

RESEARCH PAPER

Cannabinoid CB₂ receptor-mediated regulation of impulsive-like behaviour in DBA/2 mice

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BACKGROUND AND PURPOSE

This study evaluated gene expression differences between two mouse strains, characterized by opposite impulsivity-like traits and the involvement of the cannabinoid CB₂ receptor in the modulation of impulsivity.

EXPERIMENTAL APPROACH

Behavioural tests were conducted to compare motor activity, exploration and novelty seeking, attention and cognitive and motor impulsivity (delayed reinforcement task: session duration 30 min; timeout 30 s) between A/J and DBA/2 mice. Expression of genes for dopamine D₂ receptors, CB₁ and CB₂ receptors were measured in the cingulate cortex (CgCtx), caudate-putamen (CPu), accumbens (Acc), amygdala (Amy) and hippocampus (Hipp). Involvement of CB₂ receptors in impulsivity was evaluated in DBA/2 mice with a CB₂ receptor agonist (JWH133) and an antagonist (AM630).

KEY RESULTS

DBA/2 mice presented higher motor and exploratory activity, pre-pulse inhibition impairment and higher cognitive and motor impulsivity level than A/J mice. In addition, DBA/2 mice showed lower (CgCtx, Acc, CPu) D₂ receptor, lower (Amy) and higher (CgCtx, Acc, CPu, Hipp) CB₁ receptor and higher (CgCtx, Acc, Amy) and similar (CPu, Hipp) CB₂ receptor gene expressions. Treatment with JWH133 (0.5, 1, 3 mg·kg⁻¹, i.p.) reduced cognitive and motor impulsivity level, accompanied by CB₂ receptor down-regulation (CgCtx, Acc, Amy) but did not modify other behaviours. In contrast, AM630 (1, 2, 3 mg·kg⁻¹, i.p.) improved pre-pulse inhibition and reduced novelty seeking behaviour in DBA/2 mice.

CONCLUSIONS AND IMPLICATIONS

CB₂ receptors might play an important role in regulating impulsive behaviours and should be considered a promising therapeutic target in the treatment of impulsivity-related disorders.

Abbreviations

Acc, accumbens; ADHD, attention-deficit hyperactivity disorder; Amy, amygdala; CgCtx, cingulate cortex; CPu, caudate-putamen; Hipp, hippocampus; SHR, spontaneously hypertensive rats; SMART, spontaneous motor activity recording and tracking

Introduction

Impulsive behaviour is characterized by the inclination to act prematurely without forethought and, as such, has an impor-

tant role in normal behaviour, allowing individuals to adapt to uncertainty, complexity and rapidly changing environments (Pattij and Vanderschuren, 2008). On the other hand, impulsivity is present in many psychiatric disorders such as bipolar disorder, personality disorders, attention-deficit

hyperactivity disorder (ADHD) and substance use disorders (Swann *et al.*, 2004; Winstanley *et al.*, 2006; Malloy-Diniz *et al.*, 2007). There is increasing consensus that impulsivity is a heterogeneous and multi-factorial concept consisting of several distinct behavioural phenomena that are dissociable at the neuroanatomical and neuropharmacological levels. In the present study, different varieties of impulsivity such as hyperactivity (open field), high exploration (head dipping on the hole board), novelty seeking (object preference on the hole board), attention deficit (pre-pulse inhibition) and high delay discounting or low behavioural inhibition (delayed reinforcement task) have been assessed.

Several studies have considered the isogenic nature of inbred strains of mice to evaluate the contribution of genetics to different behavioural phenotypes (Crawley *et al.*, 1997). The use of different strains of mice with a homogeneous and stable genetic background has made it possible to postulate that impulsive behaviour is probably affected by genetic factors (Isles *et al.*, 2004; Otobe and Makino, 2004). In this study, two inbred strains of mice (A/J and DBA/2) were selected on the basis of previous studies showing clear motor activity and anxiety differences (Bolivar *et al.*, 2000; Moy *et al.*, 2007). We employed such a two-strain comparison to study motor and exploratory activity, anxiety, learning and memory, stress reactivity and wildness (<http://phenome.jax.org/> of the Mouse Phenome Database, The Jackson Laboratory, Bar Harbor, ME).

Dopaminergic brain circuits have been related to the neuropharmacology of impulsivity (van Gaalen *et al.*, 2006; Winstanley *et al.*, 2006). The dopaminergic system has an important role in the regulation of impulsivity since previous studies reported that activation of dopamine receptors decreased delay aversion, one of the multiple traits that characterize impulsive behaviour (van Gaalen *et al.*, 2006). A study with methamphetamine-dependent subjects, displaying high impulsivity scores, showed a negative relationship between impulsiveness and striatal dopamine D₂/D₃ receptor availability in the caudate-putamen (CPu) and accumbens (Acc) (Lee *et al.*, 2009; receptor nomenclature follows Alexander *et al.*, 2011). Furthermore, an opposite modulation of impulsive behaviour by the dopamine D₂/D₃ receptors in *core* or *shell* parts of the Acc has been recently proposed (Besson *et al.*, 2009). Another recent study related the A1 allele of the *TaqIA* polymorphism in the gene for the D₂ receptor with impulsive behaviour, concluding that this association is a vulnerability factor for the development of problematic drug use (Esposito-Smythers *et al.*, 2009).

Recently, it has been suggested that other brain systems, such as the noradrenergic, glutamatergic or cannabinoid systems, may also play a relevant role in the regulation of impulsive-like behaviours (Pattij and Vanderschuren, 2008). The endocannabinoid system has garnered much interest in the regulation of impulsivity in recent years, supported by the following studies: (i) the administration of Δ^9 -tetrahydrocannabinol increased some behavioural measures of impulsivity, suggesting that impulsivity is an assemblage of distinct components rather than a unitary process (McDonald *et al.*, 2003); (ii) impulsive subpopulations of the spontaneously hypertensive rat (SHR) strain present low densities of cannabinoid CB₁ receptor in the prefrontal cortex, and the administration of the cannabinoid agonist WIN-

55212 normalizes the impulsive behavioural profile (Adriani *et al.*, 2003); and (iii) the administration of the CB₁ receptor antagonist rimonabant (SR141716A) improved inhibitory control, and this effect was blocked by WIN-55212 without affecting other impulse measures, providing further support for distinct forms of impulsivity that can be pharmacologically dissociated (Pattij *et al.*, 2007). In addition, an association between several polymorphisms in or near the CB₁ receptor gene with impulsivity has been reported in South-west Californian Indians (Ehlers *et al.*, 2007), as well as with the risk of both drug and alcohol dependence (Zuo *et al.*, 2007).

The cannabinoid CB₂ receptor, thought to be restricted to immune cells and peripheral tissues, has been identified in the brain of certain rodents (Van Sickle *et al.*, 2005). Although immunohistochemical studies in rat brain reported CB₂ receptors at levels much lower than CB₁ receptors, the CB₂ receptors have been found in abundance in hippocampus (Hipp) and amygdala (Amy) (Gong *et al.*, 2006), specifically on postsynaptic elements (Brusco *et al.*, 2008). A recent study of our group demonstrated the distribution of CB₂ receptors in the brain of Swiss ICR and CB₂ receptor transgenic mice and pointed out the involvement of this receptor in depressive-related disorders (Garcia-Gutierrez *et al.*, 2010). A few more reports have evaluated the involvement of CB₂ receptors in psychiatric disorders, such as depression (Onaivi *et al.*, 2008; Hu *et al.*, 2009), drug abuse (Ishiguro *et al.*, 2007; Onaivi *et al.*, 2008) or schizophrenia (Ishiguro *et al.*, 2010), suggesting a role of this brain neuronal cannabinoid receptor in cognition. However, the role of this promising therapeutic target in impulsivity was still unexplored.

In the present study, motor activity, exploration, novelty seeking, attention and impulsivity were analysed in A/J and DBA/2 strains of mice, to identify the strain displaying the higher level of impulsive-like behaviours. Expression of the genes for D₂ receptors and CB₁ receptors was evaluated to identify differences that could be related with the opposing impulsivity trait between both strains, taking into account that these targets have been already related to the regulation of impulsivity. Furthermore, analysis of the expression of the gene for CB₂ receptors and pharmacological studies with a highly selective CB₂ receptor agonist (JWH133) and antagonist (AM630) were carried out to evaluate the involvement of this receptor in the regulation of certain aspects of the heightened impulsive-like behaviours displayed by DBA/2 mice.

