

## Long-term alterations in vulnerability to addiction to drugs of abuse and in brain gene expression after early life ethanol exposure

Estelle Barbier<sup>a</sup>, Olivier Pierrefiche<sup>a</sup>, David Vaudry<sup>b</sup>, Hubert Vaudry<sup>b</sup>, Martine Daoust<sup>a</sup>, Mickaël Naassila<sup>a,\*</sup>

<sup>a</sup>Equipe Région INSERM 24 (ERI24), Groupe de Recherche sur l'Alcool et les Pharmacodépendances (GRAP), Université de Picardie Jules Verne, Faculté de Pharmacie, 1 rue des Louvels, 80000 Amiens, France

<sup>b</sup>INSERM U413, IFRMP 23, University of Rouen, 76821 Mont Saint Aignan, France

### ARTICLE INFO

#### Article history:

Received 22 February 2008

Received in revised form 24 July 2008

Accepted 24 July 2008

#### Keywords:

Drug abuse

Ethanol

Prenatal

Brain development

Gene

Reward

### ABSTRACT

Exposure to ethanol early in life can have long-lasting implications on brain function and drug of abuse response later in life. The present study investigated in rats, the long-term consequences of pre- and postnatal (early life) ethanol exposure on drug consumption/reward and the molecular targets potentially associated with these behavioral alterations. Since a relationship has been demonstrated between heightened drugs intake and susceptibility to drugs-induced locomotor activity/sensitization, anxiolysis, we tested these behavioral responses, depending on the drug, in control and early life ethanol-exposed animals. Our results show that progeny exposed to early life ethanol displayed increased consumption of ethanol solutions and increased sensitivity to cocaine rewarding effects assessed in the conditioned place preference test. Offspring exposed to ethanol were more sensitive to the anxiolytic effect of ethanol and the increased sensitivity could, at least in part, explain the alteration in the consumption of ethanol for its anxiolytic effects. In addition, the sensitivity to hypothermic effects of ethanol and ethanol metabolism were not altered by early life ethanol exposure. The sensitization to cocaine (20 mg/kg) and to amphetamine (1.2 mg/kg) was increased after early life ethanol exposure and, could partly explain, an increase in the rewarding properties of psychostimulants. Gene expression analysis revealed that expression of a large number of genes was altered in brain regions involved in the reinforcing effects of drugs of abuse. Dopaminergic receptors and transporter binding sites were also down-regulated in the striatum of ethanol-exposed offspring. Such long-term neurochemical alterations in transmitter systems and in the behavioral responses to ethanol and other drugs of abuse may confer an increased liability for addiction in exposed offspring.

© 2008 Elsevier Ltd. All rights reserved.

### 1. Introduction

Ethanol use during pregnancy is one of the most common known causes of preventable birth defects, and can result in long-term deficits in physical and cognitive growth and development. When Lemoine et al. (1968) published the initial report of this syndrome in 127 cases, they described a syndrome referring to a pattern of anomalies including growth deficiency, facial abnormalities and CNS deficits, such as mental retardation and behavioral problems, occurring in children born to ethanol consuming women. This syndrome was later characterized as the fetal alcohol syndrome by Jones and Smith (1973). The combined incidence of fetal alcohol syndrome and its partial forms fetal alcohol effects and alcohol-related neurodevelopmental disorder has been estimated

to 9.1 per 1000 (Sampson et al., 1997). Brain imaging studies have identified structural changes in various brain regions of children prenatally exposed to ethanol including basal ganglia, corpus callosum, cerebellum and hippocampus that may account for the cognitive deficits (Mattson et al., 2001). Clinical studies reported that fetal ethanol exposure may increase risk for the subsequent development of dependence to ethanol and other drugs (Famy et al., 1998; Yates et al., 1998; Baer et al., 1998, 2003; Alati et al., 2006) and that children who had any prenatal ethanol exposure were 3.2 times as likely to have delinquent behavior scores (Sood et al., 2001). These studies also indicated that offspring drinking problems were predicted more effectively by prenatal ethanol exposure than by family history of alcoholism. This epidemiological evidence has also been suggested in preclinical studies. A variety of neuro-behavioral effects have been detected in animals following pre- and/or postnatal ethanol exposure and among them, early ethanol exposure can yield later enhancement of ethanol intake in rodents (see for review Spear and Molina, 2005; see Chotro et al., 2007).

\* Corresponding author. Tel.: +33 3 22 82 77 58; fax: +33 3 22 82 76 72.  
E-mail address: [mickaël.naassila@u-picardie.fr](mailto:mickaël.naassila@u-picardie.fr) (M. Naassila).

Earlier studies demonstrated altered response to the hypothermic effects of different drugs of abuse such as ethanol (Abel et al., 1981; Taylor et al., 1981) and morphine (Nelson et al., 1986), after prenatal ethanol exposure.

If the toxic effects of ethanol on brain development (Ikonomidou et al., 2000) and on cognitive performance are well characterized (Popovic et al., 2006), the long-term effects on drug vulnerability and the neurobiological substrates involved in this vulnerability remain to be elucidated, even though numerous studies identified some mechanisms involved in CNS dysfunctions observed after in utero ethanol exposure (see for review Guerri, 2002).

In order to understand the mechanisms underlying the increased vulnerability to drug abuse disorders induced by early life ethanol exposure, we used a pre- and postnatal ethanol exposure paradigm in rat (Naassila and Daoust, 2002; Dubois et al., 2006, 2008) to investigate the long-term alterations on both behavioral responses to drugs and brain gene expression. This procedure of forced 10–15% ethanol consumption by dams, before, during and after the gestation period, has been used in numerous studies (Vallés et al., 1995; Othman et al., 2002; Carneiro et al., 2005; Nowak et al., 2006; Servais et al., 2007). Using this procedure (Naassila and Daoust, 2002), dams are daily consuming about 6 g/kg ethanol before and during the gestation period and approximately 18 g/kg ethanol at the end of the lactation period. The blood alcohol concentrations range (180.5 and 10.0 mg/dl, maximum and minimum values) obtained in dams reflect the variability in ethanol intake during 24 h (unpublished data, see also Naassila and Daoust, 2002). At the behavioral level, we did not observe any alteration in the ethanol-exposed offspring neither in basal locomotor activity nor in learning and memory performances assessed in the Morris water maze and Y maze (unpublished data). Using this procedure, different neurochemicals' effects have been reported such as alterations in the developmental profile of NMDA receptor subunits expression (Naassila and Daoust, 2002), respiratory network activity (Dubois et al., 2006, 2008), cerebellar plasticity (Servais et al., 2007), hippocampal and striatal dopaminergic and muscarinic receptors binding (Carneiro et al., 2005), adenosine A1 receptor expression in cortex and cerebellum (Othman et al., 2002) and in dopamine evoked release by amphetamine (Nowak et al., 2006).

The objective of the present study was to characterize in rats that have been exposed to ethanol early in life, the changes in drugs of abuse consumption and in motivational's properties of drugs (conditioned place preference or taste aversion) and the alterations in several behavioral responses that are known to be involved in addiction such as locomotor response and locomotor sensitization. Specifically for ethanol, we also analyzed the response of the offspring to its acute hypothermic and anxiolytic effects since the sensitivity to the acute (Naassila et al., 2002, 2004) and anxiolytic (Spanagel et al., 1995) effects of ethanol are known to play a role in ethanol addiction. In addition to ethanol, we also analyzed the effects of both amphetamine and cocaine in order to test if early life ethanol exposure affects only the future response to ethanol or also alters the response to other drugs of abuse. In this regard, previous studies (Xu and Shen, 2001; Choong and Shen, 2004) have shown that psychostimulants can restore the alterations of the neuronal activity of the ventral tegmental area (a critical brain area involved in addiction) by prenatal ethanol exposure, thus suggesting that animals exposed prenatally to ethanol may respond differently to the reinforcing effects of psychostimulants. Is it noteworthy, that previous report has shown an increase in ethanol responding after prenatal exposure to cocaine in mice (Kelley and Middaugh, 1996), suggesting a potential heterosensitization.

Gene expression measurements and binding experiments have been carried out in brain structures (cortex, striatum, hippocampus, cerebellum) that have been shown to be especially susceptible to ethanol exposure during development (Guerri, 2002;

Feng et al., 2005). Among the numerous targets that have been shown to be altered by early life ethanol exposure, the present study focused principally on the neurotrophic factor BDNF and neurotransmitter receptors that have been previously shown to be important targets of the effects of prenatal ethanol exposure (Costa et al., 2000; Guerri, 2002; Feng et al., 2005). Specifically, we measured the expression of the CB1 receptor, the NMDAR1 and GABAA $\gamma$ 2 subunits, and the dopaminergic D1 and D2 receptors. CB1 receptors have been shown to play an important role in ethanol addiction (Naassila et al., 2004; Houchi et al., 2005; Warnault et al., 2007) and thus may be involved in the propensity to consume alcohol after early life ethanol exposure. In addition, we have previously shown using the same paradigm that the developmental profile of the NMDAR1 mRNA splice variants is altered during the first four weeks of life in the hippocampus of ethanol-exposed offspring (Naassila and Daoust, 2002). NMDAR1 subunits have also been shown to be necessary for the neurotoxic and teratogenic effects of prenatal alcohol exposure (Deng and Elberger, 2003). Finally we also chose to investigate the expression of the GABAA $\gamma$ 2 subunit that is the most abundant subunit in the brain and that is found in the majority of the synaptic GABAA receptors (Krystal et al., 2006). In addition a polymorphism of the  $\gamma$ 2 subunit of the GABAA receptor has been associated with genetic susceptibility to ethanol-induced motor incoordination and hypothermia, conditioned taste aversion, and withdrawal (Buck and Hood, 1998).

## 2. Materials and methods

### 2.1. Drugs

Cocaine hydrochloride, D-amphetamine and diazepam were obtained from Sigma Chemicals (Paris, France). [ $^3$ H]SCH23390, [ $^3$ H]raclopride, [ $^3$ H]mazindol, [ $^3$ H]MK-801, [ $^3$ ]CP55,940 and [ $^3$ H]muscimol were obtained from Perkin Elmer (UK). Ethanol (95% v/v) was obtained from VWR International (France). Ethanol was diluted to 20% (v/v) in physiological saline prior to the intraperitoneal (i.p.) injection. Cocaine, D-amphetamine i.p. injections were made in volumes of 0.5 ml/100 g in saline. Ethanol injections were made in volumes of 0.658 ml/100 g. Saline injections were made in volumes equal to that of the corresponding drug for each animal.

### 2.2. Animals and pre- and postnatal ethanol exposure

Adult male and virgin female Sprague–Dawley rats (200–300 g) were obtained from Charles River (France), maintained on a 12-h light/12-h dark cycle (light on between 07:00 and 19:00 h) and were used after at least 1 week of habituation in our facilities. Experiments were carried out in strict accordance with the Guide for the care and Use of Laboratory Animals (NIH) and the European Community regulations for animals use in research (Council directive #87-848, 19 October 1987 and #2001-464, 29 May 2001) laws and policies. Females were randomly assigned to two groups. The ethanol-treated group received as sole drinking fluid a 10% (v/v) ethanol solution, prepared from 95% ethanol, for 4 weeks before mating and had unlimited access to standard rat chow (UAR, France standard diet). After successful matings, the ethanol-treated group was maintained on 10% ethanol solution throughout gestation and lactation (Naassila and Daoust, 2002; Dubois et al., 2006, 2008). The ad libitum control group had unlimited access to standard rat chow and water. Three-week-old pups were weaned and group housed ( $n = 4$  per group). All behavioral experiments have been conducted with two month-old male offspring. Three hundred and sixty two naïve males born of 60 dams for the control group and 370 naïve males born of 60 dams for the ethanol-exposed group have been used in the present study. Breeders have been used only once and only one offspring per litter has been used for one type of experiment.

### 2.3. BEC analysis

Blood ethanol concentrations (BECs) were measured at different time points (30, 60, 120, 180, 240 and 360 min) after i.p. injection of 1.5 or 4.0 g/kg of ethanol. Blood was obtained from the tip of the tail in heparinized tubes from each one of the animals ( $n = 6$ /group). BECs were determined by injection of plasma into an oxygenate alcohol analyzer (Analox Instruments, London, UK). Ethanol standards were run before the samples.

### 2.4. Free choice paradigm

Fluid intake and body weight were assessed every day. Two month-old control and ethanol-exposed rats ( $n = 12$ /group) were individually housed in plastic cages with access ad libitum to standard rodent chow and habituated in their cage to

drinking from two bottles containing plain water for one week. Rats were then given access to two bottles, one containing water and the other containing ethanol. The positions of the bottles were changed every day to control for position preferences. Ethanol concentration (v/v) was increased from 3% to 10% over the course of the experiment (3% for 4 days, 6% for 2 days and 10% for 2 days). Ethanol solution was then replaced by sucrose solution. Rats received different sucrose concentrations (0.4%, 0.2% and 0.85%, w/v), respectively, during 6, 3 and 3 consecutive days. Naïve groups of rats were also tested for their psychostimulant solutions consumption, either cocaine (10, 25 and 100 mg/l) or amphetamine (10, 25 and 50 mg/l). The concentrations of both drugs were increased from the lowest to the highest dose, as done for ethanol. Since amphetamine solution is aversive, amphetamine consumption was also tested in rats that consumed cocaine (amphetamine after cocaine group) after a one-week withdrawal period (two bottles of water).

