Cocaine reverses the naltrexone-induced reduction in operant ethanol self-administration: The effects on immediate-early gene expression in the rat prefrontal cortex

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article info
Article history:
Received 29 February 2012
Received in revised form 19 May 2012
Accepted 7 June 2012

Keywords:
Naltrexone
Acamprosate
Prefrontal
C-fos
COX-2
Genetic expression

ABSTRACT
Naltrexone is a clinically approved medication for alcoholism. We aimed to investigate the effectiveness of naltrexone co-administered with cocaine and the association of these substances with immediate-early gene expression in the rat prefrontal cortex. We used chronic operant ethanol self-administration and oral treatments prescribed for alcoholism and available in pharmacies to maximise the predictive validity in humans. We performed real-time PCR analysis to determine gene expression levels in the prefrontal cortex. Only the highest dose of naltrexone (1, 3, and 10 mg/kg, p.o.) reduced the response to ethanol. Cocaine increased ethanol self-administration in a dose-dependent manner (2.5, 10, 20 mg/kg, i.p.) and reversed the naltrexone-induced reduction. Naltrexone failed to prevent the cocaine-induced increase in locomotor activity observed in these animals. Chronic self-administration of ethanol reduced the expression of the C-fos gene 4- to 12-fold and increased expression of the COX-2 (up to 4-fold) and Homer1a genes in the rat prefrontal cortex. Chronic ethanol self-administration is prevented by naltrexone, but cocaine fully reverses this effect. This result suggests that cocaine may overcome naltrexone’s effectiveness as a treatment for alcoholism. The ethanol-induced reduction in C-fos gene expression in the prefrontal cortex reveals an abnormal activity of these neurons, which may be relevant in the compulsive consumption of ethanol, the control of reward-related areas and the behavioural phenotype of ethanol addiction.

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1. Introduction
Alcoholism is a disabling addiction disorder (WHO, GISAH, 2011). An effective treatment for alcoholism remains elusive despite the advances that have been made including the development of naltrexone (i.e., Antaxone®) and acamprosate (i.e., Campral®), two clinically approved medications to treat binge ethanol consumption, ethanol abuse and dependence and to prevent relapse (Fuller and Gordis, 2001; Johnson, 2010; Mason, 2003; O’Brien et al., 1996; Spanagel and Ziegllänsberger, 1997). Treatment with oral naltrexone has been associated with a significant reduction in alcohol-related healthcare costs (Kranzler et al., 2010). Naltrexone is a non-selective opioid antagonist theorised to reduce ethanol consumption by blocking central opioid receptors that subsequently directly or indirectly modulate the effects of ethanol (Hillemacher et al., 2011; Hubbell and Reid, 1990). The mechanism of action of acamprosate is less well understood. Acamprosate is hypothesised to reduce neuronal hyperexcitability through its putative agonist-like effects at GABA receptors and its antagonist effects at the glutamate N-methyl-D-aspartate (NMDA) receptor (Kiefer and Mann, 1995; Stromberg et al., 2001). The effects of combining the two compounds on ethanol consumption have also been assessed. Stromberg et al. (2001) reported no evidence of an additive or synergistic effect resulting from such a combination nor...
was it more effective than naltrexone alone in reducing ethanol consumption by rats.

The co-abuse of ethanol and cocaine occurs with high frequency and persistence in human populations worldwide. For example, Miller et al. (1989) reported that 94% of the American patients diagnosed as cocaine-dependent were also diagnosed with an ethanol dependence. In another study, approximately 12 million members of the general population had used both ethanol and cocaine within the previous twelve months (Grant and Harford, 1990). In a more recent study in Europe, 64% of the cocaine powder users (excluding crack cocaine users) reported frequent ethanol consumption (Gossop et al., 2006), and the concomitant use of ethanol was evident by analyses of blood and urine samples in 76% of cocaine-related cases of sudden death (Lucena et al., 2010). Finally, heavy chronic alcohol use is linked to a three-fold increased risk of cocaine use (Kulaga et al., 2010). These data support the idea that cocaine use could increase the vulnerability to the development of ethanol dependence (Rubio et al., 2008) and vice versa. A careful examination of the clinical data indicates that the concurrent use of ethanol and cocaine is associated with increased mortality and morbidity resulting from cerebro- and cardiovascular complications (Camí et al., 1998; O’Connor et al., 2005; Randall, 1992; Vroegop et al., 2009) as well as hepatotoxicity and compromised mental status (Odeleye et al., 1993; Vanek et al., 1996). Taken together, these facts underscore the urgency and necessity to develop pharmacotherapeutic interventions for alcoholism and the comorbidity of alcoholism with cocaine use.

