Original Article

Binge ethanol drinking during adolescence modifies cocaine responses in mice

Journal of Psychopharmacology
2017, Vol. 31(1) 86–95

DOI: 10.1177/0269881116681457
jop.sagepub.com
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Abstract

Binge ethanol drinking is an emerging pattern of excessive consumption among adolescents and young adults. Repeated ethanol intoxication has negative consequences during critical periods of brain development. Therefore, binge ethanol intake represents a vulnerability factor that promotes subsequent manifestations of neuropsychiatric disorders. In this study, we investigated the effects of oral binge ethanol intake during adolescence on the subsequent effects of cocaine in C57BL/6 mice. Firstly, we evaluated the oral ethanol intake of two binge ethanol procedures with different ethanol concentrations (20% v/v versus 30%, v/v). The highest ethanol intake was found in mice exposed to the lower ethanol concentration (20% v/v). In a second experiment, mice exposed to binge ethanol procedure were evaluated to study the effects of cocaine on locomotor activity, behavioural sensitization, and the reinforcing effects of cocaine in the self-administration paradigm. Mice exposed to ethanol binging showed discrete detrimental effects in responses to cocaine in the different experiments evaluated. Our findings revealed that the pattern of binge ethanol consumption in adolescent mice here evaluated produced a weak facilitation of cocaine responses. The present study highlights the importance of interventions to limit the deleterious effects of binge ethanol drinking during adolescence.

Keywords

Cocaine, reward, behavioural sensitization, binge ethanol drinking, adolescent mice

Introduction

Substance use disorder is considered to be a psychiatric disorder (APA, 2014; Cuthbert, 2014), representing a public health problem (Patel et al., 2016; WHO, 2014). In recent years, binge ethanol drinking has led to an emerging pattern of high-risk consumption throughout Europe and the US, mostly among adolescents (Bekman et al., 2013; WDR-UNODC, 2015). Indeed, worldwide, approximately 16% of drinkers aged ⩾15 years engage in episodic heavy ethanol drinking (WHO, 2014). Binge ethanol intake is defined as drinking at least 5 standard drinking units of ethanol on a single occasion (over ≤2 hours), resulting in a blood ethanol concentration (BEC) of ⩾80 mg/dL (Wechsler and Nelson, 2001). In addition, ethanol intoxication during adolescence has been reported to induce brain damage (Crews et al., 2004; Guerri and Pascual, 2010; Pascual et al., 2014), and is regarded as a vulnerability factor that promotes subsequent manifestations of neuropsychiatric disorders (Casey et al., 2015; Ciudad-Roberts et al., 2015; Mateos-García et al., 2015; Pagey et al., 2010), cognitive impairment (Casey et al., 2015; Paus et al., 2008), and behavioural alterations (Spear, 2014, 2015; Spear and Swartzwelder, 2014). Furthermore, ethanol is commonly consumed together with other drugs of abuse particularly psychostimulants such as cocaine (Barrett et al., 2006; WDR-UNODC, 2015), and binge drinking is a pattern of consumption that increases the risk of drug polyconsumption (WHO, 2014).

Drug consumption usually begins during early youth and adolescence, which is considered to be a critical period for brain maturation (Crews et al., 2007; Giedd, 2008). The characteristic neuroplastic changes and neuroadaptive processes of this developmental period are accompanied by particular behaviours commonly observed in adolescence, such as impulsivity, novelty seeking and a high level of risk-taking that may be conducive to drug-consumption and addiction (Spear, 2000, 2015). In rodents, 'adolescence' is considered to broadly cover the entire postnatal period ranging, after weaning, from postnatal day (PD) 25 to early adulthood (PD 60) (Spear, 2015; Vetter-O'Hagen and Spear, 2012). This period is characterized by brain maturation, synaptic refinement and myelination (Crews et al., 2007; Squeglia et al., 2009). All these factors make the adolescent brain

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more sensitive to the deleterious effects of drug consumption and may therefore lead to altered neurocognitive functions and long-lasting vulnerability to substance use disorders (Crews et al., 2007; Mateos-Garcia et al., 2015; Molet et al., 2012, 2013; Spear, 2000, 2015; Squeglia et al., 2009).

Among illicit drugs, cocaine is the most widely used substance in addition to cannabis derivatives (WDR-UNODC, 2015). Cocaine is a powerful stimulant of the central nervous system that enhances alertness, arousal, feelings of wellbeing and energy (Dong and Nestler, 2014; Nyberg, 2014). It acts by affecting the reuptake of dopamine at synaptic terminals and inhibiting the dopamine transporter, leading to a rapid increase in dopamine neurotransmission in the brain (Buchta and Riegel, 2015; Giros et al., 1996). The mesolimbic dopamine system has been convincingly shown to have a primary role in the acute reinforcing effects of cocaine and ethanol (Dong and Nestler, 2014; Everitt and Robbins, 2016; Ozburn et al., 2015; Woolverton and Johnson, 1992).

Experimental and clinical studies support the particular susceptibility of the central nervous system to the damaging impact of drugs during adolescence, as reported by different authors (Crews et al., 2007; DeWit et al., 2000; Mateos-Garcia et al., 2015; Molet et al., 2012, 2013; Slawecki et al., 2001; Squeglia et al., 2009). In particular, the previous study by Mateos-García et al. (2015) evaluated the long-term effects of a binge pattern of ethanol exposure during adolescence on sensitivity to cocaine in mice during adulthood. In this earlier report, ethanol was administered by intraperitoneal (i.p.) route in OF1 mice. Nevertheless, no study has investigated the impact of binge ethanol intake using a voluntary ethanol intake by oral route on cocaine psychostimulant effects in C57BL/6 mice, naïve to cocaine effects. Moreover, we have compared the effects of two different ethanol concentrations (20% versus 30% v/v). In fact, using a similar ethanol intake procedure that here reported, we have recently shown that ethanol binge drinking produces reduced contextual discrimination in the fear conditioning task (Johansson et al., 2015), and cardiotoxic effects in CD1 mice (Navarro-Zaragoza et al., 2015), which highlights the alteration occurring in the central nervous system and periphery as a result of binge ethanol consumption during adolescence. Therefore, adolescents are considered to be more vulnerable to the consequences of ethanol intoxication (Quoilin et al., 2014; Slawecki et al., 2001; Spear, 2015), and although several recent studies have reported timing-specific effects within adolescence due to ethanol and other drugs such as nicotine and cannabinoids (Spear, 2015), few studies have investigated the consequences of early ethanol consumption on the subsequent effects and risks of later cocaine consumption.