Methods

Animals

All animal care and experimental procedures were in accordance with guidelines established by the European Council Directive (86/609/EEC) and were approved by the Institutional Animal Care Committee. A/J and DBA/2 strains were purchased from Harlan (Barcelona, Spain). Male mice of 8–10 weeks of age and 25 ± 2 g of weight were housed in groups of 8–10 per cage ($40 \times 25 \times 22$ cm) under controlled conditions (temperature, $23 \pm 2^\circ\text{C}$; relative humidity, $60 \pm 10\%$; 12 h light/dark cycle, lights on from 8:00 to 20:00 h.). Behavioural analyses, initiated after 1 week of acclimatization in the

Table 1

Mean (\pm SEM) percentage of body weight reduction of A/J and DBA/2 strains ($n = 12$) and DBA/2-treated mice ($n = 8$) during the delayed reinforcement task.

Group	Mean (\pm SEM) % of body weight reduction
A/J	15.77 \pm 4.19
DBA/2	16.95 \pm 2.04
DBA/2 Vehicle	14.35 \pm 3.46
DBA/2 JWH133 (0.5 mg·kg ⁻¹)	13.62 \pm 0.59
DBA/2 JWH133 (1 mg·kg ⁻¹)	16.35 \pm 4.04
DBA/2 JWH133 (3 mg·kg ⁻¹)	15.62 \pm 2.51
DBA/2 AM630 (1 mg·kg ⁻¹)	15.15 \pm 3.27
DBA/2 AM630 (2 mg·kg ⁻¹)	10.97 \pm 0.50
DBA/2 AM630 (3 mg·kg ⁻¹)	14.56 \pm 4.27
Total mean	14.82 \pm 0.59

animal room, were performed after placing the home cage in the operant-task room for 1 h before starting experiments. Standard laboratory chow and water were available *ad libitum* in all procedures, except for the delayed reinforcement task in which standard chow was restricted to only 60 min access per day. This regimen was applied from 3 days before start and during the operant task (after the end of each daily session) to guarantee the response of the mice to reinforcers. Food restriction schedule produced a weight loss in mice of around 15% from their free-feeding weight (see Table 1).

Motor activity – open field

The open field consists of a transparent square cage 25 \times 25 \times 25 cm with a white Plexiglas floor. Mice were individually placed in the centre to initiate a 30 min test that was recorded with a video camera and analysed with the SMART (Spontaneous Motor Activity Recording and Tracking) v2.5.3 software system (Panlab, Barcelona, Spain). The distance travelled and the mean speed divided into three 10 min intervals were analysed.

Exploration and novelty seeking – hole board

The exploratory level and novelty seeking behaviour were measured using an apparatus that consisted of a 40 \times 40 \times 40 cm transparent acrylic square box with a black acrylic board with four equidistant holes placed in each corner and equipped with infrared photocells to detect introductions. Animals were tested in two similar tasks. First, the number of head dips into each hole was recorded (head dipping on the hole board). The day after, a small object was introduced in two of the holes at opposite corners to measure preference as the amount of time spent head dipping in holes that had objects divided by total time spent head dipping (object preference on the hole board). Mice were placed individually in the centre to initiate a 10 min test that was video recorded and analysed with the SMART programme.

Attention – acoustic pre-pulse inhibition

Pre-pulse inhibition is the phenomenon by which a weak pre-pulse stimulus suppresses or diminishes the response to a startling stimulus showing the individual's ability to inhibit or gate the incoming inputs by adapting the amplitude of startle reflects. Animals were placed in a soundproof chamber equipped with loudspeakers (Panlab). Animals were inside a Plexiglas cylinder contacting a piezoelectric accelerometer in order to transform mice movements into digital signals. Animals were tested in the pre-pulse inhibition paradigm, using a protocol described previously (Paylor and Crawley, 1997). Each test began with a habituation phase by placing a subject undisturbed for 10 min with a constant 65 dB background noise. After that, each subject was given 80 trials over the 37 min test interval, including eight trial types in pseudorandom order: 1 \times 40 ms, 120 dB acoustic startle stimulus; 3 \times 20 ms pre-pulse stimulus (74, 82 and 90 dB); and 3 \times 20 ms pre-pulse (100 ms before the onset) plus startle stimulus trials. Finally, trials where no stimulus was presented were used to measure baseline movements. The average inter-trial interval was 15 s. The maximum startle amplitude recorded during the 100 ms sampling window was used as the dependent variable. The following formula was used to calculate % pre-pulse inhibition of a startle response: $100 - [(startle response on acoustic pre-pulse plus startle stimulus trials / startle response alone trials) \times 100]$.

Impulsivity – delayed reinforcement task

The evaluation of the delay discounting was carried out in eight modular operant chambers (Panlab) placed inside eight noise-isolation boxes (which have a fan and a light) and equipped with a chamber light, two levers, one feeder device with a magazine to drop food pellets (20 mg Dustless precision rodent pellets, Bio-Serv, Frenchtown, NJ), one stimulus light and a buzzer. In the training phase, each session began with the chamber light on, and mice lever press switched it off. One lever press delivered one food pellet (immediate lever), whereas the other lever delivered three food pellets combined with 0.5 s stimulus light and 0.5 s, 2850 Hz, 85 dB buzzer beep (delayed lever). Following food delivery, a 30 s timeout (signalled with the chamber light off) was established during which additional lever presses in either lever were recorded but without consequence. After the 30 s timeout, the chamber light was turned on indicating the start of the inter-trial interval (ITI) in which the next trial is initiated depending on each subject's spontaneous waiting before lever press. Each session lasted 30 min, and all mice performed one session per day. With to the 30 s timeout period, the maximal number of trials that an animal could theoretically complete (if responding immediately after end of the timeout) during the training phase (without delays) was 60. The length of the training phase depended on the time to achieve learning task criteria consisting of (i) reaching >75% of preference for the delayed lever, (ii) >10 reinforced trials by session and (iii) <20% deviation of number of reinforced trials, all during 3 days. Once these criteria were reached, mice continued to the test phase where a time delay was introduced between lever pressing in the delayed lever and the delivery of the three pellets. In this period, the stimulus light (not the 0.5 s buzzer beep) was turned on, and additional lever presses in either lever were recorded but without

consequence. Delay was fixed for a given daily session and increased progressively over subsequent days (0, 6, 12, 18, 24, 30, 42, 54, 66, 78, 90 s). Change in the percentage of preference for the delayed lever in relation to different delays (cognitive impulsivity) and the number of immediate lever presses during the delay time (motor impulsivity) were analysed. For pharmacological studies in DBA/2 mice, the eight mice of each treatment group were tested at the same time, and the order of treatment groups was changed day by day, by placing the treatment group that first initiated the daily session, at the end of the session on the following day. In addition, a detailed analysis of the mean number of trials that mice completed and the mean response time after the end of timeout, through the whole task, was carried out. During the last 3 days of training, mice stabilize to around 50–60 s of response time, allowing them to complete around 20 trials out of 60 (theoretical). Details of the analysis method used for each parameter are described in the Supplementary Information.

Analysis of the expression of the genes for D₂ receptors CB₁ receptors and CB₂ receptors – real-time PCR

Mice were killed 24 h after the last delayed reinforcement task session (mice were under food restriction), and brains were removed from the skull and frozen over dry ice. Coronal brain sections (500 µm), which were obtained in a cryostat (–10°C), contained the regions of interest according to Paxinos and Franklin (2001) beginning at plates 19–20 (distance from bregma: 1.42 and 1.34 mm, respectively). The cingulate cortex (CgCtx), CPu, Acc, Amy and Hipp were microdissected according to the Palkovits method (Palkovits, 1983). Total RNA was isolated from brain tissue micro-punches using Biozol reagent (Inilab, Madrid, Spain) and subsequently retrotranscribed to cDNA. Quantitative analysis of the relative abundance of D₂ receptor (Mm00438541_m1), CB₁ receptor (Mm00432621_s1) and CB₂ receptor (Mm00438286_m1) gene expressions was performed on the ABI PRISM 7700 Sequence Detector System (Applied Biosystems, Foster City, CA) on the A/J and DBA/2 strains. All reagents were obtained from Applied Biosystems, and the manufacturer's protocols were followed. The reference gene used was 18S rRNA (Hs99999901_s1), 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^{–ΔΔC_t} method (Schmittgen and Zakrajsek, 2000), so that DBA/2 or DBA/2 JWH133-treated levels were expressed relative to A/J or DBA/2 vehicle-treated levels respectively.