### 2.5. Conditioned place preference (CPP) apparatus and procedure

The CPP apparatus (BIOSEB, Chaville, France), consisted of two compartments (30 × 20 × 20 cm) separated by a guillotine door, and with distinct visual and tactile cues. One of the compartments had gray colored walls and a light-colored stainless steel floor and the opposite compartment had black and white striped walls and a dark smooth floor. Distance and time spent in each compartment were measured by computer-interfaced infrared photobeams (16 × 16 cm). Both compartments were illuminated by dim light with 40 lux brightness. The experiment consisted of three distinct phases: preconditioning phase (day 1), conditioning phase (days 2–5), and postconditioning test (day 6) as previously described (Houchi et al., 2005). To control possible innate preferences for one of the two conditioning compartments, rats underwent a single preconditioning session. Initial place preference was determined for each rat by the side in which they spent more than 600 s out a 20 min trial. Place preference conditioning was conducted using an unbiased procedure (Cunningham et al., 2003). For each group, rats were counterbalanced according to their initial preferences, so that for each group, half of the rats received drug in the initially preferred compartment and half in the least preferred one (Cunningham et al., 2006). During the conditioning phase, animals were randomly assigned to undergo either drug conditioning in the morning and saline conditioning in the afternoon, or vice versa. Animals received a total of two injections per day. Immediately after cocaine (10, 20, 25 mg/kg), ethanol (0.5, 1.0, 2.0 g/kg) or saline injection, each subject ( $n = 7–10$ /group) was confined to the appropriate compartment for 20 min (guillotine door closed). The drug- and saline-paired conditioning compartments and the time of the day of the drug or saline conditioning session (morning or afternoon) were randomly assigned and counterbalanced across all groups. Conditioning sessions were conducted twice daily for 4 days, with a minimum of 5 h between conditioning sessions. Postconditioning test was conducted by placing animals between the two compartments (guillotine door removed) and allowing free access to both conditioning compartments for 20 min. CPP was determined by comparing time spent (in s) in drug-paired compartment before (preconditioning) and after (test) conditioning session.

### 2.6. Conditioned taste aversion

Rats ( $n = 10$ /group) were individually housed with food and water ad libitum 48 h prior to the start of the conditioning procedure. The taste aversion conditioning procedure lasted for a total of 12 days and consisted of 7 days of water restriction, 4 days of conditioning and 1 test day, as previously described (Gessa et al., 2005). During water restriction animals received water for 20 min/24 h. Conditioning sessions consisted of 20 min/24 h access to one bottle with 0.2% (w/v) sucrose solution and one empty bottle. Depending on the group, rats were injected i.p. with ethanol (1.5 g/kg) or saline solution (0.9% w/v NaCl), immediately after conditioning session. The volume (ml) of sucrose solution consumed was measured for each rat. The conditioning session was repeated every day for four days. On the test day, the empty bottle was replaced by a bottle with water and no drug was injected. The volume (ml) of sucrose solution and water consumed was measured for each rat during 20 min. Preference (%) for sucrose was calculated as the ratio of sucrose consumption to total fluid intake (sucrose + water).

### 2.7. Anxiolytic effect of ethanol and diazepam measured in the elevated plus maze

The elevated plus maze apparatus was a modification of that validated by Lister (1987) and consisted of two open (50 × 10 cm) and two enclosed (50 × 10 × 40 cm) arms that extended from a common central platform (10 × 10 cm). The apparatus was constructed from black Plexiglas and elevated 50 cm above the floor. In accordance with established procedures (Rodgers and Johnson, 1995), rats ( $n = 8–17$ /group) were individually placed on the central platform of the maze facing an open arm 30 min after an i.p. injection of either saline or diazepam (3.0 mg/kg) and 10 min after i.p. injection of ethanol (1.5 g/kg). The 3.0 mg/kg dose of diazepam and the 1.5 g/kg dose of ethanol were based on previous studies in both mice and rats (Da Silva et al., 2005; Houchi et al., 2005, in press). A 5 min test duration was used, and the apparatus was thoroughly cleaned between test sessions. The conventional spatiotemporal measures (i.e. open arm time and entries) were scored. A rat was considered to have entered an arm when all four of its paws were placed in the arm.

### 2.8. Locomotor activity

Animals were confined to a 45 cm<sup>2</sup> clear acrylic plastic chamber, in which horizontal locomotion was assessed by recording the number of interruptions of infrared beams using a LE88811 IR motor activity monitor (BIOSEB, Chaville, France). Photocell beams transected the chamber 2 cm above the floor at 16 sites along each side. Test chambers were shielded from external noise and light, but each test field was illuminated with a white fluorescent light and was fully ventilated. For the study of the locomotor effects of ethanol (0.5, 0.75, 1.0 and 2.0 g/kg), rats ( $n = 5–10$ /group) were confined to chambers immediately after i.p. injections. For the study of amphetamine initial sensitivity, rats ( $n = 8$ /group) were injected i.p. with saline or 1.2 mg/kg amphetamine 30 min after habituation period and locomotor activity was recorded for 60 min. For the induction of amphetamine behavioral sensitization, each exposure to the chamber consisted of 30 min habituation period, followed by 1.2 mg/kg amphetamine injection (i.p.) and a 60 min test period. Animals were submitted to this paradigm every two days for 15 days. For the determination of maintenance of amphetamine behavioral sensitization, each animal received two challenge injections 1 and 3 weeks after repeated drug administration was stopped. The locomotor activity was recorded according to the paradigm described above. For the determination of cocaine dose effect: rats ( $n = 5–10$ /group) were injected i.p. with either saline, 5, 10 or 20 mg/kg cocaine. Locomotor activity measurements (20 min) began immediately after saline and cocaine injection. For the induction of cocaine behavioral sensitization, during the first 10 days of experiment, rats ( $n = 10$ /group) daily received saline or cocaine (10 or 20 mg/kg) injections in their home cages. On days 11–21, they remained drug-free in their home cages. For the expression of cocaine sensitization: on day 22, rats received a challenge dose of saline or cocaine (10 or 20 mg/kg). Locomotor activity was recorded during 20 min.

### 2.9. Sensitivity to ethanol-induced hypothermia

To measure hypothermia induced by ethanol (4.0 g/kg) administration (i.p.), rectal temperature was measured using a KJT thermocouple (BIOSEB, Paris, France) at room temperature (22 °C) before and after an i.p. drug injection, as previously described (Naassila et al., 2002). Rectal temperature was assessed 30, 60, 90, 120 and 180 min after drug administration ( $n = 10$ /group).

### 2.10. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

At postnatal day 14, 30, 60 and 90, offspring ( $n = 6–8$ /group) were killed, brains removed, and the structures (prefrontal cortex, hypothalamus, hippocampus, striatum and cerebellum) were dissected, quickly frozen in liquid nitrogen and stored at  $-80$  °C. Frozen tissues from individual animal were homogenized in 1.0 ml of lysate buffer (Applied Biosystems, Foster City, CA, USA) per 25 mg of tissue. Total RNAs were extracted with ABI PRISM<sup>®</sup> 6100 Nucleic Acid Prep Station according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). mRNA was eluted in 100  $\mu$ l final volume (nuclease-free water) and stored at  $-20$  °C. The reverse transcription reaction was carried out using ImProm-II<sup>™</sup> kit, according to the manufacturer's instructions (Promega, Madison, WI, USA). Each primer set (see Supplemental Table S1) was designed with the Primer Express<sup>®</sup> software (Applied Biosystems) and used at its optimal concentration with a maximal efficacy. Real-time PCR was performed on cDNA in the presence of a 1× Mastermix (Applied Biosystems) containing pre-set concentrations of dNTPs, MgCl<sub>2</sub> and the SYBR Green reporter dye along with specific primers, using the ABI Prism 7000 sequence Detection System (Applied Biosystems). RNA levels were deduced by comparison of cDNA-generated signals in samples to signals generated by a standard curve, and internally corrected with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA signal for variations in amounts of input mRNA. Specificity of PCR products was confirmed by melting curve analysis and agarose gel electrophoresis (see Supplemental Fig. 1). Means are expressed as % change of control values. Relative quantitation of gene expression levels was performed using the comparative CT method ( $2^{-\Delta\Delta CT}$ ) (Livak and Schmittgen, 2001). Using the  $2^{-\Delta CT}$  method (Livak and Schmittgen, 2001) to determine if ethanol treatment influences the expression of the GAPDH internal control gene, we did not find any significant effect of neither the ethanol treatment nor the expression level between the different brain areas at the same postnatal day. In addition, we previously demonstrated using a semi-quantitative RT-PCR technique that the GAPDH mRNA levels measured during the first month of life were not changed by early life ethanol (Naassila and Daoust, 2002).

### 2.11. Radioligand binding

Binding experiments were performed on rat striatal or hippocampal membranes from control and ethanol-exposed animals, as previously described (Houchi et al., 2005; Naassila and Daoust, 2002). Membranes were prepared from brain structures homogenized in 10 volumes ice-cold 0.32 M sucrose at 1200 rpm for 10 strokes, then centrifuged at 1000g at 4 °C for 10 min. The pellet was then homogenized and centrifuged as above. The resulting two supernatants were combined and centrifuged at 48,000g for 20 min at 4 °C. The P2 pellet was washed three times using assay buffer and the membranes were then re-suspended to approximately 1 mg/ml. The final pellet was frozen ( $-18$  °C) until use and a 10  $\mu$ l aliquot was used for protein measurement by the method of Lowry et al. (1951).

The assay conditions for each of the ligands were as follows: (i) D1R binding: [<sup>3</sup>H]SCH23390 (1.5 nM, s.a. 85 Ci/mmol) was incubated for 1 h at 30 °C in assay buffer (50 mM Tris-HCl, 4 mM MgCl<sub>2</sub>, pH 7.4). (ii) D2R binding: [<sup>3</sup>H]raclopride (1.5 nM, s.a. 80 Ci/mmol) was incubated for 1 h 30 min at 25 °C in assay buffer (50 mM Tris-HCl, 1 mM CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>, 5 mM KCl, 120 mM NaCl, 0.1% ascorbate, pH 7.4). (iii) DAT binding: [<sup>3</sup>H]mazindol (4 nM, s.a. 19.5 Ci/mmol) was incubated for 1 h 30 min at 4 °C in assay buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, pH 7.4, 20 °C). (iv) NMDAR binding: [<sup>3</sup>H]MK-801 (2.5 nM, s.a. 17.1 Ci/mmol) was incubated for 1 h 30 at 30 °C in assay buffer (50 mM Tris-HCl, pH 7.4 containing 100 μM glutamate and 30 μM glycine). (v) CB1R binding: [<sup>3</sup>H]CP55,940 (1 nM, s.a. 139.6 Ci/mmol) was incubated for 1 h at 37 °C in assay buffer (50 mM Tris-HCl, 5 mM MgCl<sub>2</sub> and 2.5 mM EDTA, 0.1% (w/v) fatty acid free BSA). (vi) GABAA binding: [<sup>3</sup>H]muscimol (2 nM, s.a. 28.5 Ci/mmol) was incubated for 30 min at 4 °C in assay buffer (50 mM Tris-citrate, pH 7.1).

One μM SCH23390, 10 μM sulpiride, 10 μM mazindol, 100 μM MK-801, 10 μM CP55,940 and 1 mM GABA were used to define nonspecific binding for D1R, D2R, DAT, NMDAR, CB1R and GABAAR, respectively. After incubation in a final volume of 500 μl assay buffer with 100–150 μg proteins, samples were filtered through Whatman GF/B (45 μm pore size) glass fibre filters pre-soaked in 0.5% polyethylenimine (PEI) and washed with an additional 2 × 5 ml assay buffer. Radioactivity was determined using 5 ml of ACS scintillation fluid and counted in a Wallac 1414 Winspectral liquid scintillation counter (Perkin Elmer, 60% efficiency for [<sup>3</sup>H]).

### 2.12. Statistical analyses

Statistical analyses were conducted using STATVIEW 5.0 software. Drug consumption and preference, locomotor activity, ethanol metabolism and hypothermia experiments were analyzed using a repeated-measure 2-way analysis of variance (RM-ANOVA) followed by a Tukey's post hoc test (factors pretreatment [control or early life ethanol] × drug concentration or time). For the CPP experiments, data were analyzed using a 3-way (pretreatment × dose × pre-post conditioning) RM-ANOVA followed by a Tukey's post hoc test. For the CTA and elevated plus maze experiments, data were analyzed using a 2-way (pretreatment × treatment) RM-ANOVA followed by a Tukey's post hoc test. No attempt was made to compare the anxiolytic effects of ethanol to the effects of diazepam. For the behavioral sensitization paradigm, data were analyzed using a 3-way (pretreatment × dose × day) RM-ANOVA followed by a Tukey's post hoc test. Quantitative real-time PCR and binding results were analyzed using Student's *t*-test. A significance level of 0.05 was used for all tests.