Currently, despite the increasing number of studies investigating the effects of naltrexone or acamprosate on ethanol/cocaine co-consumption (Hersh et al., 1998; Oslin et al., 1999; Pettinati et al., 2008a, 2008b; Sable et al., 2004; Schmitz et al., 2004, 2009; Stromberg et al., 2002; Suh et al., 2008), knowledge in this area remains incomplete. For example, a high dose of naltrexone modestly reduced heavy ethanol consumption in individuals dependent on both cocaine and ethanol (Schmitz et al., 2009). Therefore, the aim of this study was to gain deeper knowledge regarding the treatment of alcohol addiction with naltrexone and acamprosate when cocaine is co-administered. For this purpose, we used chronic operant ethanol self-administration in rats, an animal model with one of the highest levels of predictive validity in humans (Koob et al., 2003) for the development of pharmacological treatments for substance abuse disorders. Furthermore and important in this study, we investigated the expression of immediate-early genes as biomarkers of neural stimulation in the prefrontal cortex.

We focused on the prefrontal cortex because of its contribution to addictive behaviour (Lüscher and Malenka, 2011), its involvement in compulsive ethanol drinking, its demonstrated sensitivity to naltrexone and acamprosate treatment (Burattini et al., 2008; Li et al., 2010; Yu et al., 2011), and its critical role in integrating and regulating cognitive behaviour in rodents and in humans (e.g., Abernathy et al., 2010; Dayas et al., 2007; Vengeliene et al., 2009).

2. Methods

2.1. Subjects

Ninety-two male Wistar rats (Harlan, Barcelona, Spain) weighing 375–425 g at the start of the pharmacological experiments were housed in groups of 4 per cage in a temperature- and humidity-controlled environment on a 12 h reverse light/dark cycle (lights off at 07:00 h). Experimental sessions were performed during the dark phase (Fig. 1). Food and water were available ad libitum except as specified below. All research was conducted in strict adherence to the European Community Council Directive (91/441/EEC). All efforts were made to minimise animal suffering and to reduce the number of animals used.

2.2. Drugs

Ethanol solution was prepared daily as a 10% ethanol v/v solution from 99% ethanol. Acamprosate (3-Acetamidopropane-1-sulfonic acid, Campro), Naltrexone (17-(Cyclopropylmethyl)-4,5-dihydroxymorphinan-6-one, Antaxone®, Pharmazam S.A., Barcelona, Spain) were dissolved in water and administered by oral gavage (p.o.) at a volume of 3 ml/kg. Cocaine hydrochloride (Sigma–Aldrich Co., U.S.A.) was dissolved in physiological saline and injected intraperitoneally at a volume of 1 ml/kg.

2.3. Ethanol self-administration and motor experiments

2.3.1. Apparatus and procedure

The ethanol operant sessions were conducted in modular chambers enclosed in sound-attenuating cubicles (Letica, LE 850 model; Panlab, Barcelona, Spain and Med Associates Inc., St. Albans, VT, USA). The association of visual or auditory cues with lever presses or dipper presentations was avoided. The exhaust fans were inactivated because they increase the rate of ethanol evaporation. The chambers were equipped with two retractable levers located 7 cm above a grid floor on either side of a drinking reservoir positioned in the centre of the front panel of the chamber and 4 cm above the grid floor. The levers were counterbalanced to respond as the active lever (delivering 0.1 ml) or as the inactive lever. The contents of the dipper were accessible to the animal until the next lever press, at least 2.5 s later, to avoid measuring dipper presentations as lever presses.