In this context, we hypothesized that the exposure to ethanol intoxication during adolescence may increase the psychotropic effects of cocaine in adulthood, extending the risk of developing addictive disorders. Thus, the aim of the present study was to investigate the effects of voluntary and oral ethanol binge drinking during adolescence in C57BL/6 mice on the subsequent psychostimulant effects of cocaine during the juvenile period. Thus, in experiment 1, we exposed adolescent mice to a binge pattern of ethanol consumption at two different concentrations (20% versus 30% v/v), and selected the procedure producing the highest ethanol intake for subsequent studies on the effects of cocaine in juvenile mice. In experiment 2, we assessed the behavioural sensitization after cocaine administration, and the reinforcing properties of cocaine in an operant self-administration paradigm.

Material and methods

Animals

Adolescent male C57BL/6 mice purchased from Charles River (Barcelona, Spain) were used in this study. After arrival, mice were left for acclimation to the animal facilities for 7 to 10 days. During acclimation, mice were housed in groups of 4–5 individuals per cage and exposed to a reversed light–dark cycle (12:12), with lights turned off at 08.00 hours and on at 20.00 hours. After the acclimation period, mice were housed individually to begin the ethanol consumption experiments at PD30. Animal rooms were controlled for temperature (21±1°C) and humidity (55±10%). Food and water were available *ad libitum*, except during ethanol consumption and behavioural experiments. The experimental procedures were consistent with the European Communities Directive 86/609/EEC regulating animal research, and were approved by the local ethics committee (CEEA-PRBB).

Drugs

To obtain the required concentrations, ethyl alcohol (Merck Chemicals KGaA, Darmstadt, Germany) was diluted in tap water (20% and 30% v/v ethanol solutions), and cocaine hydrochloride (Alcaliber S.A., Madrid, Spain) was dissolved in 0.9% physiological saline. For cocaine-induced sensitization, cocaine was injected at a dose of 10 mg/kg. Cocaine was dissolved in an injection volume of 0.1 mL per 10g body weight, and was administered by i.p. route. For self-administration experiments, cocaine was administered by intravenous (i.v.) route at the doses of 0.5 and 0.75 mg/kg/infusion in a volume of 20 μL.

Experimental schedule

Figure 1 provides a schematic representation of the experimental schedule used in this study.

Experiment 1. To evaluate voluntary ethanol drinking during adolescence, 40 mice (starting at PD30) were subjected to two procedures with different ethanol concentrations (20% or 30% v/v ethanol; procedures B20 and B30, respectively). Half of the mice in each procedure were given water instead of ethanol solution. We evaluated total consumption calculated in volume (ml) and intake score (gEtOH intake/kg of body weight). Corresponding BEC values were also analysed on the last day of the binge procedure. We then identified the procedure that produced the highest ethanol intake (procedure B20).

Experiment 2. A separate group of 80 mice underwent procedure B20 during adolescence (starting at PD 30) and were subsequently used to evaluate cocaine-induced behavioural effects. Starting at PD 60, we assessed behavioural sensitization induced by repeated cocaine administration or the reinforcing effects of cocaine in a self-administration paradigm. Separate groups of mice were used for behavioural sensitization (40 mice) and self-administration studies (20 for control group + 20 for ethanol-exposed group mice). We allowed a minimum of 1 week between the last ethanol exposure and the start of experiments to evaluate cocaine-induced behavioural effects.

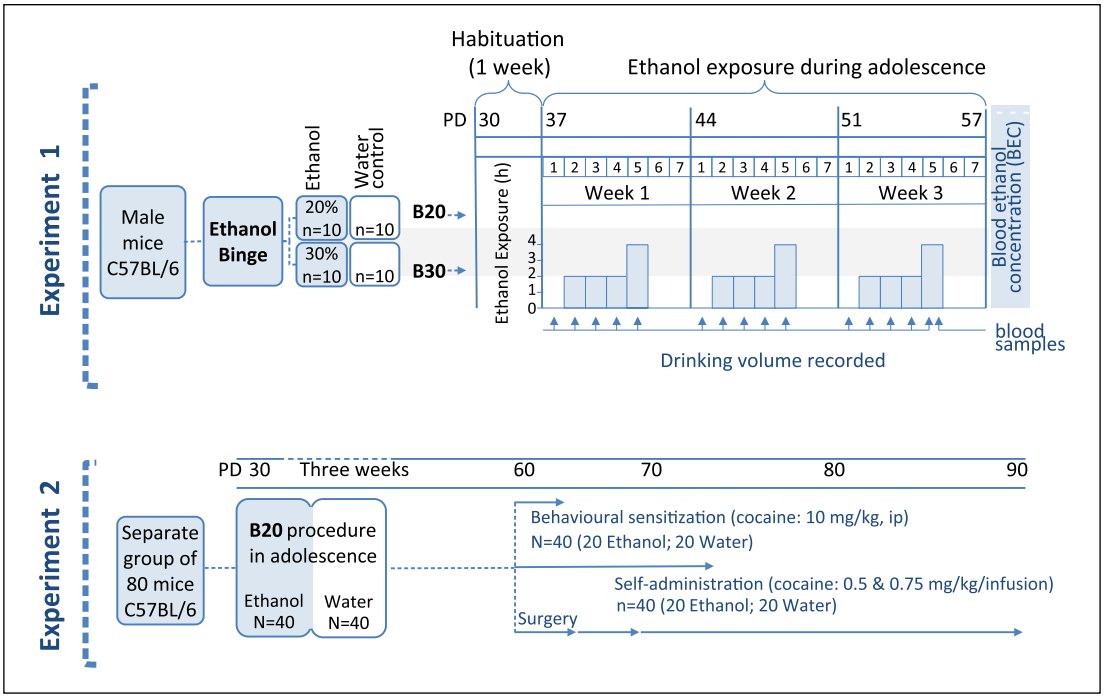

Figure 1. Schematic representation showing the schedule of ethanol intake on the basis of two previously used procedures (B20 and B30).

