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Deletion of CB_2 Cannabinoid Receptor Induces Schizophrenia-Related Behaviors in Mice

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The possible role of the CB_2 receptor (CB_2r) in psychiatric disorders has been considered. Several animal models use knockout (KO) mice that display schizophrenia-like behaviors and this study evaluated the role of CB_2r in the regulation of such behaviors. Mice lacking the CB_2r (CB_2KO) were challenged in open field, light–dark box, elevated plus-maze, tail suspension, step down inhibitory avoidance, and pre-pulse inhibition tests (PPI). Furthermore, the effects of treatment with cocaine and risperidone were evaluated using the OF and the PPI test. Gene expression of dopamine D_2 (D_2r), adrenergic-α_{2C} ($\alpha_{2C}r$), serotonergic 5-HT_{2A} and 5-HT_{2C} receptors (5-$HT_{2A}r$ and 5-$HT_{2C}r$) were studied by RT-PCR in brain regions related to schizophrenia. Deletion of CB_2r decreased motor activity in the OF test, but enhanced response to acute cocaine and produced mood-related alterations, PPI deficit, and cognitive impairment. Chronic treatment with risperidone tended to impair PPI in WT mice, whereas it 'normalized' the PPI deficit in CB_2KO mice. CB_2KO mice presented increased D_2r and $\alpha_{2C}r$ gene expressions in the prefrontal cortex (PFC) and locus coeruleus (LC), decreased 5-$HT_{2C}r$ gene expression in the dorsal raphe (DR), and 5-$HT_{2A}r$ gene expression in the PFC. Chronic risperidone treatment in WT mice left $\alpha_{2C}r$ gene expression unchanged, decreased D_2r gene expression (15 μg/kg), and decreased 5-$HT_{2C}r$ and 5-$HT_{2A}r$ in PFC and DR. In CB_2KO, the gene expression of D_2r in the PFC, of $\alpha_{2C}r$ in the LC, and of 5-$HT_{2C}r$ and 5-$HT_{2A}r$ in PFC was reduced; 5-$HT_{2C}r$ and 5-$HT_{2A}r$ gene expressions in DR were increased after treatment with risperidone. These results suggest that deletion of CB_2r has a relation with schizophrenia-like behaviors. Pharmacological manipulation of CB_2r may merit further study as a potential therapeutic target for the treatment of schizophrenia-related disorders.
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INTRODUCTION

In recent years, it has been postulated that the endocannabinoid system may be an important element involved in the development of schizophrenia. This assumption is supported by several observations: (1) cannabis use was reported in around 50% of schizophrenic patients (Barnett *et al*, 2007; Bersani *et al*, 2002); (2) cannabis use is associated with an increased risk of developing schizophrenia (Andreasson *et al*, 1987; Zammit *et al*, 2002); (3) a decrease in the age of onset of schizophrenia has been reported (Sugranyes *et al*, 2009); and (4) schizophrenia-like symptoms may develop in non-schizophrenic cannabis users (Morgan and Curran, 2008). In addition, schizophrenic patients showed a worsening of both positive and negative symptoms and cognitive deficits after administration of Δ^9-THC (D'Souza *et al*, 2005). Indeed, high levels of anandamide have been found in plasma and cerebrospinal fluid (CSF) in antipsychotic-naive first-episode paranoid schizophrenics (Leweke *et al*, 1999; Yao *et al*, 2002), and increased CB_1 receptor (CB_1r) expression in the dorsolateral prefrontal cortex (PFC) of schizophrenic patients (Dean *et al*, 2001). It is interesting to note that drugs used to treat schizophrenia modify these abnormal parameters in the endogenous cannabinoid system. In fact, the increased levels of anandamide in the CSF (Giuffrida *et al*, 2004) and plasma (De Marchi *et al*, 2003) are normalized by antipsychotic treatment. On the other hand, antipsychotic treatment decrease CB_1r immunodensity in the PFC of subjects with schizophrenia as determined in post-mortem studies (Urigüen *et al*, 2009).

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As for the anatomic location of cannabinoid receptors, CB_1r is mainly expressed in the CNS, particularly the basal ganglia, hippocampus, cerebellum, and cerebral cortex (Abood *et al*, 2010a; Herkenham *et al*, 1990, 1991). In contrast, CB_2 receptor (CB_2r) has been considered the 'peripheral' cannabinoid receptor owing to its presence in the spleen and lymphocytes (Abood *et al*, 2010b; Munro *et al*, 1993). However, considerable functional and anatomic evidence suggests that CB_2r are expressed in the nervous system. CB_2r were recently found in the brainstem of rat, mouse, and ferret (Van Sickle *et al*, 2005). Further studies in rats have identified CB_2r distributed extensively throughout different brain areas, including the spinal nucleus, hippocampus, olfactory nucleus, cerebral cortex, amygdala, striatum, thalamus, and cerebellum (Atwood and Mackie, 2010; Gong *et al*, 2006; Onaivi *et al*, 2006). In addition, CB_2r gene expression has been identified in the thalamus, periaqueductal grey matter, cervical and thoracic spinal cord, and different brain nuclei, including the caudate–putamen, nucleus accumbens, cingulate cortex, amygdala, hippocampus, ventromedial hypothalamic nucleus, arcuate nucleus, substantia nigra, and dorsal and medial raphe nuclei (Garcia-Gutierrez *et al*, 2010; Racz *et al*, 2008).

In addition, recent studies have sketched out the potential role of cannabinoid CB_2r in the neurobiology of psychiatric disorders (Ishiguro *et al*, 2010; Onaivi, 2009). Interestingly, a close relation between diminished CB_2r function (polymorphism Q63R) and increased susceptibility to schizophrenia in the presence of other risk factors has been reported (Ishiguro *et al*, 2010). This observation supports the notion of a relationship between the cannabinoid system and the development of schizophrenia. It is important to point out the role of the CB_2r in the control of fundamental neural cell processes, such as proliferation and survival (Fernandez-Ruiz *et al*, 2007). The proliferation of hippocampal neural progenitors may be modulated through CB_2r (Goncalves *et al*, 2008; Palazuelos *et al*, 2006) and has resulted defective in CB_2KO (knockout) mice (Buckley *et al*, 2000). Therefore, it can be hypothesized that deletion of the *CB_2r* gene could result in the development of neurochemical abnormalities that may underlie possible behavioral alterations in several experimental paradigms.

On the other hand, several reports have pointed out that the deletion of CB_1r produces a behavioral endophenotype consisting of a high degree of anxiety and increased vulnerability to depression-like behaviors (Martin *et al*, 2002; Urigüen *et al*, 2004) and decreased memory impairment (Bohme *et al*, 1999; Reibaud *et al*, 1999). To date, information on the impact of the elimination of the CB_1r on the development of schizophrenia/like behaviors has not been available.

The aim of this study was to determine the response of CB_2KO mice in different behavioral and cognitive experimental paradigms and gene expression alterations in brain areas related to schizophrenia. This behavioral and cognitive profile covered motor, anxiety, depression, short- and long-term memory, and attention deficit. Furthermore, the effects of treatment with risperidone were evaluated by using the pre-pulse inhibition test (PPI) in wild-type (WT) and CB_2KO mice. The gene expression studies were designed to detect changes in dopamine D_2 receptor (D_2r) in the PFC, adrenergic-α_{2C} receptor (α_{2C}r) in the PFC and locus coeruleus (LC), and serotonergic 5-HT_{2A} and 5-HT_{2C} receptors (5-HT_{2A}r and 5-HT_{2C}r) gene expression in the PFC and dorsal raphe (DR) by real-time PCR of naive WT and CB_2KO mice treated with risperidone.

MATERIALS AND METHODS

Animals

Male CB_2KO mice on a C57BL/6J congenic background (kindly provided by Nancy E Buckley, Cal State Polytechnic University, Pomona, CA) were used. CB_2KO founders crossed with outbred CD1 (Charles River, L'Arbresle Cedex, France) background (Buckley *et al*, 2000). Homozygotes from CB_2KO ($n = 119$) and age-matched WT mice ($n = 108$) were used in all experiments. Mice were 2–3 months old and weighed 25–35 g at the beginning of the experiments. All animals were kept at controlled temperature ($23 \pm 2^{\circ}$C) and light conditions (light–dark cycle switching at 0800 hours and 2000 hours). All studies were conducted in compliance with Spanish Royal Decree 223/1998 of 14 March (BOE. 8 18), the Ministerial Order of 13 October 1989 (BOE 18), and European Council Directive of 24 November 1986 (86/609/EEC) regulating the care of experimental animals. The evaluation of behaviors in the light–dark box, elevated plus-maze, tail suspension, and memory tests were made manually under blind conditions.

Drugs

Cocaine hydrochloride was obtained from the Ministry of Health and Consumer Affairs (Ministerio de Sanidad y Consumo, AGEMED, Madrid, Spain) and was dissolved in sterile 0.9% physiological saline. Mice were administered a single intraperitoneal 15 mg/kg dose 10 min before motor activity assessment. The cocaine dose was selected based on previous experiments from our laboratory not showing stereotyped behavior (data not shown). The atypical antipsychotic risperidone (STADA, Barcelona, Spain) was dissolved in sterile 0.9% physiological saline and administered *per os* in a volume of 10 ml/kg. Risperidone was administered at doses of 15, 30, and 60 μg/kg, twice a day (0830 and 1800 hours). CB_2KO ($n = 36$) and WT ($n = 36$) animals treated with risperidone or saline were used for PPI and gene expression studies.