Data analyses

Statistical analyses were performed using the Student's *t*-test to compare two groups and one or two-way ANOVA followed by the Holm–Sidak test to compare groups affected by one or two factors respectively. Two-way ANOVA with repeated measures followed by the Holm–Sidak test was used to compare two or more groups at different time points. Differences were considered significant if *P* < 0.05. SigmaStat v3.11 software (Systat

software Inc., Chicago, IL, USA) was used for all statistical analyses.

Materials

JWH133 (3-(1,1-dimethylbutyl)-1-deoxy-Δ⁸-tetrahydrocannabinol) and AM630 (6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl]-(4-methoxyphenyl)-methanone) were purchased from Tocris (Biogen, Madrid, Spain). Both were dissolved in DMSO, Tween 80 and distilled water (ratio 1:1:8). In all experiments performed with DBA/2 mice, JWH133 (0.5, 1 and 3 mg·kg^{–1}, i.p.) and AM630 (1, 2 and 3 mg·kg^{–1}, i.p.) were injected 30 min before testing in a volume of 10 ml kg^{–1}. In the delayed reinforcement task, mice received the corresponding dose on the different days of the test phase in which a delay was imposed (a total of 10 injections). Drugs were freshly prepared each day before testing. A total of seven groups of mice (eight mice per group) were employed for pharmacological studies, and therefore, we housed eight mice, corresponding to each dose of drug, per cage.

Results

Behavioural comparisons between A/J and DBA/2 mice

Motor activity. In order to assess the differences in motor activity of both strains, a 30 min open-field session divided into 10 min periods was carried out. A two-way repeated-measures ANOVA (factor 1: strain – two levels: A/J and DBA/2 and factor 2: period – three levels: 0–10, 10–20 and 20–30 min) followed by the Holm–Sidak test revealed that the travelled distance was significantly different between strains (Figures 1A-1) (strain: $F_{1,59} = 46.340$, $P < 0.001$; period: $F_{2,59} = 13.536$, $P < 0.001$; strain × period interaction: $F_{2,59} = 11.948$, $P < 0.001$) and that the mean speed values were also significantly different between A/J and DBA/2 mice (Figure 1A, A2) (strain: $F_{1,53} = 15.522$, $P = 0.004$; period: $F_{2,53} = 5.008$, $P = 0.020$; strain × period interaction: $F_{2,53} = 2.086$, $P = 0.157$). These results clearly indicate that DBA/2 mice presented higher locomotor activity than A/J mice.

Exploration and novelty seeking

Head dipping on the hole board. The numbers of head introductions performed by A/J and DBA/2 mice were evaluated to determine the difference in the level of exploratory behaviour. DBA/2 mice displayed a high exploratory phenotype associated with increased motor activity. This pattern of behaviour induced more head introductions in DBA/2 compared with A/J mice (Figures 1B-1) (Student's *t*-test: $t = -4.087$, $P < 0.001$, 18 d.f.).

Object preference on the hole board. To evaluate the novelty seeking behaviour between holes with or without an object, two objects (white stainless-steel clip and yellow plastic piece) were introduced in two of the four holes of the board, and the preference for the hole with an object over the hole without an object was assessed. A two-way ANOVA (factor 1: strain – two levels: A/J and DBA/2 and factor 2: hole type – two levels: with and without object) revealed that the number of introductions in a hole type was significantly

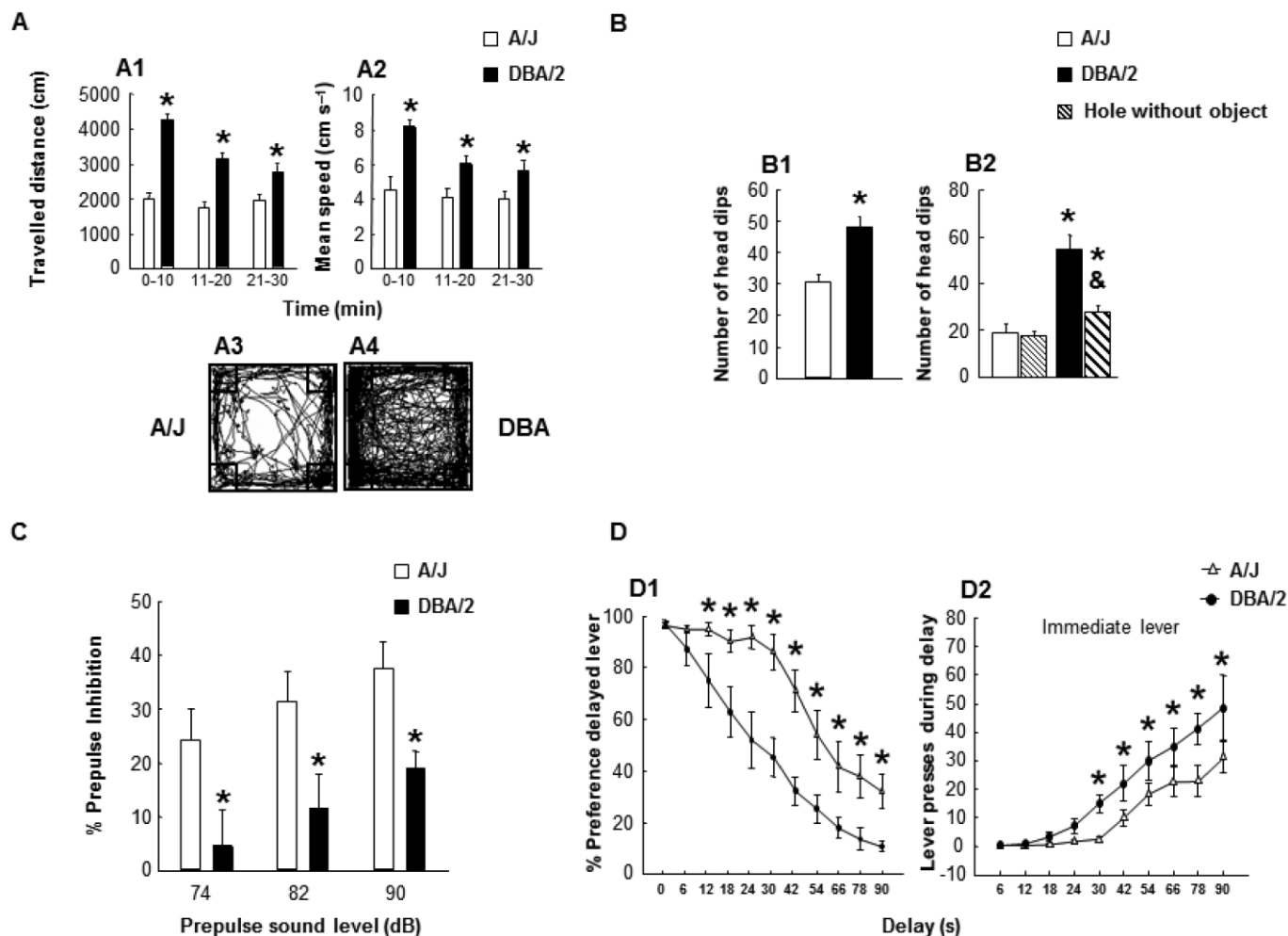


Figure 1

Behavioural assessment to distinguish opposing impulsivity patterns between A/J ($n = 10-12$) and DBA/2 mice ($n = 10-12$). (A) Spontaneous locomotor activity in the open field. (A1) Travelled distance and (A2) mean speed were measured in a 30 min session divided in 10 min periods. Data are expressed as mean \pm SEM of travelled distance (cm) and mean speed (cm s^{-1}). * $P < 0.05$, significant difference between DBA/2 mice and A/J mice. (A3 and A4) Representative images of the locomotor activity of the A/J and the DBA/2 mice (SMART tracking analysis). (B) Exploratory behaviour analysis and assessment of novelty seeking behaviour on the hole board task. Data are expressed as mean \pm SEM of (B1) number of head dips and (B2) percentage of object preference. * $P < 0.05$, significant difference between DBA/2 mice and A/J mice; & $P < 0.05$, significantly different from hole with object. (C) Pre-pulse inhibition of the acoustic startle response using 74, 82 and 90 dB pre-pulse stimuli. Data are expressed as mean \pm SEM of % pre-pulse inhibition. * $P < 0.05$, significant difference between DBA/2 mice and A/J mice. (D) Delay discounting (cognitive impulsivity) and inadequate responding (motor impulsivity) evaluation in the delayed reinforcement task. Data are expressed as mean \pm SEM of (D1) % preference for delayed reinforcement and (D2) number of immediate lever presses during time delay. * $P < 0.05$, significant difference between DBA/2 mice and A/J mice.

different between DBA/2 and A/J mice (Figure 1B, B2) (strain: $F_{1,39} = 33.013$, $P < 0.001$; hole type: $F_{1,39} = 12.023$, $P = 0.001$; strain \times hole type interaction: $F_{1,39} = 9.872$, $P = 0.003$). *Post hoc* comparisons showed that DBA/2 mice made significantly more head introductions in the holes with an object (69%), whereas A/J mice explored both holes in a similar manner (51%). The exploratory level of DBA/2 mice was higher than that displayed by A/J mice.