Binge ethanol exposure procedures

Two different procedures were used to evaluate voluntary oral ethanol intake with a binge drinking pattern of ethanol consumption using two different concentrations (20% or 30% v/v ethanol; procedures B20 and B30; Figure 1). These procedures are a modification of the drinking in the dark test previously described (Rhodes et al., 2005; Ros-Simó et al., 2012). Mice were randomly assigned to each procedure and acclimatized to the room and light cycle for 1 week before the start of the experiment. On day 1, three hours after the lights were turned off, food was removed from the home cages and water bottles were replaced with 10 mL graduated pipettes fitted with metal sipper tubes containing either 20% or 30% (v/v) ethanol solution in tap water or only tap water. During this time, the animals were individually housed in their cages. The ethanol or water cylinders remained in place for 2 h. After the 2-h period, individual intake was recorded and food and water bottles were replaced. This procedure was repeated on days 2 and 3 and fresh fluids were provided each day. On day 4, the ethanol or water presentation was conducted again, but ethanol or water cylinders remained in place for 4 h. Following the 4 h free access to fluid, and immediately after recording fluid intake, ethanol and water cylinders were replaced with water bottles. Ethanol intake was recorded for each mouse on each day of exposure, and data are expressed as g of ethanol intake/kg of body weight (gEtOH/kg). The complete procedure was repeated for three consecutive weeks. Blood samples were collected from the dorsal tail vein immediately after the last ethanol intake period (day 4, week 3). Blood samples were placed on ice for slow coagulation and were centrifuged at 10,000 g for 40 min at 4°C to obtain cell-free plasma, which was stored at −80°C prior to ethanol quantification analysis.

Behavioural sensitization

To explore behavioural sensitization induced by repeated cocaine (10 mg/kg, i.p.) administration, 40 mice were previously exposed to ethanol according to procedure B20 and at juvenile age, starting at PD60, were evaluated for locomotor sensitization induced by repeated cocaine administration (10 mg/kg, i.p.) (Martin et al., 2000). Briefly, locomotor activity was recorded in locomotor activity chambers consisting of a plastic rectangular area (24 × 24 × 24 cm^3; LE8811 IR, Panlab, S.L, Barcelona, Spain) with two crossed photocells in a low luminosity room (30 lux) with white noise. Horizontal movements were recorded for 20 min, as previously described (Gracia-Rubio et al., 2016). On day 1, mice were injected with cocaine (10 mg/kg, i.p.) or saline, and were immediately placed in the locomotor activity boxes for 30 min. The locomotor activity of the mice was then registered for the last 20 min in the boxes. Using the same procedure, cocaine (10 mg/kg, i.p.) or saline was administered daily for five consecutive days and locomotor activity was recorded at the same time each day to control for circadian rhythm effects. Cocaine or saline administration was then withdrawn for 7 days, after which mice were given a challenge injection of cocaine (10 mg/kg, i.p.) or saline on day 12 to evaluate locomotor sensitization. Locomotor activity was measured as the number of times the mouse crossed the photo beam during the evaluation period.

Operant self-administration procedure

Mice were trained to receive cocaine infusions for 1 h per day for 10 consecutive days under a fixed ratio 1 (FR1) schedule of reinforcement. Cocaine doses (0.5 or 0.75 mg/kg/infusion) were selected according to previous studies performed in our laboratory

(Soria et al., 2005; Touriño et al., 2012). For the self-administration experiments, surgical implantation of the catheter into the jugular vein was performed following anaesthetization with a mixture of ketamine hydrochloride (100 mg/ml; Imalgène®1000, Rhône Mérieux, Lyon, France) and xylazine hydrochloride (20 mg/kg; Sigma Chemical Co., Madrid, Spain). Both compounds were dissolved in distilled water to obtain a final concentration of 5 mg/mL of ketamine and 1 mg/mL of xylazine. The anaesthetic solution was injected in a volume of 0.15 mL per 10 g body weight, i.p. (Soria et al., 2005; Touriño et al., 2012).

Following surgery, mice were housed individually and allowed to recover for at least 4 days prior to the first self-administration session. For self-administration procedures, we used eight operant chambers with two nose-pokes (Model ENV-307A-CT, Med Associates, Inc. Cibertec. Madrid. Spain). Active and inactive nose-pokes were selected randomly. Cocaine was delivered in a 20 µL injection over 2 s via a syringe mounted on a microinfusion pump (PHM-100A, Med-Associates, Georgia, VT, USA) connected via Tygon tubing (0.96 mm outer diameter, Portex Fine Bore Polythene Tubing, Portex Limited, UK) to a single-channel liquid swivel (375/25, Instech Laboratories, Plymouth Meeting, PA, USA) and the mouse's intravenous catheter. All sessions started with a priming injection of cocaine. When mice responded on the reinforcing nose-pokes, the stimulus lights (one located inside the nose-poke and the other above it) lit up for 4 s and a cocaine infusion was delivered automatically over 2 s. The number of reinforces was limited to 50 infusions per session. Each infusion was followed by a 30 s time-out period in which an active nose-poke had no consequences. After each session, mice were returned to their home cages. Mice were considered to have acquired stable self-administration when the following criteria were met on three consecutive days: (i) 80% stability in reinforcements (the number of reinforces on each day deviated by <20% from the mean number of reinforces over the three consecutive days); (ii) ⩾70% of responses were received at the active nose-poke; and (iii) ⩾5 responses were received at the active nose-poke (excluding priming reinforcement).

