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RESEARCH PAPER

Pregabalin- and topiramate-mediated regulation of cognitive and motor impulsivity in DBA/2 mice

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BACKGROUND AND PURPOSE

Impulsivity is a core symptom in many neuropsychiatric disorders. The main objective of this study was to evaluate the effects of topiramate and pregabalin on the modulation of different impulsivity dimensions in DBA/2 mice.

EXPERIMENTAL APPROACH

The effects of acute and chronic administration of pregabalin (10, 20 and 40 mg·kg^{-1}) and topiramate (12.5, 25 and 50 mg·kg^{-1}) were evaluated in the light–dark box (LDB), hole board test (HBT) and delayed reinforcement task (DRT). α_{2A}-Adrenoceptor, D_2-receptor and TH gene expression were evaluated by real-time PCR in the prefrontal cortex (PFC), accumbens (ACC) and ventral tegmental area (VTA), respectively.

KEY RESULTS

Acute pregabalin administration showed a clear anxiolytic-like effect (LDB) but did not modify novelty-seeking behaviour (HBT). In contrast, topiramate produced an anxiolytic effect only at the highest dose, whereas it reduced novelty seeking at all doses tested. In the DRT, acute pregabalin had no effect, whereas topiramate only reduced motor impulsivity. Chronically, pregabalin significantly increased motor impulsivity and topiramate diminished cognitive impulsivity. Pregabalin decreased α_{2A}-adrenoceptor and D_2-receptor gene expression in the PFC and ACC, respectively, and increased TH in the VTA. In contrast, chronic administration of topiramate increased α_{2A}-adrenoceptor and D_2-receptor gene expression in the PFC and ACC, respectively, and also increased TH in the VTA.

CONCLUSIONS AND IMPLICATIONS

These results suggest that the usefulness of pregabalin in impulsivity-related disorders is related to its anxiolytic properties, whereas topiramate modulates impulsivity. These differences could be linked to their opposite effects on α_{2A}-adrenoceptor and D_2-receptor gene expression in the PFC and ACC, respectively.

Abbreviations

ACC, accumbens nucleus; ADHD, attention-deficit hyperactivity disorder; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; D_2-receptor, D_2 dopamine receptor; DRT, delayed reinforcement task; HBT, hole board test; LDB, light–dark box; PFC, prefrontal cortex; VTA, ventral tegmental area

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Introduction

Impulsivity constitutes a complex and multidimensional personality trait (Evenden, 1999a,b) that can be studied in both humans and animals by a wide range of methods (Winstanley *et al.*, 2006). Behavioural disinhibition (motor impulsivity), manifested by poor inhibitory control of pre-potent responses, and impulsive choice (cognitive impulsivity), which refers to the preference for smaller immediate rewards over larger delayed rewards, are the most representative dimensions (Dougherty *et al.*, 2003; Otobe and Makino, 2004; Adriani *et al.*, 2010). In addition, there are other behavioural dimensions such as novelty-seeking behaviour closely related to impulsivity (Petry, 2001; James *et al.*, 2007; Evren *et al.*, 2012). Although impulsivity is a normal behaviour in healthy humans allowing adaptation to uncertainty (Marazziti *et al.*, 2010), there are several neuropsychiatric disorders such as ADHD (attention-deficit hyperactivity disorder), drug abuse, pathological gambling, bipolar disorder, obsessive compulsive disorder, aggression, anorexia/bulimia nervosa, suicide, trichotillomania, intermittent explosive disorder, self-injurious behaviour or kleptomania, presenting a high level of impulsivity as a core symptom (Rapport *et al.*, 1985; August and Garfinkel, 1989; Jensen *et al.*, 1990; Fahy and Eisler, 1993). Therefore, novel pharmacological strategies that alleviate impulsive behaviours could be very helpful in the management of these disorders.

In recent years, there has been an increase in the use of anticonvulsant drugs in the treatment of distinct neuropsychiatric disorders characterized by impulse control problems. Carbamazepine was one of the first to be used and it enabled a reduced dose of other antipsychotic drugs to be effective in the treatment of agitation and disruptive behaviours, such as aggressiveness, impulsivity, perversity or suicidal attempts (Vogelaer, 1981). Valproate has been widely used in the management of personality disorders, improving some symptoms like aggression, irritability and high impulsivity (Wilcox, 1994; 1995; Kavoussi and Coccaro, 1998).

Some of the so-called new anticonvulsants that appeared in the 1990s (Bourgeois, 1996; Wilson and Brodie, 1996) have demonstrated efficacy in the treatment of drug abuse disorders by alleviating withdrawal symptoms (Zullino *et al.*, 2004), reducing craving (urge to consume) (Furieri and Nakamura-Palacios, 2007; Vengeliene *et al.*, 2007; Miranda *et al.*, 2008; Reis *et al.*, 2008) or attenuating the pleasurable effects of drug intake, thus avoiding relapse (Bisaga *et al.*, 2006; Martinotti *et al.*, 2007). Among these new antiepileptic drugs, topiramate stands out in substance abuse intervention (mainly alcohol dependence) due to its ability to reduce consumption and relapse (Kampman *et al.*, 2004; Cubells, 2006; Nguyen *et al.*, 2007; Kenna *et al.*, 2009; Johnson and Ait-Daoud, 2010). Topiramate has a complex and not well known mechanism of action, but its main effects include the modulation of voltage-gated sodium channels (Zona *et al.*, 1997; Taverna *et al.*, 1999), an increase in GABA neurotransmission (White *et al.*, 1997; 2000) and the blockade of α-amino-3-hydroxy-5-methylisoxozole-proprionic acid (AMPA)/kainate receptors (Gibbs *et al.*, 2000; Poulsen *et al.*, 2004). Although it has been hypothesized that topiramate's usefulness in the management of drug abuse may be related to its anti-craving effect diminishing the pleasurable effects of drugs mediated by modulation of the dopaminergic mesolimbic pathways (Johnson *et al.*, 2003; Johnson, 2004b), it has been proposed that topiramate could also modulate impulsive behaviours (Smathers *et al.*, 2003; Dolengevich Segal *et al.*, 2006; Rubio *et al.*, 2009).

Another anticonvulsant, pregabalin, which is indicated for the treatment of generalized anxiety disorders and neuropathic pain, is emerging as a potential therapeutic tool in the field of alcoholism. This drug ameliorates alcohol withdrawal symptoms (Martinotti *et al.*, 2008; Di Nicola *et al.*, 2010; Oulis and Konstantakopoulos, 2010) and relapse through a mechanism less related to alcohol craving and more associated with the treatment of the comorbid psychiatric symptomatology such as an increased anxiety level (Martinotti *et al.*, 2010). In addition, a very recent study shows for the first time that pregabalin is able to reduce alcohol consumption (Stopponi *et al.*, 2011). Pregabalin acts as a presynaptic inhibitor of the release of excessive levels of excitatory neurotransmitters by selectively binding to the α2-δ subunit of voltage-gated calcium channels (Stahl, 2004). Through this mechanism, it has been proposed that pregabalin reduces the increase in dopamine in the nucleus accumbens resulting from acute morphine administration (Andrews *et al.*, 2001).