Attention. Mice were tested in a pre-pulse inhibition apparatus, and their startle response was analysed with or without a prior pre-pulse stimulus. The presentation of the 74, 82 and

90 dB pre-pulse stimuli revealed a significant difference between strains (Figure 1C): two-way ANOVA (factor 1: strain – two levels: A/J and DBA/2 and factor 2: pre-pulse sound level – three levels: 74, 82 and 90 dB) followed by the Holm–Sidak test (strain: $F_{1,59} = 19.129$, $P < 0.001$; pre-pulse sound level: $F_{2,59} = 33.13$, $P = 0.044$; strain \times pre-pulse sound level interaction: $F_{2,59} = 0.0166$, $P = 0.984$). These results suggest that the DBA/2 mice had a sensorimotor gating disturbance that probably reflected an attention deficit.

Impulsivity. The A/J and DBA/2 mice were evaluated in the delayed reinforcement task. The delayed discounting rate

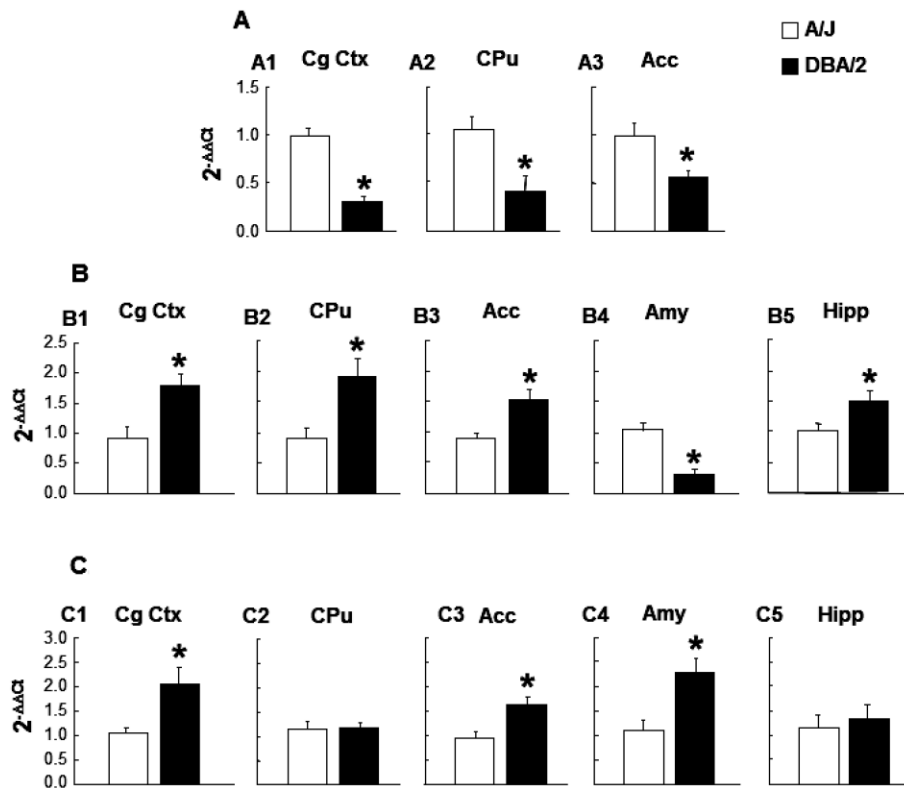


Figure 2

Gene expression analyses by real time-PCR in A/J and DBA/2 mice. Gene expression in DBA/2 mice is expressed relative to A/J. Data are expressed as mean \pm SEM of $2^{-\Delta\Delta C_t}$. (A) D₂ receptor gene expression in (A1) CgCtx (A/J $n = 5$, DBA/2 $n = 6$) (A2) CPu (A/J $n = 9$, DBA/2 $n = 6$) and (A3) Acc (A/J $n = 6$, DBA/2 $n = 6$). (B) CB₁ receptor gene expression in (B1) CgCtx (A/J $n = 5$, DBA/2 $n = 6$) (B2) CPu (A/J $n = 8$, DBA/2 $n = 6$) (B3) Acc (A/J $n = 7$, DBA/2 $n = 7$) (B4) Amy (A/J $n = 8$, DBA/2 $n = 8$) and (B5) Hipp (A/J $n = 9$, DBA/2 $n = 8$). (C) CB₂ receptor gene expression in (C1) CgCtx (A/J $n = 9$, DBA/2 $n = 7$) (C2) CPu (A/J $n = 10$, DBA/2 $n = 10$) (C3) Acc (A/J $n = 9$, DBA/2 $n = 9$) (C4) Amy (A/J $n = 8$, DBA/2 $n = 9$) and (C5) Hipp (A/J $n = 10$, DBA/2 $n = 9$). * $P < 0.05$, significant difference between DBA/2 mice and A/J mice.

(change in the percentage of preference for the delayed lever) and inadequate responding (immediate lever presses during delay time) were assessed. Since no delay was imposed on test day 1, no differences were found between strains starting with a similar preference level. On subsequent days, DBA/2 mice discounted delayed rewards more markedly than A/J mice. The differences between the percent preference values in both strains reached statistical significance from the second up to the last delay (Figure 1D, D1): two-way repeated measures ANOVA (factor 1: strain – two levels: A/J and DBA/2 and factor 2: delay – 10 levels: 6, 12, 18, 24, 30, 42, 54, 66, 78 and 90 s) following by the Holm–Sidak test (strain: $F_{1,248} = 12.640$, $P = 0.002$; delay: $F_{10,248} = 66.366$, $P < 0.001$; strain \times delay interaction: $F_{10,248} = 3.752$, $P < 0.001$). On the other hand, inadequate pressing of the immediate lever during the delay onset increased progressively, as expected, in both strains, rising significantly ($P < 0.05$) above the zero at 24 s for DBA/2 and at 42 s for A/J mice. DBA/2 mice performed significantly more inadequate lever presses compared with A/J mice, suggesting a higher motor impulsivity level (Figure 1D, D2): two-way repeated-measures ANOVA, with the Holm–Sidak test (strain: $F_{1,259} = 5.383$, $P = 0.030$; delay: $F_{10,259} = 30.654$, $P < 0.001$; strain \times delay interaction: $F_{10,259} = 1.643$, $P = 0.096$). Results for the mean number of trials that mice completed

and the mean response time after the end of timeout through the whole task are described in Supplementary Information (Figures S1, S2 and S3).

Expression of the genes for D2 receptors, CB1 receptors and CB2 receptors in selected brain regions. The statistical analyses revealed that D₂ receptor gene expression was significantly reduced in the CgCtx (–69%) (Student's t -test: $t = 7.866$, $P < 0.001$, 9 d.f.), CPu (–61%) (Student's t -test: $t = 3.087$, $P = 0.009$, 13 d.f.) and Acc (–43%) (Student's t -test: $t = 3.015$, $P = 0.013$, 10 d.f.) of DBA/2 mice compared with A/J mice (Figure 2A).

CB₁ receptor gene expression was significantly higher in the CgCtx (92%) (Student's t -test: $t = -3.139$, $P = 0.012$, 9 d.f.), CPu (110%) (Student's t -test: $t = -2.958$, $P = 0.012$, 12 d.f.), Acc (66%) (Student's t -test: $t = -2.922$, $P = 0.013$, 12 d.f.), and Hipp (47%) (Student's t -test: $t = -2.599$, $P = 0.020$, 15 d.f.) of DBA/2 mice, whereas the expression of this gene was markedly lower in the Amy (–68%) (Student's t -test: $t = 5.611$, $P < 0.001$, 14 d.f.) compared with A/J mice (Figure 2B).