Motor Activity

Open field test. The open field consists of a transparent square cage $25 \times 25 \times 25\,cm^3$ with a white Plexiglas floor (Urigüen *et al*, 2004). Mice were individually placed in the center to initiate a 20-min test that was recorded with a video camera and analyzed with the SMART (Spontaneous Motor Activity Recording and Tracking) v.2.5.3 software system (Panlab, Barcelona, Spain). Traveled distance and mean speed were analyzed. At 10 min after cocaine administration, motor activity was measured for 20 min. Results obtained in this test were analyzed in the whole 20-min period and also divided into 5-min periods.

Anxiety-Like Behavior

Light–dark box test. This model (Crawley and Goodwin, 1980) consisted of two methacrylate boxes $20 \times 20 \times 15\,cm^3$, one transparent and one black and opaque, linked by an

opaque tunnel (4 cm). Light from a 60 W desk lamp located 25 cm above the light box provided room illumination. Mice were individually placed facing the black box and tested in 5-min sessions. The time spent in the lighted area and the number of transitions was recorded. A mouse that introduced three paws into the opposite side of the box was counted as a transition.

Elevated plus-maze test. This paradigm consisted of two open arms and two enclosed horizontal perpendicular arms 50 cm above the floor (Lister, 1987). The junction of four arms formed a central squared platform ($5 \times 5\,cm^2$). The test began with the animal being placed in the center of the apparatus facing one of the enclosed arms and allowed to explore freely for 5 min. We counted as arm entries the introduction of four paws into the arm. The time spent in the open arms and the number of open-arm entries was recorded.

Depression-Like Behavior

Tail suspension test. Mice were individually suspended by the tail at the edge of a lever suspended above the table top (the distance to the table surface was 35 cm), and affixed with adhesive tape placed approximately 1–2 cm from the tip of the tail (Vaugeois *et al*, 1997). The duration of immobility was measured for 6 min. In this situation, mice develop escape-oriented behaviors interspersed with increasingly longer bouts of immobility.

Evaluation of Short- and Long-Term Memory

Step down inhibitory avoidance. The apparatus is a $31 \times 19 \times 15\,cm^3$ acrylic box with a platform located next to a grid. Mice were placed on the platform and their latency to step down on the grid with all four paws was measured; a modified protocol was followed (Izquierdo *et al*, 1998). During the training session, immediately after stepping down on the grid, the animals received a 2.0-s, 0.4-mA scrambled foot shock. Retention tests were procedurally identical, except that no foot shock was given and the latency to step down in these conditions was taken as a measure of emotional memory. A ceiling of 180 s was imposed, that is, animals with a test latency of more than 180 s were counted as 180 s. Each animal was tested at 1 and 3 h after training (short-term memory) and at 24 h (long-term memory).

Sensorimotor Gating

Acoustic pre-pulse inhibition. Pre-pulse inhibition refers to the reduction in amplitude of the startle reflex that occurs when a brief, sub-threshold stimulus immediately precedes a startle stimulus (Hoffman and Ison, 1980). Startle responses were measured using the CIBERTEC REST 141 system (Madrid, Spain). The testing chamber consisted of a plastic adjustable cover mounted on a platform. Movement of the mice within the cover was detected by a piezoelectric accelerometer attached below the platform. A loudspeaker mounted 15 cm above the cover provided background white noise and both acoustic pulses and pre-pulses. The entire apparatus was housed in a ventilated enclosure. Presentation of acoustic pulse and pre-pulse stimuli was controlled by the MONRS software and interface system, which also digitized, rectified, and recorded the responses from the accelerometer. Mean startle amplitude was determined by averaging ten 100 ms readings taken from the beginning of the pulse stimulus onset. Test sessions consisted of no stimulus, pulse-only, and pre-pulse trials. Each 'pre-pulse' trial consisted of a 20-ms 68, 71, or 77 dB non-startling pre-pulse followed 100 ms later by a 40-ms startling pulse of 120 dB. In contrast, 'pulse-only' trials consisted of the 120-dB stimulus only, and 'no-stimulus' trials contained background noise only. To allow acclimatizing, 3 days before the performance of the test sessions, mice were placed each day in the apparatus for 5 min without background noise. Test sessions began with a 5-min acclimatization period using a background noise of 65 dB. The test sessions consisted of a series of 11 pulse-only trials for habituation purposes, followed by 10 trials of each pre-pulse intensity plus pulse, and 10 no-stimulus trials, all presented in a pseudorandom order with a 7–23 s inter-trial variable interval. The percentage of pre-pulse inhibition was defined as ((startle amplitude on pulse alone trials−startle amplitude on pre-pulse trials) $\times$ 100)/startle amplitude on pulse alone trials.

Experimental design. Pre-pulse inhibition experiments were carried out in two steps. Firstly, the PPI response was determined under baseline conditions ($n = 36$). After confirming a significant difference in the PPI response, the effect of chronic oral risperidone treatment (twice a day for 12 days) was tested in both CB_2KO ($n = 36$) and WT ($n = 36$) mice. Animals were randomly assigned to each treatment group (saline, risperidone 15, 30, and 60 μg/kg) in each genotype. PPI response was evaluated after 4, 8, and 12 days of treatment. Each PPI test session was conducted between 1200 and 1500 hours. At 1 h after the last test session, animals were killed and brains were removed for gene expression studies. The number of animals used for statistical analyses ($n = 32$ per genotype) was slightly lower than the initial number of treated animals owing to software failure ($n = 4$), escape from PPI restraint ($n = 3$), and accidental death after oral administration ($n = 1$).

Gene Expression Analyses

Gene expression studies focused on the main targets of the mechanism of action of risperidone, which is characterized by potent blockade of 5-HT_{2A}r coupled with the relatively weaker antagonism of the dopamine D_2r. In addition, this drug displays high affinity for serotonin 5-HT_{2C}r and adrenergic α_{2C}r (Schotte *et al*, 1995).

Real-time PCR. Mice were killed and brains were removed from the skull and frozen over dry ice. Coronal brain sections (500 μm) beginning at plates 19–20 (Paxinos and Franklin, 2001) were obtained in a cryostat (−10°C). The PFC, LC, and DR were microdissected according to a modification of the Palkovits method (Palkovits, 1983) as described previously (Garcia-Gutierrez *et al*, 2010). Total RNA was isolated from brain tissue micropunches using Trizol reagent (Invitrogen, Madrid, Spain) and subsequently retrotranscribed to cDNA. Quantitative analysis of

the relative abundance of 5-HT_{2A}r, 5-HT_{2C}r, D_2r, and α_{2C}r gene expressions was performed on the ABI PRISM 7700 Sequence Detector System (Applied Biosystems, Foster City, CA). All reagents were obtained from Applied Biosystems and the manufacturer protocols were followed. The reference gene used was 18S rRNA, detected using Taqman ribosomal RNA control reagents. All primer–probe combinations were optimized and validated for relative quantification of gene expression. Briefly, data for each target gene were normalized to the endogenous reference gene, and the fold change in target gene mRNA abundance was determined using the $2^{-\Delta\Delta Ct}$ method (Schmittgen *et al*, 2000). This quantification method involves comparing the Ct values of the samples of interest with a control or calibrator, such as a non-treated sample or RNA from normal tissue. The Ct values of both the calibrator and the samples of interest are normalized to an appropriate endogenous housekeeping gene (*18S rRNA*). CB_2KO ($n=10$) and WT ($n=8$) intact animals were used to study receptor gene expression under baseline conditions. Not all the samples analyzed resulted in useful data owing to failed reactions during RT-PCR processing. From the initial CB_2KO ($n=36$) and WT ($n=36$) mice treated with risperidone, eight samples were used per treatment group.

Statistical Analyses

In the open field, light–dark box, elevated plus-maze and tail suspension tests, amplitude of acoustic startle response baseline determination, and gene expression studies under baseline conditions, statistical analysis was performed using the Student's *t*-test for comparing two groups. One-way analysis of variance (ANOVA) with repeated measures was carried out for both 5-min periods in the open field and step down inhibitory avoidance tests, and pre-pulse inhibition first determination. When appropriate, *post hoc* individual differences between groups were determined using the Student–Newman–Keuls test. Two-way ANOVA was carried out to evaluate the dose–response effects of risperidone treatment on gene expression. When appropriate, *post hoc* individual differences between groups were determined using the Student–Newman–Keuls test. Two-way ANOVA with repeated measures was used to analyze the temporal course and dose–response effects of risperidone treatment on the amplitude of the acoustic startle response. Two-way ANOVA with two repeated measures was used to analyze the temporal course and dose–response effects of risperidone treatment on %PPI. Differences were considered significant if the probability of error was less than 5%. SigmaStat v3.11 and SPSS v17 software was used for all statistical analyses.

RESULTS

Assessment of Motor Activity: Open Field Test

Analyses of 20-min periods: CB_2KO mice ($n=14$) presented significantly shorter traveled distances compared with their respective controls ($n=10$) (Student's *t*-test, $t=2.482$, $p=0.021$, 22 d.f.) (Figure 1a). Interestingly, the administration of cocaine (15 mg/kg, intraperitoneal) significantly increased the traveled distance in CB_2KO mice ($n=14$) compared with WT mice ($n=10$) (Student's *t*-test, $t=-5.410$, $p<0.001$, 22 d.f.) (Figure 1c).