Training was conducted using a modification of the method used by López-Moreno et al. (2004). Briefly, the rats were placed on a restricted water intake schedule ranging from 2 to 4 days to facilitate the training in lever pressing. The length of the water restriction depended on the animal’s rate of learning; animals that did not learn the first or second day were restricted for 4 days. During the first 4 days of training, 76 animals received a 1% saccharin solution in the dipper. Thereafter, the following sequence was followed on a fixed-ratio schedule of...
reinforcement: 0.2% saccharin for three sessions, 0.2% saccharin and 0.2% ethanol for three sessions, 0.16% saccharin and 2% ethanol for three sessions, 0.12% saccharin and 4% ethanol for three sessions, 0.08% saccharin and 6% ethanol for three sessions, 0.04% saccharin and 8% ethanol for three sessions, 0.02% saccharin and 10% ethanol for three sessions and 1% ethanol for the remaining sessions. An additional group of 16 animals that had access only to saccharin and did not receive any pharmacological treatment during the study was used as the control group for the genetic expression experiment (the calibrator group). The baseline (obtained at day 0) corresponded to the average number of ethanol responses obtained on the final 5 days before the first experiment (from the 59th day to the 63rd day); the number of responses varied by 15% or less. All the ethanol operant sessions lasted 30 min under a fixed-ratio 1 schedule for the entire study. The locomotor activity of the rats was assessed using 6 custom-made 40 × 35 × 35 cm rectangular boxes; the boxes were equipped with 8 photocells arranged in 2 lines (4 and 8 cm above the floor) that detected the locomotor activity as beam breaks. The animals were evaluated in a counterbalanced manner during a four-day period and in a single 20 min trial immediately following an injection with cocaine or the vehicle. The horizontal and rearing activity of the rats was recorded.

2.3.2. Experiment 1: the effects of subchronic treatment with naltrexone or acamprosate on the response to ethanol

Experiment 1 was designed to identify which drug (naltrexone or acamprosate) more efficiently reduced ethanol self-administration and to select one of the drugs for further behavioural studies concerning alcohol/cocaine co-administration. To this end, we evaluated the effects of varying doses of naltrexone (0, 1, 3 and 10 mg/kg, p.o.) and acamprosate (0, 35, 75, and 210 mg/kg, p.o.) on the number of limited-access responses to ethanol. Naltrexone and acamprosate were dissolved in water and administrated by oral gavage 60 min prior to the ethanol operant session daily for 5 consecutive days. The route of administration (p.o.) was chosen to strengthen the ecological validity of the study because it most closely resembled the setting for human patients. The rats were deprived of food 12 h prior to the pharmacological treatment. Two groups were used: (a) the control group received saline; (b) the three cocaine groups were treated orally with water and with 2.5, 10 or 20 mg/kg, i.p. cocaine and (c) the treatment group received saline and then injected with saline; (b) the cocaine groups were administered with saline; (c) the treatment group received saline and then injected with saline; (d) the cocaine groups were treated orally with water and with 2.5, 10 or 20 mg/kg, i.p. cocaine. The treatment schedule is depicted in Fig. 1b.

2.3.4. Experiment 3: the effects of cocaine and naltrexone and their co-administration on locomotor activity

In this experiment, we aimed to evaluate whether naltrexone would prevent cocaine-induced motor sensitisation. As described above, naltrexone was administered 1 h before the administration of cocaine to the animals treated with a combination of naltrexone and cocaine (Fig. 1b).

2.4. Genetic expression experiments

2.4.1. Apparatus and procedure

Real-time PCR was performed using a LightCycler 480-II machine (Roche) with primers and assays designed by the Universal Probe Library (UPL) from Roche (see Table 1). A 10-fold dilution series of the template was employed to amplify every gene to validate the efficiency of each assay and to confirm that the amplification efficiencies of the target and reference genes were comparable (indicated by a near-zero slope value for both the target and reference genes). The 18S ribosomal RNA gene was used as an internal control for normalisation. The saccharin-vehicle group (the non-ethanol-treated group) was used as a calibrator (an untreated control), and the 2−ΔΔCT method was used to analyse the expression data (Schmittgen and Livak, 2008).

The prefrontal cortex, including the frontal association cortex and the more rostral/anterior regions of the lateral–ventral–dorsal–medial areas of the orbital cortex, prelimbic cortex and secondary motor cortex (Paxinos and Watson, 1998 for rat), was immediately dissected on ice and quickly frozen on dry ice at −80 °C. Total RNA was isolated from the prefrontal cortex of each rat using TriPure Isolation Reagent (Roche) and stored at −80 °C. One microgram of total RNA was reverse transcribed into cDNA using the Transcriptor First Strand cDNA Synthesis Kit (Roche).