The efficacy of pregabalin or topiramate in impulsive-related disorders (mainly drug abuse) remains poorly understood. In the present study, we evaluated anxiety-like behaviour [light–dark box (LDB)], novelty seeking [hole board test (HBT)] and cognitive and motor impulsivity [delayed reinforcement task (DRT)] in DBA/2 mice, a strain with a high endogenous impulsivity level (Helms *et al.*, 2006; Patel *et al.*, 2006; Navarrete *et al.*, 2012). Dopaminergic and adrenergic key targets gene expression analyses were focused in brain regions from the mesolimbic and mesocortical pathways [ventral tegmental area (VTA), nucleus accumbens (ACC) and prefrontal cortex (PFC)] due to their involvement in impulsive behaviour (Wang *et al.*, 2002; Basar *et al.*, 2010; Kim and Lee, 2010). Tyrosine hydroxylase (TH) and the type 2 dopamine receptor (D_2-receptor) were analysed in dopaminergic cell bodies (VTA) and in terminals (ACC), respectively. On the other hand, the α_{2A}-adrenoceptor was studied in the PFC. The main purpose of this study was to elucidate if topiramate and/or pregabalin regulate certain impulsivity dimensions (novelty seeking or intolerance to delay) and if this regulation involves changes in dopaminergic and/or adrenergic pathways. Furthermore, the effects of both anticonvulsants on the high anxiety-like behaviour expressed by DBA/2 mice were evaluated in order to make a better distinction between pregabalin and topiramate.

Methods

Animals

DBA/2 OlaHsd mice were purchased from Harlan (Barcelona, Spain). Male mice between 8 and 10 weeks old and 20–25 g in weight were housed in groups of eight per cage (40 × 25 × 22 cm) under controlled conditions (temperature, 23 ± 2°C; relative humidity, 60 ± 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 animal room, were performed placing the home cage in the operant-task room 1 h before starting experiments. Standard laboratory chow (commercial diet for rodents A04 Panlab, Barcelona, Spain) and water were available *ad libitum* in all procedures, except for the DRT in which standard chow was restricted to only 60 min access per day. This food restriction regimen was applied from 3 days before starting and during the operant task (after the end of each daily session) to guarantee mice response to reinforcers. The food restriction schedule produced weight loss in mice of around 15% from their free-feeding weight. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). All experiments were in accordance with guidelines established by the European Council Directive (86/609/EEC) and approved by the Institutional Animal Care Committee.

Drugs

Pregabalin (Lyrica® by Pfizer, Madrid, Spain) and topiramate (Topamax® by Janssen-Cilag, Madrid, Spain) were dissolved in distilled water. In all the experiments, pregabalin (10, 20 and 40 mg·kg^{-1}, p.o.) and topiramate (12.5, 25, 50 mg·kg^{-1}, p.o.) were administered in a volume of 10 mL·kg^{-1}. In the LDB and HBT, mice received a unique dose 60 min before the start of the probe. In the DRT, two different administration schedules, acute and chronic, were used. In the acute schedule, mice received the corresponding unique daily dose 60 min before the beginning of each session. This dose was given only during the 10 days of the test phase, in which a delay was imposed (a total of 10 doses). In the chronic schedule, during the 7 day pretreatment the same acute dose was administered twice a day (approximately every 12 h); this preceded the DRT. During testing, the same administration protocol was followed; the morning dose was administered 60 min before each session (27 day treatment for a total of 54 doses). Drugs were freshly prepared each day before testing. A total of seven groups of mice (eight mice per group) were employed for each administration schedule (single, acute and chronic), and therefore, a total of 168 mice were tested in the present study. Although there are no pregabalin or topiramate pharmacokinetic specific data in mice, according to human studies (Shank and Maryanoff, 2008; Bockbrader *et al.*, 2010) and taking into consideration the metabolizing differences between mice and humans (Siefert *et al.*, 1999; Yang and Bankir, 2005), both drugs probably present good gastrointestinal absorption, rapidly reaching maximum and maintained plasma levels in humans. Furthermore, it could be hypothesized that with the acute schedule of administration used for the DRT, there was no drug accumulation since both pregabalin and topiramate would be totally excreted before the subsequent day's dose.

Anxiety-like behaviour – LDB test

The LDB model (Crawley and Goodwin, 1980) consisted of two methacrylate boxes 20 × 20 × 15 cm, 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 whose three paws were in the opposite box was considered a transition.

Impulsive-like behaviour

Novelty seeking – HBT Novelty-seeking behaviour was measured using an apparatus that consisted of a 40 × 40 × 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 head dips. First, there was a training phase in which the animals were introduced inside the apparatus for habituation. The number of head dips into each hole was recorded to discard mice with unconditioned preference for any hole. The day after, a small object was introduced into 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 the total time spent head dipping (object preference). Mice were individually placed in the centre to initiate a 10 min test.

Delay discounting – DRT The evaluation of the delay discounting was carried out in eight modular operant chambers (Panlab) placed inside eight soundproof 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 a lever press switched 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 time out (signalled with the chamber light off) was established, during which additional lever presses of either lever were recorded but without consequence. After the 30 s time out, the chamber light was turned on, indicating the start of the intertrial interval (ITI) in which the next trial is initiated depending on each subject's spontaneous waiting before the lever press. All mice performed one session of 30 min per day. According to the 30 s time out period, the maximal number of trials that an animal could theoretically complete (in the case of response immediately after the end of the timeout) during the training phase (without delays) was 60. The length of the training phase depended on the time to achieve the learning task criteria consisting of (1) reaching >75% of preference for the delayed lever; (2) >10 reinforced trials by session and (3) <20% deviation in the number of reinforced trials, all during 3 days. Once these criteria were reached, mice followed with the test phase where a time delay was introduced between lever pressing in the delayed lever and the delivery of the three pellets. During this period, the stimulus light (not the 0.5 s buzzer beep) was turned on, and additional lever presses of either lever were recorded but without consequence. The delay was fixed for a given daily session and progressively increased 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. Ineffective responses in

the immediate lever increased as a function of the delay time imposed. Each treatment group was tested at the same time, and the treatment group starting order was counterbalanced, placing the treatment group that first initiated a daily session at the end on the following day.

TH, D_2–receptor and α_{2A}-adrenoceptor gene expression analysis – real-time PCR

Mice were killed 24 h after the last DRT 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 the bregma: 1.42 and 1.34 mm respectively). The PFC, ACC and VTA were microdissected according to the method of Palkovits (Palkovits, 1983). Total RNA was isolated from micropunches of brain tissue using TRI Reagent® (Applied Biosystems, Madrid, Spain) and subsequently retrotranscribed to cDNA. Quantitative analysis of the relative abundance of α_{2A}-adrenoceptors (Mm00845383_s1), D_2–receptor (Mm00438541_m1) and TH (Mm00447546_m1) gene expression was performed on the ABI PRISM 7700 Sequence Detector System (Applied Biosystems, Foster City, CA) between the treatment and control groups. All reagents were obtained from Applied Biosystems, and the manufacturers' 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 (Livak and Schmittgen, 2001) so that treatment group levels were expressed relative to control group levels. Not all the mice used in the behaviour tests were included in the statistical analyses of real-time PCR studies due to the following reasons: low quantity of total RNA isolated and lack of real-time PCR amplification.

