Arias M, Maldonado C, Vidal-Infer A, Guerri C, Aguilar MA, Miñarro J (2011) Intermittent ethanol exposure increases long-lasting behavioral and neurochemical effects of MDMA in adolescent mice. Psychopharmacology 218:429–442. doi:10.1007/s00213-011-2329-x

Rodriguez-Arias M, Navarrete F, Daza-Losada M, Navarro D, Aguilar MA, Berbel P, Miñarro J, Manzanares J (2013) CB1 cannabinoid receptor-mediated aggressive behavior. Neuropharmacology 75:172–180. doi:10.1016/j.neuropharm.2013.07.013

Rydén E, Thase ME, Straht D, Aberg-Wistedt A, Bejerot S, Landen M (2009) A history of childhood attention-deficit hyperactivity disorder (ADHD) impacts clinical outcome in adult bipolar patients regardless of current ADHD. Acta Psychiatr Scand 120:239–246. doi:10.1111/j.1600-0447.2009.01399.x

Schmittgen TD, Zakrajsek BA, Mills AG, Gorn V, Singer MJ, Reed MW (2000) Quantitative reverse transcription-polymerase chain reaction to study mRNA decay: comparison of endpoint and real-time methods. Anal Biochem 285:194–204. doi:10.1006/abio.2000.4753

Scott AL, Bortolato M, Chen K, Shih JC (2008) Novel monoamine oxidase A knock out mice with human-like spontaneous mutation. Neuroreport 19:739–743. doi:10.1097/WNR.0b013e3282fd6e88

Singh JP, Volavka J, Czobor P, Van Dorn RA (2012) A meta-analysis of the Val158Met COMT polymorphism and violent behavior in schizophrenia. PLoS One 7, e43423. doi:10.1371/journal.pone.0043423

Smoothy R, Brain PF, Berry MS, Haug M (1986) Alcohol and social behaviour in group-housed female mice. Physiol Behav 37:689–694. doi:10.1016/0031-9384(86)90173-3

Soyka M, Zill P, Koller G, Samochowiec A, Grzywacz A, Preuss UW (2015) Val158Met COMT polymorphism and risk of aggression in alcohol dependence. Addict Biol. doi:10.1111/adb.12098

Stetler DA, Davis C, Leavitt K, Schriger I, Benson K, Bhakta S, Wang LC, Oben C, Watters M, Haghnegahdar T, Bortolato M (2014) Association of low-activity MAOA allelic variants with violent crime in incarcerated offenders. J Psychiatr Res 58:69–75. doi:10.1016/j.jpsychires.2014.07.006

Sulcova E, Mechoula R, Fride E (1998) Biphasic effects of anandamide. Pharmacol Biochem Behav 59:347–353. doi:10.1016/S0091-3057(97)00422-X

Takahashi A, Quadros IM, de Almeida RM, Miczek KA (2011) Brain serotonin receptors and transporters: initiation vs. termination of escalated aggression. Psychopharmacology 213:183–212. doi:10.1007/s00213-010-2000-y

Takahashi A, Quadros IM, de Almeida RM, Miczek KA (2012) Behavioral and pharmacogenetics of aggressive behavior. Curr Top Behav Neurosci Behav Pharmacogenet Aggress Behav 12:76–138. doi:10.1007/7854_2011_191

Ueki S, Fujiwara M, Ogawa N (1972) Mouse-killing behavior (muricide) induced by D9-tetrahydrocannabinol in the rat. Physiol Behav 9:585–587. doi:10.1016/0031-9384(72)90016-9

Urigüen L, Pérez-Rial S, Ledent C, Palomo T, Manzanares J (2004) Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB1 receptors. Neuropharmacology 46:966–973. doi:10.1016/j.neuropharm.2004.01.003

Valverde O, Torrens M (2012) CB1 receptor-deficient mice as a model for depression. Neuroscience 204:193–206. doi:10.1016/j.neuroscience.2011.09.031

Valzelli L (1985) Animal models of behavioral pathology and violent aggression. Methods Find Exp Clin Pharmacol 4:189–193

Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, Stella N, Makriyannis A, Piomelli D, Davison JS, Marnett LJ, Di Marzo V, Pittman QJ, Patel KD, Sharkey KA (2005) Identification and functional characterization of brainstem cannabinoid CB2 receptors. Science 310:329–332. doi:10.1126/science.1115740

Volavka J (2013) Violence in schizophrenia and bipolar disorder. Psychiatr Danub 25:24–33