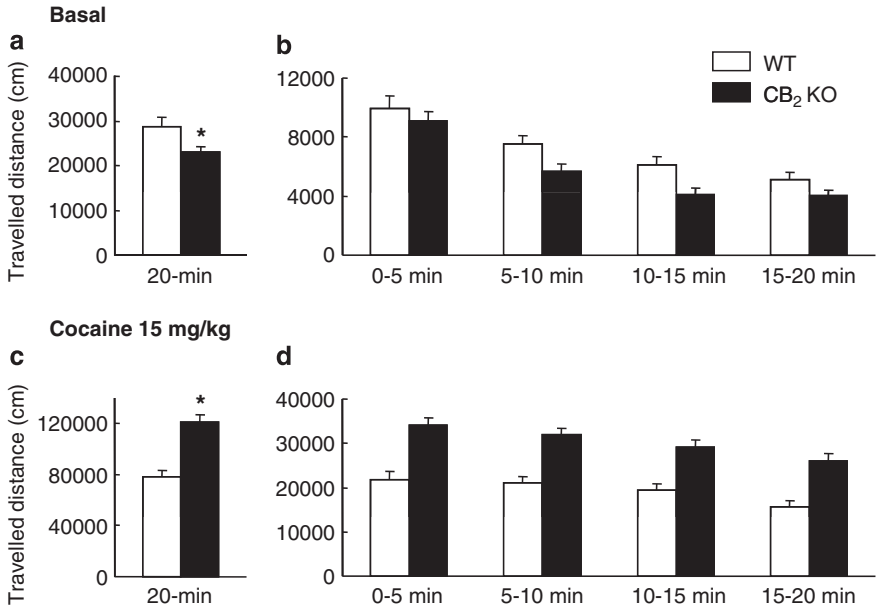

Figure 1 Evaluation of spontaneous motor activity in wild-type (WT) and CB_2KO (knockout) mice. Effect of cocaine on motor activity in both genotypes. The assessment of motor activity was determined measuring the traveled distance (cm) in the open field test during 20 min. In (a), the columns represent the means and the vertical lines represent the 1 ± standard error of mean (SEM) of the traveled distance (cm) by CB_2KO compared with WT mice, under baseline conditions. In (c), WT and CB_2KO mice received a single cocaine dose (15 mg/kg) and 10 min after the traveled distance was measured during 20 min. Columns represent the means and the vertical lines represent the 1 ± SEM of the traveled distance (cm) in mice treated with cocaine (15 mg/kg). In (b) and (d), the columns represent the means and the vertical lines represent the 1 ± SEM of the traveled distance (cm) by CB_2KO compared with WT mice analyzed in 5-min periods. *Values from CB_2KO mice that differ significantly from values in WT mice (Student's *t*-test, $p<0.05$).

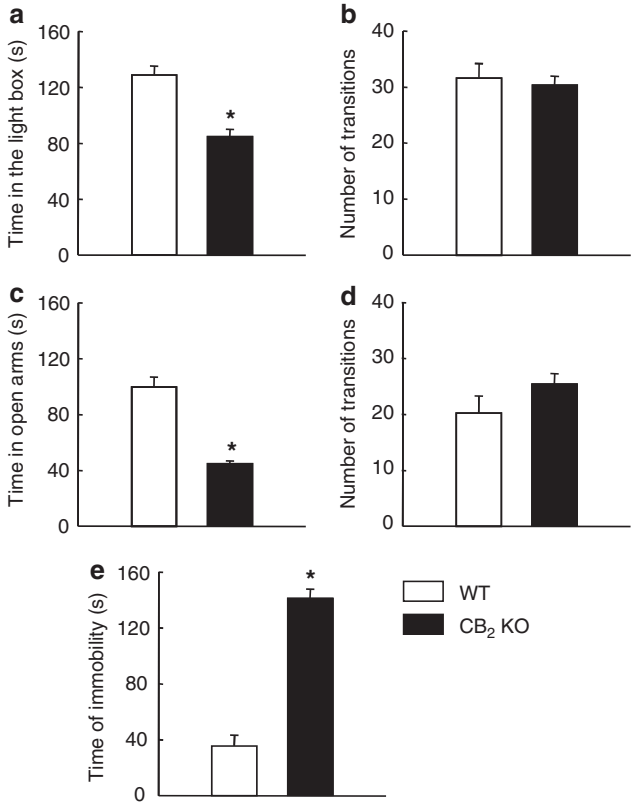

Figure 2 Evaluation of anxiogenic- and depressive-like behaviors in wild-type (WT) and CB_2KO (knockout) mice. The assessment of anxiogenic-like behaviors in WT and CB_2KO mice was carried out by using the light–dark box and the elevated plus maze. Columns represent the means and vertical lines represent the 1 ± standard error of mean (SEM) of the time spent in the light side of the light–dark box and the time spent in the open arms of the elevated plus maze (s), (a) and (c) respectively. Columns represent the means and vertical lines represent the 1 ± SEM of the number of transitions in the light–dark box and in the open arms, (b) and (d) respectively. In (e), the assessment of depressive-like behaviors in WT and CB_2KO mice in tail suspension. Columns represent the means and vertical lines represent the 1 ± SEM of the time of immobility (s). *Values from CB_2KO that differ significantly from WT values (Student's *t*-test, $p<0.05$).

Analyses of 5-min periods: Under baseline conditions, CB_2KO mice ($n=14$) presented significantly shorter traveled distances compared with their corresponding control ($n=10$) in the open field test. One-way ANOVA with repeated measures showed the significant main effects of genotype ($F_{(1,22)}=6.150$, $p=0.021$) and time course ($F_{(1,22)}=124.922$, $p<0.001$), but not significant effect of the genotype × time-course interaction ($F_{(1,22)}=0.097$, $p=0.759$) (Figure 1b). The administration of cocaine (15 mg/kg, intraperitoneal) significantly increased the traveled distance in CB_2KO ($n=14$) compared with WT mice ($n=10$). One-way ANOVA with repeated measures showed significant main effects of genotype ($F_{(1,22)}=29.271$, $p<0.001$) and time course ($F_{(1,22)}=36.851$, $p<0.001$), but no significant effect of the genotype × time-course interaction ($F_{(1,22)}=0.773$, $p=0.389$) (Figure 1d).

Assessment of Anxiety-Like Behaviors

CB_2KO mice ($n=12$) spent significantly less time in the light box compared with WT mice ($n=14$) (Student's *t*-test, $t=4.367$, $p<0.001$, 24 d.f.) (Figure 2a). No differences were observed between the two genotypes in the number of transitions (Student's *t*-test, $t=0.341$, $p=0.736$, 24 d.f.) (Figure 2b).

In the elevated plus-maze test, the percentage of time spent in the open arms significantly decreased in CB_2KO ($n=12$) compared with WT mice ($n=14$) (Student's *t*-test, $t=5.440$, $p<0.001$, 24 d.f.) (Figure 2c). No differences were observed in the number of transitions between compartments (Student's *t*-test, $t=-1.024$, $p=0.316$, 24 d.f.) (Figure 2d).

Assessment of Depressive-Like Behaviors: Tail Suspension Test

Exposure to the tail suspension induced a higher immobility time in CB_2KO mice ($n=8$) than in WT littermates ($n=10$) (Student's *t*-test, $t=-10.047$, $p<0.001$, 16 d.f.), revealing increased despair behavior in the mice that lacked CB_2r (Figure 2e).

Assessment of Memory Impairment: Step Down Inhibitory Avoidance

No difference was observed in the pre-training session between CB_2KO ($n=11$) and WT ($n=8$) mice. Interestingly, the evaluation of representative parameters of short-term memory revealed that CB_2KO presented a shorter latency time at 1 and 3 h after scrambled foot shock. Moreover, CB_2KO mice had a lower latency time at 24 h (long-term memory) (one-way ANOVA with repeated measures followed by Student–Newman–Keuls test, genotype $F_{(1,17)}=7.446$, $p=0.014$; time $F_{(1,17)}=6.297$, $p=0.023$; genotype × time $F_{(1,17)}=8.069$, $p=0.011$) (Figure 3).

Assessment of Sensorimotor Gating: Pre-pulse Inhibition

CB_2KO mice ($n=36$) did not show differences in the acoustic startle response amplitude compared with WT mice ($n=36$) (Student's *t*-test, $t=0.265$, $p=0.792$, 70 d.f.) (Figure 4a). On the other hand, PPI was significantly decreased in CB_2KO compared with WT mice. One-way ANOVA with repeated measures revealed the significant main effects of genotype ($F_{(1,70)}=16.762$, $p<0.001$) and pre-pulse intensity ($F_{(1,70)}=230.285$, $p<0.001$), whereas the genotype × pre-pulse intensity interaction was not significant ($F_{(1,70)}=0.155$, $p=0.695$) (Figure 4b). Therefore, the reduction of PPI observed in CB_2KO mice was independent of the pre-pulse intensity tested.

Time Course and Dose Response of the Effects of Treatment with Risperidone on Acoustic Startle Response Amplitude

Risperidone treatment had a statistically significant effect, whereas genotype factor ($n=32$) did not alter the acoustic startle response amplitude (Figure 5, Table 1). The time of treatment induced significant changes in startle amplitude.

Time Course and Dose Response of the Effects of Treatment with Risperidone on %PPI

The complexity of this experimental design did not allow the main factors of variation, genotype, and risperidone

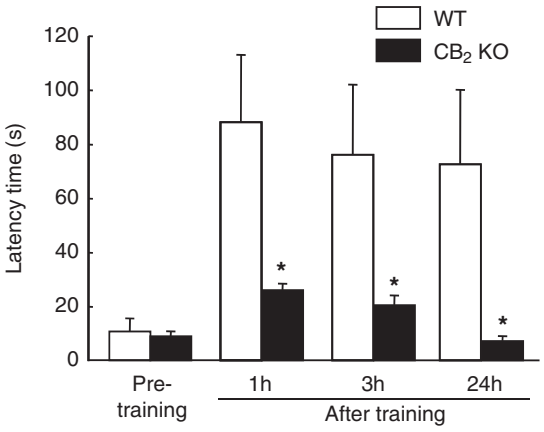

Figure 3 Evaluation of short- and long-term memory in wild-type (WT) and CB_2KO (knockout) mice. The memory assessment of WT and CB_2KO mice was evaluated using the step down inhibitory avoidance task. Columns represent the means and vertical lines represent the 1 ± standard error of mean (SEM) of latency time before descent from the platform at four time points (pre-training, 1, 3, and 24 h). *Values from CB_2KO mice that differ significantly from values in WT mice (Student–Newman–Keuls test, $p<0.05$).