2.4.2. Experiment 4: the effects of ethanol, cocaine and naltrexone on the immediate-early gene expression in the prefrontal cortex

The goal of experiment 4 was to map the brain prefrontal activity related to our behavioural results. For this purpose, we assessed the immediate-early gene expression of two regulatory transcription factors (C-fos and Zif-268) and five effector immediate-early genes (Bdnf, Homer1a, Rheb and COX-2) to maximise the association between the prefrontal activity and the animal’s state before the operant ethanol session, the animals were sacrificed rapidly by decapitation during the period they would have been placed into the ethanol operant chamber for the 15th day of ethanol operant self-administration (see Fig. 1a).

2.5. Statistical analysis

The data from the two first experiments were analysed using a one-way ANOVA with treatment as the between-subject factor followed by Tukey’s post hoc test. A two-way ANOVA (cocaine × treatment as the between-subject factors) was used to analyse the data from the third experiment. Similarly, a two-way ANOVA (gene × treatment as the between-subject factors) was used for the fourth experiment, but in this case, the Bonferroni post hoc test was used because of the greater number of multiple comparisons. After confirming the significant main effects by ANOVA,

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Details of the primers used and cycle numbers required to reach the crossing point (threshold) in the saccharin group in the prefrontal cortex.</th>
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<tbody>
<tr>
<td>Gene</td>
<td>Name</td>
</tr>
<tr>
<td>18S</td>
<td>18S ribosomal RNA gene</td>
</tr>
<tr>
<td>Rheb</td>
<td>Ras homologue enriched in brain</td>
</tr>
<tr>
<td>Zif-268</td>
<td>Transcription factor Zif268</td>
</tr>
<tr>
<td>C-fos</td>
<td>Proto-oncogene c-Fos</td>
</tr>
<tr>
<td>Homer1</td>
<td>Homer homologue 1</td>
</tr>
<tr>
<td>Arc</td>
<td>Activity-regulated cytoskeleton-associated protein</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclooxygenase-2</td>
</tr>
<tr>
<td>Bdnf</td>
<td>Brain-derived neurotrophic factor</td>
</tr>
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A sample’s “Crossing Point” (Cp) depends on the initial concentration of CDNA in the sample. Samples with a lower initial concentration of target DNA require more amplification cycles to reach the Cp. A sample with a higher concentration requires fewer cycles. The quantity of DNA doubles every cycle, and 3.32 cycles represent a 10-fold increase assuming 100% PCR efficiency.
a significance level of $p < 0.05$ was applied to all statistical analyses. The SPSS statistical software package (version 17.0) for Windows (Chicago, IL) was used for all statistical analyses.

3. Results

3.1. Experiment 1: the effects of subchronic treatment with naltrexone or acamprosate on the response to ethanol

The goal of the first experiment was to establish dose–response curves for naltrexone (0, 1, 3 and 10 mg/kg, p.o.) and acamprosate (0, 35, 75 and 210 mg/kg, p.o.) on operant ethanol self-administration ($n = 10 – 12$ per group). This was performed over five consecutive days (subchronic treatment). The ANOVA and post hoc data analyses indicated that only the highest dose of naltrexone (10 mg/kg) significantly suppressed the total number of ethanol responses [$F(3,39) = 10.25, p < 0.001$], as shown in Fig. 2a. Ethanol intake levels were between $0.61 \pm 0.4$ and $0.74 \pm 0.5$ g/kg (mean $\pm$ SEM) in the vehicle group and between $0.32 \pm 0.3$ and $0.40 \pm 0.3$ g/kg in the group treated with 10 mg/kg naltrexone. Doses of 1 and 3 mg/kg of naltrexone failed to reduce the number of ethanol responses. Treatment with acamprosate at 35, 70 or 210 mg/kg produced no significant changes in the number of ethanol responses [$F(3,40) = 0.29, p = 0.831$], as shown in Fig. 2b. There were no significant differences in the activity towards the inactive lever. This experiment allowed us to select naltrexone for future experiments and eliminate acamprosate.