Quantification of plasma ethanol

Frozen plasma samples were allowed to reach room temperature before processing. 5 µL aliquots of each sample were transferred to autosampler microvials, combined with 5 µL of internal standard solution (*tert*-butanol) and 15 µL of ultrapure water, mixed thoroughly for 20 s and analysed by GC-MS in a 6890 gas chromatograph equipped with a 7683 autosampler and coupled to an Agilent 5973 mass selective detector. 1 µl of sample was injected (split ratio 1:50) into a HP-Innowax column (15 m × 0.25 mm i.d.; film thickness 0.5 µm; J&W Scientific, Folsom, CA, USA). The carrier gas was helium at a constant flow of 1.2 mL/min. The GC temperature was ramped as follows: initial 40°C held for 2.5 min, increased to 100°C at 70°C/min, and held for 0.3 min. For optimal sensitivity, acquisition was performed in SIM mode by acquiring the characteristic ions *m*/*z* 45 and *m*/*z* 31 for ethanol, and *m*/*z* 59 and *m*/*z* 41 for *tert*-butanol. The injector and transfer line were maintained at 250°C, the MS source at 230°C, and the quadrupole at 150°C. Quantification was based on the analyte integral, and internal standard peaks and a calibration curve were constructed before and after the batch.

Statistical analysis

In experiment 1, for the ethanol intake experiments, a two-way analysis of variance (ANOVA) with repeated measures was performed, with days and ethanol intake as factors of variation. One-way ANOVA (between subjects) was used to compare total ethanol intake and BEC values between the two procedures.

In experiment 2, for the locomotor sensitization study, a three-way ANOVA with repeated measures was performed, with pre-exposure to ethanol/water, cocaine treatment and days as factors of variation. To analyse the acquisition of cocaine self-administration during the 10-day training, a three-way ANOVA was used, with nose-poke (active or inactive), pre-exposure to ethanol/water, and day as factors of variation.

In all the studies, following the main effects of the ANOVA, Bonferroni post-hoc tests were calculated whenever required. Correlations between ethanol intake (gEtOH/kg) and BEC were calculated using Pearson's rank correlation for each ethanol intake procedure (B20 and B30) and to calculate correlations between ethanol intake (gEtOH/kg) and behavioural responses. Data are presented as mean ± SEM. A p-value <0.05 was considered statistically significant. The Bonferroni's correction was applied to calculate p-values after multiple comparisons. Analyses were performed using SPSS v19.

Results

Experiment 1

C57BL/6 mice show higher ethanol consumption when using a drinking solution of ethanol at 20% (v/v). Figure 2 shows the levels of ethanol intake (gEtOH/kg body weight) for the ethanol procedures evaluated. Two-way ANOVA with repeated measures showed a significant effect of day during the three weeks of alcohol exposure ($F_{(11, 198)} = 74.250$, $p < 0.01$), and an effect of the ethanol concentrations ($F_{(1, 18)} = 8.725$, $p < 0.01$) with no interaction between both factors ($F_{(11, 198)} = 1.224$, NS). The highest ethanol consumption was observed in mice exposed to the B20 procedure (Figure 2). One-way ANOVA calculated to compare the BEC of each procedure revealed no differences between groups ($F_{(1, 18)} = 0.814$, NS) (Figure 3). Pearson's correlation calculated between ethanol intake (gEtOH/kg) and BEC for each procedure (B20 and B30) showed no significant correlation for any of the procedures (B20: $r = 0.285$; B30: $r = -0.049$). However, all mice exposed to the B20 procedure achieved BEC higher than 80 mg/dL (10 out of 10 mice), whereas in the case of the B30 procedure, only 7 out of 10 mice achieved intoxication BEC levels. Considering such results and taking into account that the higher ethanol consumption was observed in mice exposed to the B20 procedure, this procedure was selected to carry out experiment 2.

Experiment 2

The means of ethanol intake for the three binge episodes, corresponding to the 4 h-access day of each week, were: 7.3 ± 0.3 gEtOH/kg (first week); 6.8 ± 0.4 gEtOH/kg (second week) and 8.1 ± 0.3 gEtOH/kg (third week), respectively. No significant differences were shown between the levels of ethanol intake for the different procedures (B20) carried out in experiments 1 and 2.

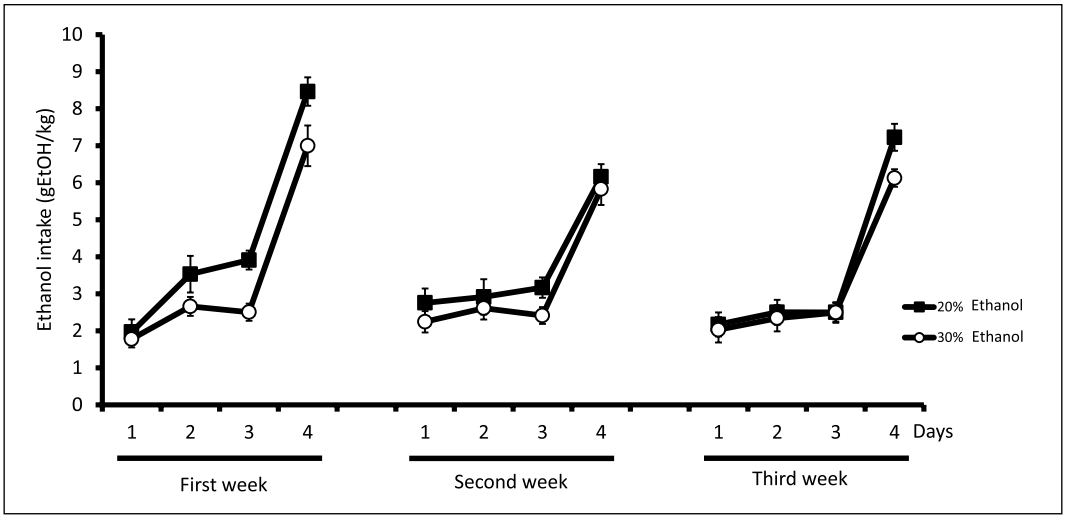

Figure 2. Ethanol intake (gEtOH/kg) in both procedures (B20 and B30) used in experiment 1. The procedures were performed for three consecutive weeks. Data are presented as mean ± SEM. (n = 10 mice per group). Black squares represent data from procedure B20; white circles represent data from procedure B30.

