Pharmacology, Biochemistry and Behavior 100 (2012) 630–638

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Early maternal separation affects ethanol-induced conditioning in a nor-BNI insensitive manner, but does not alter ethanol-induced locomotor activity

Ricardo Marcos Pautassi [a,c,*], Michael E. Nizhnikov [b], Ma. Carolina Fabio [a,c], Norman E. Spear [b]

[a] Instituto de Investigación Médica M. y M. Ferreyra (INIMEC–CONICET), Córdoba, C.P 5000, Argentina
[b] Center for Development and Behavioral Neuroscience, Binghamton University, Binghamton, NY 13902-6000, USA
[c] Facultad de Psicología, Universidad Nacional de Córdoba, Córdoba, C.P 5000, Argentina

ARTICLE INFO

Article history:
Received 10 August 2011
Received in revised form 29 October 2011
Accepted 7 November 2011
Available online 13 November 2011

Keywords:
Maternal separation
Ethanol
Second-order conditioning
Norbinaltorphimine

ABSTRACT

Early environmental stress significantly affects the development of offspring. This stress has been modeled in rats through the maternal separation (MS) paradigm, which alters the functioning of the HPA axis and can enhance ethanol intake at adulthood. Infant rats are sensitive to ethanol's reinforcing effects, which modulate ethanol seeking and intake. Little is known about the impact of MS on sensitivity to ethanol's appetitive and aversive effects during infancy. The present study assessed ethanol-induced conditioned place preference established through second-order conditioning (SOC), spontaneous or ethanol-induced locomotor activity and ethanol intake in preweanling rats that experienced normal animal facility rearing (AFR) or daily episodes of maternal separation (MS) during postnatal days 1–13 (PDs 1–13). Low-ethanol dose (0.5 g/kg) induced appetitive conditioned place preference (via SOC) in control rats given conventional rearing but not in rats given maternal separation in early infancy, whereas 2.0 g/kg ethanol induced aversive conditioned place preference in the former but not the latter. The administration of a kappa antagonist at PD 1 or immediately before testing did not alter ethanol-induced reinforcement. High (i.e., 2.5 and 2.0 g/kg) but not low (i.e., 0.5 g/kg) ethanol dose induced reliable motor stimulation, which was independent of early maternal separation. Ethanol intake and blood alcohol levels during conditioning were unaffected by rearing conditions. Pups given early maternal separation had lower body weights than controls and showed an altered pattern of exploration when placed in an open field. These results indicate that, when assessed in infant rats, earlier maternal separation alters the balance between the appetitive and aversive motivational effects of ethanol but has no effect on the motor activating effects of the drug.

1. Introduction

Early environmental stress (EES) significantly affects the development of the offspring. An alteration in the quality or quantity of maternal care is perhaps the most critical source of EES and can lead to enhanced basal and stress-related responsivity of the HPA axis (Michaels and Holtzman, 2008), fearfulness to novel environments (Huot et al., 2001) and alterations in the functioning of several transmitter systems, notably dopamine (Meaney et al., 2002), noradrenaline (Caldji et al., 2000) and the dynorphin/kappa opioid system (Vazquez et al., 2005). Most of these results have been gathered through the maternal separation (MS) paradigm (Plotsky and Meaney, 1993), in which animals experience daily episodes of maternal separation for varying periods of time (usually 180 or 360 min each day) during the first weeks of life.

Maternal separation stress resulted in higher vulnerability to initiate self-administration of ethanol (Huot et al., 2001; Cruz et al., 2008) and enhanced morphine-induced conditioned place preference (CPP) but reduced spiradoline-induced conditioned place aversion (Michaels and Holtzman, 2008). Maternal separation also facilitates the expression of ethanol-induced behavioral sensitization in female mice (Kawakami et al., 2007). These studies tested the effects of MS only at adulthood, as have most other studies that have tested the effects of early maternal deprivation. It is still unknown if MS affects the sensitivity of an organism to pharmacological unconditioned effects of ethanol at earlier ontogenetic stages, such as before weaning.

To our knowledge no study has analyzed ethanol-mediated appetitive or aversive learning after early maternal separation. These motivational effects of ethanol are dependent on the integrity of the opioid system (Nizhnikov et al., 2009) and significantly modulate ethanol seeking and intake (Pautassi et al., 2009). A growing body of literature also indicates that ethanol exposure early in development, which is surprisingly prevalent, enhances the risk for alcohol abuse and dependence later in life (Spear and Molina, 2005; Dewit et al., 2000). It is therefore important to assess if EES affects

* Corresponding author at: Instituto de Investigación Médica M. y M. Ferreyra (INIMEC–CONICET), Friuli 2434, Córdoba, C.P 5000, Argentina. Tel.: +54 351 4681465; fax: +54 351 4695163.
E-mail address: rpautassi@gmail.com (R.M. Pautassi).