3.2. Experiment 2: the effects of the chronic administration of cocaine, naltrexone and their co-administration on the response to ethanol

The purpose of this experiment was two-fold: first, to establish a dose–response curve for cocaine (2.5, 10 and 20 mg/kg, i.p.) on the response to ethanol, and second, to investigate whether the effective dose of naltrexone (10 mg/kg, p.o.) could prevent operant ethanol self-administration even with the co-administration of cocaine, which is the most common pattern of human cocaine use (e.g., Gossop et al., 2006; Miller et al., 1989). This experiment was performed over 14 consecutive days (chronic treatment). Cocaine caused a dose-dependent increase in the number of responses to ethanol [$F(2,44) = 6.42, p < 0.001$], as shown in Fig. 3a ($n = 10 – 12$ per group). A post hoc analysis revealed that (a) the highest dose of cocaine (20 mg/kg) significantly increased the total number of ethanol responses and (b) the number of ethanol responses from the group treated with the lowest dose of cocaine (2.5 mg/kg) was lower than those of the groups receiving the other two doses of cocaine. There were no significant differences in the activity towards the inactive lever (between 1 and 7 responses). Fig. 3b shows the evolution of the number of ethanol responses averaged over two days during the 14 days of treatment with cocaine.

Chronic treatment with naltrexone (10 mg/kg, p.o.) significantly reduced the total number of responses to ethanol compared with the number of responses made by the vehicle group [$F(3,40) = 19.84, p < 0.001$], as shown in Fig. 4a ($n = 10 – 12$ per group). This supports our results from the subchronic experiment. Further, the post hoc analysis revealed that both cocaine doses (10 and 20 mg/kg, i.p.) fully reversed the naltrexone-induced reduction in operant ethanol self-administration. Moreover, the number of ethanol responses made by the groups treated with both cocaine doses was increased compared with the number made by the vehicle group. Fig. 4b shows the responses averaged over two days for alcohol self-administration during the 14 days of treatment with naltrexone and cocaine.

3.3. Experiment 3: the effects of cocaine, naltrexone and their co-administration on locomotor activity

The aim of the third experiment was to evaluate locomotor activity after treatment with cocaine, with the effective dose of naltrexone (10 mg/kg, p.o.) and with a combination of the two drugs (Fig. 5). With this information, we intended to determine whether cocaine-induced motor sensitisation would be associated with operant ethanol responses and, more importantly, whether cocaine-induced motor sensitisation would be prevented by naltrexone. An ANOVA [cocaine $F(2,44) = 28.37, p < 0.001$; naltrexone $F(6,609) = 1.44, p = 0.006$] and a post hoc analysis showed that only the 10 and 20 mg/kg i.p. doses of cocaine increased the total locomotor activity (horizontal and vertical) compared with the responses of the vehicle-control group. Naltrexone had no effect on locomotor activity ($n = 10 – 12$ per group). Also, naltrexone failed to prevent cocaine-induced locomotor activity: the locomotor activity of the groups of rats treated with naltrexone and 10 or 20 mg/kg of cocaine was significantly higher than that of the vehicle-control group. Finally, there were no

**Fig. 2.** Naltrexone significantly reduces operant ethanol self-administration in rats. The total number of lever presses during the 5 day period of treatment is shown. (A) Subchronic treatment with naltrexone (10 mg/kg, p.o., Antaxone®) decreased the self-administration of ethanol. (B) Subchronic treatment with Acamprosate (p.o., Campral®) did not cause any change. Values are expressed as the mean $\pm$ SEM of $n = 10 – 12$. ***$p < 0.001$ compared with the vehicle. ###$p < 0.01$ compared with 1 and 3 mg/kg of naltrexone. A one-way ANOVA was followed by Tukey’s post-hoc test.
significant differences between the groups treated with cocaine alone and the groups treated with a combination of naltrexone and cocaine.