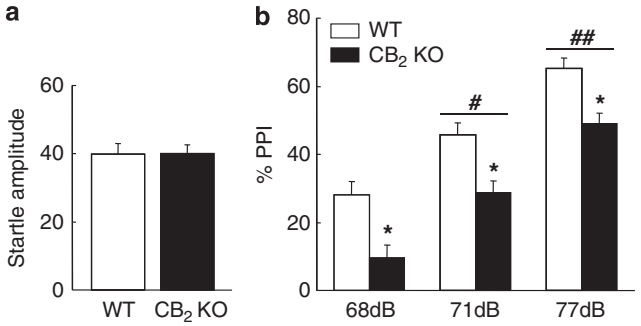

Figure 4 Amplitude and pre-pulse inhibition of the acoustic startle response in wild-type (WT) and CB_2KO (knockout) mice. Startle amplitude was measured using 120-dB pulse trials in WT and CB_2KO mice. In (a), the columns represent the means and the vertical lines represent the 1 ± SEM of the startle amplitude. Pre-pulse inhibition of the acoustic startle response was measured using 68, 71, and 77-dB pre-pulse stimuli in WT and CB_2KO mice. In (b), the columns represent the means and the vertical lines represent the 1 ± SEM of % pre-pulse inhibition. *Values from CB_2KO mice that differ significantly from the values in WT mice (one-way analysis of variance (ANOVA) with repeated measures, genotype, $p<0.001$). #Values obtained at 71 dB that differ significantly from those obtained at 68 dB; and ##values obtained at 77 dB that differ significantly from those obtained at 68 and 71 dB (one-way ANOVA with repeated measures, intensity, $p<0.001$).

treatment to reach statistical significance (Figure 6, Table 2). However, the two factors of repetition, pre-pulse intensity and time of treatment, have emerged as relevant factors of variation. In the case of pre-pulse intensity, more intense pre-pulse induced a higher %PPI value. In addition, the significant interaction between these two factors and genotype indicates that the evolution of %PPI differed in WT and CB_2KO mice. Thus, WT mice treated with saline showed a tendency toward lower %PPI (10–15%) over the course of treatment. In contrast, CB_2KO mice treated with saline showed a tendency toward higher %PPI (5–15%) in the same period. Although CB_2KO mice had a lower %PPI in the baseline determination, this slight increase could indicate that these mice maintain capacity to improve pre-attention. The differences between genotypes in the temporal course of %PPI were also observed in risperidone-treated animals. Thus, risperidone treatment enhanced the reduction of %PPI observed in saline-treated WT mice. The lowest and medium doses resulted in a 12–17%, decrease, and the highest dose in a 16–21% decrease. Consequently, risperidone treatment tended to reduce pre-pulse inhibition. On the other hand, risperidone treatment increased %PPI in CB_2KO mice. The percentage PPI increased by 25–36% (15 μg/kg), 25–30% (30 μg/kg), and 32–34% (60 μg/kg). In CB_2KO mice, risperidone treatment tended to improve PPI response (Figure 6, Table 2).

Evaluation of D_2r, α_{2C}r, 5-HT_{2C}r, and 5-HT_{2A}r Gene Expressions

Dopamine D_2r gene expression was studied in the PFC under baseline conditions and after 12 days of treatment with risperidone (15, 30, and 60 μg/kg, *per os*) or saline in WT and CB_2KO mice. D_2r gene expression was significantly increased in the PFC of CB_2KO mice compared with WT mice (Student's *t*-test, $t=-2.933$, $p=0.013$, 12 d.f.) ($n=6$–8) (Figure 7a). After 12 days, risperidone treatment modified D_2r gene expression in the PFC (two-way ANOVA, genotype: $F_{(1,63)}=5.397$, $p=0.024$; risperidone treatment: $F_{(3,63)}=6.805$, $p<0.001$; genotype × treatment interaction: $F_{(3,63)}=6.856$, $p<0.001$). In WT mice ($n=8$ per group), only the 15 μg/kg risperidone dose significantly reduced D_2r gene expression in the PFC (Student–Newman–Keuls test, $p<0.05$) (Figure 7b). In CB_2KO mice ($n=8$ per group), treatment with all risperidone doses (15, 30, and 60 μg/kg, *per os*) significantly reduced D_2r gene expression in the PFC (Student–Newman–Keuls test, $p<0.05$) (Figure 7b). Saline treatment did not modify the observed differences (under baseline conditions) in D_2r gene expression between CB_2KO and WT mice (Student–Newman–Keuls test, $p<0.05$) (Figure 7b). D_2r gene expression at doses of 30 and 60 μg/kg of risperidone was significantly reduced in CB_2KO compared with risperidone-treated WT mice (Student–Newman–Keuls test, $p<0.05$) (Figure 7b).

Secondly, α_{2C}r gene expression was analyzed in the PFC and the LC under baseline conditions and after 12 days of treatment with risperidone (15, 30, and 60 μg/kg, *per os*) or saline in WT and CB_2KO mice. The α_{2C}r gene expression was significantly increased in the PFC (Student's *t*-test, $t=-4.262$, $p=0.003$, 9 d.f.) ($n=5$–6) (Figure 8a) and LC (Student's *t*-test, $t=-2.420$, $p=0.039$, 10 d.f.) ($n=6$) (Figure 8c) of CB_2KO compared with WT mice under baseline conditions. In the PFC, risperidone or saline treatment did not modify the α_{2C}r gene expression found under baseline conditions (two-way ANOVA, genotype: $F_{(1,63)}=960.581$, $p<0.001$; risperidone treatment: $F_{(3,63)}=0.426$, $p=0.735$; genotype × treatment interaction: $F_{(3,63)}=1.447$, $p=0.239$) (Figure 8b). In the LC, risperidone treatment reduced α_{2C}r gene expression only in CB_2KO mice ($n=8$ per group), whereas it failed to alter α_{2C}r gene expression in WT mice ($n=8$ per group) (two-way ANOVA, genotype: $F_{(1,63)}=114.593$, $p<0.001$; risperidone

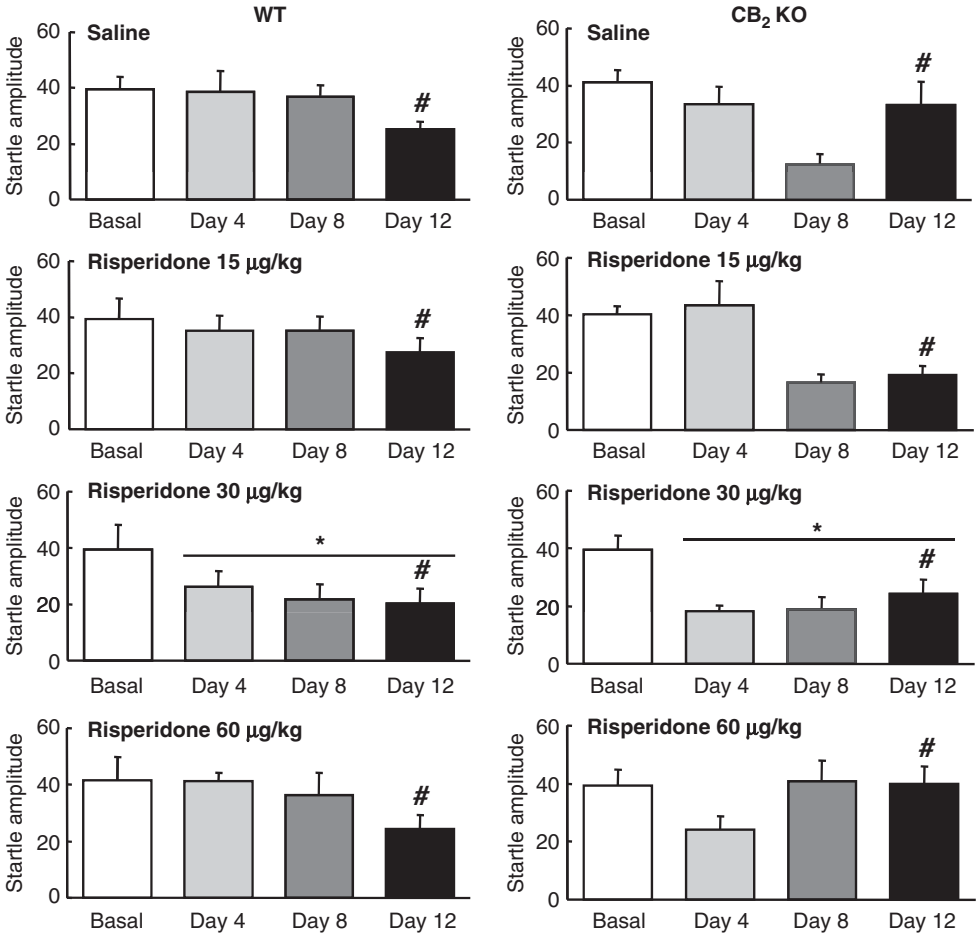

Figure 5 Effects of risperidone on the amplitude of the acoustic startle response in wild-type (WT) and CB_2KO (knockout) mice. The effect of oral chronic treatment with risperidone on the amplitude of the acoustic startle response was measured using 120 dB pulse trials in WT and CB_2KO mice. Determinations were performed before (baseline), and after 4, 8, and 12 days of treatment. Each panel corresponds with different groups of treatment with saline or risperidone (15, 30, and 60 μg/kg, *per os*). Columns represent the means and vertical lines represent the 1 ± standard error of mean (SEM) of % startle amplitude. *Values from risperidone-treated CB_2KO and WT mice that differ significantly from values in saline-treated CB_2KO and WT mice (two-way analysis of variance (ANOVA) with repeated measures, treatment, $p<0.05$). #Values from day 12 that differ significantly from baseline (two-way ANOVA with repeated measures, time, $p<0.001$).