## 3. Results

### 3.1. Ethanol consumption by dams

The average daily ethanol consumption expressed as pure ethanol intake (g/kg body weight, g/kg bwt) was stable before mating and during the gestation period reaching 6.3 g/kg bwt and increased during the lactation period reaching 17 g/kg bwt during the last week of lactation (Table 1). This level of ethanol consumption is consistent with our previous study and this procedure has been shown to produce pharmacologically significant blood ethanol concentrations (Naassila and Daoust, 2002) that are comparable to what is found in human pregnant chronic

**Table 1**  
Ethanol consumption, dam weight gain, litter size and pup weight

Measure	Ethanol-exposed group	Control group
Daily ethanol consumption (g ethanol/kg body weight)		
Before gestation (4 weeks)	6.3 ± 0.28	–
Gestation	6.2 ± 0.49	–
Lactation week 1	8.9 ± 0.86	–
Lactation week 2	12.9 ± 0.05	–
Lactation week 3	17.9 ± 0.1	–
Mean weight gain (g)	107.4 ± 6.77*	142 ± 11.22
Litter size	13.33 ± 1.0	13.19 ± 0.70
Mean offspring weight (g)		
Day 8	15.1 ± 1.3	14.6 ± 1.2
Day 10	18.1 ± 1.6	18.5 ± 1.5
Day 12	23.0 ± 2.1	21.8 ± 1.5
Day 15	24.5 ± 2.2	26.2 ± 1.4
Day 18	30.6 ± 0.8	29.5 ± 2.1
Day 21	36.7 ± 2.4	41.6 ± 5.3

\**p* < 0.05 compared to control group.

drinkers (Halmesmaki, 1988) and can be considered as moderate (Eckardt et al., 1998). Blood ethanol concentrations determined from the blood samples collected at 08:00 h on day 14 of gestation and day 14 of lactation reached 86.3 ± 15.3 and 111.7 ± 13.5 mg/dl, respectively (Naassila and Daoust, 2002).

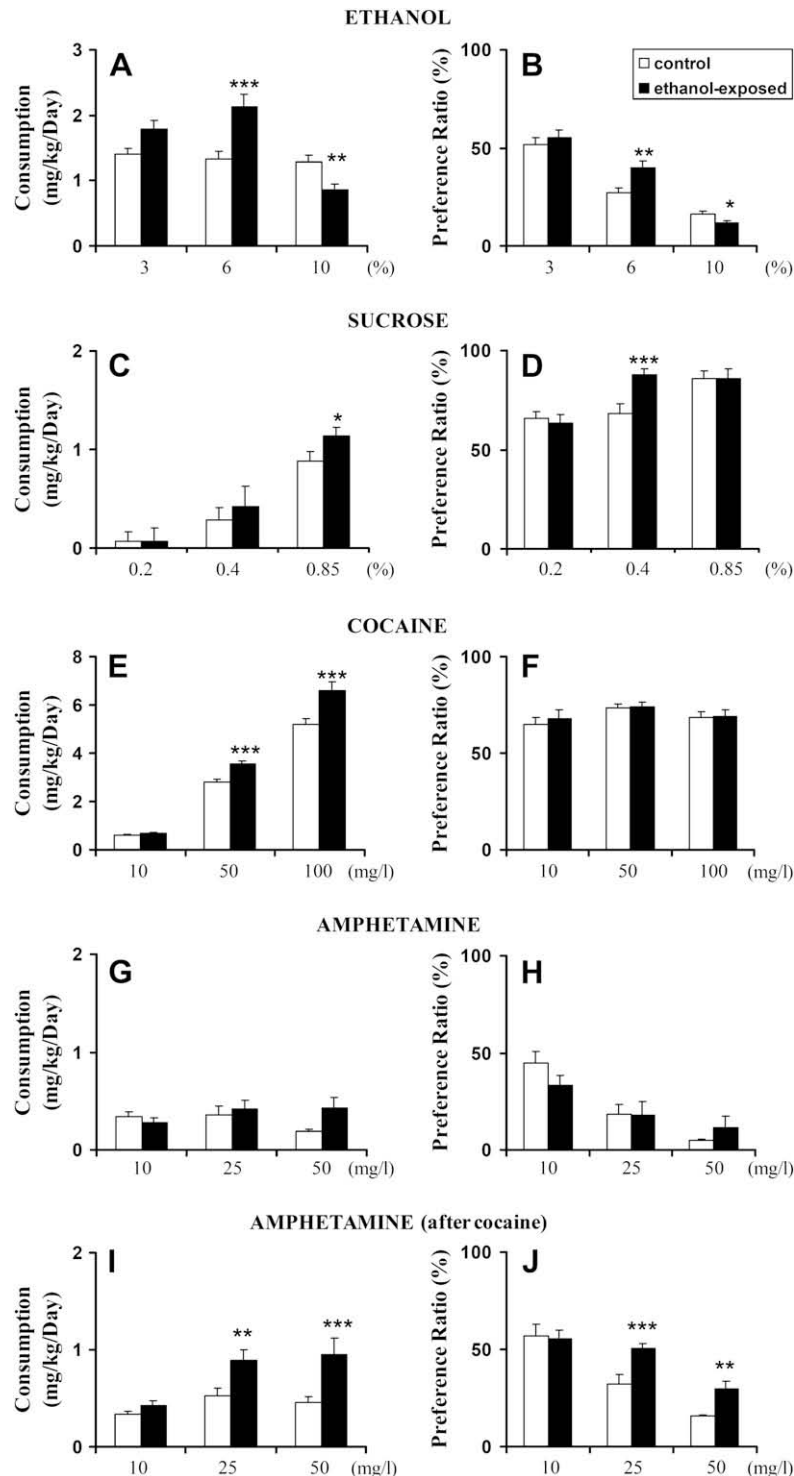
### 3.2. Fluid consumption

Early life ethanol exposure altered both ethanol consumption and preference for the 6% and 10% ethanol concentrations (consumption: main effect of pretreatment ( $F(1,399) = 4.2, p < 0.05$ ) and significant interaction ( $F(2,399) = 11.78, p < 0.001$ ); preference: main effect of pretreatment ( $F(1,399) = 2.88, p < 0.05$ ) and significant interaction ( $F(2,399) = 6.30, p < 0.005$ )) (Fig. 1A and B). Tukey's post hoc analysis revealed a significant increase in both ethanol consumption ( $p < 0.001$ ) and preference ( $p < 0.001$ ) for the 6% ethanol concentration and a significant decrease for the 10% ethanol concentration (consumption ( $p < 0.01$ ) and preference ( $p < 0.05$ )). There were no differences between control and early life ethanol-exposed rats in total intake of fluid (water + ethanol) (no main effect of pretreatment ( $F(1,399) = 0.92, p > 0.05$ ) and no significant interaction ( $F(2,399) = 1.79, p > 0.05$ )) (Table 2).

Early life ethanol-exposed rats also displayed an increase in both sucrose consumption (main effect of pretreatment  $F(1,215) = 9.45, p < 0.005$ ; main effect of dose  $F(2,215) = 163.97, p < 0.001$ ; and significant interaction  $F(2,215) = 2.95, p = 0.05$ ) and sucrose preference (no main effect of pretreatment  $F(1,215) = 3.04, p > 0.05$ ; main effect of dose  $F(2,215) = 14.83, p < 0.001$ ; and significant interaction  $F(2,215) = 4.49, p < 0.05$ ) (Fig. 1C and D). Tukey's post hoc analysis revealed a significant increase of consumption for the 0.85% sucrose solution ( $p < 0.001$ ) and preference ( $p < 0.001$ ) for the 0.4% sucrose solution. There were no differences between control and early life ethanol-exposed rats in total intake of fluid (water + sucrose) (no main effect of pretreatment ( $F(1,215) = 1.47, p > 0.05$ ) and no significant interaction ( $F(2,215) = 1.34, p > 0.05$ )) (Table 2). This increase in consumption of sucrose, another substance known to increase DAergic neurons activity, indicates that ethanol-exposed rats are not experiencing anhedonia and that drug intake is positively correlated to sucrose intake. In this regard, previous studies have shown that individual difference in oral sucrose consumption is predictive of ethanol and psychostimulant consumption (Carroll et al., 2007; DeSousa et al., 2000).

Cocaine consumption was significantly increased in ethanol-exposed animals (main effect of pretreatment  $F(1,324) = 23.9, p < 0.001$ ; main effect of dose  $F(1,324) = 389.6, p < 0.001$ ; and significant interaction  $F(2,324) = 5.43, p < 0.005$ ) (Fig. 1C). Tukey's post hoc analysis revealed a significant increase at 50 and 100 mg/l ( $p < 0.005$ ). This increased cocaine consumption was not associated to a significant increase in preference (Fig. 3F) and there was a significant difference in overall fluid intake (main effect of pretreatment  $F(1,324) = 17.98, p < 0.001$ ; main effect of dose  $F(2,324) = 9.90, p < 0.001$ ; and no significant interaction  $F(2,324) = 0.29, p > 0.05$ ), Table 2.

Amphetamine consumption and preference were not significantly altered by early life ethanol consumption (Fig. 1G and H) (consumption: no main effect of pretreatment  $F(1,143) = 1.45, p > 0.05$ ; no main effect of dose  $F(1,143) = 0.68, p > 0.05$ ; preference: no main effect of pretreatment  $F(1,143) = 0.25, p > 0.05$ ; main effect of dose  $F(1,143) = 22.88, p < 0.001$  and no significant interaction  $F(2,143) = 1.89, p > 0.05$ ). When amphetamine consumption and preference were measured in rats that consumed cocaine (amphetamine after cocaine group), significant increase in both amphetamine consumption (main effect of pretreatment  $F(1,143) = 15.28, p < 0.001$ ; main effect of dose  $F(1,143) = 7.14, p < 0.001$ ; and significant interaction  $F(2,143) = 3.14, p < 0.05$ ) (Fig. 1I) and preference (main effect of pretreatment  $F(1,143) = 5.15,$



**Fig. 1.** Ethanol (3%, 6% and 10%) ( $n = 12$  per group) (A, B), consumption (g/kg/day) and preference in control (□) and ethanol-exposed groups (■). Sucrose (0.2%, 0.4% and 0.85%) ( $n = 12$  per group) (C, D), cocaine (10, 50 and 100 mg/l) ( $n = 16$  per group) (E, F) and amphetamine (10, 25 and 50 mg/l) ( $n = 8$  per group) (G, H) consumption and preference ratio in control (□) and ethanol-exposed groups (■). Amphetamine consumption and preference ratio were also measured after one week after cocaine consumption was stopped (I, J). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to control group.

$p < 0.05$ ; main effect of dose  $F(1,143) = 17.3$ ,  $p < 0.001$ ; and significant interaction  $F(2,143) = 4.38$ ,  $p < 0.05$  (Fig. 1J) were observed in ethanol-exposed animals. Tukey's post hoc analysis revealed a significant increase at 25 and 50 mg/l (consumption:  $p < 0.01$ ,  $p < 0.001$ , respectively; preference:  $p < 0.001$ ,  $p < 0.01$ , respectively). This increased consumption and preference for amphetamine solutions (25 and 50 mg/l) were not associated to an increase in overall fluid intake (Table 2).

### 3.3. Conditioned place preference and conditioned taste aversion

Neither control nor early life ethanol-exposed rats showed place preference with the saline treatment in both compartments (Fig. 2A). Three-way RM-ANOVA revealed significant main effects of dose ( $F(2,116) = 3.71$ ,  $p < 0.05$ ), session ( $F(1,116) = 8.36$ ,  $p < 0.005$ ) and a significant dose  $\times$  session interaction ( $F(2,116) = 6.87$ ,  $p < 0.005$ ), Fig. 2A. Tukey's post hoc analysis

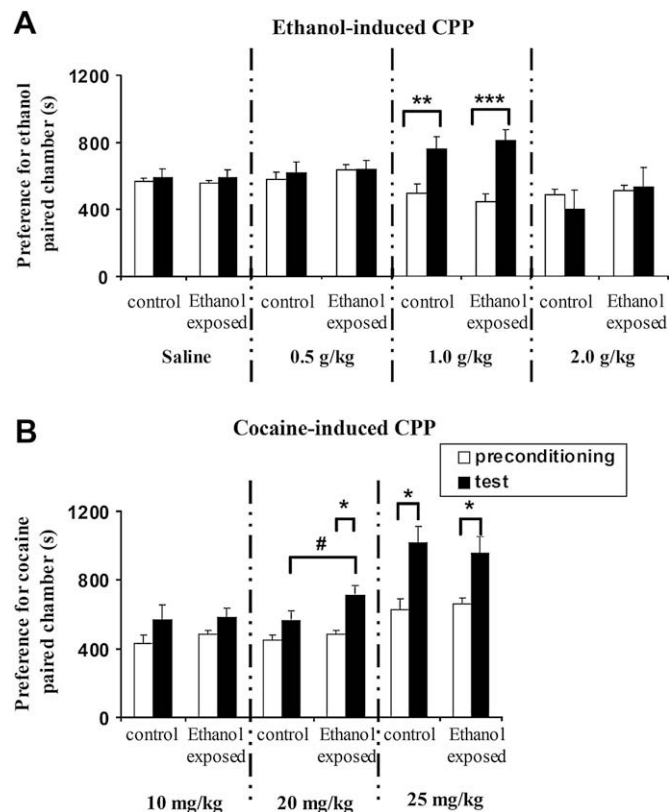
**Table 2**  
Total fluid consumption for each drug solution at different concentrations

Drug	Concentration	Total consumption (ml)	
		Control	Ethanol exposed
Ethanol	3%	38.1 ± 1.03	39.4 ± 1.32
	6%	36.5 ± 0.69	35.3 ± 0.70
	10%	47.3 ± 1.27	44.2 ± 1.18
Sucrose	0.2%	39.5 ± 1.07	39.1 ± 1.09
	0.45%	43.4 ± 1.47	44.1 ± 1.85
	0.85%	54.9 ± 3.31	61.8 ± 3.91*
Cocaine	10 mg/l	40.6 ± 1.42	42.8 ± 1.72
	50 mg/l	33.7 ± 1.32	38.7 ± 1.20
	100 mg/l	37.2 ± 1.05	44.2 ± 1.55
Amphetamine	10 mg/l	34.3 ± 1.3	37.3 ± 1.0
	25 mg/l	38.0 ± 1.6	40.2 ± 1.1
	50 mg/l	35.3 ± 1.1	36.8 ± 1.3
Amphetamine after cocaine	10 mg/l	33.8 ± 0.97	38.0 ± 1.24*
	25 mg/l	37.5 ± 3.41	39.7 ± 3.73
	50 mg/l	34.0 ± 0.97	35.8 ± 1.66

\* $p < 0.05$  compared to respective control.

indicated that both control and early ethanol-exposed groups demonstrated an ethanol-induced CPP for the 1.0 g/kg dose ( $p < 0.01$  and  $p < 0.001$ , respectively) and there was no difference between the two groups.