Statistical analysis

Statistical analysis was performed using two-way ANOVA with repeated measures followed by Student–Newman–Keul's test to compare the treatment and control groups at different time points in the DRT. One-way ANOVA followed by Student–Newman–Keul's test was employed when comparing the effects of pregabalin or topiramate on anxiety-like behaviour, novelty seeking or gene expression between the treatment and control groups. Differences were considered significant if the probability of error was less than 5%. SigmaStat v3.11 software (Systat Software Inc., Chicago, IL) was used for all statistical analysis.

Results

Effects of pregabalin and topiramate on anxiety-like behaviour

Anxiety-like behaviour was evaluated with the LDB paradigm, administering one single dose of each anticonvulsant drug 60 min before the test. Pregabalin fully reduced the high anxiety level expressed by DBA/2 mice, increasing the permanence time in the lighted side at all doses tested (Figure 1A: one-way ANOVA followed by Student–Newman–Keul's method, $F_{(3,31)} = 9.549$, $P < 0.001$). In contrast, topiramate decreased anxiety only with the highest dose (Figure 1C: one-way ANOVA followed by Student–Newman–Keul's method, $F_{(3,31)} = 4.261$, $P = 0.013$). The number of transitions between compartments was not affected by either pregabalin (Figure 1B: one-way ANOVA followed by Student–Newman–Keul's method, $F_{(3,31)} = 0.255$, $P = 0.857$) or topiramate (Figure 1D: one-way ANOVA followed by the Student–Newman–Keul's method, $F_{(3,31)} = 0.641$, $P = 0.595$).

Effects of pregabalin and topiramate on impulsive-like behaviours

Novelty seeking HBT was used to analyse the effects of pregabalin and topiramate, single-dose administration 60 min before the task, on novelty-seeking behaviour. The natural preference for the exploration of two of the four holes with a novel object expressed by DBA/2 mice, was not modified by pregabalin (Figure 2A: one-way ANOVA followed by Student–Newman–Keul's method, $F_{(3,31)} = 0.0366$, $P = 0.990$). On the other hand, topiramate significantly and dose-dependently reduced the object preference (Figure 2B: one-way ANOVA followed by Student–Newman–Keul's method, $F_{(3,31)} = 6.415$, $P = 0.002$)

Delay discounting

Acute administration schedule

Cognitive impulsivity In order to evaluate the effects of acute administration of both pregabalin and topiramate on cognitive impulsivity, DBA/2 mice were challenged in the DRT and were given the drug only during the test phase. Statistical analysis of the % preference change along time delays (delay discounting) indicated that neither pregabalin (Figure 3A: two-way ANOVA with RM followed by the Student–Newman–Keul's method, pregabalin dose $F_{(3,319)} = 0.645$, $P = 0.593$; delay $F_{(10,319)} = 89.305$, $P < 0.001$; pregabalin dose × delay interaction $F_{(30,319)} = 0.435$, $P = 0.996$) nor topiramate (Figure 3B: two-way ANOVA with RM followed by the Student–Newman–Keul's method, topiramate dose $F_{(3,319)} = 1.054$, $P = 0.385$; delay $F_{(10,319)} = 44.541$, $P < 0.001$; topiramate dose ×delay interaction $F_{(30,319)} = 1.416$, $P = 0.081$) were able to significantly modify the % preference with respect to the control group at any dose tested. Therefore, when administered acutely only during the test phase, pregabalin and topiramate failed to modulate cognitive impulsivity.

Motor impulsivity The number of ineffective responses during delay onset (not rewarded), reflecting motor dimension of impulsivity, was measured. Pregabalin did not modify the increasing number of immediate lever presses during delays (Figure 4A: two-way ANOVA with RM followed by the Student–Newman–Keul's method, pregabalin dose $F_{(3,319)} = 0.829$, $P = 0.489$; delay $F_{(9,319)} = 37.147$, $P < 0.001$; pregabalin dose × delay interaction $F_{(27,319)} = 1.015$, $P = 0.448$). However, topiramate significantly reduced DBA/2 motor impulsivity, mainly at the highest dose (Figure 4B: two-way ANOVA with RM followed by the Student–Newman–Keul's method, topiramate dose

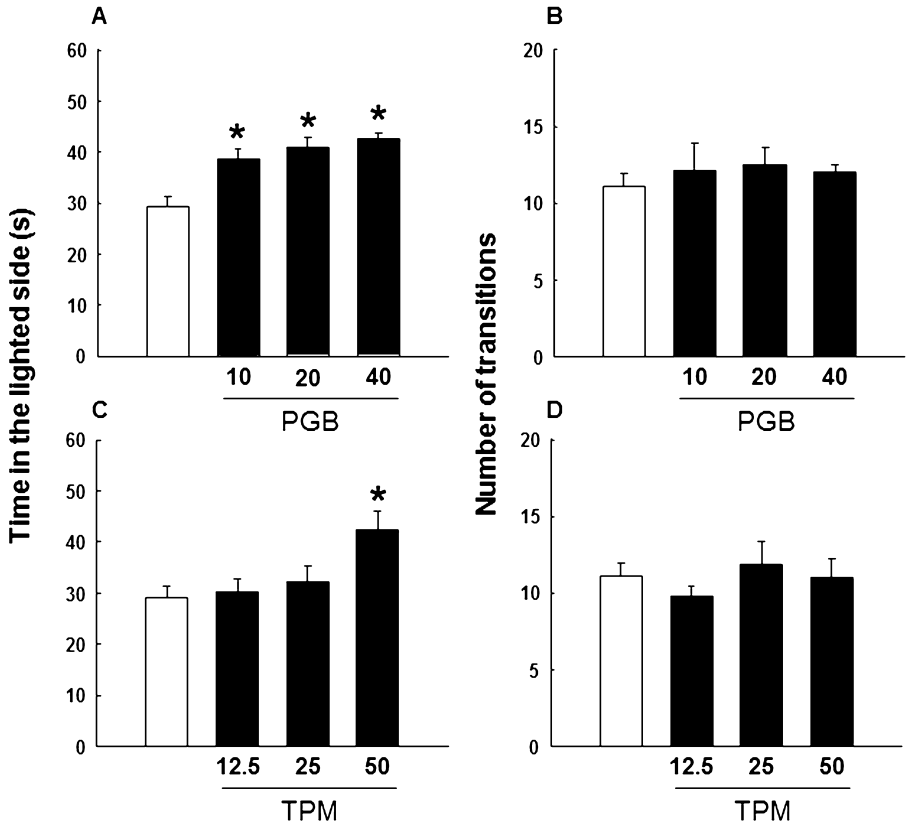

Figure 1

Evaluation of anxiety-like behaviour in DBA/2 mice treated with pregabalin (10, 20 or 40 mg·kg^{-1}, p.o., 1 h before testing) or topiramate (12.5, 25 or 50 mg·kg^{-1}, p.o., 1 h before testing) in the LDB paradigm. Columns represent the means and vertical lines ± SEM of the time spent in the lighted side (A,C) and the number of transitions (B,D). *Values of drug-treated DBA/2 mice that are significantly different ($P < 0.05$) from its corresponding vehicle group.