CB₂ receptor gene expression was also investigated in the CgCtx, CPu, Acc, Amy and Hipp. The statistical analyses revealed pronounced increases in the CgCtx (95%) (Student's t -test: $t = -2.962$, $P = 0.010$, 14 d.f.), Acc (70%) (Student's

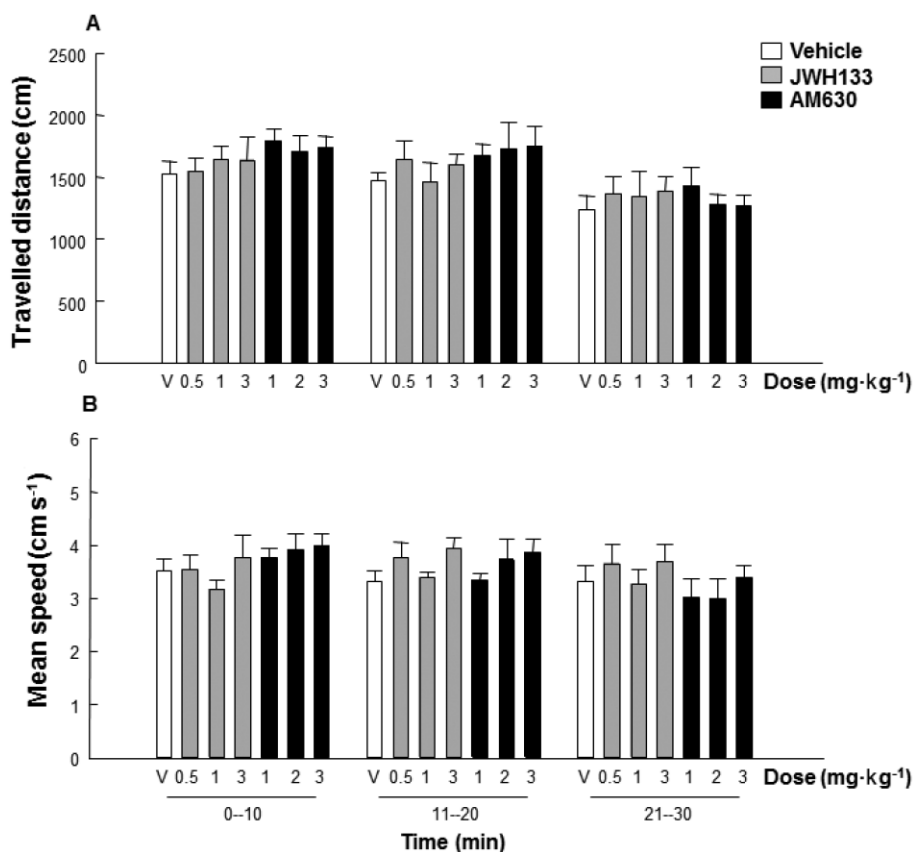


Figure 3

Analysis of motor activity in DBA/2 mice after acute administration of JWH133 (0.5, 1 and 3 mg·kg⁻¹) or AM630 (1, 2 and 3 mg·kg⁻¹) in the open-field test ($n = 8$ per group). Data are expressed as mean \pm SEM of (A) travelled distance (cm) and (B) mean speed (cm s⁻¹). No differences were found between treatment groups.

t -test: $t = -3.244$, $P = 0.005$, 16 d.f.), and Amy (105%) (Student's t -test: $t = -3.184$, $P = 0.006$, 15 d.f.) of DBA/2 mice. However, no differences were found in relative gene expression in the CPu (Student's t -test: $t = -0.099$, $P = 0.922$, 18 d.f.) or Hipp (Student's t -test: $t = -0.455$, $P = 0.655$, 17 d.f.) (Figure 2C).

Effects of JWH133 and AM630 on impulsive-like behaviours in DBA/2 mice

Locomotor activity. In the open field, the administration of JWH133 (0.5, 1 and 3 mg·kg⁻¹) or AM630 (1, 2 and 3 mg·kg⁻¹) to DBA/2 mice did not modify travelled distance (Figure 3A): two-way repeated-measures ANOVA (factor 1: treatment – seven levels: vehicle, JWH133 0.5, JWH133 1, JWH133 3, AM630 1, AM630 2 and AM630 3 mg·kg⁻¹ and factor 2: period – three levels: 0–10, 10–20 and 20–30 min) (treatment: $F_{6,167} = 0.683$, $P = 0.664$; period: $F_{2,167} = 15.355$, $P < 0.001$; treatment \times period interaction: $F_{12,167} = 0.407$, $P = 0.958$); nor mean speed (Figure 3B): two-way repeated-measures ANOVA (treatment: $F_{6,167} = 1.281$, $P = 0.283$; period: $F_{2,167} = 3.442$, $P = 0.036$; treatment \times period interaction: $F_{12,167} = 0.749$, $P = 0.701$).

Exploratory level and novelty seeking behaviour. The exploratory level on the hole board (number of head dips) did not

differ between treatment groups of DBA/2 mice (Figure 4A) (one-way ANOVA, factor treatment – seven levels: vehicle, JWH133 0.5, JWH133 1, JWH133 3, AM630 1, AM630 2 and AM630 3 mg·kg⁻¹, $F_{6,55} = 0.910$, $P = 0.495$). The treatment with single doses of JWH133 (0.5, 1 or 3 mg·kg⁻¹) did not change the percentage of object preference in DBA/2 mice (Figure 4B), whereas the administration of AM630 (1, 2 and 3 mg·kg⁻¹) dose-dependently blocked significantly the novelty seeking behaviour (one-way ANOVA followed by the Holm–Sidak test, factor treatment, $F_{6,55} = 4.335$, $P < 0.001$).

Attention deficit. The effects of the acute administration of JWH133 (0.5, 1 and 3 mg·kg⁻¹) and AM630 (1, 2 and 3 mg·kg⁻¹) on the pre-pulse inhibition of DBA/2 mice were measured according to the same protocol described previously (Figure 5). JWH133 did not significantly modify the underlying attention deficit displayed by DBA/2 mice at any pre-pulse sound. On the other hand, AM630 produced a significant improvement of the pre-pulse inhibition of DBA/2 mice at all the pre-pulses ($P < 0.05$) at the intermediate and highest administered doses (one-way ANOVA, factor treatment – seven levels: vehicle, JWH133 0.5, JWH133 1, JWH133 3, AM630 1, AM630 2 and AM630 3 mg·kg⁻¹, followed by the Holm–Sidak test, 74 dB: $F_{6,55} = 2.995$, $P = 0.014$; 82 dB: $F_{6,54} = 2.768$, $P = 0.022$; 90 dB: $F_{6,54} = 4.896$, $P < 0.001$).

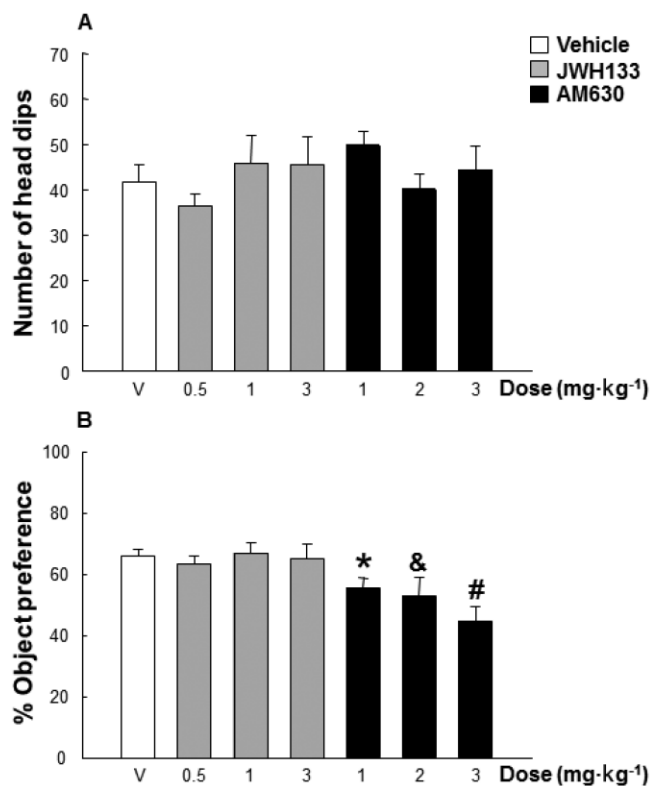


Figure 4

Effects of JWH133 (0.5, 1 and 3 mg·kg⁻¹) or AM630 (1, 2 and 3 mg·kg⁻¹) on exploratory level and novelty seeking behaviour of DBA/2 mice ($n = 8$ per group) on the hole board task. Data are expressed as mean \pm SEM of (A) number of head dips and (B) percentage of object preference. *, &, #, $P < 0.05$, significantly different from the vehicle group, for AM630 (1, 2 or 3 mg·kg⁻¹) treated mice respectively.