Cocaine induced a significant CPP in both groups and 3-way RM-ANOVA revealed a significant cocaine dose  $\times$  session  $\times$  pretreatment interaction ( $F(2,112) = 3.33$ ,  $p < 0.05$ , Fig. 2B). There were significant



**Fig. 2.** Ethanol (A) and cocaine (B) induced conditioned place preference (CPP) ( $n = 8$ – $10$  per group). Data are expressed as mean time spent ( $\pm$ SEM) in the drug-paired chambers during the 20-min preconditioning ( $\square$ ) and 20-min test phases ( $\blacksquare$ ). (A) Control and ethanol-exposed groups ( $n = 8$ /group) were injected i.p. with 1.0 and 2.0 g/kg ethanol. (B) Control and ethanol-exposed groups ( $n = 8$ – $10$ /group) were injected i.p. with 10, 20 or 25 mg/kg cocaine. \* $p < 0.05$ , \*\* $p < 0.01$  compared to respective preconditioning session; # $p < 0.05$  compared to respective control group.

main effects of dose ( $F(2,112) = 14.24$ ,  $p < 0.0001$ ), session ( $F(2,112) = 34.96$ ,  $p < 0.0001$ ) and pretreatment ( $F(2,112) = 3.38$ ,  $p < 0.05$ ) and also dose  $\times$  pretreatment ( $F(2,112) = 2.98$ ,  $p < 0.05$ ) and dose  $\times$  session ( $F(2,112) = 3.11$ ,  $p < 0.05$ ) interactions. Tukey's post hoc analyses indicated that cocaine 25 mg/kg induced significant CPP in both groups ( $p < 0.05$ ) while the dose of 20 mg/kg induced a significant CPP only in the early life ethanol-exposed group ( $p < 0.05$ ).

In the CTA test, both control and early ethanol-exposed groups demonstrated an aversion for 1.5 g/kg ethanol (main effect of ethanol:  $F(1,39) = 15.09$ ,  $p < 0.001$ ), but no significant difference of ethanol aversive properties between the two groups was observed (no main effect of pretreatment  $F(1,39) = 0.034$ ,  $p > 0.05$  and no interaction, data not shown).

#### 3.4. Anxiety-like behavior and effects of diazepam and ethanol

In the elevated plus maze test, early life ethanol-exposed rats showed no difference compared to control rats in the time spent in open arms (Fig. 3A) nor in the number of open arms' entries (Fig. 3B), revealing no significant difference of spontaneous anxiety-related behaviors between the two groups after saline injection. An anxiolytic effect of diazepam (3.0 mg/kg, i.p.) was observed in both groups of rats (main effect of treatment, time:  $F(1,19) = 30.79$ ,  $p < 0.001$ ; entries:  $F(1,19) = 23.24$ ,  $p < 0.001$ ) and there was no difference between the two groups.

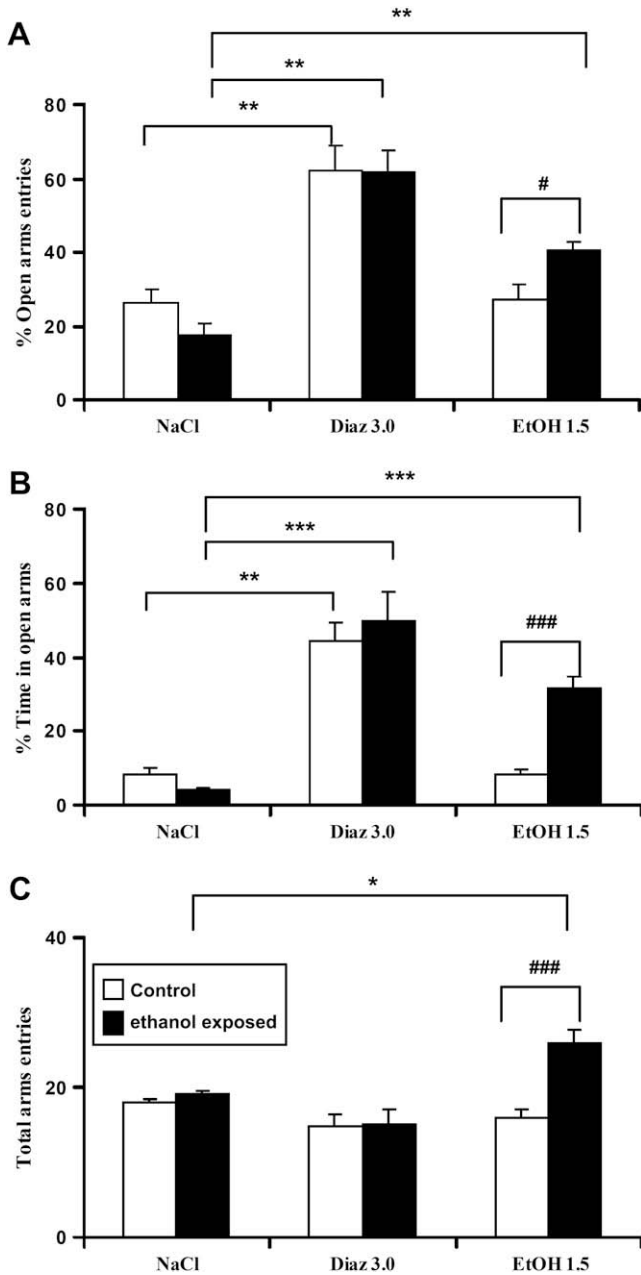
Two-way ANOVA revealed a main effect of ethanol ( $F(1,22) = 6.89$ ,  $p < 0.05$ ), no main effect of pretreatment ( $F(1,22) = 0.25$ ,  $p > 0.05$ ); and a significant interaction ( $F(1,22) = 5.71$ ,  $p < 0.05$ ) for the number of open arm entries (Fig. 3A); and main effects of pretreatment ( $F(1,22) = 18.41$ ,  $p < 0.009$ ), ethanol ( $F(1,292) = 17.28$ ,  $p < 0.001$ ) and a significant interaction ( $F(1,22) = 17.45$ ,  $p < 0.001$ ) for the time spent in open arms (Fig. 3B). Tukey's post hoc analysis indicated a significant increase in these parameters compared to the control group and to the respective NaCl group (as indicated in Fig. 3).

There was no difference in the number of total entries in arms between early-life ethanol-exposed rats and controls, revealing no impact of early ethanol exposure on locomotor activity evaluated in this test (Fig. 3C). There was also no alteration in the number of total entries in arms after diazepam (3.0 mg/kg) and no difference between the two groups (no main effects of treatment:  $F(1,19) = 2.64$ ,  $p > 0.05$  and pretreatment:  $F(1,19) = 0.06$ ,  $p > 0.05$ ). Ethanol (1.5 g/kg) increased the number of total entries in arms in early life ethanol exposed animals, 2-way ANOVA revealed a main effect of pretreatment ( $F(1,22) = 8.04$ ,  $p < 0.05$ ) and a significant interaction ( $F(1,22) = 5.38$ ,  $p < 0.05$ ) (Fig. 3C). Tukey's post hoc analysis indicated a significant increase compared to the control group ( $p < 0.001$ ) and to respective NaCl group ( $p < 0.05$ ).

#### 3.5. Locomotor responses to cocaine and ethanol and sensitivity to amphetamine- or cocaine-induced behavioral sensitization

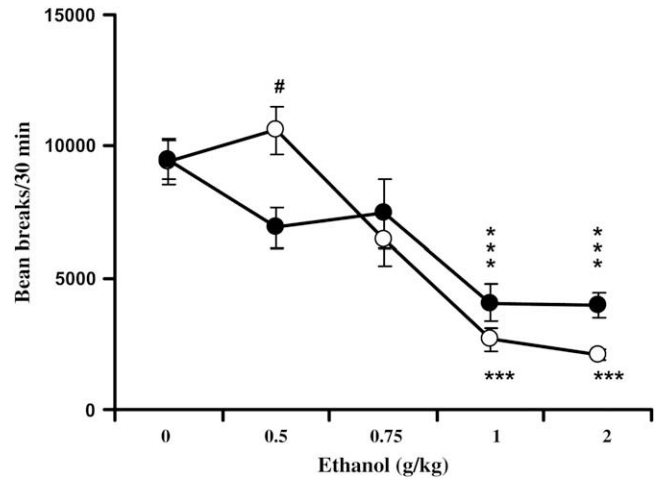
Two-way ANOVA revealed no difference between control and early life ethanol-exposed animals in locomotor response to increasing doses of cocaine (treatment:  $F(1,65) = 0.18$ ,  $p > 0.05$ ; dose:  $F(3,65) = 9.52$ ,  $p < 0.001$  and no interaction) (data not shown). In contrast, the locomotor response to ethanol was slightly altered by early life ethanol exposure, 2-way ANOVA revealed a main effect of ethanol dose ( $F(3,87) = 22.58$ ,  $p < 0.001$ ) and a significant dose  $\times$  pretreatment interaction ( $F(4,87) = 3.88$ ,  $p < 0.01$ ) (Fig. 4). Tukey's post hoc analysis indicated a significant difference in sensitivity to lower dose of ethanol (0.5 g/kg) ( $p < 0.01$ ).

Three-way ANOVA revealed that cocaine induced significant locomotor sensitization after repeated injections (main effects of day of treatment  $F(1,103) = 26.08$ ,  $p < 0.001$ ; dose  $F(2,103) = 9.73$ ,

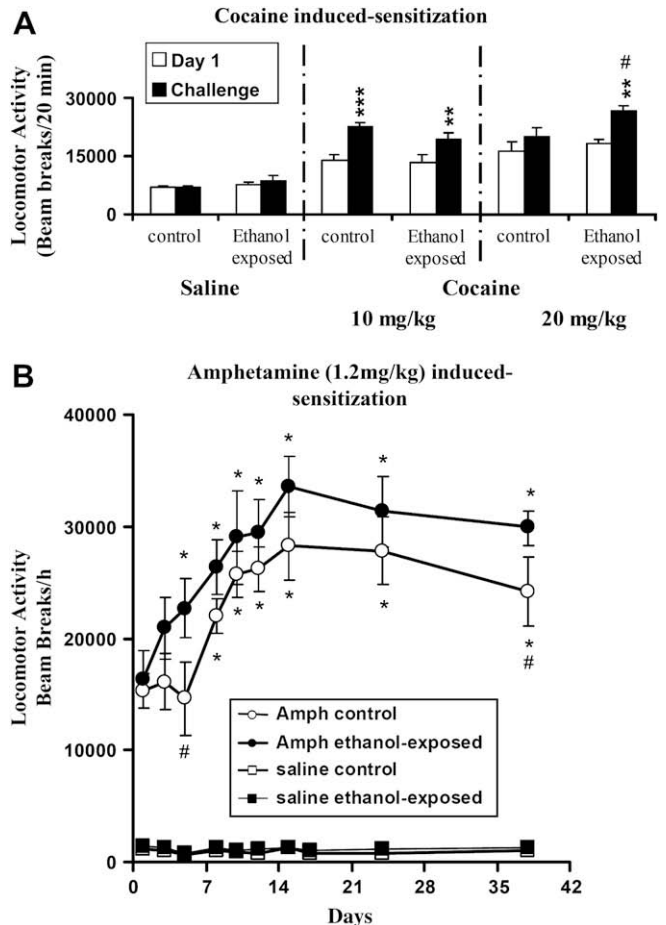


**Fig. 3.** Behavioral analysis in the elevated plus maze. The anxiolytic effects of diazepam (3.0 mg/kg) ( $n=8$  per group) or ethanol (1.5 g/kg) ( $n=17$  per group) were measured in control (□) and early life ethanol-exposed (■) groups. Open arms entries, time in open arms and total arms entries, (A, B and C, respectively).