### 3.4. Experiment 4: the effects of ethanol, cocaine and naltrexone on immediate-early gene expression in the rat prefrontal cortex

In the fourth and final experiment, we explored the genetic expression of the most common immediate-early genes used to map brain activity associated with behaviour (Kubik et al., 2007). There is ample evidence that the prefrontal cortex is involved in drug addiction (e.g., Lüscher and Malenka, 2011) and, more specifically, in ethanol operant self-administration (Dayas et al., 2007). Fig. 6 emphasizes the results of a two-way ANOVA analysis of C-fos and COX-2 gene expression [treatment $F(7,609) = 7.46$, $p < 0.001$; gene $F(6,609) = 54.81$, $p < 0.001$; interaction $F(42,609) = 5.64$, $p < 0.001$ and the subsequent Bonferroni post hoc analysis. Operant ethanol self-administration significantly reduced the expression of the C-fos gene in all groups ($p < 0.001$) except for the group treated with 2.5 mg/kg cocaine when compared with the saccharin group (calibrator group). Furthermore, ethanol treatment increased the expression of the COX-2 ($p < 0.001$) and Homer1a ($p = 0.007$, data not shown) genes. All treatments blocked the increased COX-2 gene expression induced by ethanol ($p < 0.001$). Zif-268, Bdnf, Arc and Rheb gene expression was not altered by operant ethanol self-administration.

The right column of Table 1 shows the estimated amount of DNA of each gene in the prefrontal cortex of each rat in the saccharin-untreated group (the calibrator group). The quantity of DNA of each gene, assuming a 100% PCR efficiency, doubles every cycle; every 3.32 cycles represent a 10-fold increase in the amount of DNA. The genes are listed according to their estimated expression in the prefrontal cortex in the saccharin group.

### 4. Discussion

#### 4.1. Naltrexone treatment for ethanol dependence

Opioid antagonists have been widely studied for the treatment of ethanol dependence (Hillemacher et al., 2011; Johnson, 2006, 2008; Soyka and Rösner, 2008; Walker and Koob, 2008; Walker et al., 2011). Of these antagonists, naltrexone seems to be one of the safest and most effective by multiple routes of administration (Johnson et al., 2004; Rösner et al., 2010). We selected the doses based on the results of previous studies (Blumberg and Ikeda, 1978; Czachowski and DeLory, 2009; Stromberg et al., 1998), and we...
adapted them to the pharmacokinetic and pharmacodynamic characteristics of oral administration, that is, oral naltrexone achieves its peak plasma levels 60 min after administration (Verebey et al., 1976) and the presence of food decreases the pharmacological activity of acamprosate by decreasing its rate of absorption to less than 10% during the first 24 h (Spanish Agency for Medicines and Health Products (AEMPS), Online Medicines Information Center; FDA Approved Drug Products (Drugs@FDA)).

Our data extend previous reports of animal models that show that naltrexone attenuates context-induced ethanol seeking (Marinelli et al., 2007), blocks the increased ethanol consumption following a period of abstinence known as the ethanol deprivation effect (Heyser et al., 2003), increases the extinction of responses to ethanol and attenuates the cue-induced reinstatement of ethanol-reinforced behaviour (Bienkowski et al., 1999). Furthermore, we believe that our results have a higher predictive validity for humans because we treated the animals orally with the liquid naltrexone available in pharmacies under the brand name of Antaxon®. Here, a dose of 10 mg/kg (p.o.) was found effective. However, our results exhibit distinct differences from previous studies using animal models. It has been reported that lower doses of naltrexone, 0.05–0.45 mg/kg (ji et al., 2008) or 0.1–1 mg/kg (Walker and Koob, 2008), decreased the number of lever presses for ethanol and ethanol intake in the two-bottle free-choice paradigm. The most plausible explanation for these discrepancies is that those authors subcutaneously administered naltrexone, a route approximately 30-fold more potent than an intraperitoneal injection (Williams and Broadbridge, 2009); naltrexone is expected to be even less potent administered orally as it was in this study than by intraperitoneal injection.