Cognitive and motor impulsivity levels. Learning criteria reflecting preference for the large reinforcement which was immediately available as the small reinforcement in the first training phase were achieved after 10 training days. Drug testing began from first delay session with the administration of JWH133 (0.5, 1 and 3 mg·kg⁻¹) or AM630 (1, 2 and 3 mg·kg⁻¹). The administration of JWH133 increased, in a dose-dependent way, the percent preference with respect to the control group for the large reinforcement over delays, which reached statistical significance at 24 s (0.5 mg·kg⁻¹), from 12 to 30 s (1 mg·kg⁻¹) and from 12 to 66 s (3 mg·kg⁻¹) time delays (Figure 6A) (two-way repeated-measures ANOVA, factor 1 treatment – seven levels: vehicle, JWH133 0.5, JWH133 1, JWH133 3, AM630 1, AM630 2 and AM630 3 mg·kg⁻¹ and factor 2 delay – 10 levels: 6, 12, 18, 24, 30, 42, 54, 66, 78 and 90 s, followed by the Holm–Sidak test, treatment: $F_{3,351} = 2.617$, $P = 0.071$; delay: $F_{10,351} = 159.611$, $P < 0.001$; treatment \times delay interaction: $F_{30,351} = 0.972$, $P = 0.513$). The administration of the antagonist AM630 (1, 2 and 3 mg·kg⁻¹) did not change the percent preference compared with the vehicle group (Figure 6C) (two-way repeated-measures ANOVA followed by the Holm–Sidak test, treatment: $F_{3,351} = 0.406$, $P = 0.750$; delay: $F_{10,351} =$

99.920, $P < 0.001$; treatment \times delay interaction: $F_{30,351} = 0.434$, $P = 0.996$).

JWH133 treatment significantly reduced the number of immediate lever presses of DBA/2 mice (Figure 6B) (two-way repeated-measures ANOVA followed by the Holm–Sidak test, treatment: $F_{3,319} = 9.575$, $P < 0.001$; delay: $F_{9,319} = 57.289$, $P < 0.001$; treatment \times delay interaction: $F_{27,319} = 2.851$, $P < 0.001$), but AM630 did not have any effect on motor impulsivity (Figure 6D) (two-way repeated-measures ANOVA followed by the Holm–Sidak test, treatment: $F_{3,319} = 2.995$, $P = 0.048$; delay: $F_{9,319} = 65.977$, $P < 0.001$; treatment \times delay interaction: $F_{27,319} = 0.782$, $P = 0.773$).

Expression of the genes for CB₂ receptors after sub-chronic JWH133 treatment. The sub-chronic administration of JWH133 (0.5, 1 and 3 mg·kg⁻¹) during the delay phase of the delayed reinforcement task reduced the CB₂ receptor gene expression in the brain regions of high-impulsive DBA/2 mice in which there was a higher basal level in comparison with the low-impulsive A/J mice (Figure 7) (one-way ANOVA, factor treatment – four levels: vehicle, JWH133 0.5, JWH133 1 and JWH133 3 mg·kg⁻¹, followed by the Holm–Sidak test, CgCtx: $F_{3,23} = 5.934$, $P = 0.005$; Acc: $F_{3,27} = 3.311$, $P = 0.037$; Amy: $F_{3,25} = 4.478$, $P = 0.013$). *Post hoc* comparisons revealed that this reduction achieved statistical significance only with the highest dose of JWH133 (3 mg·kg⁻¹).

Discussion

The results of the present study show that differences in the expression of the genes for D₂ receptors, CB₁ receptors and CB₂ receptors could underlie the differences in impulsivity levels between A/J and DBA/2 strains and suggest for the first time that the CB₂ receptor is involved in the regulation of impulsive-like behaviours, as treatment with a highly selective CB₂ receptor agonist and antagonist was able to modulate certain impulsive markers. These conclusions were based on several findings. First, DBA/2 mice presented motor hyperactivity, increased exploration, novelty seeking behaviour, attention deficit and greater delay discounting and behavioural disinhibition than A/J mice. Significant changes in D₂ receptor, CB₁ and CB₂ receptor gene expressions were found in specific brain regions closely related with impulsive behaviour between A/J and DBA/2 mice. Treatment with the CB₂ receptor antagonist AM630 reduced object preference on the hole board task and improved attention in DBA/2 mice. Finally, sub-chronic treatment with the CB₂ receptor agonist JWH133 decreased impulsive choice and the number of inadequate responses during the delay phase in DBA/2 mice, an effect that was accompanied by a normalization of the CB₂ receptor gene expression (down-regulation).

In the present study, a greater cognitive and motor impulsivity level with increased motor/exploratory activity and impairment of attentional processes was found in the DBA/2 strain. Travelled distance and average speed in the open field test as well as the number of head dips on the hole board were significantly higher in DBA/2 compared to A/J mice. In addition, DBA/2 mice displayed significant impairments in attentional processes in the pre-pulse inhibition paradigm at all

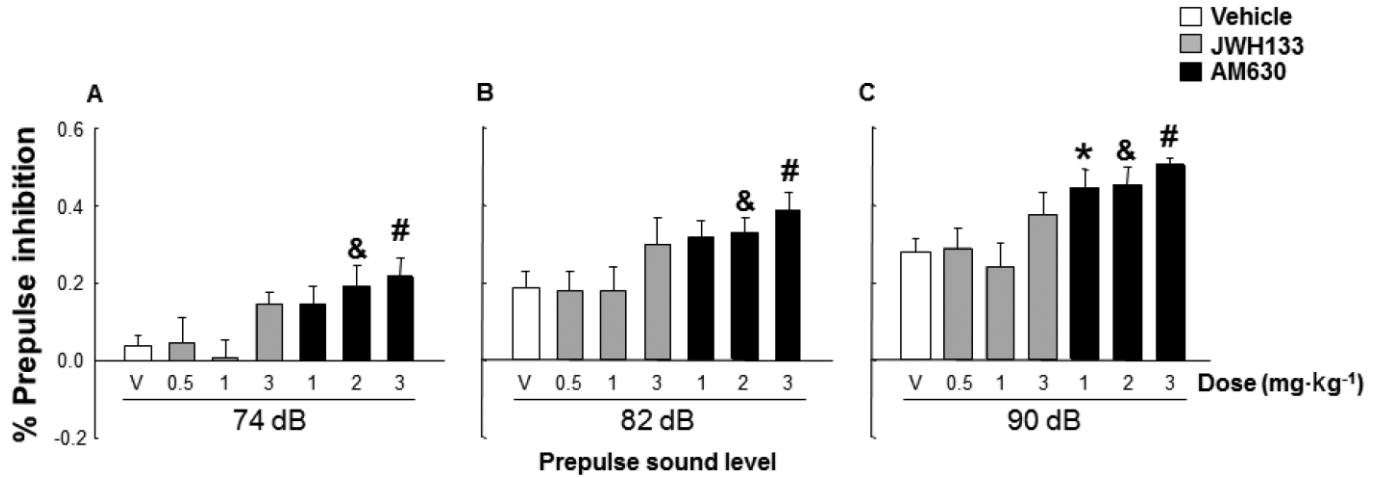


Figure 5

Evaluation of effects of JWH133 (0.5, 1 and 3 mg·kg⁻¹) or AM630 (1, 2 and 3 mg·kg⁻¹) on pre-pulse inhibition of DBA/2 mice ($n = 8$ per group) in the pre-pulse inhibition paradigm. Data are expressed as mean \pm SEM of percentage pre-pulse inhibition at (A) 74 dB (B) 82 dB and (C) 90 dB pre-pulse sound levels. *, &, #, $P < 0.05$, significantly different from the vehicle group, for AM630 (1, 2 or 3 mg·kg⁻¹) treated mice respectively.