$p < 0.005$  and significant dose  $\times$  day  $\times$  pretreatment  $F(2,103) = 3.14$ ,  $p < 0.05$  and dose  $\times$  pretreatment  $F(2,103) = 5.49$ ,  $p < 0.05$  interactions (Fig. 5A). Tukey's post hoc analysis indicated that both groups displayed significant sensitization at the 10 mg/kg dose of cocaine ( $p < 0.001$  and  $p < 0.01$  compared to day 1 for control and ethanol-exposed groups, respectively) while a significant sensitization at the 20 mg/kg dose of cocaine was observed only in the ethanol-exposed group ( $p < 0.01$  compared to day 1 and  $p < 0.05$  compared to the control group). Repeated administrations of cocaine produced locomotor sensitization in ethanol-exposed animals at all cocaine doses tested whereas they are associated with an inverted U-shaped function of locomotor activity in control rats, as previously described (Post and Weiss, 1988). This decrease in locomotion might be linked to behavior disruption due to stereotypies induced by the highest dose in controls. Saline-



**Fig. 4.** Locomotor activity of control (○) and ethanol-exposed groups (●) in response to saline to increasing doses of ethanol (0.5, 0.75, 1.0 and 2.0 g/kg) ( $n=6-14$  per group). \*\*\* $p < 0.001$  compared to respective saline; # $p < 0.01$  compared to respective ethanol dose.



**Fig. 5.** Early life ethanol exposure increased locomotor sensitization to psychostimulants. (A) Rats daily received cocaine injections in their home cage to induce cocaine sensitization. Effects of cocaine (10 or 20 mg/kg) ( $n=10$  per group) or saline injections on locomotor activity were evaluated the first day (day 1) of the repeated cocaine administration period (daily injection for 10 days) and 12 days after the end of the repeated cocaine administration period (day 22). Values represent mean ( $\pm$ SEM) of horizontal activity counts during 20 min. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to day 1; # $p < 0.05$  compared to respective control group. (B) Effects of saline and amphetamine (1.2 mg/kg) ( $n=8$  per group) injections on locomotor activity during repeated amphetamine administration and three weeks after withdrawal in control (○) and ethanol-exposed groups (●). \* $p < 0.05$  compared to day 1; # $p < 0.05$  compared to respective control group.

injected controls that were cocaine challenged did not display enhanced locomotor activity when challenged with cocaine in the two groups (control and ethanol-exposed, Fig. 5A).

Development of behavioral sensitization to amphetamine (1.2 mg/kg) was observed in both groups and there was a significant difference between the two groups, main effects of pretreatment ( $F(1,142) = 3.59$ ,  $p < 0.05$ ), time ( $F(1,142) = 6.44$ ,  $p < 0.001$ ) and a significant pretreatment  $\times$  time interaction ( $F(8,142) = 5.60$ ,  $p < 0.001$ ), Fig. 5B. Tukey's post hoc analysis revealed that sensitization was faster and more pronounced at the end of the test in the early life ethanol-exposed group compared to control group, with a significant increase of locomotion starting at the third day in the early life ethanol-exposed animals ( $p < 0.05$ ) and at the eighth day in the control group ( $p < 0.05$ ). The last day of behavioral testing, i.e. 2 weeks after the end of the repeated-injections period the response to amphetamine challenge was higher in early life ethanol-exposed rats compared to controls ( $p < 0.05$ ) (Fig. 5B). Saline-injected controls that were amphetamine challenged did not display enhanced locomotor activity when challenged with amphetamine in the two groups (control and ethanol-exposed) (day:  $F(3,39) = 0.02$ ,  $p > 0.05$ ). Ethanol-exposed animals were more sensitive to the locomotor effect of the amphetamine challenge, 3 weeks after the end of repeated administration, thus indicating that the expression of sensitization is increased by early life ethanol exposure.

### 3.6. Ethanol induced hypothermia and ethanol metabolism

There was no difference between control and early life ethanol-exposed animals for their sensitivity to ethanol (4.0 g/kg)-induced hypothermia ( $F(9,149) = 0.20$ ,  $p > 0.05$ , data not shown). Blood ethanol levels measured after i.p. injection of 4.0 and 1.5 g ethanol/kg doses did not differ significantly between the two groups (RM-ANOVA  $F(7,48) = 3.29$ ,  $p > 0.05$  and  $F(4,79) = 1.13 > 0.05$ , respectively) (data not shown). Thus, differences in ethanol consumption are not associated with altered ethanol metabolism.

### 3.7. Gene expression

We addressed the possibility that the behavioral alterations of drug of abuse responses may involve transcriptional regulation events in key brain areas. Our study shows that early life ethanol exposure has long-term pleiotropic effects on neurotransmission systems. These alterations are very specific and cannot be attributed to a global change in synaptic proteins or in the number of neurons. mRNA levels were measured 14, 30, 60 and 90 days after birth in various brain regions (frontal cortex, hippocampus, striatum, thalamus/hypothalamus and cerebellum) and concerned neurotransmitter systems, with the NMDAR1 subunit of the NMDA receptor, the cannabinoid CB1 receptor, the  $\gamma 2$  subunit of the GABAA receptor, the D1 and D2 dopamine receptors and the neuronal growth factor BDNF. Early life ethanol exposure induced differential effects depending on targeted mRNA, time point and brain structure (Fig. 6). Thus, for example at postnatal day 60 and in all analyzed brain regions, the expression of NMDAR1 was downregulated while that of BDNF was upregulated and that of the  $\gamma 2$  subunit of GABAA receptor unaffected. The results show that alterations of gene expression can last over a very long period since some changes are still detected at 90 days of age, i.e. 9 weeks after the end of ethanol exposure (Fig. 6). In the five brain areas investigated, the downregulated genes (50–100% decrease) at 2 months of age were involved in neurotransmission (NMDAR1 subunit, CB1 receptor) (Fig. 6B and C). In contrast, expression of the neurotrophin BDNF that is involved in both neuronal growth and plasticity, was increased in all structures (150–450% increase) (Fig. 6A). Dopamine (DA) D2 receptor (D2R) mRNA levels were greatly increased (200–500%) specifically in both thalamus/hypothalamus

and striatum (Fig. 6F). At 90 days of age, both NMDAR1 and CB1 receptors expression was still decreased in several brain areas such as thalamus/hypothalamus, striatum, hippocampus and cerebellum. At 30 days of age, i.e. one week after the end of ethanol exposure (during lactation period), expression of different genes (CB1 and D1R and BDNF) was increased (100–200%) in specific brain structures. Only slight modifications of gene expression were observed during the ethanol exposition, i.e. at day 14 of the lactation period. NMDAR1 expression was significantly increased in cerebellum. NMDAR1, CB1 and D1R and BDNF were decreased in frontal cortex. GABAA $\gamma 2$  subunit expression was not altered by early life ethanol exposure.

When the developmental profiles of expression are analyzed, an increase of the level of expression from postnatal day 14 to postnatal day 60 (no variation between day 60 and 90) is observed for the hippocampal (not shown) and striatal (Fig. 7A) NMDAR1 subunit and early life ethanol blocked (or delayed) this upregulation. This alteration is also observed for the striatal CB1 mRNA level (Fig. 7B). In contrast, the BDNF mRNA level is increased in ethanol-exposed animal compared to the control group at postnatal day 60 (Fig. 7C).

### 3.8. Radioligand binding

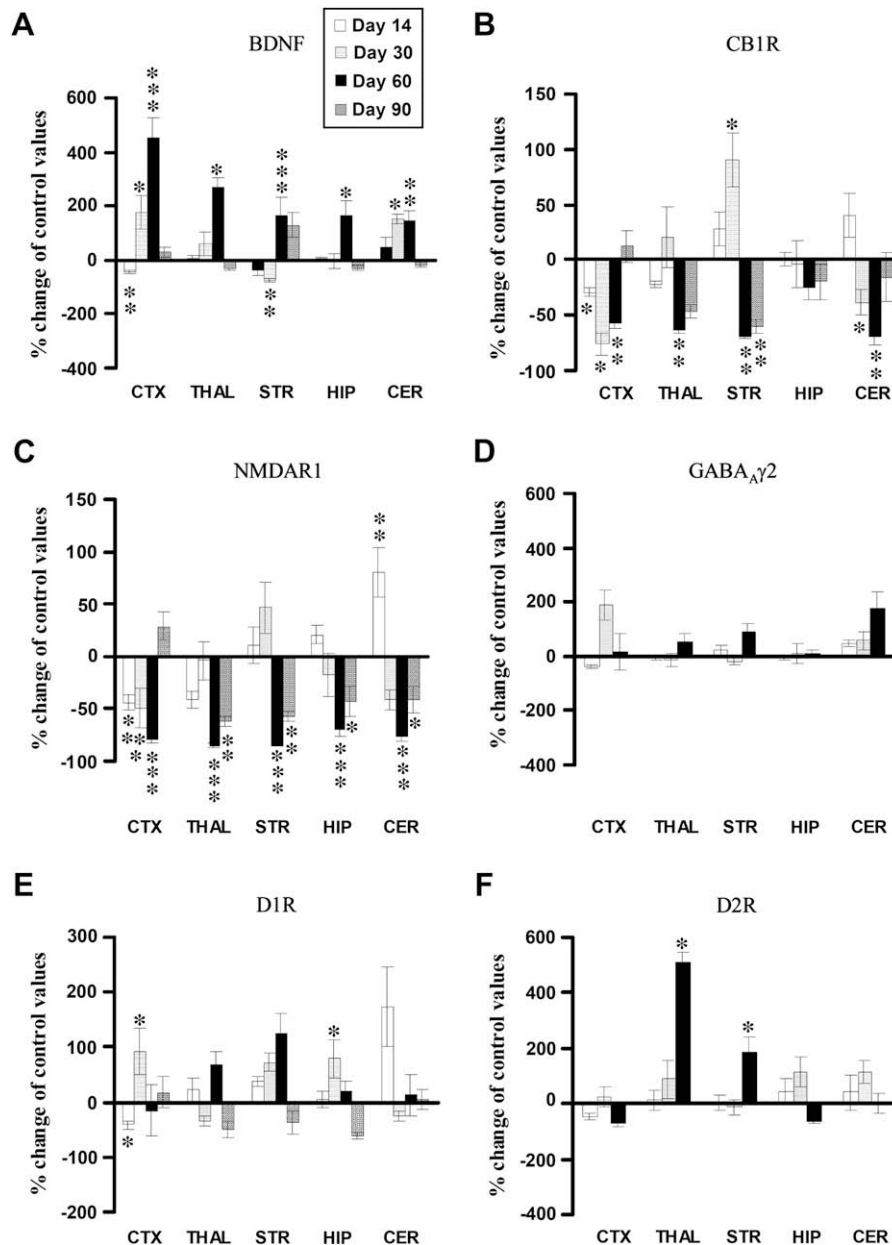
Fig. 8 shows the results of the radioligand binding studies. In the striatum, the 1 month-old early life ethanol-exposed rats had a significant decrease in (D1: 37%,  $p < 0.001$ ; D2: 31%,  $p < 0.005$ ) DAergic receptor binding and no significant alteration of dopamine transporter binding (Fig. 8A). In the striatum, the 2 month-old early life ethanol exposed animals had a significant decrease in D1R binding (37%,  $p < 0.001$ ) and dopamine transporter binding (32%,  $p < 0.001$ ) and no significant alteration in D2R binding (Fig. 8B). In the hippocampus, early life ethanol exposure did not alter neither CB1, nor NMDA, nor GABAA receptors binding (Fig. 8C). Our data show that early life ethanol exposure did not alter neither striatal D1R mRNA despite the decrease in [ $^3$ H]SCH23390 binding. In addition, no correlation was found between D2R mRNA level and [ $^3$ H]raclopride binding as well as for NMDAR1 subunit and CB1R mRNA levels and [ $^3$ H]MK-801 and [ $^3$ H]CP55,940 binding, respectively. This is in agreement with previous study that has shown a dissociation between mRNA and protein expression and that the ethanol-induced changes are not strictly parallel (Sheela Rani and Ticku, 2006).

## 4. Discussion

The long-term alterations of behavioral responses to drugs of abuse and the neurochemical changes observed in ethanol-exposed rats demonstrate that early life ethanol exposure has lasting neurobiological consequences potentially involved in vulnerability to addiction to drugs of abuse.