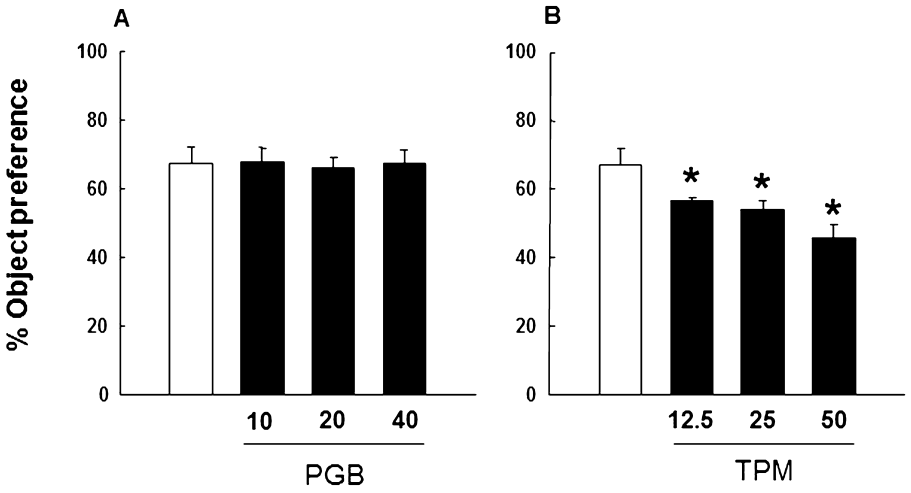

Figure 2

Analysis of novelty-seeking behaviour in DBA/2 mice treated with pregabalin (10, 20 or 40 mg·kg^{-1}, p.o., 1 h prior testing) or topiramate (12.5, 25 or 50 mg·kg^{-1}, p.o., 1 h before testing) on the HBT. Columns represent the means and vertical lines ± SEM of the % preference to explore holes containing an object with pregabalin (A) or topiramate (B). *Values of drug-treated DBA/2 mice that are significantly different ($P < 0.05$) from its corresponding vehicle group.

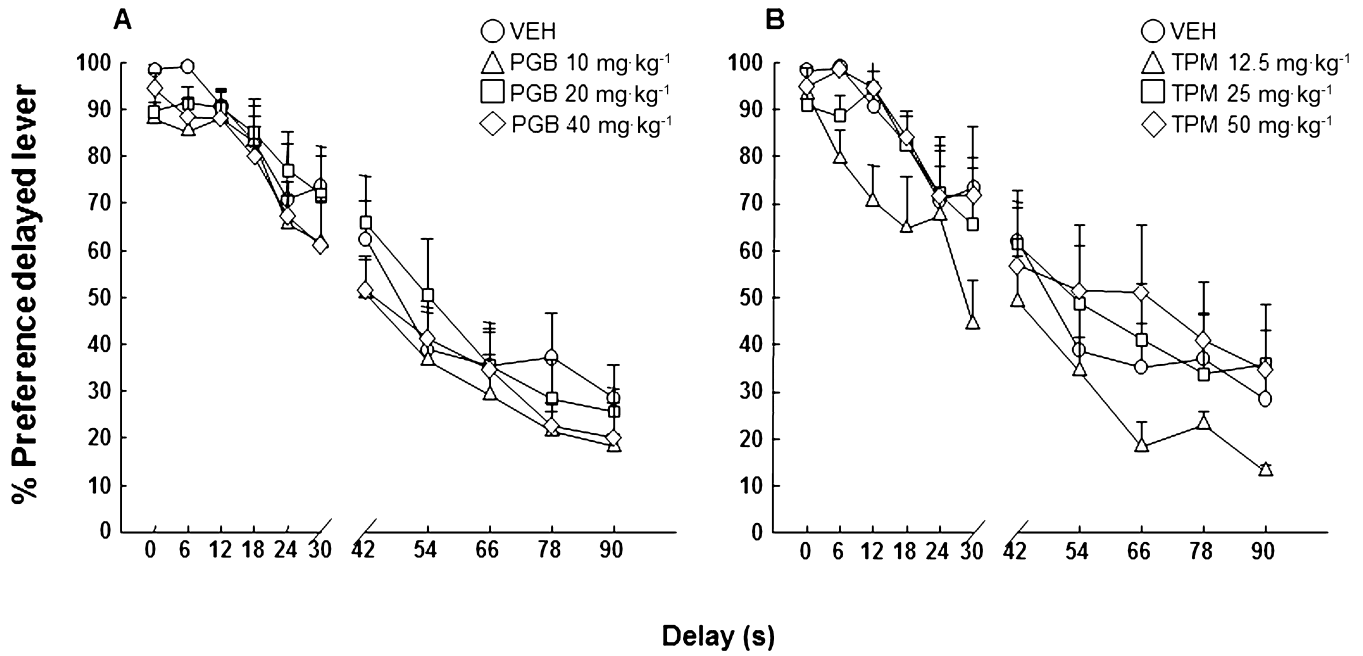

Figure 3

Assessment of cognitive impulsivity (delay discounting) in DBA/2 mice treated with pregabalin (10, 20 or 40 mg·kg^{-1}, p.o., for 10 days and 1 h before testing) or topiramate (12.5, 25 or 50 mg·kg^{-1}, p.o., for 10 days and 1 h before testing) in the DRT. Dots represent the means and vertical lines ± SEM of % preference for delayed reinforcement with pregabalin (A) or topiramate (B) treatment.