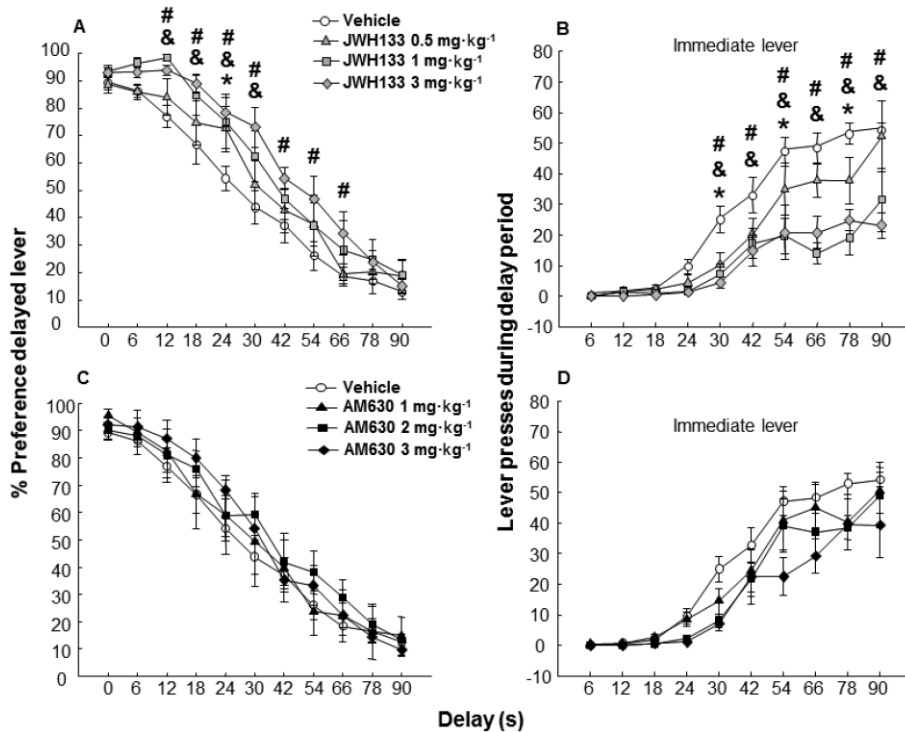


Figure 6

Effects of JWH133 (0.5, 1 and 3 mg·kg⁻¹) or AM630 (1, 2 and 3 mg·kg⁻¹) on high cognitive and motor impulsivity level of DBA/2 mice ($n = 8$ per group) in the delayed reinforcement task in which progressive time delays associated with the large reinforcer were imposed on subsequent days. Data are expressed as mean \pm SEM of (A, C) percentage preference for the delayed lever and (B, D) number of inadequate immediate lever presses during time delay. *, &, #, $P < 0.05$, significantly different from the vehicle group, for JWH133 (0.5, 1 and 3 mg·kg⁻¹) treated mice respectively.

three pre-pulses tested. One of the circuits that probably plays a major role in the regulation of motor activity is the nigrostriatal dopaminergic system, which has cell bodies in the substantia nigra that project their axons to the CPu. This

region contains a high density of D₂ receptors regulating mainly motor activity. The results of this study showed that the most active strain of mice (DBA/2) displayed 61% less D₂ receptor gene expression in the CPu compared with A/J mice.

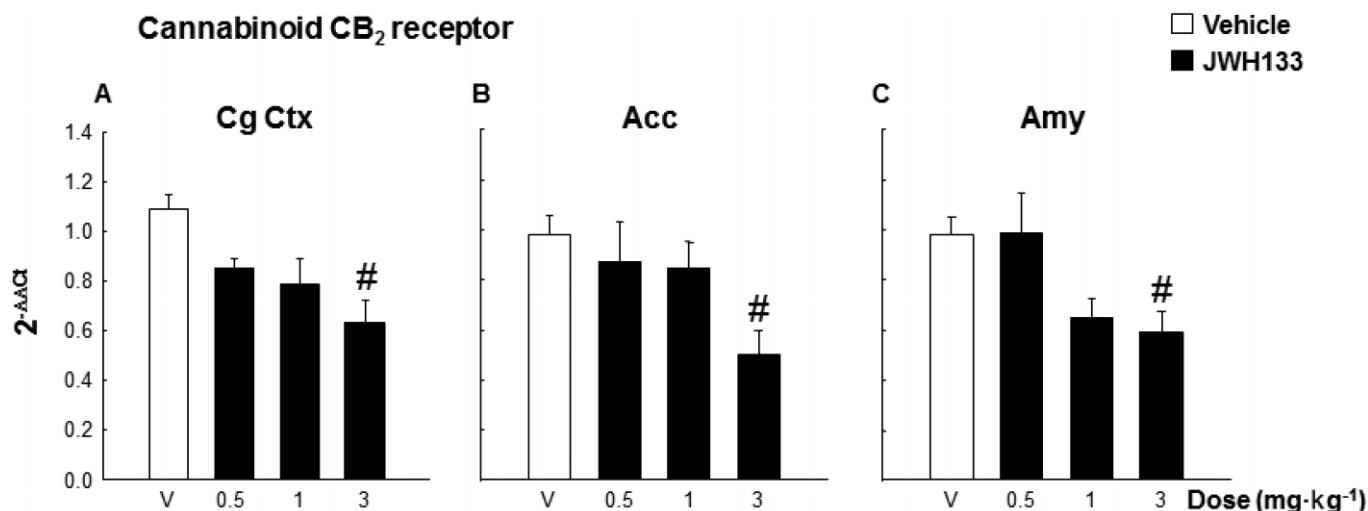


Figure 7

Effects of JWH133 (0.5, 1 and 3 mg kg⁻¹) on CB₂ receptor gene expression of DBA/2 mice ($n = 8$ per group) after sub-chronic treatment during delay phase of the delayed reinforcement task in (A) CgCtx (B) Acc and (C) Amy. Data are expressed as mean \pm SEM of 2^{-ΔΔCt}. # $P < 0.05$, significantly different from the vehicle group.

The involvement of D₂ receptors in impulsive behaviour is based on several findings. Several D₂ receptor gene variants were strongly correlated with impulsiveness in ADHD and addictive compulsive behaviours (Blum *et al.*, 1995; Eisenberg *et al.*, 2007). In addition, D₂ receptor availability was significantly reduced in the Acc (ventral striatum) of high-impulsive rats using micro-PET (Dalley *et al.*, 2007). In methamphetamine-dependent subjects displaying high impulsivity scores, there was a negative relationship between impulsiveness and striatal D₂/D₃ receptor availability in the CPu and Acc, measured through PET scanning with [¹⁸F]fallypride (Lee *et al.*, 2009). Our results, showing decreased (43%) D₂ receptor gene expression in the Acc of DBA/2 mice, are in agreement with this previous study. In addition, trait impulsivity in healthy human volunteers was inversely correlated with D₂/D₃ receptor availability in the substantia nigra/ventral tegmental area (Buckholtz *et al.*, 2010). Similarly, in the CgCtx, a brain region closely involved in higher-order cognitive functions such as reward-related learning, D₂ receptor gene expression was significantly lower (69%) in DBA/2 compared with A/J mice. Furthermore, it has been reported that pharmacological modulation by D₂ receptor antagonists induced impulsive choice, suggesting that these receptors normally promote choice of the delayed reinforcement (Wade *et al.*, 2000), which is consistent with the results obtained in the delayed reinforcement task and the opposing D₂ receptor gene expression patterns observed in A/J and DBA/2 mice.

Recently, it has been established that the endocannabinoid system plays an important role in the control of motor activity (Zimmer *et al.*, 1999), anxiety and cognition (Wotjak, 2005; Lafenetre *et al.*, 2007), emotional behaviour (Valverde, 2005) and memory (Varvel *et al.*, 2002). Cannabinoid neurotransmission has also been implicated in impulsivity (Pattij and Vanderschuren, 2008), since different forms of impulsive behaviours may be pharmacologically

dissociated in rats by using selective agonist or antagonist of the CB₁ receptor (Pattij *et al.*, 2007). The results of our study revealed a significant increase of CB₁ receptor gene expression in CgCtx, Acc, CPu and Hipp, and decreased expression of this gene in the Amy of DBA/2 mice. In contrast, Adriani and collaborators pointed out a reduction in CB₁ receptor density in the prefrontal cortex of the SHR (a model of ADHD) (Adriani *et al.*, 2003; Adriani and Laviola, 2004). There are several possible explanations of this discrepancy, as summarised here. First, although rats and mice present similar CB₁ receptor brain distribution (Gatley *et al.*, 1996), there are no studies evaluating differences in CB₁ receptor gene and protein expression between these species that could be related with behavioural and brain functional differences. Adriani *et al.* used a gross general prefrontal cortex dissection, whereas in our present study, the CgCtx sub-region of DBA/2 mice was microdissected from 500 μm-thick coronal brain slices using a Ø 22 μm needle. In SHR rats, membrane CB₁ receptor protein expression was determined by Western blotting, whereas we measured the CB₁ receptor mRNA in DBA/2 mice, by real time-PCR. The different sensitivities of these techniques and intracellular metabolic alterations, such as protein synthesis regulation at a post-transcriptional level or translocation of mRNA transcripts to another neuronal compartment, which can affect mRNA translation rate, could account for differences between gene transcription and final receptor expression on cell membranes.