### 4.1. Consumption and sensitivity to rewarding/aversive effects of drugs of abuse

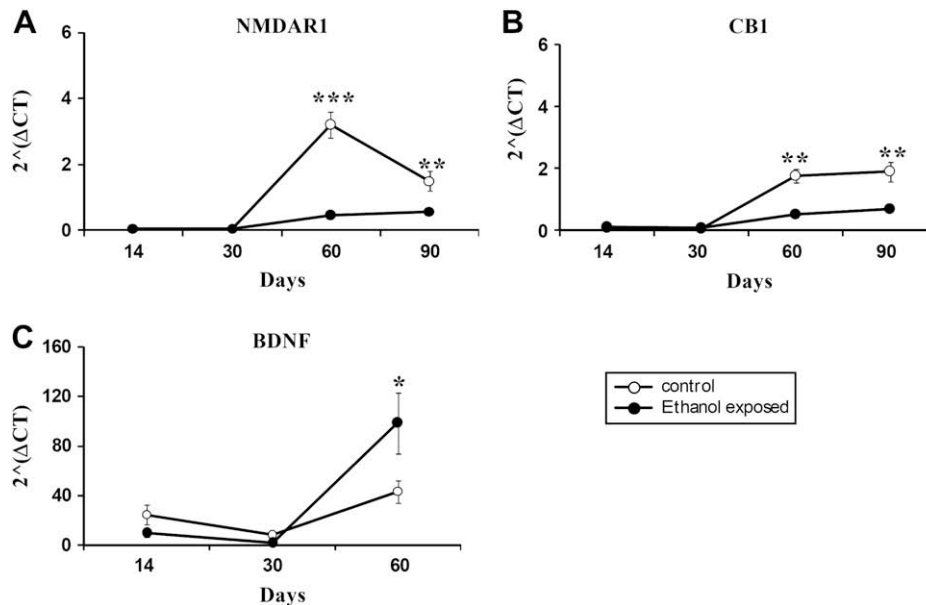
Numerous studies have directly examined the effects of pre- and/or postnatal ethanol exposure on later ethanol consumption in mice and rats, but yielded conflicting results (for review see Spear and Molina, 2005). The results of the present study confirm the increased ethanol intake and preference previously observed in rats after different paradigms of early life ethanol exposure (Bond and DiGiusto, 1976; Phillips and Stainbrook, 1976; Holloway and Tapp, 1978; Randall et al., 1983; Molina et al., 1987) and specially studies that showed increased consumption of only low ethanol concentration solution (3–6%) (Bond and DiGiusto, 1976; Holloway and Tapp, 1978). Our results are also in accordance with a more recent study showing that prenatal ethanol exposure increases 6% ethanol



**Fig. 6.** Effects of early ethanol exposure on mRNA levels for BDNF (A), CB1R (B), NMDAR1 subunit (C), GABA<sub>A</sub>2 subunit (D), DA DR1 receptor (E) and DA DR2 receptor (F). Bar graphs represent mean changes of control group values (%  $\pm$  SEM; a 100% increase represents a 2-fold change and a 50% decrease represents a 2-fold down regulation over control, respectively) within different ages and cerebral structures (prefrontal cortex: CTX, thalamus/hypothalamus: THAL, striatum: STR, hippocampus: HIP and cerebellum: CER). \* $p$  < 0.05, \*\* $p$  < 0.01, \*\*\* $p$  < 0.001 compared to respective control group (Student's  $t$  test).  $n$  = 5–8 per group.

consumption in adolescent rats (Chotro and Arias, 2003). At the 10% concentration, ethanol consumption and preference were decreased in early life ethanol-exposed animals compared to controls, suggesting that the sensitivity to aversive ethanol solution was increased. However, no difference in the sensitivity to the aversive properties of ethanol was observed in our CTA paradigm when ethanol is i.p. injected. The relative contribution of rewarding and aversive effects of ethanol in each procedure is not clear (Chester and Cunningham, 2002) and numerous studies have shown no correlation between ethanol consumption and sensitivity to ethanol-induced CTA or CPP (Risinger et al., 2001; Hill et al., 2003; Blednov et al., 2006, 2007). It is noteworthy that a recent review indicated that a negative correlation between CTA and ethanol drinking and a modest positive correlation between CPP and ethanol drinking were found in the literature (Green and Grahame, 2008).

Our results also show that early life ethanol exposure facilitated the induction of place preference by cocaine. Again the results on cocaine intake are not correlated to those on cocaine-induced CPP. Although the maximum dose–response function was not defined in the cocaine-induced CPP experiments, the data suggest a leftward shift in the dose–response function for the ethanol-exposed animals. In our experimental conditions, controls did not show significant cocaine-induced CPP at the 20 mg/kg dose. These conditions may have allowed the effect early life ethanol exposure to be detected. The modification of the sensitivity to the rewarding effects of cocaine is not associated with alteration of preference for cocaine solution. The increase in cocaine consumption of rats exposed to early life ethanol could be related to the significant decrease in the density of striatal DA transporter binding sites that may affect its pharmacological sensitivity. In addition, early life ethanol exposure did not alter consumption of amphetamine solution that is aversive with



**Fig. 7.** Developmental profile of NMDAR1 subunit (A), CB1 receptor (B) and BDNF (C) mRNA levels in the striatum of control (○) and ethanol-exposed groups (●), expressed as  $2^{-\Delta CT}$  values. \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to respective ethanol-exposed group and \* $p < 0.05$  compared to respective control group (Student's *t* test).

increasing concentrations (Vazquez et al., 2006). The different profile of ethanol effects on cocaine and amphetamine consumption may be explained by their gustative and postdigestive properties and/or by their different mechanisms of action on monoaminergic systems. In this regard, another study has previously shown that difference in amphetamine consumption was not associated with an alteration in DA transporter expression (Vazquez et al., 2006). The stimulant and rewarding effects of cocaine imply a blockade of DA transporter whereas those of amphetamine involve an increase in extracellular monoamine levels. Differences in amphetamine and cocaine consumption may also be dependent upon their manner in which they regulate DA transporter expression (reviewed in Zahniser and Sorkin, 2004). Interestingly, when rats were given amphetamine one week after cocaine, amphetamine solution was less aversive and ethanol exposed animals consumed significantly more amphetamine compared to control rats. Thus, suggesting that early life ethanol-exposed animals have been sensitized by cocaine pre-exposure and displayed specific alterations in expression and/or functioning of DA transporter.

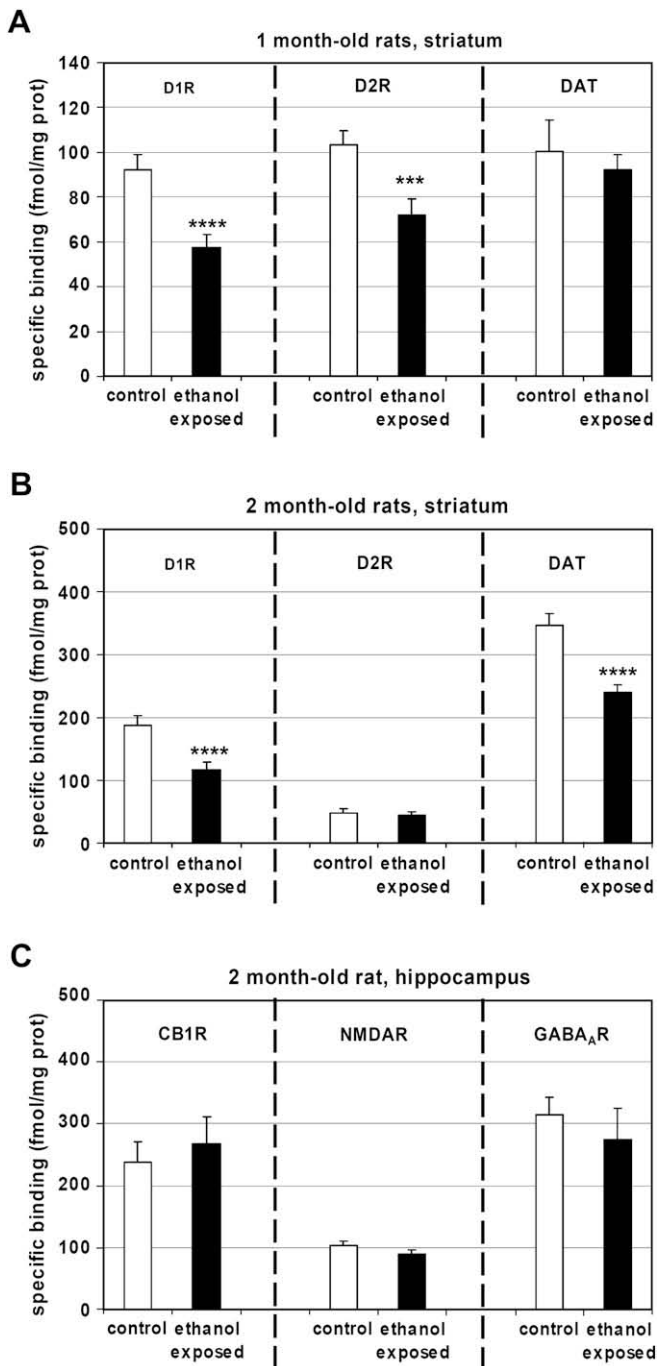
#### 4.2. Sensitivity to locomotor and hypothermic effects of drugs of abuse

There was no difference in the sensitivity to the locomotor effects of acute cocaine or amphetamine, as suggested previously (Blanchard et al., 1987; Randall and Hannigan, 1999). These data indicate that the altered sensitivity to the rewarding effects of cocaine is not associated with an alteration of the sensitivity to the acute locomotor effects of cocaine, that is consistent with data indicating that the drug's rewarding properties are not dependent on locomotor stimulation (Carr et al., 1988). Our results also demonstrate that the decreased striatal DAT binding (−34%) after early life ethanol exposure is not associated with an alteration of the locomotor stimulating and rewarding effects of cocaine. This is in accordance with previous data showing that genetically modified mouse strains with a reduction in DAT expression retain the stimulating and rewarding effects of cocaine (Tilley et al., 2007).

In contrast, control and ethanol-exposed rats did differ in the pattern of locomotor sensitization to psychostimulants. The sensitization to locomotor effect of amphetamine was increased in

ethanol-exposed animals compared to controls and cocaine induced significant sensitization at both low and high doses in early life ethanol-exposed animals while it induced sensitization only at the 10 mg/kg dose in control animals. These data suggest that early life ethanol exposure affects the mesolimbic DA system. In this regard, previous results demonstrated that prenatal ethanol exposure may alter adaptations in DAergic function after chronic psychostimulant treatment, thus altering the sensitization to subsequent stimulant exposure that may increase substance abuse risk (Shen et al., 1999).

Our findings indicate that among the acute responses to ethanol that were tested (hypothermia, locomotion, anxiety), only the sensitivity to the anxiolytic effects of ethanol was changed by early life ethanol exposure. In this regard, our results show only a slight modification of the locomotor response to ethanol after early life ethanol exposure and no modification of the sensitivity to the hypothermic effects of a high dose of ethanol. In ethanol-exposed offspring, the locomotor stimulating effect following a single challenge of low-dose of ethanol has been reported to be either augmented (Rockman et al., 1989) or blunted (Becker et al., 1993). In addition, previous studies have shown contradictory results on the sensitivity to ethanol-induced hypothermia that has been shown to be reduced (Abel et al., 1981) or increased (Taylor et al., 1981) in prenatally ethanol-exposed animals. Prenatal ethanol exposure has previously been shown to either increase (Hofmann et al., 2005; Dursun et al., 2006) or decrease (Carneiro et al., 2005) anxiety-like behavior. Our results do not demonstrate that the increased ethanol consumption is associated with a significant increase in anxiety-like behavior. The present binding study also indicate that neither the hippocampal [ $^3$ H]muscimol nor the [ $^3$ H]flunitrazepam (data not shown) binding sites are altered by early life ethanol exposure. Anxiety has been implicated in alcohol consumption and addiction. According to the 'tension reduction' hypothesis (Cappell and Herman, 1972), elevated levels of anxiety may lead to an increased predisposition to alcohol addiction. Ethanol-exposed animals were more sensitive to the anxiolytic effects of 1.5 g/kg ethanol. Since the anxiolytic effects of ethanol were increased, we also checked the sensitivity to the anxiolytic effects of diazepam and the results showed that ethanol-exposed animals were specifically more sensitive to ethanol. Interestingly, recent work using knockin mice



**Fig. 8.** Dopamine D1 ( $[^3\text{H}]\text{SCH23390}$ ), D2 ( $[^3\text{H}]\text{raclopride}$ ) and transporter (DAT) ( $[^3\text{H}]\text{mazindol}$ ) measurements in the striatum from 1-month-old (A) and 2-month-old (B) rats. (C) Endocannabinoids CB1 receptor (CB1R) ( $[^3\text{H}]\text{CP55,940}$ ), NMDA receptor (NMDAR) ( $[^3\text{H}]\text{MK-801}$ ) and GABA<sub>A</sub> receptor (GABA<sub>A</sub>R) ( $[^3\text{H}]\text{muscimol}$ ) measurements in the hippocampus from 2-month-old rats. \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$  compared to respective control group (Student's  $t$  test).  $n = 10$  per group.

with point mutations in the GABA<sub>A</sub> receptor  $\alpha 1$  subunit has shown that these mice displayed an increased sensitivity to the anxiolytic effects of ethanol and no difference in the sensitivity to the anxiolytic effects of diazepam (Weiner et al., 2008). These results suggest that early life ethanol exposure might induce changes in GABA<sub>A</sub> receptor subunit expression potentially associated with altered GABA<sub>A</sub> receptor function and pharmacological sensitivity. These data also suggest that the increased response to the anxiolytic effects of ethanol in early life ethanol-exposed animals may drive higher ethanol consumption. Further studies are needed to

investigate if the binding sites affinity and other GABA<sub>A</sub> receptor subunits are altered by ethanol exposure.