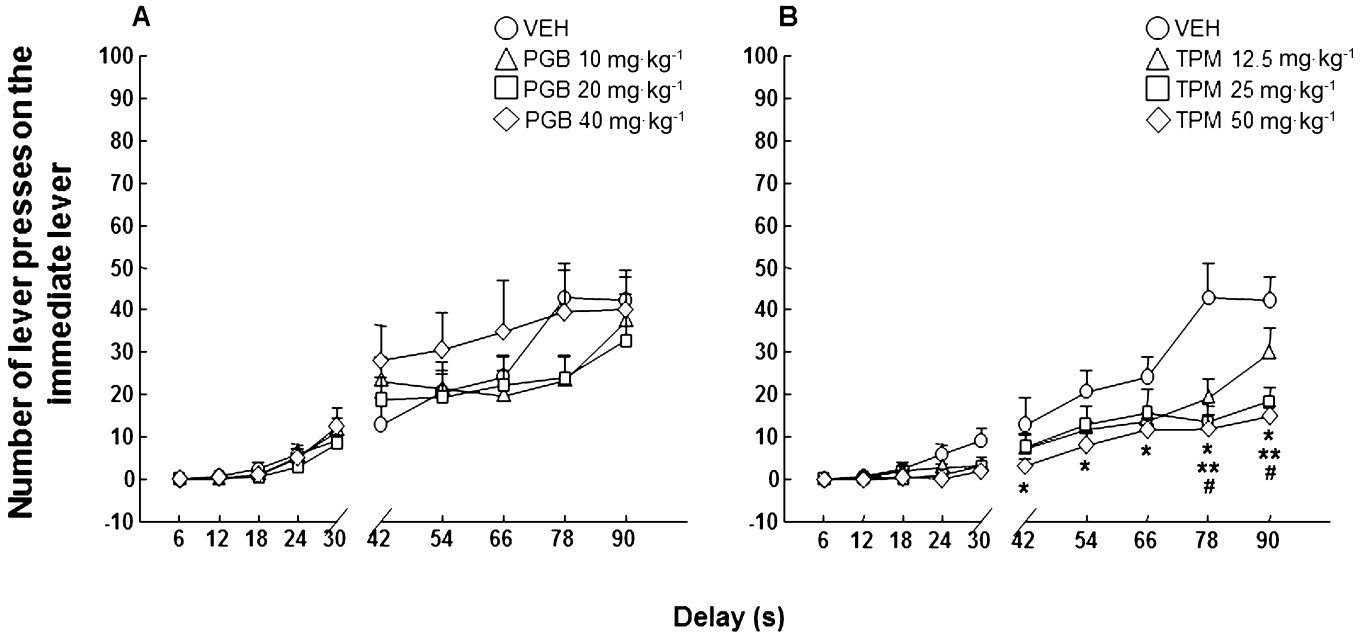

Figure 4

Motor impulsivity evaluation (ineffective responding) in DBA/2 mice treated with pregabalin (10, 20 or 40 mg·kg^{-1}, p.o., for 10 days and 1 h before testing) or topiramate (12.5, 25 or 50 mg·kg^{-1}, p.o., for 10 days and 1 h before testing) in the DRT. Dots represent the means and vertical lines ± SEM of number of lever presses in the immediate lever during delay onset with pregabalin (A) or topiramate (B) treatment. *Values for topiramate 50 mg·kg^{-1} group that are significantly different ($P < 0.05$) from its corresponding vehicle group; **values for topiramate 25 mg·kg^{-1} group that are significantly different ($P < 0.05$) from its corresponding vehicle group; #values for topiramate 12.5 mg·kg^{-1} group that are significantly different ($P < 0.05$) from its corresponding vehicle group.

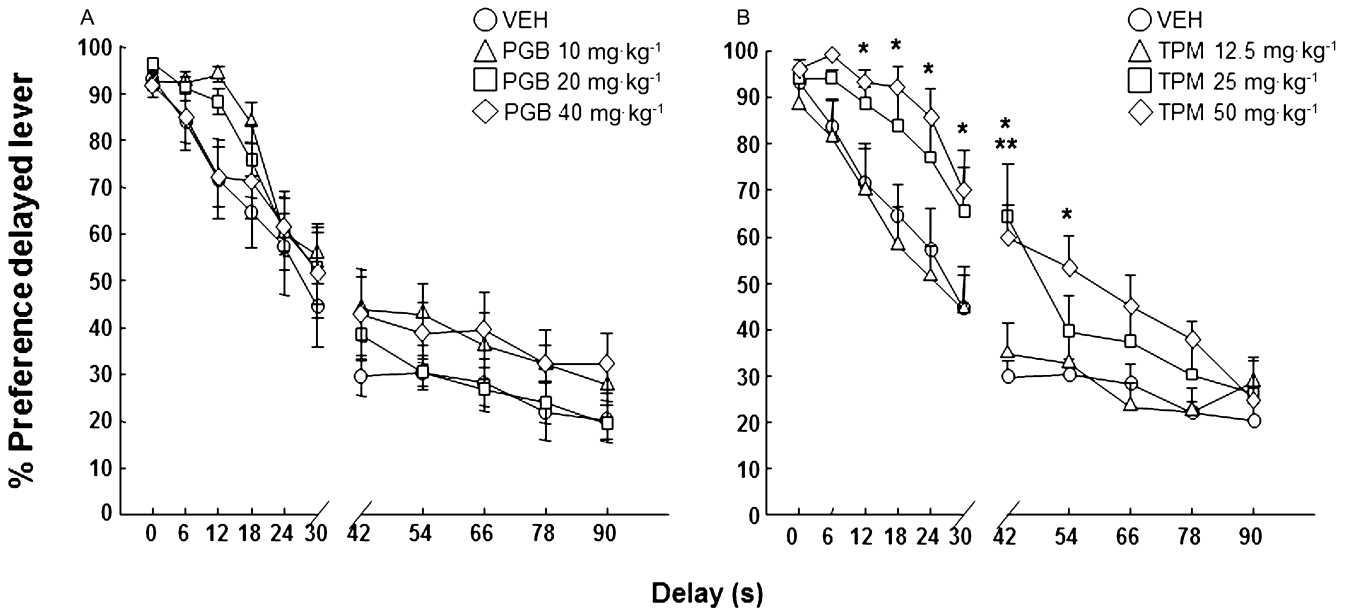

Figure 5

Assessment of cognitive impulsivity (delay discounting) in DBA/2 mice treated with pregabalin (10, 20 or 40 mg·kg^{-1}, p.o., twice a day for 27 days) or topiramate (12.5, 25 or 50 mg·kg^{-1}, p.o., twice a day for 27 days) in the DRT. Dots represent the means and vertical lines ± SEM of % preference for delayed reinforcement with pregabalin (A) or topiramate (B) treatment. *Values from topiramate 50 mg·kg^{-1} treated mice that are significantly different ($P < 0.05$) from its corresponding vehicle group; **values from topiramate 25 mg·kg^{-1} treated mice that are significantly different ($P < 0.05$) from its corresponding vehicle group.

$F_{(3,319)} = 5.091$, $P = 0.006$; delay $F_{(9,319)} = 57.026$, $P < 0.001$; topiramate dose × delay interaction $F_{(27,319)} = 3.954$, $P < 0.001$).

Chronic administration schedule

Cognitive impulsivity Cognitive impulsivity was evaluated after chronic treatment twice a day (7 day pretreatment plus treatment during the DRT). Chronic pregabalin did not modify DBA/2 delay discounting at any dose tested (Figure 5A: two-way ANOVA with RM followed by Student–Newman–Keul's method, pregabalin dose $F_{(3,319)} = 1.580$, $P = 0.217$; delay $F_{(10,319)} = 105.594$, $P < 0.001$; pregabalin dose × delay interaction $F_{(30,319)} = 0.894$, $P = 0.629$). On the other hand, topiramate significantly improved DBA/2 cognitive impulsivity, producing a dose-dependent decrease in delay discounting (Figure 5B: two-way ANOVA with RM followed by Student–Newman–Keul's method, topiramate dose $F_{(3,319)} = 4.140$, $P = 0.015$; delay $F_{(10,319)} = 131.747$, $P < 0.001$; topiramate dose × delay interaction $F_{(30,319)} = 1.855$, $P = 0.006$).