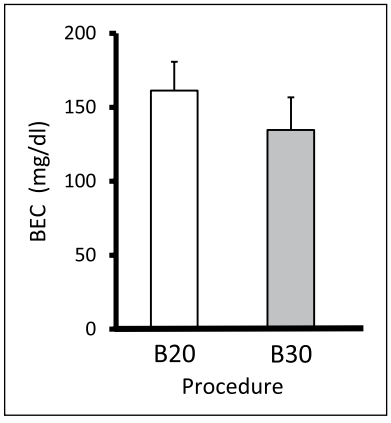

Figure 3. BEC values in procedures B20 and B30. BEC values (mg/dL) calculated from blood samples collected on the last day of ethanol intake (n = 10 mice per group). Data are presented as mean ± SEM.

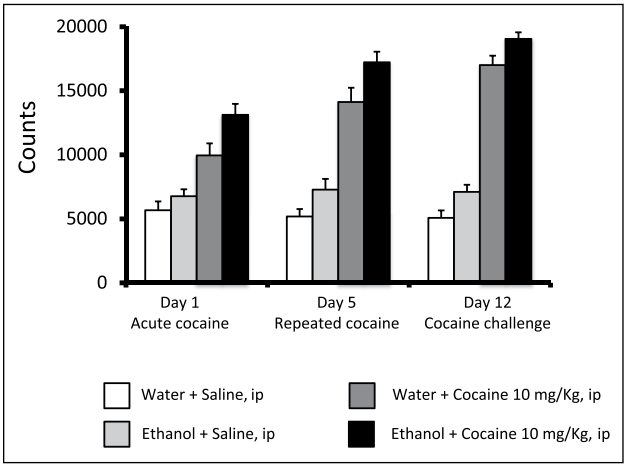

Figure 4. Behavioural sensitization induced by repeated cocaine administration (10 mg/kg, i.p.) in mice exposed to ethanol according to the B20 procedure. Day 1 represents acute cocaine (10 mg/kg) or saline effects. Day 5 represents the effects of repeated cocaine (or saline), and Day 12 represents the effect of a cocaine (or saline) challenge after 7 days with no cocaine (or saline) treatment. White bars represent water + saline groups; light grey bars represent ethanol + saline groups; dark grey bars represent water + cocaine groups and black bars represent ethanol + cocaine groups. Data are presented as mean ± SEM (n = 10 mice per group).

Binge ethanol consumption slightly modified locomotor sensitization induced by cocaine. A group of 20 mice were previously exposed to ethanol and a group of 20 mice were exposed to water, on the basis of the B20 procedure. At PD60, mice were evaluated for behavioural sensitization. Mice were exposed to repeated injections of cocaine (10 mg/kg) or saline according to the described behavioural sensitization procedure (Figure 4). Three-way ANOVA calculated for cocaine-induced behavioural sensitization showed an effect of ethanol/water intake ($F_{(1, 36)}$=11.736, $p < 0.01$); effect of cocaine treatment ($F_{(1, 36)}$=182.021, $p < 0.01$; an effect of the day ($F_{(2, 72)}$= 49.523, $p < 0.01$), with interaction between days and cocaine treatment ($F_{(2, 72)}$=53.204, $p < 0.01$), with no interaction between cocaine treatment and ethanol/water intake ($F_{(1, 36)}$=0,609, NS), with no interaction between day and ethanol/water intake ($F_{(2, 72)}$= 0,424, NS) and with no interaction between day, cocaine treatment and ethanol/water intake ($F_{(2, 72)}$= 1.272, NS) (Figure 4). Pearson's correlation calculated between ethanol intake (gEtOH/kg) and locomotor activity after acute cocaine (day 1) showed a significant positive correlation (r = 0.679, $p < 0.05$), whereas the correlation calculated between ethanol intake (gEtOH/kg) and the challenge of cocaine (day 12) showed no significant correlation (r = 0.366, NS).

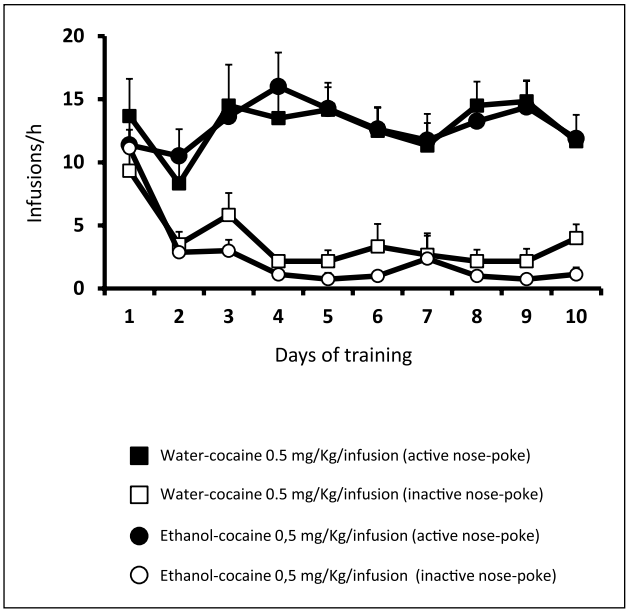

Figure 5. Acquisition of cocaine (0.5 mg/kg/infusion) self-administration in mice exposed to ethanol/saline according to procedure B20. Number of nose-pokes into the active and the inactive nose-pokes in 1 h session. Black squares represent activations into the active nose-pokes in the water + cocaine group. White squares represent activations into the inactive nose-pokes in the water + cocaine group. Black circles represent activations into the active nose-pokes in the ethanol + cocaine group. White circles represent activations into the inactive nose-pokes in the ethanol + cocaine group. Data are presented as mean ± SEM (n = 6 per group).