#### 4.3. Alteration of brain gene expression

We found decreased CB1 receptor and NMDAR1 subunit mRNA levels and increased BDNF and D2R mRNA levels in brain of young adult rats. Since infusion of BDNF into the VTA facilitates the development of sensitization to repeated cocaine exposure, and genetic deletion of the BDNF gene in mice results in delayed locomotor sensitization to cocaine (Horger et al., 1999) it is possible that the increased level of BDNF expression observed in the present study could explain, at least in part, the increased expression of psychostimulant sensitization in early life ethanol-exposed animals. Other studies have shown contradictory results on BDNF mRNA and protein levels, measured in cortex, hippocampus, striatum and cerebellum during the first three weeks of life, after prenatal ethanol exposure (Heaton et al., 2000; Feng et al., 2005). In the various brain structures, our results on BDNF show that mRNA levels are decreased or not changed at postnatal day 14 and then increased at postnatal day 30 or 60 and returned to control levels at postnatal day 90. Our results showing increased expression of BDNF are consistent with an earlier study in which protein levels of nerve growth factor and BDNF were transiently enhanced in cortical/striatal regions after pre- and postnatal ethanol exposure (Heaton et al., 2000). It has been hypothesized that this increase in neurotrophins, may play a protective role against ethanol neurotoxicity or conversely may have adverse effects by disturbing the balance of normal expression patterns of these substances (Heaton et al., 2000).

In the present study, few modifications of GABA<sub>A</sub> $\gamma 2$  subunit, DA D1 and D2 mRNA were observed irrespective of the age studied. Alterations induced by early life ethanol exposure are observed during the first postnatal weeks or in mature brain. In this regard, D1 receptor mRNA levels were increased in adolescent cortex and hippocampus while D2 receptor mRNA levels were enhanced in mature striatum and thalamus/hypothalamus. For the other targets, from a developmental point of view, alterations are generally observed starting on postnatal day 30 and changes remained on postnatal 60 and 90.

Our data indicate that early life ethanol exposure decreased NMDAR1 subunit mRNA expression in all tested brain regions, starting on postnatal day 14 in cortex and on postnatal day 60 in other structures. The decreased expression of NMDAR1 subunit is in agreement with earlier studies in which the density of low affinity  $[^3\text{H}]\text{MK801}$  binding sites were assessed in hippocampus. Contrarily to our present results and our previous study (Naassila and Daoust, 2002) in which we measured high affinity binding sites, other studies have consistently found reductions in  $[^3\text{H}]\text{MK-801}$  binding in the hippocampus of the offspring of ethanol-exposed dams (e.g. Diaz-Granados et al., 1997). It should be noted that alterations in the levels of NMDAR1 subunits are difficult to interpret since a large intracellular pool of unassembled NMDAR1 subunits has been demonstrated (Huh and Wenthold, 1999) and thus the mRNA levels may not be correlated to the protein levels. Altogether, these results suggest that this altered expression could be involved in the neurodevelopmental disorders associated with fetal ethanol exposure.

Earlier studies have also suggested that both CB1 and DA D2 receptors levels and BDNF are associated with ethanol consumption (Naassila et al., 2004; McGough et al., 2004; Thanos et al., 2004). We showed in this study that in two-month-old ethanol-exposed rats, the D2R expression is increased in striatum and thalamus/hypothalamus, 2-fold and 5-fold, respectively, suggesting a profound alteration of the DAergic system. Together, these findings suggest that BDNF and the endocannabinoid, glutamatergic

and DAergic systems may have key role in drugs of abuse vulnerability after early life ethanol exposure.

#### 4.4. Neurochemical alterations

Our binding experiments demonstrated that early life ethanol exposure significantly decreased striatal DAergic D1 and D2 receptors in one-month old rats and decreased DAergic D1R and transporter in two-month old rats. Thus, indicating that behavioral alterations are also associated with neurochemical/functional modifications.

Our results show that striatal D1, D2 DAergic and transporter binding is decreased in ethanol-exposed animals. These results agree with others (Carneiro et al., 2005; Druse et al., 1990). Decreased striatal D2R binding to DA synthesis ratio has also been found in Rhesus monkeys after moderate and continuous ethanol exposure during gestation (Schneider et al., 2005). Our data show that the decreased striatal DAergic receptors binding in adolescent brain (one-month-old) is also observed in young adult brain (2-month-old) with a decrease in DAergic D1R and DA transporter binding.

These DAergic alterations may have important repercussions on normal functioning. Disrupted DAergic transmission causes addiction (Berke and Hyman, 2000) and Volkow et al. (2004) suggested that decreased striatal D2R availability might reduce sensitivity to nondrug-related stimuli, such as hedonic positive stimuli, which would impair inhibitory control and increase the compulsive drive to take drugs.

In conclusion, our study shows that early life ethanol exposure alters the expression of a well-defined set of genes in association with permanent changes in behavioral responses to drugs of abuse. The aberrant DA function observed in the present study may have important implications in the later behavioral responses to drugs and propensity for drug abuse. Importantly, the present data indicate for the first time that early life exposure to ethanol increases the propensity for later drugs self-administration and behavioral responses to drugs of abuse. These findings may have important implications for understanding the development of vulnerability to addiction to drugs of abuse after early life ethanol exposure and for its clinical management.

#### Acknowledgments

This work is supported by INSERM/Mildt and Conseil Régional de Picardie fundings. EB is supported by a doctoral Fellowship from the French Ministry of National Education and Research/Technology (MENRT). We thank M. Ludovic Didier for taking care of the animals and for technical support and Dr. Catherine Vilpoux for helpful discussions and critical reading of the manuscript. Part of the results of the present study has been presented at the 2006 ISBRA and 2007 ESBRA meetings.

#### Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.neuropharm.2008.07.030.

#### References

Abel, E.L., Bush, R., Dintcheff, B.A., 1981. Exposure of rats to alcohol in utero alters drug sensitivity in adulthood. *Science* 212, 1531–1533.  
 Alati, R., Al Mamun, A., Williams, G.M., O'Callaghan, M., Najman, J.M., Bor, W., 2006. In utero alcohol exposure and prediction of alcohol disorders in early adulthood: a birth cohort study. *Arch. Gen. Psychiatry* 63, 1009–1016.  
 Baer, J.S., Sampson, P.D., Barr, H.M., Connor, P.D., Streissguth, A.P., 2003. A 21-year longitudinal analysis of the effects of prenatal alcohol exposure on young adult drinking. *Arch. Gen. Psychiatry* 60, 377–385.

Baer, J.S., Barr, H.M., Bookstein, F.L., Sampson, P.D., Streissguth, A.P., 1998. Prenatal alcohol exposure and family history of alcoholism in the etiology of adolescent alcohol problems. *J. Stud. Alcohol* 59, 533–543.  
 Becker, H.C., Hale, R.L., Boggan, W.O., Randall, C.L., 1993. Effects of prenatal ethanol exposure on later sensitivity to the low-dose stimulant actions of ethanol in mouse offspring: possible role of catecholamines. *Alcohol. Clin. Exp. Res.* 17, 1325–1336.  
 Berke, J.D., Hyman, S.E., 2000. Addiction, dopamine, and the molecular mechanisms of memory. *Neuron* 25, 515–532.  
 Blanchard, B.A., Hannigan, J.H., Riley, E.P., 1987. Amphetamine-induced activity after fetal alcohol exposure and undernutrition in rats. *Neurotoxicol. Teratol.* 9, 113–119.  
 Blednov, Y.A., Walker, D., Martinez, M., Harris, R.A., 2006. Reduced alcohol consumption in mice lacking preprodynorphin. *Alcohol* 40, 73–86.  
 Blednov, Y.A., Cravatt, B.F., Boehm 2nd, S.L., Walker, D., Harris, R.A., 2007. Role of endocannabinoids in alcohol consumption and intoxication: studies of mice lacking fatty acid amide hydrolase. *Neuropsychopharmacology* 32, 1570–1582.  
 Bond, N.W., DiGiusto, E.L., 1976. Effects of prenatal alcohol consumption on open-field behaviour and alcohol preference in rats. *Psychopharmacology* 46, 163–165.  
 Buck, K.J., Hood, H.M., 1998. Genetic association of a GABAA receptor 2 subunit variant with severity of acute physiological dependence on alcohol. *Mamm. Genome* 9, 975–978.  
 Cappell, H., Herman, C.P., 1972. Alcohol and tension reduction. A review. *Q. J. Stud. Alcohol* 33, 33–64.  
 Carneiro, L.M., Diógenes, J.P., Vasconcelos, S.M., Aragão, G.F., Noronha, E.C., Gomes, P.B., Viana, G.S., 2005. Behavioral and neurochemical effects on rat offspring after prenatal exposure to ethanol. *Neurotoxicol. Teratol.* 27, 585–592.  
 Carr, G.D., Phillips, A.G., Fibiger, H.C., 1988. Independence of amphetamine reward from locomotor stimulation demonstrated by conditioned place preference. *Psychopharmacology (Berl.)* 94, 221–226.  
 Carroll, M.E., Anderson, M.M., Morgan, A.D., 2007. Regulation of intravenous cocaine self-administration in rats selectively bred for high, (HiS) and low (LoS) saccharin intake. *Psychopharmacology (Berl.)* 190, 331–341.  
 Chester, J.A., Cunningham, C.L., 2002. GABA(A) receptor modulation of the rewarding and aversive effects of ethanol. *Alcohol* 26, 131–143.  
 Choong, K.C., Shen, R.Y., 2004. Methylphenidate restores ventral tegmental area dopamine neuronal activity in prenatal ethanol-exposed rats by augmenting dopamine neurotransmission. *J. Pharmacol. Exp. Ther.* 309, 444–451.  
 Chotro, M.G., Arias, C., 2003. Prenatal exposure to ethanol increases ethanol consumption: a conditioned response? *Alcohol* 30, 19–28.  
 Chotro, M.G., Arias, C., Laviola, G., 2007. Increased ethanol intake after prenatal ethanol exposure: studies with animals. *Neurosci. Biobehav. Rev.* 31, 181–191.  
 Costa, E.T., Savage, D.D., Valenzuela, C.F., 2000. A review of the effects of prenatal or early postnatal ethanol exposure on brain ligand-gated ion channels. *Alcohol. Clin. Exp. Res.* 24, 706–715.  
 Cunningham, C.L., Ferree, N.K., Howard, M.A., 2003. Apparatus bias and place conditioning with ethanol in mice. *Psychopharmacology* 170, 409–422.  
 Cunningham, C.L., Gremel, C.M., Groblewski, P.A., 2006. Drug-induced conditioned place preference and aversion in mice. *Nat. Protoc.* 1, 1662–1670.  
 Da Silva, G.E., Vendruscolo, L.F., Takahashi, R.N., 2005. Effects of ethanol on locomotor and anxiety-like behaviors and the acquisition of ethanol intake in Lewis and spontaneously hypertensive rats. *Life Sci.* 77, 693–706.  
 Deng, J., Elberger, A.J., 2003. Corpus callosum and visual cortex of mice with deletion of the NMDA-NR1 receptor. II. Attenuation of prenatal alcohol exposure effects. *Brain Res. Dev. Brain Res.* 144, 135–150.  
 DeSousa, N.J., Bush, D.E., Vaccarino, F.J., 2000. Self-administration of intravenous amphetamine is predicted by individual differences in sucrose feeding in rats. *Psychopharmacology (Berl.)* 148, 52–58.  
 Diaz-Granados, J.L., Spuhler-Phillips, K., Lilliquist, M.W., Amsel, A., Leslie, S.W., 1997. Effects of prenatal and early postnatal ethanol exposure on [<sup>3</sup>H]MK-801 binding in rat cortex and hippocampus. *Alcohol. Clin. Exp. Res.* 21, 874–881.  
 Druse, M.J., Tajuddin, N., Kuo, A., Connerty, M., 1990. Effects of in utero ethanol exposure on the developing dopaminergic system in rats. *J. Neurosci. Res.* 27, 233–240.  
 Dubois, C., Naassila, M., Daoust, M., Pierrefiche, O., 2006. Early chronic ethanol exposure in rats disturbs respiratory network activity and increases sensitivity to ethanol. *J. Physiol.* 576, 297–307.  
 Dubois, C., Houchi, H., Naassila, M., Daoust, M., Pierrefiche, O., 2008. Blunted response to low oxygen of rat respiratory network after perinatal ethanol exposure: involvement of inhibitory control. *J. Physiol.* 586, 1413–1427.  
 Dursun, I., Jakubowska-Dogru, E., Uzbay, T., 2006. Effects of prenatal exposure to alcohol on activity, anxiety, motor coordination, and memory in young adult Wistar rats. *Pharmacol. Biochem. Behav.* 85, 345–355.  
 Eckardt, M.J., File, S.E., Gessa, G.L., Grant, K.A., Guerri, C., Hoffman, P.L., Kalant, H., Koob, G.F., Li, T.K., Tabakoff, B., 1998. Effects of moderate alcohol consumption on the central nervous system. *Alcohol. Clin. Exp. Res.* 22, 998–1040.  
 Famy, C., Streissguth, A.P., Unis, A.S., 1998. Mental illness in adults with fetal alcohol syndrome or fetal alcohol effects. *Am. J. Psychiat.* 155, 552–554.  
 Feng, M.J., Yan, S.E., Yan, Q.S., 2005. Effects of prenatal alcohol exposure on brain-derived neurotrophic factor and its receptor tyrosine kinase B in offspring. *Brain Res.* 1042, 125–132.  
 Gessa, G.L., Serra, S., Vacca, G., Carai, M.A., Colombo, G., 2005. Suppressing effect of the cannabinoid CB1 receptor antagonist, SR147778, on alcohol intake and motivational properties of alcohol in alcohol-preferring sp rats. *Alcohol. Clin. Exp. Res.* 40, 46–53.  
 Green, A.S., Grahame, N.J., 2008. Ethanol drinking in rodents: is free-choice drinking related to the reinforcing effects of ethanol? *Alcohol* 42, 1–11.