Human studies support the notion of CB₁ receptor gene disruption and potential alterations in impulsive behaviours. Indeed, increased impulsiveness was significantly associated with the six-repeat allele of the triplet repeat polymorphism (AATn/A6), as well as four single nucleotide polymorphisms in or near the CB₁ receptor gene in Southwest Californian Indians (Ehlers *et al.*, 2007). Recently, it has been proposed that several CB₁ receptor polymorphisms

are associated with central effects of the endocannabinoid system, including impulsivity and substance addiction (Dinu *et al.*, 2009). Furthermore, an association between CB₁ and childhood ADHD in Spanish male alcoholic patients was also reported, suggesting an important role of this gene in novelty seeking (Ponce *et al.*, 2003), a behaviour that appears to be strongly related to impulsivity (Van Laere *et al.*, 2009) and drug abuse (Kelly *et al.*, 2006). DBA/2 mice showed a clear object preference as evaluated through the head dipping task on the hole board, whereas A/J mice explored holes with or without a novel object equally.

The results of this study revealed that DBA/2 mice have a higher CB₂ receptor gene expression in the CgCtx, Acc and Amy compared with A/J mice, but no changes were found in the CPu and Hipp between both strains. Brain regions presenting high CB₂ receptor gene expression have been closely related with impulsive behaviour. Furthermore, human studies found evidence for the relevance of the Amy in decision-making processes, considered as a part of an 'impulsive system' that triggers emotional responses to immediate outcomes (Bechara, 2005). On the other hand, lesions and functional brain imaging studies have demonstrated a critical involvement of the Acc pattern of neuronal activity in impulsivity (Basar *et al.*, 2010). A few reports suggest enhancement of CB₂ receptor gene expression in the brain after chronic mild stress (Onaivi *et al.*, 2008) or reduction of the expression after consumption of ethanol or other drugs of abuse (Ishiguro *et al.*, 2007). Therefore, it appears that functional alterations of the CB₂ receptor gene in the brain may be associated with increased vulnerability for anxiety, depressive-like behaviours and consumption of drugs of abuse, pathological states closely related with impulsive behaviour.

As several earlier studies have revealed the involvement of dopamine D₂ receptors and CB₁ receptors in impulsivity, this study was focused on the potential role of the CB₂ receptor in this personality trait. Consequently, the effects of the CB₂ receptor agonist JWH133 (0.5, 1 and 3 mg·kg⁻¹) and antagonist AM630 (1, 2 and 3 mg·kg⁻¹) were explored in DBA/2 mice, a strain displaying a high impulsive-like behaviour, employing behavioural paradigms that measure distinct varieties of impulsivity (hyperactivity, high exploration, novelty seeking, attention deficit, high delay discounting and low behavioural inhibition).

Neither travelled distance and mean speed in the open field nor the number of head dips on the hole board parameters were significantly modified by JWH133 or AM630 treatment, excluding any effect on motor or exploratory activity that could interfere with the interpretation of the rest of measured behaviours. Object preference on the hole board task was not affected by the administration of the agonist JWH133, whereas the antagonist AM630 blocked this impulsive-related behaviour in a significant and dose-dependent way. There are no previous studies about the effects of these drugs on novelty seeking behaviour, but the role of the endocannabinoid system in this impulsivity aspect has been recently evaluated through the CB₁ receptor. Low baseline CB₁ receptor availability in human brains was related to a high novelty seeking personality (Van Laere *et al.*, 2009), emphasizing that this negative correlation is most pronounced in the amygdala in accordance with the results obtained with DBA/2 mice.

The administration of JWH133 did not significantly change the percentage of pre-pulse inhibition with respect to the vehicle group, whereas AM630 significantly attenuated the attention deficit in DBA/2 mice. Several studies have shown that the activation of the endocannabinoid system through synthetic or natural ligands that mainly activate CB₁ receptors, impairs sensorimotor gating (Schneider and Koch, 2002; Wegener *et al.*, 2008; Long *et al.*, 2009). On the other hand, the administration of CB₁ receptor antagonists produces an opposite effect (Ballmaier *et al.*, 2007). The results of the present study point out that the notable role of the endocannabinoid system in the modulation of sensorimotor gating is not only mediated by CB₁ receptors but also by CB₂ receptors, as blockade of the latter receptors improved the pre-pulse inhibition of DBA/2 mice.

In the present study, treatment with JWH133 during the delay phase decreased the degree of impulsive choice in DBA/2 mice at the beginning of the task with the intermediate dose (1 mg·kg⁻¹) and also during 42, 54 and 66 s delay times with the highest dose (3 mg·kg⁻¹). On the other hand, AM630 failed to alter DBA/2 cognitive impulsivity level at all tested doses. In addition, JWH133 dose-dependently decreased the number of inadequate responses at the final time delays. Unexpectedly, AM630 treatment showed a similar tendency without reaching statistical significance. It is possible that the increased CB₂ receptor gene expression in CgCtx, Acc and Amy found in DBA/2 mice was related to the level of impulsive behaviour. On this basis, acute blockade of CB₂ receptors would tend to reduce impulsivity level (as observed in the object preference and the pre-pulse inhibition tasks), whereas the acute activation of the receptor would enhance this behaviour. However, in the delayed reinforcement task, administration of JWH133 improved impulsivity level, whereas AM630 was without effects. These apparently opposite effects of the CB₂ receptor agonist JWH133 may be related to the neuroadaptive receptor regulation occurring after repeated treatment (10 days' treatment during the test phase) that would produce a down-regulation. As expected, treatment with the CB₂ receptor agonist JWH133 was able to down-regulate CB₂ receptor gene expression in CgCtx, Acc and Amy, reaching statistical significance only with the highest dose (3 mg·kg⁻¹). These results seem to indicate that the modulation of cognitive and motor impulsivity achieved with JWH133 treatment would be related to the regulation of CB₂ receptors. However, it is possible that, despite using highly selective CB₂ receptor ligands, these effects might not be exclusively mediated by CB₂ receptors, and further studies combining both ligands (agonist and antagonist) are necessary to confirm the specific participation of the CB₂ receptor. In addition, the precise molecular mechanisms by which CB₂ receptor ligands are regulating impulsive behaviours remains to be further explored.

In conclusion, the present study provides new information about gene expression differences underlying opposing impulsivity patterns and the involvement of the CB₂ receptor in the modulation of different varieties of impulsive behaviour, such as novelty seeking, attention deficit or impulsive choice/response, suggesting the possible therapeutic usefulness of this target in impulsivity-related disorders.

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Conflicts of interest

All authors report no biomedical financial interests or potential conflicts of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Mean number of completed trials and mean response time analysis between AJ and DBA/2 mouse strains in the training and test phases of the delayed reinforcement task. Data are expressed as mean \pm SEM of (A) mean number of completed trials during each 30 min session and (B) mean waiting spontaneous time during ITI before initiating a new trial (A/J $n = 12$, DBA/2 $n = 12$). *, values from DBA/2 mice that are significantly different ($P < 0.05$) from A/J mice.

Figure S2 Evaluation of the effects of JWH133 on the mean number of completed trials and mean response time parameters between JWH133-treated and vehicle groups in the training and test phases of the delayed reinforcement task. Data are expressed as mean \pm SEM of (A) the mean number of completed trials during each 30 min session and (B) the mean waiting spontaneous time during ITI before initiating a new trial (vehicle group: $n = 8$; JWH133 groups (0.5, 1 and 3 mg·kg⁻¹): $n = 8$).

Figure S3 Evaluation of the effects of AM630 on the mean number of completed trials and mean response time parameters between AM630-treated and vehicle groups in the training and test phases of the delayed reinforcement task. Data are expressed as mean \pm SEM of (A) mean number of

completed trials during each 30 min session and (B) mean waiting spontaneous time during ITI before initiate a new trial (vehicle group: $n = 8$; AM630 groups (1, 2 and 3 mg·kg⁻¹): $n = 8$). **, values from DBA/2 AM630 2 mg·kg⁻¹ treated mice significantly different ($P < 0.05$) from the vehicle group.

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