In this study, animals were also orally treated with acamprosate (Camporal®), but none of the doses tested reduced the number of operant ethanol responses. Acamprosate is prescribed to maintain abstinence in individuals who are already abstinent at the initiation of treatment (Mason, 2005), whereas naltrexone is effective in reducing ethanol intake and relapse but less effective in supporting the maintenance of abstinence (Garbutt, 2010). These facts suggest that the specific neurobiology of naltrexone (opioid neurotransmission) and acamprosate (the amino acid neurotransmitters GABA and glutamate) may be linked to different stages of alcoholism; thus, it may be useful to integrate these treatments. Accordingly, some research using animal models shows that a combination of naltrexone and acamprosate could be more effective than either compound alone to prevent the alcohol deprivation effect in rats (Heyser et al., 2003) or in mice in a limited access alcohol model (Kim et al., 2004). In humans, the combined administration of the compounds has been proven to be behaviourally and pharmaco-logically safe (Johnson et al., 2003; Kiefer and Wiedemann, 2004).

4.2. Cocaine increases operant ethanol self-administration and reverses the naltrexone-induced reduction

Cocaine at 20 mg/kg increased the number of responses to obtain ethanol. Interestingly, this effect results from long-lasting neuroadaptations because cocaine was administered 6 h after the daily operant ethanol self-administration session. Thus, the animals did not have access to ethanol until approximately 17 h after the cocaine injection (see Fig. 1b). We followed this extended schedule because we wanted to avoid (a) cocaine-induced place-conditioned motor sensitisation and motor hyperactivity in the ethanol operant chambers (Antoniou et al., 1998; Stromberg and Mackler, 2005), (b) a putative cocaine-induced aversion to ethanol (this effect has been repeatedly demonstrated with saccharin by several authors, e.g., Davis and Riley, 2007; Ferrari et al., 1991; Goudie et al., 1978), (c) the formation of cocaethylene, a psychoactive compound that results from the co-administration of cocaine and ethanol (Hedaya and Pan, 1997; Laizure et al., 2003; Landry, 1992; Schechter, 1995), and (d) other non-controllable variables resulting from the concomitant exposure to cocaine and ethanol. For example, cocaine (1–30 mg/kg) administered 15 min prior to the test session suppresses lever-pressing for food (Ross and Schaal, 2002), and a similar reduction
in lever press rates for an ethanol solution has been observed with 10 and 30 mg/kg cocaine (van Haaren and Anderson, 1994). Therefore, we assume that the changes that we would observe in ethanol response should reflect long-lasting cocaine-induced effects rather than acute cocaine-induced effects.

In contrast, it could be argued that cocaine could cause an increase of lever presses as a result of the stereotypical behaviour induced by cocaine (Aliane et al., 2009), but the lack of effect on the activity towards the inactive lever makes this explanation unlikely. Another explanation that we consider more plausible is that this effect could be mediated by cocaine withdrawal. It has been recently demonstrated that functional and morphological changes occur in the brain after repeated injections of cocaine even in the absence a prolonged withdrawal period (Dobi et al., 2011). If this is the case, our results may indicate that such neuroadaptations induced by cocaine withdrawal alter the sensitivity to the effects of ethanol, resulting in an increased number of ethanol responses.

There also appears to be a complementary relationship between the two drugs wherein exposure to ethanol alters the response to cocaine (Bernier et al., 2011; Mierzejewski et al., 2003); even rats that prefer ethanol are more sensitive to the reinforcing effects of cocaine self-administered to the brain (Katner et al., 2011). Subchronic and chronic treatment with naltrexone reduced operant ethanol self-administration. The reason naltrexone was totally ineffective in the presence of cocaine remains unclear. One reasonable explanation is that the neurobiological changes induced by cocaine overcome those induced by naltrexone. Naltrexone exhibits an affinity 10–25 times higher for the mu opioid receptors than for the kappa or delta receptors (Mannelli et al., 2011). The activation of mu opioid receptors indirectly causes excitatory effects in dopaminergic neurons by inhibiting the release of the inhibitory neurotransmitter GABA in critical brain areas regulating euphoria and reward (Corbett et al., 2006; Guy et al., 2011; Nestler, 2001).

Consequently, naltrexone blocks the activation of mu opioid receptors by ethanol as well as the synthesis and release of endogenous opioids induced by ethanol (Herz, 1997). In contrast, cocaine directly increases the extracellular levels of dopamine in brain-rewarding areas by two main mechanisms: inhibiting the dopaminergic transporter and enhancing its exocytotic release (Venton et al., 2006). This abundant release of dopamine is followed by the increased production of genetic transcription factors, which causes synaptic modifications such as dendrite sprouting and other intra-cellular changes to explain short- and long-term behavioural effects (Nestler, 2005). Therefore, it seems that the cocaine-induced neuroadaptations were unaffected by naltrexone treatment.