Motor impulsivity Surprisingly, the change from acute to chronic administration of pregabalin and topiramate produced an opposite response schedule when evaluating motor impulsivity in the DRT. Pregabalin significantly and dose-dependently increased the number of ineffective responses (Figure 6A: two-way ANOVA with RM followed by Student–Newman–Keul's method, pregabalin dose $F_{(3,319)} = 9.055$, $P < 0.001$; delay $F_{(9,319)} = 69.405$, $P < 0.001$; pregabalin dose × delay interaction $F_{(27,319)} = 1.611$, $P = 0.032$), whereas different topiramate doses were without effects (Figure 6B: two-way ANOVA with RM followed by the Student–Newman–Keul's method, topiramate dose $F_{(3,319)} = 0.818$, $P = 0.495$; delay $F_{(9,319)} = 61.252$, $P < 0.001$; topiramate dose × delay interaction $F_{(27,319)} = 0.624$, $P = 0.928$).

Gene expression changes after chronic pregabalin and topiramate administration

Real time-PCR gene expression analyses revealed that the chronic administration of pregabalin significantly reduced α_{2A}-adrenoceptor mRNA levels in the PFC of DBA/2 mice (Figure 7A: one-way ANOVA followed by Student–Newman–Keul's method, $F_{(3,27)} = 10.304$, $P = 0.001$) and also decreased D_2–receptor gene expression in the ACC (Figure 7B: one-way ANOVA followed by Student–Newman–Keul's method, $F_{(3,31)} = 3.091$, $P = 0.043$). Conversely, in the VTA, pregabalin dose-dependently increased TH gene expression (Figure 7C: one-way ANOVA followed by Student–Newman–Keul's method, $F_{(3,31)} = 3.894$, $P = 0.019$). On the other hand, chronic topiramate up-regulated α_{2A}-adrenoceptors in the PFC (Figure 7D: one-way ANOVA followed by Student–Newman–Keul's method, $F_{(3,28)} = 4.192$, $P = 0.016$) and D_2-receptors in the ACC (Figure 7E: one-way ANOVA followed by Student–Newman–Keul's method, $F_{(3,31)} = 3.153$, $P = 0.040$). In addition, topiramate increased TH gene expression in the VTA (Figure 7F: one-way ANOVA followed by the Student–Newman–Keul's method, $F_{(3,30)} = 6.005$, $P = 0.003$).

Discussion

To our knowledge, the results of the present study provide information for the first time about the differential effects of

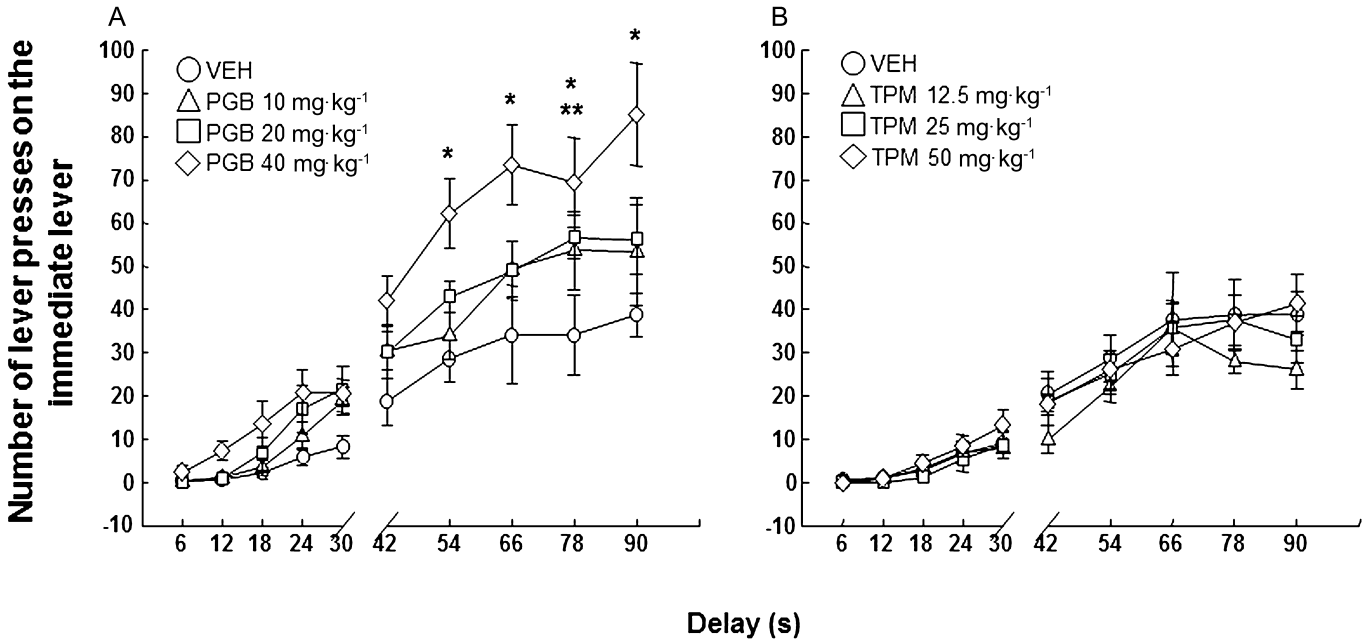

Figure 6

Motor impulsivity evaluation (ineffective responding) in DBA/2 mice treated with pregabalin (10, 20 or 40 mg·kg^{-1}, p.o., twice a day for 27 days) or topiramate (12.5, 25 or 50 mg·kg^{-1}, p.o., twice a day for 27 days) in the DRT. Dots represent the means and vertical lines ± SEM of number of lever presses in the immediate lever during delay onset with pregabalin (A) or topiramate (B) treatment. *Values from pregabalin 40 mg·kg^{-1} group that are significantly different ($P < 0.05$) from its corresponding vehicle group; **values from pregabalin 20 mg·kg^{-1} group that are significantly different ($P < 0.05$) from its corresponding vehicle group.

pregabalin and topiramate on anxiety- and impulsive-like behaviours employing different administration schedules. Topiramate reduced novelty seeking and as expected, according to previous clinical studies by our group (Rubio *et al.*, 2009), acutely modulated motor impulsivity and chronically modulated cognitive impulsivity in the DBA/2 strain of mice with a high-impulsive basal level (Navarrete *et al.*, 2012). On the other hand, pregabalin did not have any effect on either object preference or acutely in the DRT, whereas when administered chronically, it exacerbated motor impulsivity levels in DBA/2 mice. In addition, anxiety-like behaviour evaluation showed that pregabalin has a clear anxiolytic profile in comparison with topiramate, suggesting that the therapeutic usefulness of pregabalin in drug dependence management is more related to this emotional aspect. Furthermore, real-time PCR analyses clearly showed that both drugs modulated α_{2A}-adrenoceptors, D_2-receptors and TH gene expressions differently in the cortico-mesolimbic pathway, providing novel insight about the neurochemical modulatory effects of pregabalin and topiramate and their possible relationship with impulsivity regulation.