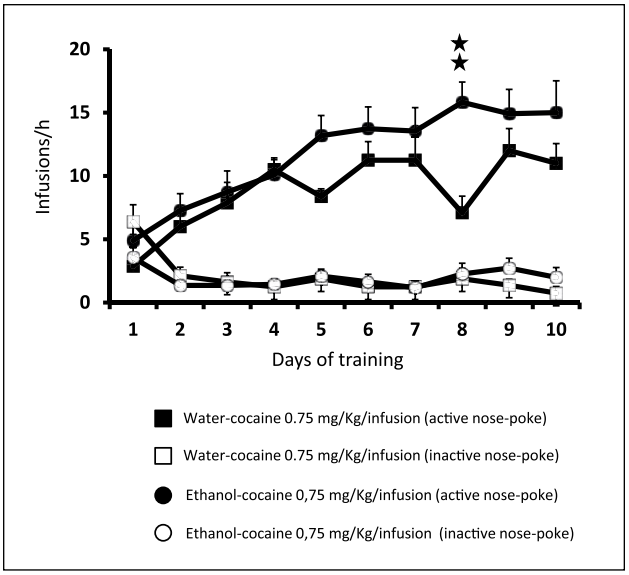

Figure 6. Acquisition of cocaine (0.75 mg/kg/infusion) self-administration in mice exposed to ethanol intake accordingly to procedure B20. Number of nose-pokes into the active and the inactive nose-pokes in 1 h session. Black squares represent activations into the active nose-pokes in the water + cocaine group. White squares represent activations into the inactive nose-pokes in the water + cocaine group. Black circles represent activations into the active nose-pokes in the ethanol + cocaine group. White circles represent activations into the inactive nose-pokes in the ethanol + cocaine group. Data are presented as mean ± SEM (n = 8–11 per group). Two black stars $p < 0.01$ for cocaine effects in groups of mice exposed to ethanol versus groups of mice exposed to water (Bonferroni post-hoc test).

Cocaine self-administration was slightly modified in juvenile mice exposed to binge ethanol during adolescence. At PD60, mice exposed to ethanol or water were trained for 10 days to self-administer cocaine (0.5 or 0.75 mg/kg/infusion) under a FR1 schedule of reinforcement (Figures 5 and 6). Regarding 0.5 mg/kg/infusion cocaine self-administration, three-way ANOVA results showed a significant effect of the nose-pokes (active/inactive) ($F_{(1, 20)} = 69.050$, $p < 0.01$) an effect of the day ($F_{(9, 180)} = 5.448$, $p < 0.01$), with no effect of the ethanol/water intake ($F_{(1, 20)} = 1.003$, NS), with interaction between nose-pokes and day ($F_{(9, 180)} = 8.838$, $p < 0.01$), with no interaction between ethanol/water exposure and day effects ($F_{(9, 180)} = 1.153$, NS), no interaction between ethanol/water exposure and nose-poke effects ($F_{(1, 20)} = 1.012$, NS) or between ethanol/water exposure, nose-poke and day effects ($F_{(9, 180)} = 1.253$, NS). The acquisition criterion was achieved by 85.7% of mice exposed to water and 88.8% of mice exposed to ethanol, respectively (Figure 5). No difference in the number of cocaine infusions between mice exposed to ethanol or water was observed (Figure 5). No difference was observed in the number of sessions required to achieve the acquisition criterion between mice exposed to ethanol (5.1 ± 1.1 days) and those exposed to water (5.5 ± 1.2 days). Pearson's correlation calculated between ethanol intake (gEtOH/kg) and total cocaine intake during the self-administration procedure showed no significant effect ($r = -0.236$, NS).

Three-way ANOVA values for cocaine at 0.75 mg/kg/infusion showed a significant effect of the nose-pokes (active/inactive) ($F_{(1,32)} = 85.938$, $p < 0.01$), an effect of the day ($F_{(9, 288)} = 7.683$, $p < 0.01$), with no effect of the ethanol/water intake ($F_{(1, 32)} = 1.351$, NS), with interaction between nose-pokes and day ($F_{(9, 288)} = 15.809$, $p < 0.01$), with interaction between ethanol/water exposure and day effects ($F_{(9, 288)} = 1.286$, $p < 0.05$), with no interaction between ethanol/water exposure and nose-poke effects ($F_{(1, 32)} = 1.286$, NS) and interaction between ethanol/water exposure, nose-poke and day effects ($F_{(9, 288)} = 2.052$, $p < 0.05$). The Bonferroni post-hoc test showed differences in the number of cocaine infusions between mice exposed to ethanol and those exposed to water, only on day 8 ($p < 0.01$) (Figure 6). From the total of mice, 72.7% and 84.6% of mice exposed to water or ethanol, respectively, reached the acquisition criterion. The Bonferroni post-hoc analysis to compare differences between active and inactive nose-pokes showed that mice exposed to water during adolescence discriminated between active and inactive nose-pokes on different days ($p < 0.01$, days 2 to 7 and day 10), whereas mice exposed to ethanol during adolescence discriminated between nose-pokes from day 2 to 10 ($p < 0.01$, in all cases) (Figure 6). However, we observed no significant difference in the number of sessions required to achieve the acquisition criteria between mice exposed to ethanol (6.73 ± 0.38 days) and those exposed to water (6.13 ± 0.33 days). Pearson's correlation calculated between ethanol intake (gEtOH/kg) and total cocaine intake during the self-administration procedure showed no significant correlation ($r = 0.034$, NS).

Discussion

Our study shows that oral binge ethanol intake in adolescent mice produced a modest facilitation of the psychostimulant effects of cocaine at a juvenile age, including locomotor activity and the reinforcing effects of cocaine in the self-administration paradigm, whereas almost no differences were found in cocaine-induced behavioural sensitization. Moreover, our study also shows that the two concentrations of ethanol solutions used (20% v/v and 30% v/v) affected the total amount of ethanol consumed (gEtOH/kg) by adolescent mice, but did not produce differences between the BEC obtained in both procedures.