Table 1 Results of Two-way ANOVA with Repeated Measures Performed on Startle Amplitude Data

Factors	d.f.	F	*p*-value
Between subject			
Genotype	1, 59	1.447	0.234
Treatment	3, 59	3.380	<0.05
Within subject			
Time	1, 59	33.348	<0.001
Time × genotype	1, 59	0.319	0.574
Time × treatment	3, 59	1.477	0.230

treatment: $F_{(3,63)}=5.204$, $p=0.003$; genotype × treatment interaction: $F_{(3,63)}=5.077$, $p=0.004$) (Figure 8d). Treatment with all the doses studied of risperidone in CB_2KO significantly reduced α_{2C}r gene expression in the LC (Student–Newman–Keuls test, $p<0.05$) (Figure 8d). Although treatment with all the doses of risperidone studied reduced gene expression level in CB_2KO, these levels were significantly higher than those observed in WT mice (Student–Newman–Keuls, $p<0.05$) (Figure 8d).

Thirdly, 5-HT_{2C}r gene expression was examined in the PFC and DR under baseline conditions and after 12 days of risperidone treatment (15, 30, and 60 μg/kg, *per os*) or saline in WT and CB_2KO mice. Under baseline conditions, no difference was observed between the two genotypes in the PFC (Student's *t*-test, $t=-0.025$, $p=0.981$, 10 d.f.) ($n=6$) (Figure 9a). However, risperidone treatment reduced 5-HT_{2C}r gene expression in the PFC in both genotypes (two-way ANOVA, genotype: $F_{(1,63)}=237.027$, $p<0.001$; risperidone treatment: $F_{(3,63)}=36.346$, $p<0.001$; genotype × treatment interaction: $F_{(3,63)}=37.532$, $p<0.001$). In WT mice ($n=8$ per group), oral risperidone treatment (15, 30, and 60 μg/kg, *per os*) significantly reduced 5-HT_{2C}r gene expression in the PFC (Student–Newman–Keuls test, $p<0.05$) (Figure 9b). In CB_2KO mice ($n=8$ per group), only risperidone at the dose of 60 μg/kg (*per os*) significantly reduced 5-HT_{2C}r gene expression in the PFC

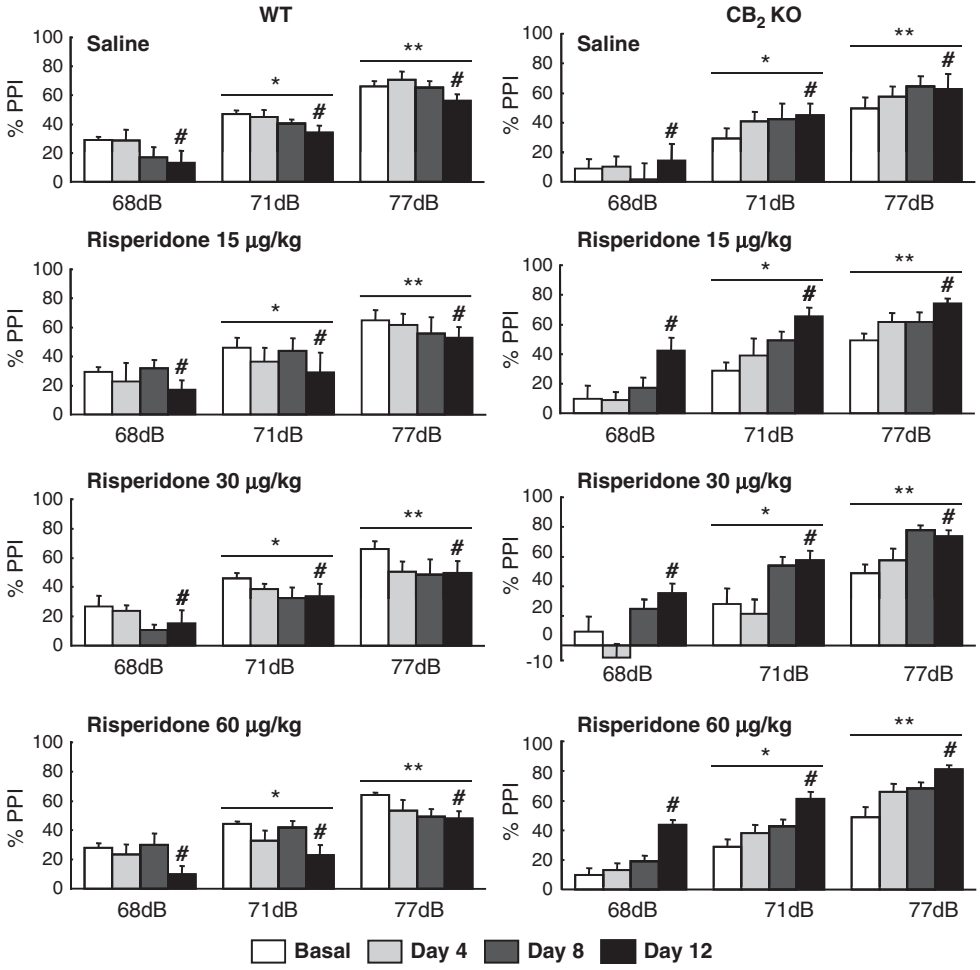

Figure 6 Effects of risperidone on pre-pulse inhibition test (PPI) of the acoustic startle response in wild-type (WT) and CB_2KO (knockout) mice. The effect of oral chronic treatment with risperidone on pre-pulse inhibition of the acoustic startle response was measured using 68, 71, and 77 dB pre-pulse stimuli in WT and CB_2KO mice. Determinations were performed before (baseline), and after 4, 8, and 12 days of treatment. Each panel corresponds to different groups of treatment with saline or risperidone (15, 30, and 60 μg/kg, *per os*). Columns represent the means and vertical lines represent the 1 ± standard error of mean (SEM) of % pre-pulse inhibition. *Values obtained at 71 dB that differ significantly from those obtained at 68 dB; **values obtained at 77 dB that differ significantly from those obtained at 68 and 71 dB (two-way analysis of variance (ANOVA) with two repeated measures, intensity, $p<0.001$). #Values from day 12 that differ significantly from baseline (two-way ANOVA with two repeated measures, time, $p<0.05$).

compared with the saline group (Student–Newman–Keuls test, $p<0.05$) (Figure 9b). The reduction observed in the risperidone-treated WT mice was significant compared with risperidone-treated CB_2KO mice at all doses (Student–Newman–Keuls test, $p<0.05$) (Figure 9b). On the other hand, under baseline conditions, CB_2KO presented significantly reduced 5-HT_{2C}r gene expression in the DR compared with WT mice (Student's *t*-test, $t=2.919$, $p=0.011$, 14 d.f.) ($n=7$–9) (Figure 9c). The effect of risperidone treatment on 5-HT_{2C}r gene expression in the DR was different in WT and CB_2KO mice (two-way ANOVA, genotype: $F_{(1,63)}=4.901$, $p=0.031$; risperidone treatment: $F_{(3,63)}=4.164$, $p=0.010$; genotype × treatment interaction: $F_{(3,63)}=14.026$, $p<0.001$). In WT mice ($n=8$ per group), 30 and 60 μg/kg risperidone doses significantly reduced 5-HT_{2C}r gene expression in the DR (Student–Newman–Keuls test, $p<0.05$) (Figure 9d). In contrast, in CB_2KO mice ($n=8$ per group) the 60 μg/kg risperidone dose significantly increased 5-HT_{2C}r gene expression in the DR (Student–Newman–Keuls test, $p<0.05$) (Figure 9d). Significant differences between CB_2KO and WT mice were observed in saline-treated and 30 and 60 μg/kg risperidone-treated mice (Student–Newman–Keuls test, $p<0.05$) (Figure 9d).

Finally, 5-HT_{2A}r gene expression was examined in the PFC and DR under baseline conditions and after 12 days of risperidone (15, 30, and 60 μg/kg, *per os*) or saline treatment in WT and CB_2KO mice. Under baseline conditions, gene expression of 5-HT_{2A}r was significantly reduced in the PFC of CB_2KO compared with WT mice (Student's *t*-test, $t=2.502$, $p=0.024$, 15 d.f.) ($n=7$–10) (Figure 10a). Risperidone treatment had not significant effect of on 5-HT_{2A}r gene expression in the PFC (two-way ANOVA, genotype: $F_{(1,63)}=13.838$, $p<0.001$; risperidone treatment: $F_{(3,63)}=2.756$, $p=0.051$; genotype × treatment interaction: $F_{(3,63)}=0.274$, $p=0.844$). The differences observed in CB_2KO and WT mice treated with risperidone ($n=8$ per group) were due to the genotype factor (Figure 10b). In the DR under baseline conditions, no significant difference was observed in 5-HT_{2A}r gene expression between CB_2KO and WT mice (Student's *t*-test, $t=1.533$, $p=0.164$, 8 d.f.) ($n=5$) (Figure 10c). However, risperidone treatment induced different effects on 5-HT_{2A}r gene expression in the DR of

WT and CB_2KO mice (two-way ANOVA, genotype: $F_{(1,63)} = 36.216$, $p < 0.001$; risperidone treatment: $F_{(3,63)} = 4.012$, $p = 0.012$; genotype × treatment interaction: $F_{(3,63)} = 23.454$, $p < 0.001$). In WT mice ($n = 8$ per group), treatment with risperidone doses of 30 and 60 μg/kg significantly reduced 5-HT_{2A}r gene expression in the DR (Student–Newman–Keuls test, $p < 0.05$) (Figure 10d). In CB_2KO mice ($n = 8$ per group), the 60 μg/kg risperidone dose significantly increased 5-HT_{2A}r gene expression in the DR (Student–Newman–Keuls test, $p < 0.05$) (Figure 10d). Significant differences between CB_2KO and WT were observed in mice treated with 30 and 60 μg/kg risperidone (Student–Newman–Keuls test, $p < 0.05$) (Figure 10d).