- Guerra, C., 2002. Mechanisms involved in central nervous system dysfunctions induced by prenatal ethanol exposure. *Neurotox. Res.* 4, 327–335.
- Halmesmaki, E., 1988. Alcohol counselling of 85 pregnant problem drinkers: effect on drinking and fetal outcome. *Br. J. Obstet. Gynaecol.* 95, 243–247.
- Heaton, M.B., Mitchell, J.J., Paiva, M., Walker, D.W., 2000. Ethanol-induced alterations in the expression of neurotrophic factors in the developing rat central nervous system. *Dev. Brain Res.* 121, 97–107.
- Hill, K.G., Alva, H., Blednov, Y.A., Cunningham, C.L., 2003. Reduced ethanol-induced conditioned taste aversion and conditioned place preference in GIRK2 null mutant mice. *Psychopharmacology (Berl.)* 169, 108–114.
- Hofmann, C.E., Patyk, I.A., Weinberg, J., 2005. Prenatal ethanol exposure: sex differences in anxiety and anxiolytic response to a 5-HT<sub>1A</sub> agonist. *Pharmacol. Biochem. Behav.* 82, 549–558.
- Holloway, J.A., Tapp, W.N., 1978. Effects of prenatal and/or early postnatal exposure to ethanol on offspring of rats. *Alcohol Tech. Rep.* 7, 108–115.
- Horger, B.A., Iyasere, C.A., Berhow, M.T., Messer, C.J., Nestler, E.J., Taylor, J.R., 1999. Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. *J. Neurosci.* 19, 4110–4122.
- Houchi, H., Babovic, D., Pierrefiche, O., Ledent, C., Daoust, M., Naassila, M., 2005. CB1 receptor knockout mice display reduced ethanol-induced conditioned place preference and increased striatal dopamine D2 receptors. *Neuro-psychopharmacology* 30, 339–349.
- Houchi, H., Warnault, V., Barbier, E., Dubois, C., Pierrefiche, O., Ledent, C., Daoust, M., Naassila, M., 2008. Involvement of A2A receptors in anxiolytic, locomotor and motivational properties of ethanol in mice. *Genes Brain Behav.*, in press.
- Huh, K.H., Wenthold, R.J., 1999. Turnover analysis of glutamate receptors identifies a rapidly degraded pool of the N-methyl-D-aspartate receptor subunit, NR1, in cultured cerebellar granule cells. *J. Biol. Chem.* 274, 151–157.
- Ikonomidou, C., Bittigau, P., Ishimaru, M.J., Wozniak, D.F., Koch, C., Genz, K., Price, M.T., Stefovskova, V., Horster, F., Tenkova, T., Dikranian, K., Olney, J.W., 2000. Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. *Science* 287, 1056–1060.
- Jones, K.L., Smith, D.W., 1973. Recognition of the fetal alcohol syndrome in early infancy. *Lancet* 2, 999–1001.
- Kelley, B.M., Middaugh, L.D., 1996. Ethanol self-administration and motor deficits in adults C57BL/6J mice exposed prenatally to cocaine. *Pharmacol. Biochem. Behav.* 55, 575–584.
- Krystal, J.H., Staley, J., Mason, G., Petrakis, I.L., Kaufman, J., Harris, R.A., Gelernter, J., Lappalainen, J., 2006. Gamma-aminobutyric acid type A receptors and alcoholism: intoxication, dependence, vulnerability, and treatment. *Arch. Gen. Psychiatry* 63, 957–968.
- Lemoine, P., Harousseau, H., Borteyru, J.P., Menuet, J.C., 1968. Les enfants de parents alcooliques: Anomalies observées: à propos de 127 cas. *Quest Méd.* 6, 476–482.
- Lister, R.G., 1987. The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology (Berl.)* 92, 180–185.
- Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>(-Delta Delta C(T))</sup> Method. *Methods* 25, 402–408.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- Mattson, S.N., Schoenfeld, A.M., Riley, E.P., 2001. Teratogenic effects of alcohol on brain and behavior. *Alcohol Res. Health* 25, 185–191.
- McGough, N.N., He, D.Y., Logrip, M.L., Jeanblanc, J., Phamluong, K., Luong, K., Kharazia, V., Janak, P.H., Ron, D., 2004. RACK1 and brain-derived neurotrophic factor: a homeostatic pathway that regulates alcohol addiction. *J. Neurosci.* 24, 10542–10552.
- Molina, J.C., Hoffmann, H., Spear, L.P., Spear, N.E., 1987. Sensorimotor maturation and alcohol responsiveness in rats prenatally exposed to alcohol during gestational day 8. *Neurotoxicol. Teratol.* 9, 121–128.
- Naassila, M., Daoust, M., 2002. Effect of prenatal and postnatal ethanol exposure on the developmental profile of mRNAs encoding NMDA receptor subunits in rat hippocampus. *J. Neurochem.* 80, 850–860.
- Naassila, M., Ledent, C., Daoust, M., 2002. Low ethanol sensitivity and increased ethanol consumption in mice lacking adenosine A2A receptors. *J. Neurosci.* 22, 10487–10493.
- Naassila, M., Pierrefiche, O., Ledent, C., Daoust, M., 2004. Decreased alcohol self-administration and increased alcohol sensitivity and withdrawal in CB1 receptor knockout mice. *Neuropharmacology* 46, 243–253.
- Nelson, L.R., Lewis, J.W., Kokka, N., Branch, B.J., Taylor, A.N., 1986. Prenatal exposure to ethanol potentiates morphine-induced hypothermia in adult rats. *Neuro-behav. Toxicol. Teratol.* 8, 469–474.
- Nowak, P., Dabrowska, J., Bortel, A., Izabela, B., Kostrzewa, R.M., Brus, R., 2006. Prenatal cadmium and ethanol increase amphetamine-evoked dopamine release in rat striatum. *Neurotoxicol. Teratol.* 28, 563–572.
- Othman, T., Legare, D., Sadri, P., Lauth, W.W., Parkinson, F.E., 2002. A preliminary investigation of the effects of maternal ethanol intake during gestation and lactation on brain adenosine A(1) receptor expression in rat offspring. *Neurotoxicol. Teratol.* 24, 275–279.
- Phillips, D.S., Stainbrook, G.L., 1976. Effects of early alcohol exposure upon adult learning ability and taste preference. *Physiol. Psychol.* 4, 473–475.
- Popovic, M., Caballero-Bleda, M., Guerra, C., 2006. Adult rat's offspring of alcoholic mothers are impaired on spatial learning and object recognition in the Can test. *Behav. Brain Res.* 174, 101–111.
- Post, R.M., Weiss, S.R.B., 1988. Sensitization and kindling: implications for the evolution of psychiatric symptomatology. In: Kalivas, P.W., Barnes, C.D. (Eds.), *Sensitization in the Nervous System*. Telford Press, Caldwell, pp. 257–291.
- Randall, S., Hannigan, J.H., 1999. In utero alcohol and postnatal methylphenidate: locomotion and dopamine receptors. *Neurotoxicol. Teratol.* 21, 587–593.
- Randall, C.L., Hughes, S.S., Williams, C.K., Anton, R.F., 1983. Effect of prenatal alcohol exposure on consumption of alcohol and alcohol-induced sleep time in mice. *Pharmacol. Biochem. Behav.* 18, 325–329.
- Risinger, F.O., Freeman, P.A., Greengard, P., Fienberg, A.A., 2001. Motivational effects of ethanol in DARPP-32 knock-out mice. *J. Neurosci.* 21, 340–348.
- Rockman, G.E., Markert, L.E., Delrizzo, M., 1989. Effects of prenatal ethanol exposure on ethanol-induced locomotor activity in rats. *Alcohol* 6, 353–356.
- Rodgers, R.J., Johnson, N.J., 1995. Factor analysis of spatiotemporal and ethological measures in the urine elevated plus-maze test of anxiety. *Pharmacol. Biochem. Behav.* 52, 297–303.
- Sampson, P.D., Streissguth, A.P., Bookstein, F.L., Little, R.E., Clarren, S.K., Dehaene, P., Hanson, J.W., Graham Jr., J.M., 1997. Incidence of fetal alcohol syndrome and prevalence of alcohol-related neurodevelopmental disorder. *Teratology* 56, 317–326.
- Schneider, M.L., Moore, C.F., Barnhart, T.E., Larson, J.A., DeJesus, O.T., Mukherjee, J., Nickles, R.J., Converse, A.K., Roberts, A.D., Kraemer, G.W., 2005. Moderate-level prenatal alcohol exposure alters striatal dopamine system function in rhesus monkeys. *Alcohol. Clin. Exp. Res.* 29, 1685–1697.
- Servais, L., Houze, R., Bearzatto, B., Gall, D., Schiffmann, S.N., Cheron, G., 2007. Purkinje cell dysfunction and alteration of long-term synaptic plasticity in fetal alcohol syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 104, 9858–9863.
- Sheela Rani, C.S., Ticku, M.K., 2006. Comparison of chronic ethanol and chronic intermittent ethanol treatments on the expression of GABA<sub>A</sub> and NMDA receptor subunits. *Alcohol* 38, 89–97.
- Xu, C., Shen, R.Y., 2001. Amphetamine normalizes the electrical activity of dopamine neurons in the ventral tegmental area following prenatal ethanol exposure. *J. Pharmacol. Exp. Ther.* 297, 746–752.
- Shen, R.Y., Hannigan, J.H., Kapatos, G., 1999. Prenatal ethanol reduces the activity of adult midbrain dopamine neurons. *Alcohol. Clin. Exp. Res.* 23, 1801–1807.
- Sood, B., Delaney-Black, V., Covington, C., Nordstrom-Klee, B., Ager, J., Templin, T., Janisse, J., Martier, S., Sokol, R.J., 2001. Prenatal alcohol exposure and childhood behavior at age 6 to 7 years: I. dose-response effect. *Pediatrics* 108, E34.
- Spanagel, R., Montkowski, A., Allingham, K., Stöhr, T., Shoaib, M., Holsboer, F., Landgraf, R., 1995. Anxiety: a potential predictor of vulnerability to the initiation of ethanol self-administration in rats. *Psychopharmacology (Berl.)* 122, 369–373.
- Spear, N.E., Molina, J.C., 2005. Fetal or infantile exposure to ethanol promotes ethanol ingestion in adolescence and adulthood: a theoretical review. *Alcohol. Clin. Exp. Res.* 29, 909–929.
- Taylor, A.N., Branch, B.J., Liu, S.H., Wiechmann, A.F., Hill, M.A., Kokka, N., 1981. Fetal exposure to ethanol enhances pituitary-adrenal and temperature responses to ethanol in adult rats. *Alcohol. Clin. Exp. Res.* 5, 237–246.
- Thanos, P.K., Taintor, N.B., Rivera, S.N., Umegaki, H., Ikari, H., Roth, G., Ingram, D.K., Hitzemann, R., Fowler, J.S., Gately, S.J., Wang, G.J., Volkow, N.D., 2004. DRD2 gene transfer into the nucleus accumbens core of the alcohol preferring and nonpreferring rats attenuates alcohol drinking. *Alcohol. Clin. Exp. Res.* 28, 720–728.
- Tilley, M.R., Cagniard, B., Zhuang, X., Han, D.D., Tiao, N., Gu, H.H., 2007. Cocaine reward and locomotion stimulation in mice with reduced dopamine transporter expression. *BMC Neurosci.* 8, 1–7.
- Vallés, S., Felipe, V., Montoliu, C., Guerra, C., 1995. Alcohol exposure during brain development reduces 3H-MK-801 binding and enhances metabotropic-glutamate receptor-stimulated phosphoinositide hydrolysis in rat hippocampus. *Life Sci.* 56, 1373–1383.
- Vazquez, V., Giros, B., Daugé, V., 2006. Maternal deprivation specifically enhances vulnerability to opiate dependence. *Behav. Pharmacol.* 17, 715–724.
- Volkow, N.D., Fowler, J.S., Wang, G.J., Swanson, J.M., 2004. Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. *Mol. Psychiatry* 9, 557–569.
- Warnault, V., Houchi, H., Barbier, E., Pierrefiche, O., Vilpoux, C., Ledent, C., Daoust, M., Naassila, M., 2007. The lack of CB1 receptors prevents neuro-adaptations of both NMDA and GABA(A) receptors after chronic ethanol exposure. *J. Neurochem.* 102, 741–752.
- Weiner, J.L., Ariwodola, O.J., Jones, K.M., Chappell, A.M., Werner, D.F., Homanics, G.E., 2008. Paradoxical increases in behavioral and synaptic sensitivity to ethanol in genetically-engineered mice with ethanol-insensitive alpha1 GABA<sub>A</sub> receptors. *Alcohol* 42 (P93), 330.
- Yates, W.R., Cadoret, R.J., Troughton, E.P., Stewart, M., Giunta, T.S., 1998. Effect of fetal alcohol exposure on adult symptoms of nicotine, alcohol, and drug dependence. *Alcohol. Clin. Exp. Res.* 22, 914–920.
- Zahniser, N.R., Sorkin, A., 2004. Rapid regulation of the dopamine transporter: role in stimulant addiction. *Neuropharmacology* 47, 80–91.