C57BL/6 mice were exposed to a daily limited-access ethanol intake model for three consecutive weeks, on the basis of previous studies (Ozburn et al., 2015; Rhodes et al., 2005; Ros-Simó et al., 2012), in order to assess a possible increased vulnerability to cocaine abuse related to binge drinking behaviour. Our data show that the lowest concentration of ethanol (20%) gave rise to higher levels of ethanol intake than the higher concentration (30%), most probably due to the lower palatability of strong ethanol concentration solutions, consistent with previous studies (Rhodes et al., 2005). Both ethanol concentrations induced elevated BEC levels, high enough to be considered binge ethanol consumption, but no significant correlations were found between ethanol intake and BEC levels for either procedure (B20 and B30). Different factors may contribute to this fact: (i) blood samples to analyse BEC were extracted after the last 4 h drinking period and the pattern of ethanol intake tended to be different for each mouse during the 4 h period of free access to ethanol. Consequently, the rate of ethanol intake and ethanol metabolised and excreted also tended to be different for each mouse, producing a significant variability in ethanol intake and BEC levels, which made it difficult to obtain significant correlations. (ii) Mice were fed until the ethanol intake period began and gastric content tended to be different for each animal. Gastric content strongly influences ethanol absorption and subsequent BEC levels (Erickson and Byers, 1989). In the case of the B20 procedure, all mice reached ethanol intoxication levels in blood (>80 mg/dL), whereas, in the B30 procedure, 7 out of 10 animals showed levels of ethanol intoxication. Our study shows that the ethanol-intake procedure used may induce different episodes of intoxication during adolescence, and could be responsible for the subsequent additive effect on cocaine responses observed later in juvenile mice. There is compelling evidence that adolescence is a brain maturation period characterised by neuroplastic and dynamic processes, leading to the refinement of certain brain areas, neural circuits, neurotransmitter systems and hormonal secretion (Guerri and Pascual, 2010).

Ethanol binge drinking during adolescence increased the acute locomotor effects induced by cocaine in mice. Locomotor effects after cocaine in animals with ethanol binge-drinking experience were higher than those observed in animals receiving water. Few studies have evaluated the influence of ethanol on psychostimulant effects of ethanol. Accordingly, DBA/2J juvenile male mice that received intragastric administration of ethanol exhibited an increased effect on locomotion (Molet et al., 2013). In the same line, the psychostimulant mephedrone increased locomotor activity in CD1-mice, which was further enhanced when combined with ethanol administered by i.p. route (Ciudad-Roberts et al., 2015). A recent study (Mateos-Garcia et al., 2015) showed that female mice pre-treated with ethanol showed increased locomotion following a low-dose cocaine injection (1 mg/kg). However, no changes in locomotor activity were observed in male mice exposed to the same conditions. In that study, ethanol was administered i.p. (2.5 mg/kg) with a different treatment schedule, and locomotor activity was evaluated three weeks after the last ethanol administration using a different strain of mice (OF1 mice). The study by Mateos-Garcia et al. (2015) also revealed the different sex-dependent sensitivity to the effects of abused drugs, in agreement with those reported for the behavioural effects of psychostimulants that were modified by sex and the hormonal milieu (Martini et al., 2014). The mentioned differences in the experimental conditions of the previous studies could contribute to the apparent discrepancies found between the corresponding findings observed.

The administration of repeated doses of cocaine (10 mg/kg) produced behavioural sensitization to the hyperlocomotor effects of the psychostimulant. Under the present experimental conditions, mice exposed to ethanol showed a weak increase in locomotor activity compared with mice exposed to drinking water after acute and repeated cocaine administration. Hence, this enhanced effect seems to be additive rather than potentiating, as the increase in locomotion observed in mice exposed to ethanol remained unchanged in cocaine-treated mice. In fact, the ethanol and water groups treated with acute cocaine started at different levels of locomotor activity on day 1, indicating that ethanol intake seems to affect the acute response to cocaine, but there were no differences in the trajectory of changes between those two groups after repeated cocaine, as is evidenced by the lack of interaction between ethanol intake, cocaine treatment and day effects when calculated the ANOVA. Accordingly, a significant correlation was found between ethanol intake (gEtOH/kg) and cocaine locomotor effects on day 1, but this correlation was not observed at the endpoint of the behavioural sensitization, on day 12, after the administration of a challenge of cocaine. Interestingly, mice exposed to ethanol and treated with saline exhibited a trend to increase in locomotion when compared with mice exposed to water and administered saline, from day 1, suggesting a progressive development of a conditioned hyperlocomotion in this group of mice, probably due to ethanol exposure during adolescence, which could be attributed to changes in context reactivity or anxiety due to alcohol exposure (Varlinskaya et al., 2016). Consequently, the history of oral ethanol intake produced only modest changes on behavioural sensitization of cocaine-induced locomotor responding, mainly revealed on the acute effects of the psychostimulant. However, we cannot discard that oral ethanol intake with a more prolonged or intense pattern of consumption could produce alterations in the sensitivity to cocaine effects. Therefore, high rates of binge drinking that are particular prevalent late in adolescence seem to confer increased vulnerability for alcohol abuse (Molet et al., 2012) and neurocognitive alterations (Spear, 2015; Spear and Swartzwelder, 2014).

Acute cocaine effects are assumed to depend primarily on the inhibition of the dopamine transporters at synaptic terminals that increase dopaminergic neurotransmission in limbic areas (Buchta and Riegel, 2015; Giros et al., 1996), whereas behavioural sensitization to cocaine has been described as a neuroadaptive process characterized by progressively enhanced locomotor responses following repeated exposure to psychoactive drugs such as cocaine, and is related to the compulsive pattern of drug-seeking

behaviour (Berridge and Kringelbach, 2015; Robinson and Berridge, 1993). Our data seem to confirm that previous exposure to oral binge ethanol consumption appears to increase sensitivity to acute cocaine effects but did not affect to the neuroadaptive responses developed by chronic cocaine as revealed by the changes after acute locomotor activity but not during behavioural sensitization.