When administered acutely, none of the doses of pregabalin tested (10, 20 and 40 mg·kg^{-1}) or topiramate (12.5, 25 and 50 mg·kg^{-1}) modified the delay discounting progression of DBA/2 mice compared to the corresponding vehicle group. This lack of effect suggests that this schedule of administration (each session during the delay phase of the task) was not adequate to produce any effect on cognitive impulsivity. On the other hand, when motor impulsivity was evaluated by counting the number of immediate lever presses during the delay onset, pregabalin did not produce any effect, but topiramate clearly enhanced behavioural inhibition, mainly at the highest dose (50 mg·kg^{-1}). A possible explanation for this discrepancy may be related to differences in their mechanisms of action. Pregabalin modulates voltage-gated calcium channels binding to the α2-δ subunit mainly in hyperexcitability states (Taylor *et al.*, 2007), whereas topiramate acts through several mechanisms leading to a potent inhibitory state (White *et al.*, 1997; Zona *et al.*, 1997; Reis *et al.*, 2002; Braga *et al.*, 2009) independent of neuronal excitability. This fact may contribute to a more efficacious behavioural inhibition in DBA/2 mice, lowering the number of ineffective responses during the time delay. In the DRT, mice consistently learn to make a response (lever press) to achieve a reward (food). Development of such automatic processes seems to depend on glutamatergic neurotransmission through the activation of N-methyl D-aspartate receptors (Kelley *et al.*, 1997) and the activation of AMPA receptors is needed for their expression (Backstrom and Hyytia, 2003). Topiramate, acting as an AMPA receptor antagonist may improve the ability to these mice to wait, so reducing the number of ineffective (not rewarded) responses. In the same way, this mechanism could also explain the significant reduction in novelty-seeking behaviour of DBA/2 mice that was not achieved with pregabalin. Novelty seeking has been associated with drug abuse (Lange *et al.*, 2010; Cummings *et al.*, 2011). Hence, topiramate's ability to reduce novelty exploration behaviour may account for its usefulness as a drug-dependence treatment.

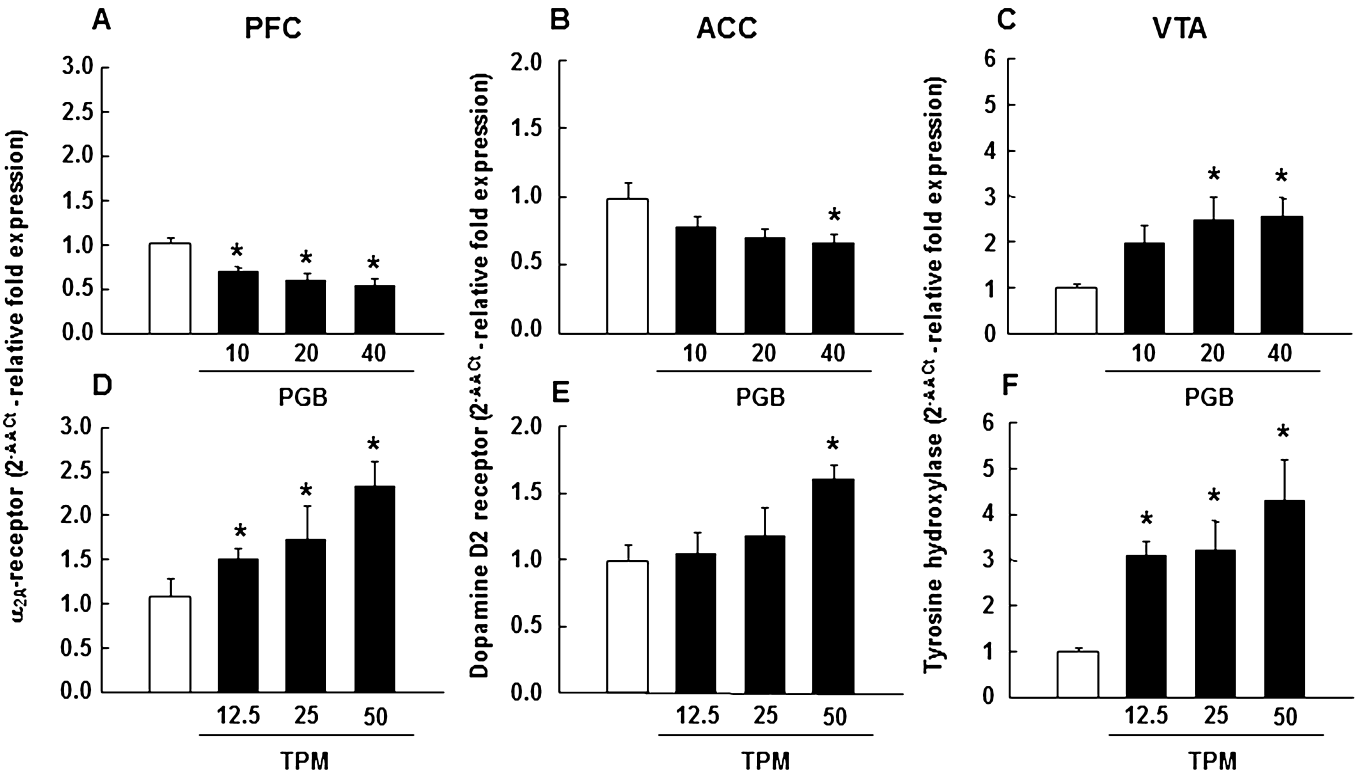

Figure 7

α_{2A}-adrenoceptor, D_2-receptor and TH relative gene expressions evaluation in the PFC, ACC and VTA, respectively, of pregabalin (10, 20 or 40 mg·kg^{-1}, p.o., twice a day for 27 days)- or topiramate (12.5, 25 or 50 mg·kg^{-1}, p.o., twice a day for 27 days)-treated mice by real time-PCR. Columns represent the means and vertical lines ± SEM of relative ($2^{-\Delta\Delta Ct}$ method) α_{2A}-adrenoceptor gene expression in PFC (A,D), D_2-receptor gene expression in ACC (B,E) and TH gene expression in VTA (C,F) of DBA/2 treated mice. *Values of drug-treated DBA/2 mice that are significantly different ($P < 0.05$) from its corresponding vehicle group.

Since acute administration of either drug did not alleviate the high cognitive impulsivity level in DBA/2 mice, it was hypothesized that chronic administration with a pretreatment phase before the beginning of the DRT and the administration of the drug twice a day would be appropriate to identify whether pregabalin or topiramate is able to modulate delay discounting. Although chronic administration of topiramate was without effect on motor impulsivity, the medium (25 mg·kg^{-1}) and highest (50 mg·kg^{-1}) doses of topiramate significantly reduced delay discounting in DBA/2-treated mice. The percentage of preference for the delayed lever was maintained significantly higher than in the control group from 12 s until 54 s of delay. This effect was not present in the final stages of the experiment, probably due to a tolerance effect. These results suggest that the schedule of dosing and duration of the treatment play a crucial role in the modulatory effect of topiramate on impulsive choice. Indeed, depending on the administration schedule, this drug modulated either motor or cognitive impulsivity behaviours. In contrast, pregabalin failed to alter the preference for the delayed lever and even significantly increased motor impulsivity when administered chronically at a 40 mg·kg^{-1} dose. This effect could be related to the anxiolytic effect of pregabalin (Lauria-Horner and Pohl, 2003; Frampton and Foster, 2006). A decrease in the anxiety level in spontaneously anxious DBA/2 mice (Griebel *et al.*, 2000; Ohl *et al.*, 2003; Yilmazer-Hanke *et al.*, 2003) may be responsible for behavioural disinhibition, leading to an increase in the number of immediate lever presses. The inability of pregabalin to diminish cognitive or motor impulsivity seems to indicate that its potential beneficial effects on drug abuse may be due to the regulation of other behavioural mechanisms such as co-morbid psychiatric symptomatology (Martinotti *et al.*, 2010). Data shown in Figure 1 clearly indicate that pregabalin presents a potent anxiolytic effect, increasing the time spent in the lighted and open side at all doses tested, supporting the previous hypothesis. Indeed, recent data from a study by our group demonstrated that pregabalin reduces the increase in the anxiety level produced by spontaneous cannabinoid withdrawal in mice (Aracil-Fernandez *et al.*, 2011).