DISCUSSION

The results of this study provide new information about a possible role of the CB_2r in the regulation of schizophrenia-like behaviors. This claim is supported by several observations: (1) CB_2KO mice exhibited decreased spontaneous motor activity and increased sensitivity to the motor stimulant effects of acute cocaine administration in the open field test; (2) deletion of *CB_2r* gene produced an anxiogenic-like response in the light–dark box and elevated plus-maze tests, and a depressogenic-like response in the tail suspension test; (3) CB_2KO mice showed disrupted short- and long-term memory consolidation in the step down inhibitory avoidance paradigm; (4) the PPI of the acoustic startle response was significantly lower in CB_2KO mice compared with WT mice; (5) PPI was markedly enhanced after chronic oral treatment with the antipsychotic drug risperidone in CB_2KO mice, but was not affected in WT mice; (6) deletion of CB_2r's increased D_2r and α_{2C}r gene expression in the PFC and LC and decreased 5-HT_{2C}r gene expression in the DR and 5-HT_{2A}r gene expression in the PFC of CB_2KO compared with WT mice; (7) oral risperidone treatment of WT mice did not affect α_{2C}r gene expression, decreased D_2r (15 μg/kg) in the PFC, and decreased 5-HT_{2C}r and 5-HT_{2A}r in the PFC and DR; and (8) treatment with risperidone in CB_2KO mice reduced gene expressions of D_2r in the PFC, α_{2C}r in the LC, and 5-HT_{2C}r and 5-HT_{2A}r in the PFC; risperidone treatment increased 5-HT_{2C}r and 5-HT_{2A}r gene expression in the DR.

It is known that schizophrenia is associated with brain abnormalities induced during CNS development (Rapoport *et al*, 2005; Ross *et al*, 2006). A number of findings suggest a pro-neurogenic role of CB_2r in the control of fundamental neural cell processes (Galve-Roperh *et al*, 2008; Harkany *et al*, 2007; Katona and Freund, 2008). Therefore, it can be hypothesized that the lack of CB_2r might impair neural development, thus inducing relevant alterations in several brain areas. In this context, it can be postulated that these alterations could be the substrate underlying the behavioral modifications observed in CB_2KO mice. However, the existence of developmental compensatory mechanisms related to the lack of this receptor cannot be excluded. In addition, it is well known that the systemic administration of receptor antagonists does not mimic the situation of a KO mouse for the same receptor. The blockade of the CB_2r has been involved in the prevention of alcohol preference development (Ishiguro *et al*, 2006), the inhibition of food consumption, and the enhancement of spontaneous activity and stereotyped behavior in C57BL/6 and DBA/2 mice (Onaivi *et al*, 2008). In contrast, pre-pulse inhibition or

Table 2 Results of Two-way ANOVA with Two Repeated Measures Performed on PPI Data

Factors	d.f.	F	*p*-value
Between subject			
Genotype	1, 59	0.160	0.690
Treatment	3, 59	0.417	0.741
Within subject			
Pre-pulse intensity	1, 59	925.419	<0.001
Pre-pulse intensity × genotype	1, 59	16.421	<0.001
Pre-pulse intensity × treatment	3, 59	2.163	0.102
Time	1, 59	5.264	<0.05
Time × genotype	1, 59	55.211	<0.001
Time × treatment	3, 59	0.986	0.406
Pre-pulse intensity × time	1, 59	0.022	0.884
Pre-pulse intensity × time × genotype	1, 59	0.041	0.840
Pre-pulse intensity × time × treatment	3, 59	1.113	0.351

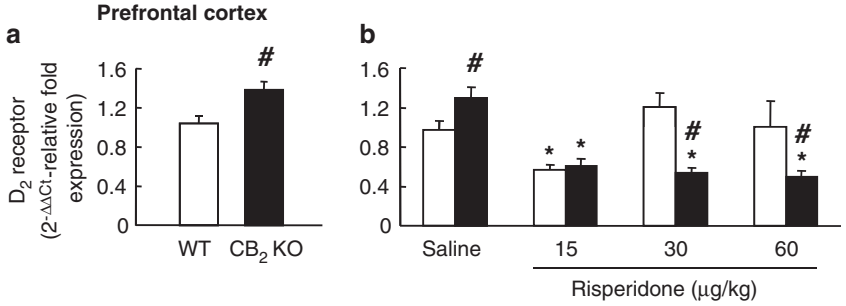

Figure 7 Evaluation of dopamine D_2 receptor (D_2r) gene expression in the prefrontal cortex. In (a), columns represent the means and vertical lines represent the 1 ± standard error of mean (SEM) of $2^{-\Delta\Delta Ct}$ of relative D_2r gene expression in WT and CB_2KO mice under baseline conditions. In (b), WT and CB_2KO mice received risperidone (15, 30, or 60 μg/kg/day; 12 days; *per os*; 0.3 ml per mice) or saline. Columns represent the means and vertical lines represent the 1 ± SEM of $2^{-\Delta\Delta Ct}$ of relative D_2r gene expression on day 12. #Values from CB_2KO mice that differ significantly from values in WT mice (Student–Newman–Keuls, $p < 0.05$). *Values from WT and CB_2KO mice treated with risperidone that differ significantly from values in saline-treated mice (Student–Newman–Keuls, $p < 0.05$).

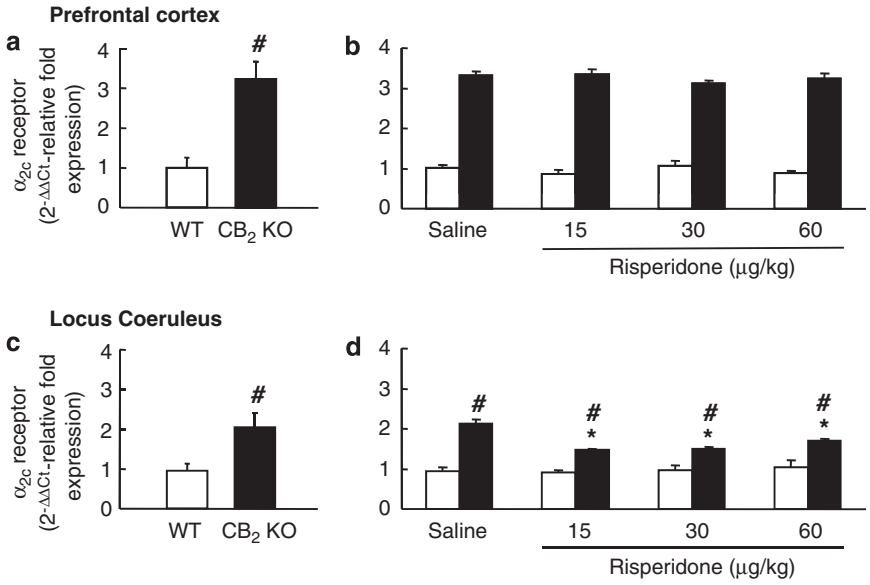

Figure 8 Evaluation of adrenergic α_{2C} receptor (α_{2c}r) gene expression in the prefrontal cortex (PFC) and locus coeruleus (LC). In (a) and (c), columns represent the means and vertical lines represent the 1 ± standard error of mean (SEM) of $2^{-\Delta\Delta Ct}$ of relative α_{2C}r gene expression in the PFC and LC, respectively, of wild-type (WT) and CB_2KO (knockout) mice under baseline conditions. In (b) and (d), WT and CB_2KO mice received risperidone (15, 30, or 60 μg/kg/day; 12 days; *per os*; 0.3 ml per mice) or saline. Columns represent the means and vertical lines represent the 1 ± SEM of $2^{-\Delta\Delta Ct}$ of relative α_{2C}r adrenergic gene expression in the PFC and LC on day 12. #Values from CB_2KO mice that differ significantly from values in WT mice (Student's *t*-test, $p < 0.05$). *Values from CB_2KO mice treated with risperidone that differ significantly from values in saline-treated CB_2KO mice (Student–Newman–Keuls, $p < 0.05$).

locomotor activity was not affected in mice (Ishiguro *et al*, 2010). Indeed, transient inactivation of the receptor by CB_2r antagonist use in WT mice does not induce the behavioral abnormalities observed in CB_2KO mice.