4.3. Naltrexone fails to block cocaine-induced locomotor activity

We can conclude that we replicated previous findings showing that cocaine increases locomotor activity ( Ago et al., 2008). This occurred even in the absence of a context-dependent sensitisation (Mattson et al., 2008; Weiss et al., 1989) as far as the animals were tested only once on the locomotor apparatus. Despite the fact that naltrexone can attenuate amphetamine-induced conditioned locomotor sensitisation (Häggkvist et al., 2011), it did not alter cocaine-induced locomotor activity. This fact agrees with other reports (e.g., Smith et al., 2009), and our interpretation is that cocaine-induced neuroadaptations are minimally affected by naltrexone treatment.

4.4. Operant ethanol self-administration regulates the expression of immediate-early C-fos and COX-2 genes in the rat prefrontal cortex

The expression of the C-fos gene in the prefrontal cortex was significantly reduced in all the groups of animals following ethanol operant self-administration except for the group treated with the lowest dose of cocaine. Because C-fos is considered a marker of brain activation that demarcates neuronal populations undergoing long-term changes in function (Biały and Kaczmarek, 1996; Morgan and Curran, 1991), our results suggest that there is an abnormal functioning of the prefrontal cortex during the time the animal is placed in the ethanol operant chamber. This has important implications. The prefrontal cortex sends neural projections to the nucleus accumbens and the ventral tegmental area, providing feedback and controlling these areas of the brain (Feil et al., 2010; López-Moreno et al., 2008). A loss of this feedback would impair the ability to overcome drives to obtain pleasure through unsafe actions ( Kosten and George, 2002) and would make weighing options and self-control difficult ( Nestler, 2005). Intriguingly, the conditioned stimulus-induced reinstatement of extinguished ethanol seeking in rats has been associated with increased C-fos expression within the prefrontal cortex that is not attenuated by naltrexone treatment (Dayas et al., 2007). These results strongly suggest that changes in C-fos expression within the prefrontal cortex are a useful biomarker of the behavioural phenotype of ethanol addiction.

One of our more interesting findings was that the expression of the COX-2 gene was altered. Operant ethanol self-administration increased its expression up to four-fold. Because the animals were sacrificed at the point at which they would have been re-introduced into the operant chambers on day 15, it could be hypothesised that COX-2 over-expression is associated with ethanol craving. The following evidence in the literature would not support this hypothesis: (a) the COX-2 gene contributes to synapse-specific plasticity; it is a major mediator of inflammation, and it is over-expressed in many types of cancer (Aparicio-Gallego et al., 2007; Harper and Tyson-Capper, 2008; Kubik et al., 2007; Lanahan and Worley, 1998); and (b) previous reports have shown that chronic ethanol consumption increases COX-2 mRNA levels in the hippocampus (Simonyi et al., 2002), the liver (Nanji et al., 1997) and the cerebral cortex of rats (Vallés et al., 2004), participating in the response to oxidative stress and ethanol-induced inflammation. Therefore, our results suggest that the over-expression of the COX-2 gene may be associated with ethanol-induced neurotoxicity and is a consequence of ethanol exposure rather than a cause of ethanol intake. This is the first report to show that operant ethanol self-administration can modulate the activity of this gene. However, further studies are required to investigate why every treatment, cocaine, naltrexone and their combined administration, blocked the ethanol-induced over-expression of the COX-2 gene. Currently, no study is exploring whether cocaine or naltrexone modulates the expression of this gene.

4.5. Final comments

The present work provides support for three ideas. First, our results, derived from an experimental set-up designed to maximise its predictive validity in humans, suggest that pharmacological treatment with naltrexone for alcoholism may be impaired when cocaine is co-administered. Second, our data reveal that daily chronic operant ethanol self-administration is associated with dramatic changes in gene expression in the rat prefrontal cortex. Third, naltrexone failed to block the ethanol-induced alterations of C-fos and COX-2 gene expression. It would be of great interest to find a genetic biomarker of neural activation that could predict the behavioural efficacy of a pharmacological treatment for alcoholism.

Statement of interest

None.