It is important to note that the measurement of motor impulsivity in the DRT is different from the evaluation in the five-choice serial reaction time or Go/NoGo tasks. The former evaluates the inability to wait until the reinforcement is delivered (a response that does not have consequences) and the latter the inability to withhold a prepotent response (a response that has negative consequences). The analysis of motor impulsivity in animal experimental models has been classically developed in tasks in which the animal has to refrain from responding to achieve a goal (reward). In the

present study, the number of lever presses during the delay onset would determine the level of restlessness in mice. As stated by other authors, this behavioural parameter also takes part in the definition of motor impulsivity (Dellu-Hagedorn, 2006; Boes *et al.*, 2009). Indeed, the Barratt Impulsiveness Scale (BIS-11), a widely used and well-validated tool to measure human impulsivity, considers motor impulsiveness as 'acting without thinking and restlessness' (Patton *et al.*, 1995).

Gene expression analyses were focused on dopaminergic and adrenergic neurotransmission systems. There is much evidence for the critical involvement of dopamine in impulsive behaviour (van Gaalen *et al.*, 2006; Buckholtz *et al.*, 2010) and special attention has been paid to the role of D_2-receptors in this effect (Dalley *et al.*, 2007; Hamidovic *et al.*, 2009; Lee *et al.*, 2009). On the other hand, PFC adrenergic circuit involvement in decision making is well known (Dalley *et al.*, 2008; Kim and Lee, 2010). Agonists of α_{2A}-adrenoceptors have been shown to be useful in the treatment of inattention, hyperactivity and impulsiveness in ADHD (Scahill, 2009); and, recently, the α_{2A}-adrenoceptor agonist guanfacine was found to ameliorate impulsive choice behaviours in primates (Kim *et al.*, 2011). For these reasons, in the present study we investigated whether the effects of pregabalin and topiramate on impulsivity dimensions are related to their modulation of α_{2A}-adrenoceptor, D_2-receptor and TH gene expression. These studies were carried out in the mesolimbic–mesocortical pathways for three reasons: (1) the critical involvement of this pathway in the regulation of impulsive behaviours (van Gaalen *et al.*, 2006; Dalley *et al.*, 2007; Lee *et al.*, 2009; Basar *et al.*, 2010; Buckholtz *et al.*, 2010; Kim and Lee, 2010); (2) its crucial role in reinforcement effects of drugs of abuse (Phillips and Fibiger, 1973; Leshner and Koob, 1999; Hyman and Malenka, 2001); and (3) dopaminergic and adrenergic tone are both modulated by pregabalin (Andrews *et al.*, 2001; Gajraj, 2005; Takeuchi *et al.*, 2007) and topiramate (Johnson, 2004a,b). The neuropharmacological action of topiramate includes facilitation of GABA-mediated neurotransmission and blockade of AMPA/kainate glutamate receptors. According to Johnson's hypothesis (Johnson *et al.*, 2003), because mesocorticolimbic dopamine release is under tonic inhibitory control via GABAergic neurons and excitatory control via glutamatergic neurons, topiramate may inhibit dopamine release and consequently reduce receptor activation. Maintenance of this effect with chronic administration could produce a compensatory effect. Real-time PCR results support this hypothesis since topiramate dramatically increased TH gene expression in the VTA and also up-regulated D_2-receptors in the ACC. Furthermore, it is widely accepted that low D_2-receptor availability in the brains of animals or humans is related with a high impulsivity level (Dalley *et al.*, 2007; Lee *et al.*, 2009), probably due to a high basal dopaminergic tone. Indeed, it has been reported that pharmacological modulation by D_2-receptor antagonists induced impulsive choice, suggesting that these receptors normally promote choice of the delayed reinforcement (Wade *et al.*, 2000). DBA/2 mice present low D_2-receptor gene expression in comparison with a low-impulsive strain (Navarrete *et al.*, 2012). Therefore, it seems that the enhancement of D_2-receptor expression in the ACC, achieved with the chronic administration of topiramate, could be closely associated with the cognitive impulsivity modulation. On the other hand, pregabalin showed no effect on cognitive impulsivity, a fact that could be partially explained by a distinct dopaminergic modulation that entails an opposite effect on D_2-receptor gene expression and a smaller increase in TH in the VTA in comparison with topiramate.

Interestingly, the administration of topiramate up-regulated the α_{2A}-adrenoceptor gene expression in the PFC dose-dependently, which would fit with a direct/indirect adrenergic blockade not previously described in the literature for this drug. Genetic variants of the α_{2A}-adrenoceptor are involved in drug abuse (Feng *et al.*, 1998; Prestes *et al.*, 2007) and ADHD (Xu *et al.*, 2001; Schmitz *et al.*, 2006). Furthermore, α_{2A}-adrenoceptor gene expression in the PFC has been inversely correlated with lever pressing to obtain a reward (Pickering *et al.*, 2007), suggesting that animals with a low responding rate present higher α_{2A}-adrenoceptor gene expression levels. This finding seems to agree with the chronic pregabalin effect on motor impulsivity since the dose-dependent increase in the number of ineffective responses is associated with a dose-dependent decrease in α_{2A}-adrenoceptor gene expression in the PFC. In the same way, it could be hypothesized that the lack of effect of chronic topiramate on behavioural inhibition in comparison with the acute schedule may be related to the significant increase in α_{2A}-adrenoceptor mRNA levels in the PFC.

In conclusion, the present study demonstrates that the chronic administration of topiramate regulated cognitive impulsivity, whereas acute drug treatment regulated motor impulsivity expressed by DBA/2 mice. These results point out the relevance of the administration schedule to regulate distinct dimensions of impulsive behaviour. In addition, topiramate reduced novelty-seeking behaviour, which is closely associated with drug abuse vulnerability. These findings suggest that the therapeutic utility of topiramate in addictive behaviours, such as alcohol-dependence, may be due to its ability to control impulsive-like behaviours. The impulsivity modulation showed by topiramate seems to be associated with differential gene expression changes in mesolimbic–mesocortical dopaminergic and adrenergic neurotransmission. The present results suggest that the up-regulation of D_2-receptor gene expression induced by topiramate could be the main mechanism responsible for the reduction in novelty seeking and cognitive impulsivity in DBA/2 mice. On the other hand, the therapeutic utility of pregabalin in impulsive-related disorders appears to be more associated with its ability to regulate other behavioural aspects such as anxiety, since no beneficial effects were achieved in either the HBT or in the DRT.

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Conflicts of interest

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

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