In recent years, a considerable number of susceptibility genes for schizophrenia have been described (Desbonnet *et al*, 2009). The generation of mice with targeted mutation of these genes was focused on the development of genetic models of the putative regulation of the pathophysiological mechanisms (Gray *et al*, 2009; Halene *et al*, 2009; Han *et al*, 2009; Perry *et al*, 2009; Powell *et al*, 2008; Rojas *et al*, 2007; Sakae *et al*, 2008; Tanda *et al*, 2009; Wiedholz *et al*, 2008) and genetic models of risk for schizophrenia (Bégou *et al*, 2008; Dyck *et al*, 2009; Karl *et al*, 2007; Kvajo *et al*, 2008; Willi *et al*, 2010). Schizophrenia includes abnormalities in coordination and movement including extrapyramidal paradigms (tending toward higher motor activity) and catatonia (usually lower motor activity) (Peralta *et al*, 2010). Schizophrenia manifests a spectrum of different levels of motor activity observable as endophenotypes of this disease. Mice with mutations in the genes of susceptibility to schizophrenia exhibited increased (Gray *et al*, 2009; Powell *et al*, 2008; Sakae *et al*, 2008; Wiedholz *et al*, 2008), decreased (Hattori *et al*, 2008), or failure to alter (Wolinsky *et al*, 2007) motor activity. The results found in this study revealed that deletion of the *CB_2r* gene significantly reduced spontaneous motor activity in the open field and increased sensitivity to the motor stimulant effects of acute cocaine administration. Similarly, PKCI/HINT1 KO mice presented diminished spontaneous motor activity with increased sensitivity to the motor action of amphetamine (Barbier *et al*, 2007). In contrast, mice that were genetically modified at the NMDA or dopamine receptors presented increased spontaneous motor activity and reduced reactivity to dizocilpine, phencyclidine, amphetamine, or cocaine (Gainetdinov *et al*, 1999; Mohn *et al*, 1999). The limitations of this paradigm seem to be related to the lack of specificity and predictive value of the effects on negative or cognitive symptoms. In addition, it has been reported that individuals with bipolar disorder and schizophrenia have distinctive profiles of exploratory behavior (Perry *et al*, 2009). Patients with bipolar mania present high motor activity and increased object interaction, whereas patients with schizophrenia exhibit normal object interaction (Perry *et al*, 2009). As patients with bipolar disorder present reduced PPI (Giakoumaki *et al*, 2007) and increased motor activity (Perry *et al*, 2009), it is possible that mutant mice with motor hyperactivity and reduced PPI are more closely related to animal models of bipolar disorder than to schizophrenia.

The response of CB_2KO mice has been studied in animal models of anxiety and depression. It is important to note that schizophrenic patients report depression and anxiety as the most frequent early signs and symptoms occurring before the first psychotic episode (Iyer *et al*, 2008). Thus, the characterization of clinically important phenomena observed before the onset of psychosis could be important in the diagnostic process. CB_2KO mice display increased anxiogenic-like response in the light–dark box and elevated plus-maze tests. Similarly, mutant mice like the heterozygous YWHAE and homozygous sandy show moderately enhanced anxiety-like behavior in the elevated plus-maze test (Hattori *et al*, 2008; Ikeda *et al*, 2008), whereas other mice with deletion of schizophrenia-related genes did not show any variation in anxiety-like behaviors in the light–dark box and elevated plus-maze tests compared with WT mice (Barbier and Wang, 2009; Hsu *et al*, 2007; Wolinsky *et al*, 2007). On the other hand, mice with

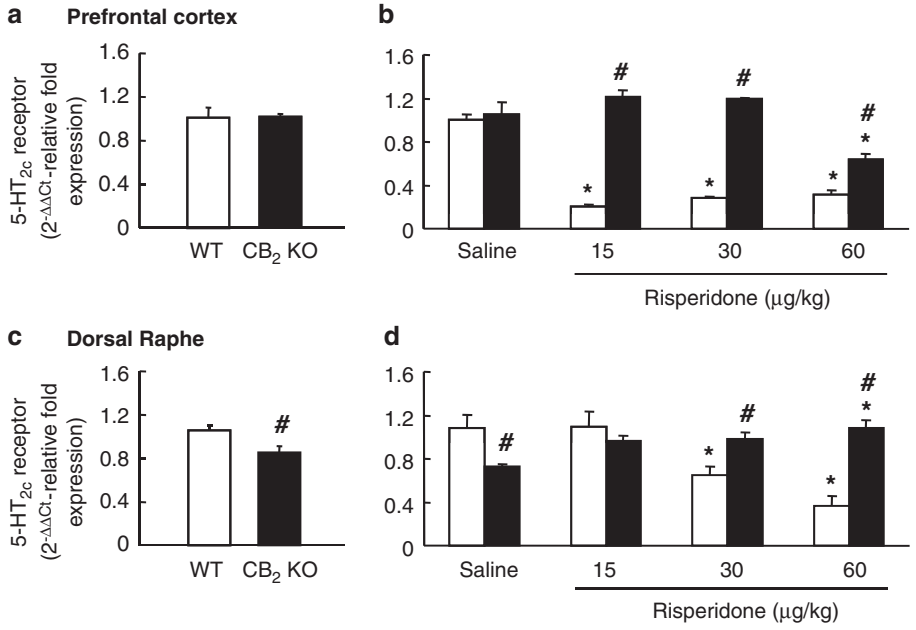

Figure 9 Evaluation of serotonergic 5-HT_{2C} receptor (5-HT_{2C}r) gene expression in the prefrontal cortex (PFC) and dorsal raphe (DR). In (a) and (c), columns represent the means and vertical lines represent the 1 ± standard error of mean (SEM) of $2^{-\Delta\Delta Ct}$ of 5-HT_{2C}r gene expression in the PFC and DR, respectively, of wild-type (WT) and CB_2KO (knockout) mice. In (b) and (d), WT and CB_2KO mice received risperidone (15, 30, or 60 μg/kg/day; 12 days; *per os*; 0.3 ml per mice) or saline. Columns represent the means and vertical lines represent the 1 ± SEM of $2^{-\Delta\Delta Ct}$ of 5-HT_{2C}r gene expression in the PFC and DR on day 12. #Values from CB_2KO mice that differ significantly from values in WT mice (Student's *t*-test, $p<0.05$). *Values from WT and CB_2KO mice treated with risperidone that differ significantly from the values in the saline-treated mice (Student–Newman–Keuls, $p<0.05$).

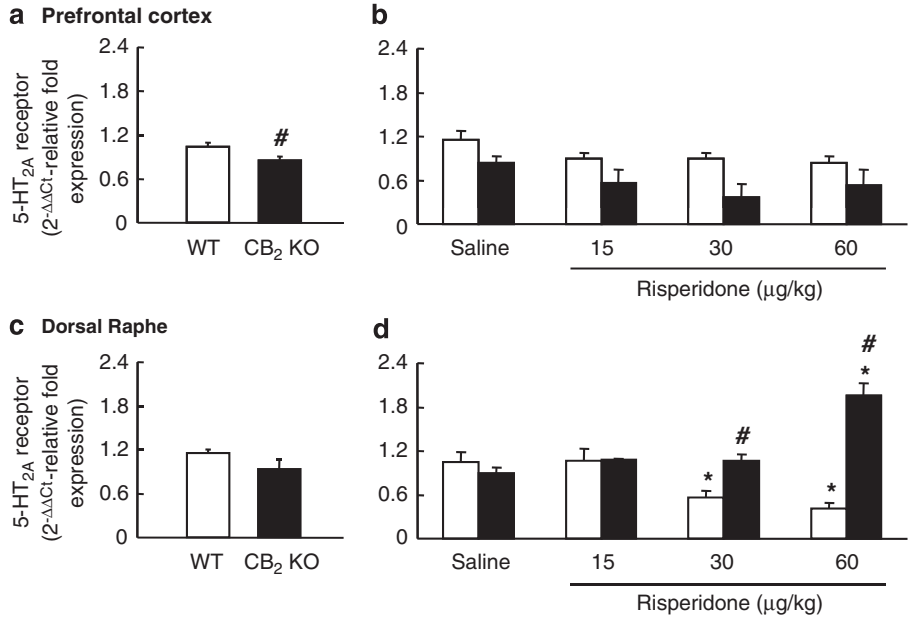

Figure 10 Evaluation of serotonergic 5-HT_{2A} receptor (5-HT_{2A}r) gene expression in the prefrontal cortex (PFC) and dorsal raphe (DR). In (a) and (c), columns represent the means and vertical lines represent the 1 ± standard error of mean (SEM) of $2^{-\Delta\Delta Ct}$ of relative 5-HT_{2A}r gene expression in the PFC and DR, respectively, of wild-type (WT) and CB_2KO (knockout) mice. In (b) and (d), WT and CB_2KO mice received risperidone (15, 30, or 60 μg/kg/day; 12 days; *per os*; 0.3 ml per mice) or saline. Columns represent the means and vertical lines represent the 1 ± SEM of $2^{-\Delta\Delta Ct}$ of relative 5-HT_{2A}r gene expression in the PFC and DR on day 12. #Values of CB_2KO mice that differ significantly from the values in WT mice (Student's *t*-test, $p<0.05$). *Values from WT and CB_2KO mice treated with risperidone that differ significantly from the values in saline-treated mice (Student–Newman–Keuls, $p<0.05$).

overexpression of CB_2r presented reduced anxiety-like behaviors using the same experimental paradigm (García-Gutiérrez and Manzanares, 2011). Deletion of the *CB_2r* gene induced depressive-like responses in the tail suspension test. Similarly, Homer1-KO mice and STOP-null mice show increased depressive-like behavior in the Porsolt test (Delotterie *et al*, 2010; Szumlinski *et al*, 2005). However, other animal models based on schizophrenia susceptibility

gene mutations showed an antidepressant-like response (Barbier and Wang, 2009; Perona *et al*, 2008; Sakae *et al*, 2008; Tanda *et al*, 2009; Yamasaki *et al*, 2008). In contrast, overexpression of CB_2r resulted in decreased depressive-like behaviors in acute models (tail suspension and novelty-suppressed feeding test) and in exposure to chronic mild stress, suggesting that pharmacological manipulation of this receptor may be an interesting therapeutic target in depression-related behaviors (Garcia-Gutierrez *et al*, 2010).