Ethanol oral binge drinking weakly increased the reinforcing effects of cocaine within the self-administration paradigm. As in previous studies (Soria et al., 2005; Touriño et al., 2012), two different doses of cocaine (0.5 and 0.75 mg/kg/infusion) were evaluated. No between-group differences were found with the lower dose of cocaine, (0.5 mg/kg/infusion), whereas a significant effect of treatment was revealed for the higher dose of cocaine (0.75 mg/kg/infusion), suggesting a moderate increase in the reinforcing effects of cocaine in mice exposed to ethanol during adolescence. This increase was statistically significant on day 8, and the fact that mice exposed to water showed a lower number of responses on this particular day also contributed to the observed significant differences. Considering our results, the most plausible interpretation is that ethanol pre-treated mice developed a slightly increase in cocaine reinforcing potential under our experimental conditions. Another possible interpretation may be that cocaine has discrete lower reinforcing potential in ethanol pre-treated mice. Thus, the animals consume a greater amount of cocaine in the self-administration paradigm due to a lower sensitivity to such effects. To separate these two possible interpretations, a full dose-response function is required in order to ascertain how the curve is shifted (left or right or down) (Soria et al., 2005). Moreover, the self-administration task is a learning process that requires motivation and the participation of cognitive capabilities including attention. We could speculate that cognitive alterations due to ethanol exposure during adolescence, including attention, arousal alterations and impulsivity, may also modify the performance of the animals in the self-administration paradigm (Sanchez-Roige et al., 2014).

Recent studies with cocaine and other drugs of abuse proposed that early exposure to ethanol increases sensitivity to the rewarding effects of the drug in adult mice (Do Couto et al., 2011; Hutchison and Riley, 2012; Mateos-García et al., 2015; Molet et al., 2012, 2013; Spear, 2015; Spear and Swartzwelder, 2014). For instance, adolescent mice exposed to ethanol or to the psychostimulant 3,4-methylenedioxymethamphetamine (MDMA) prolonged rewarding effects of MDMA and increased monoamine levels after a priming of MDMA in adulthood (Do Couto et al., 2011). In the same line, a facilitation of the rewarding effects of cocaine was revealed in the conditioned place preference paradigm in rats previously treated with ethanol i.p. (Hutchison and Riley, 2012). Moreover, juvenile DBA/2J mice exposed to ethanol showed increased rewarding effects following cocaine intake in adulthood, through a mechanism that includes positive modulation of striatal ERK signalling (Molet et al., 2013). Interestingly, activation of the ERK pathway has been shown to require stimulation of dopamine D1 receptors that have repeatedly been reported to play a key role in acute and adaptive responses to cocaine and other psychostimulants (Pascoli et al., 2014; Valjent et al., 2010).

Despite limited research to date about the interaction between pre-exposure to ethanol on cocaine addictive effects, the study by Mateos-Garcia et al. (2015) showed that repeated ethanol injections increased cocaine self-administration in male and female OF1 mice. In fact, female mice treated with ethanol performed a higher number of infusions than those mice treated with saline on different days of the training period while significant differences between males treated with ethanol were only observed on a single day (Mateos-García et al., 2015). This previous study also revealed that ethanol pre-treated animals developed rewarding effects in the conditioned place preference induced with a low dose of cocaine. However, the same set of mice was tested in both conditioned place preference and self-administration paradigm. Hence, one might considerer that mice exposed to cocaine during the conditioned place preference sensitized when exposed to the self-administration experiments (Mateos-García et al., 2015). Apparent discrepancies between this earlier study and our findings could be due to the fact that in the present study, mice were naïve to cocaine when exposed to the self-administration study. Additionally, under our experimental conditions, a contingent effect between drinking behaviour and the psychotropic effects of ethanol may be progressively developed throughout the ethanol intake procedure. Consequently, the contingency between drinking behaviour and the reinforcing effects of ethanol may spread the salience attribution to ethanol as a reinforcer, increasing drug-taking behaviour and the rewarding effects of cocaine, thus producing sensitization in neural circuits responsible for reward (Everitt and Robbins, 2016; Ozburn et al., 2015).

The observed discrete facilitation of the reinforcing effects of cocaine in mice exposed to ethanol may be due to the effect of ethanol on the dopamine neurotransmitter system, in limbic areas, which undergoes critical maturation steps during adolescence, through a mechanism involving both D1 and D2 dopamine receptor subtypes (Tarazi and Baldessarini, 1999). Consistently with this statement, ethanol induces enhanced dopamine release in juvenile rodents, which remains high throughout adulthood, contributing to elevated ethanol consumption (Molet et al., 2012; Pascual et al., 2009) or increased vulnerability to other drugs, such as cocaine.

Taken together, our results demonstrate that the reinforcing effects of cocaine are weakly facilitated as a consequence of voluntary intermittent ethanol binge drinking during adolescence under our experimental conditions. Notably, the model of voluntary ethanol binge drinking used in our study was able to produce the alterations discussed above. Although most of our results reveals a modest impact of ethanol intake on cocaine effects, we cannot discard that a more intense or prolonged exposure to binge ethanol consumption during adolescence could facilitate the development of neurobiological changes that would enhance the sensitivity of brain reward circuits to the effects of psychostimulant drugs like cocaine.

Acknowledgements

The Spanish Health National System is acknowledged for the OJP contract (MS10/00576). AEA, IGR and LC were supported by pre-doctoral fellowships from the Spanish Ministry of Education and the Spanish Ministry of Economy.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was

supported by the UE MedBioinformatics project (grant number 634143), the Spanish Ministry of Economy and Innovation and FEDER (grant numbers SAF2013-41761-R and PSI2014-51847-R), the Generalitat de Catalunya (grant numbers 2014SGR34 and 2014SGR14) and the Spanish Ministry of Health, Social Affairs and Equality (grant numbers PNSD 2014-020 and 2014-007) (Red Temática de Investigación Cooperativa en Salud – ISCIII - FEDER - RETIC-Trastornos Adictivos RTA, RD12/0028/0024 and RD12/0028/0005), and the Generalitat de Valencia (PROMETEO II/2014/063).

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