Cognitive dysfunction is one of the three main clusters of symptoms in schizophrenia and rodent models involving susceptibility genes related to schizophrenia manifesting as significant deficits in working memory and spatial learning (Gainetdinov *et al*, 1999; Ikeda *et al*, 2008; Yamasaki *et al*, 2008). Using the step down inhibitory avoidance task, CB_2KO mice showed disrupted short- and long-term memory consolidation of the task. Other genetically modified mice, proposed as animal models of behavioral and biochemical alterations implicated in schizophrenia, show deficits in short-term working and spatial memory (Bégou *et al*, 2008; Gray *et al*, 2009) and deficits in retention of emotional or spatial memory (Rojas *et al*, 2007; Tanda *et al*, 2009). However, not all these mutant mice present memory impairment; for instance, trace amine 1 receptor KO mice show no difference on a working memory task compared with WT mice (Wolinsky *et al*, 2007).

Impaired sensorimotor gating has been proposed as a common feature of the cognitive dysfunction observed in schizophrenia (Braff *et al*, 2001). The presence of PPI deficit has thus been considered an important behavioral trait in rodent models of schizophrenia. For instance, a clear attention deficit has been observed in mice with mutations in dopamine or glutamate receptors (Ralph *et al*, 2001; Wiedholz *et al*, 2008; Yamashita *et al*, 2006). In this study, baseline PPI was significantly lower in CB_2KO mice than WT mice; however, there is little information about the role of CB_2r in the regulation of PPI in mice. Previous studies have reported that blockade of the CB_2r fails to alter the response to PPI, but increases the attention deficit induced by MK-801 (Ishiguro *et al*, 2010). Disruption of PPI in rats is at least partly due to activation of D_2r (Swerdlow *et al*, 1991), suggesting that increased activity at these receptors might also be a substrate for PPI deficits in schizophrenia. In this sense, patients with schizophrenia present significant differences in the acoustic startle reflex, habituation to startle stimuli, and several PPI levels compared with healthy controls (Moriwaki *et al*, 2009), and antipsychotic treatment with olanzapine, aripiprazole, and risperidone improve PPI, but do not modify the acoustic startle reflex or habituation (Kishi *et al*, 2010; Wynn *et al*, 2007). Another study using a longitudinal within-subjects design reported improved results after switching from the conventional antipsychotic zuclopenthixol to long-acting injectable risperidone (Martinez-Gras *et al*, 2009). On the other hand, the administration of antipsychotics increases PPI or reverses the PPI disruptions induced by psychostimulants in animal studies (Egashira *et al*, 2005; Gray *et al*, 2009; Nagai *et al*, 2006; Powell *et al*, 2008; Swerdlow *et al*, 1991; Thomsen *et al*, 2010). Acute administration of clozapine, risperidone, quetiapine, and haloperidol (Egashira *et al*, 2005; Powell *et al*, 2008; Thomsen *et al*, 2010), and chronic administration of clozapine (Gray *et al*, 2009) reverse the PPI deficit observed in different genetically manipulated mice. In addition, the administration of haloperidol, clozapine, and risperidone reverses drug-induced PPI deficits (Nagai *et al*, 2006; Swerdlow *et al*, 1991). The results of this study are consistent with these reports as chronic oral treatment with the atypical antipsychotic risperidone (at doses commonly used in the treatment of schizophrenia in patients) markedly attenuated the PPI deficits observed in CB_2KO mice. In contrast, risperidone tended to disrupt PPI progressively over the course of treatment in WT mice. In particular, the dose of 60 μg/kg markedly decreased PPI after 12 days of treatment (21% at 71 dB). On the other hand, risperidone reduced startle amplitude in both genotypes, being more evident in the CB_2KO mice.

The exploration of neurochemical changes in CB_2KO mice potentially related with the schizophrenia-like behaviors observed revealed alterations in the expression of dopaminergic, adrenergic, and serotonergic receptor genes. It is important to note that variations in the amount of gene transcript do not necessarily mean that concomitant changes in protein occur. These functional modifications could underlie the attention deficit, at least in part, as treatment with risperidone improved the PPI response and tended to 'normalize' some of the genes that are altered in CB_2KO mice. In contrast, treatment with risperidone failed to improve the PPI response in WT mice, but slightly impaired PPI response at the highest dose and longest duration of treatment. In addition, the effects of risperidone induced different alterations in receptor gene expression compared with CB_2KO mice.

The genes and the brain regions studied were selected based on two criteria: (1) previously reported alterations in gene expression associated with schizophrenia-like behaviors in genetically modified mice (Willi *et al*, 2010) and (2) a relation between the main receptor targets and the mechanism of action of risperidone. D_2r, 5-HT_{2A}r, 5-HT_{2C}r, and α_{2C}r satisfied these criteria (Abi-Dargham and Laruelle, 2005; McCormick *et al*, 2010; Meltzer and Huang, 2008).

Deletion of the CB_2r increased D_2r gene expression in the PFC. This situation could be related with increased dopaminergic tone, thus favoring deficient sensorimotor gating as occurs in DAT KO mice that present a chronic hyperdopaminergic state and deficient sensorimotor gating in the PPI (Ralph *et al*, 2001). The increased gene expression of this dopamine receptor was significantly reduced after treatment with risperidone, suggesting that the effectiveness of this drug on the PPI deficit could be at least partly related to this reduction in gene expression and its ability to block the receptor. The reduced effect of risperidone treatment on D_2r gene expression in the PFC of WT mice could be related with the lack of improvement in %PPI observed in these mice.

CB_2KO mice presented significantly reduced 5-HT_{2C}r gene expression in the DR nucleus. This reduction could be related with a decrease in the inhibitory control of DR serotonergic neurons played by 5-HT_{2C}r (Quérée *et al*, 2009), which may result in increased serotonin release in the PFC. Furthermore, deletion of the CB_2r significantly reduced 5-HT_{2A}r gene expression in PFC. Taking into account that PFC activity is modulated by serotonin through 5-HT_{2A}r (Puig *et al*, 2010), it can be hypothesized

that decreased gene expression of 5-HT_{2A}r could be a compensatory mechanism induced by persistently increased serotonin release by the DR terminals in the PFC. In CB_2KO mice, the effects of risperidone tend to restore 5-HT_{2C}r gene expression in the DR to the levels observed in WT mice, whereas risperidone treatment tends to reduce gene expression of this serotonergic receptor in the PFC. Typical and atypical antipsychotics show a similar effect of reducing 5-HT_{2C}r gene expression in the cortex (Buckland *et al*, 1997; Huang *et al*, 2006). Therefore, if decreased gene expression of 5-HT_{2A}r in the PFC reflects a possible compensatory mechanism in response to increased serotonin release from DR terminals, it can be hypothesized that risperidone treatment contributes to this mechanism by reducing 5-HT_{2A}r gene expression in the PFC even more at the same time that it increases 5-HT_{2A}r gene expression in the DR. Similarly, clozapine reduces 5-HT_{2A}r gene expression in the frontal cortex (Burnet *et al*, 1996). In WT mice risperidone treatment reduces 5-HT_{2A}r and 5-HT_{2C}r gene expression in the PFC and DR. In the PFC, CB_2KO 5-HT_{2A}r and 5-HT_{2C}r were reduced, whereas they were increased in the DR. In addition, risperidone treatment in these CB_2KO mice markedly improves the PPI deficit. In summary, CB_2KO mice present alterations in the serotonergic pathway from the DR to the PFC that could be responsible, at least in part, for the observed PPI deficit. It seems that the effect of risperidone treatment on this serotonergic pathway could be related to its beneficial effect on PPI.

The α_{2C}r plays an important role in cognitive processing in the PFC. The activation of the α_{2C}r by an α_2 agonist impairs performance of a spatial delayed alternation task, as seen in α_{2A} KO mice (Franowicz *et al*, 2002). In this sense, we found increased α_{2C}r gene expression in both the PFC and LC of CB_2KO mice. This alteration could thus be related with the observed PPI deficit. However, results obtained in studies made in two types of α_{2C} mutant mice do not support this explanation. Overexpression of α_{2C}r results in enhanced PPI response (Sallinen *et al*, 1998). On the other hand, α_{2C} KO mice show reduced PPI response (Sallinen *et al*, 1998). Despite this discrepancy, it may be speculated that increased expression of the α_{2C} receptor contributes somewhat to the PPI deficit in CB_2KO mice. This contribution could be related to the effects of increased α_{2C}r expression in the LC. Noradrenaline release in the PFC by LC terminals could be reduced. As risperidone treatment in CB_2KO mice reduced α_{2C}r expression in the LC, this effect could restore noradrenaline release in the PFC, thus contributing to PPI improvement. In WT mice, risperidone did not significantly modify α_{2C}r gene expression.

In summary, deletion of the cannabinoid *CB_2r* gene produced behavioral alterations that are commonly expressed in preclinical animal models of schizophrenia, namely altered locomotor activity, anxiety-like and depressive-like behaviors, and cognitive deficits including impaired sensorimotor gating. Gene expression studies in the PFC, DR, and LC revealed alterations in different dopaminergic, serotonergic, and noradrenergic receptors. Chronic treatment with the atypical antipsychotic risperidone reduced the PPI deficit, an effect that could be associated with modifications in the biochemical alterations observed in CB_2KO mice. These results suggest that CB_2r deletion was related to the observed schizophrenia-like behaviors. Pharmacological manipulation of CB_2r may be further explored as a potential therapeutic target for the treatment of schizophrenia-related disorders.

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DISCLOSURE

The authors declare no conflict of interest.

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