0091-3057/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2011.11.005

responsiveness to the motivational effects of ethanol in infancy. Previous studies conducted in our lab assessed ethanol-mediated learning in infancy (Pautassi et al., 2009) and analyzed how alterations in maternal behavior affected responsiveness to ethanol's ataxic effects and ethanol intake during adolescence (Ponce et al., 2011). The present study assessed ethanol-induced reinforcement – in terms of second-order conditioning (SOC, Experiments 1 and 2) – in preweanling rats that experienced repeated episodes of maternal isolation during the first two weeks of life (postnatal days 1 to 13, PDs 1–13). Second-order conditioning is a variant of CPP in which animals are given pairings of ethanol and an intraoral conditional stimulus (CS_1), followed by pairings of CS_1 and a texture CS_2. In other words, during phase 1 (i.e., first-order conditioning) the unconditional stimulus (ethanol) is associated with intraoral water (CS_1) and in phase 2 (i.e., second-order conditioning) CS_1 is associated with CS_2 (sandpaper). Ethanol reinforcement is then reflected in response to CS_2. This procedure has proven useful in revealing ethanol-mediated learning in infant (Molina et al., 2006, 2007) and adolescent (Pautassi et al., 2011a) rats, when it was not evident in terms of first-order conditioning. The main hypothesis in the present study was that MS would alter ethanol-induced reinforcement. Specifically, the expectation was that the stress of maternal separation would potentiate alcohol's positive reinforcing capabilities.

The kappa opioid receptor (KOR) system is thought to mediate some of the aversive consequences of stress (Land et al., 2009). Therefore, an additional hypothesis was that KOR antagonism [by the administration of norbinaltorphimine (nor-BNI)], given before onset of the MS treatment (Experiment 1) would counteract the presumably stressful effects of repeated maternal isolation. Nor-BNI has been observed to inhibit stress-induced potentiation of ethanol-reinforcement in adults (Sperling et al., 2010) and to alter ethanol-induced taste conditioning in infant rats (Nizhnikov et al., in press). The acquisition of ethanol-induced SOC took place 24 h after the last episode of maternal separation on PD13. It was conceivable, therefore, that lingering effects of this stressful situation affected SOC. Also, training and testing involved substantial maternal deprivation of its own that perhaps could have interacted with the previous episodes of isolation and altered ethanol-mediated conditioning. These hypotheses imply acute stress effects likely sensitive to kappa antagonism. Experiment 2 tested the effects of proximal treatment with nor-BNI [24 h before conditioning] on ethanol-induced SOC.

Spontaneous and ethanol-induced open field exploration (Experiments 1 and 3) and ethanol intake (Experiment 1) were also measured. Ethanol-induced locomotor activity (LMA) has been proposed as an index, albeit indirect, of ethanol reinforcement (Acevedo et al., 2010) and a relationship between them is predicted by several theories (e.g., Robinson and Berridge, 1993). Based on previous studies (Meaney et al., 2002; Huot et al., 2001), we expected MS pups to display heightened ambulation during the onset of testing but reduced overall locomotion scores, as well as heightened ethanol intake. Blood alcohol levels (BALs) after normal or MS rearing were also assessed.

The rationale for choosing infant rats was the scarcity of information about the effects of maternal separation during the preweanling period and the possibility that the effects of MS might emerge only after further brain development. There are few studies that, instead of imposing a substantial delay until testing on adulthood, analyzed the effects of chronic maternal separation immediately after termination of this treatment during infancy (Arnold and Siviy, 2002) or adolescence (Spivey et al., 2008).

2. Materials and methods

2.1. Experimental designs

Experiment 1 assessed ethanol-mediated second order conditioning in subjects that, during the first two postnatal weeks, experienced normal animal facility rearing (AFR) or daily episodes of maternal separation (MS). Experiment 1 was defined by a 2 (sex: male or female) × 2 (early rearing conditions: AFR or MS) × 3 (nor-BNI treatment at PD1: 0, 10 or 30 mg/kg) × (ethanol treatment at second order conditioning: 0.0 [water vehicle], 0.5 or 2.0 g/kg) factorial. Each of the groups was composed of 4–6 animals. Second-order conditioning and testing occurred on PD 14 and 15, respectively. On PD16 the pups were assessed for spontaneous open field exploration and ethanol intake.

Experiment 2 assessed ethanol-induced second order conditioning in animals that experienced maternal isolation during the first two postnatal weeks and were given a KOR antagonist (or its vehicle) shortly before conditioning. A 2 (sex: male or female) × 2 (nor-BNI treatment 24 h before conditioning: 0 or 10 mg/kg) × 2 (ethanol treatment (0.0 [water vehicle] or 2.0 g/kg)) factorial was employed, with 5–6 animals in each group.

Experiments 3a and 3b analyzed ethanol-induced locomotor activity in AFR and MS animals that experienced short (Experiment 3a) or long (Experiment 3b) maternal deprivation immediately before testing. Experiment 3a analyzed ethanol-induced locomotor activity 90 min after removal of the home cage in MS and AFR animals. This temporal gap between home cage removal and testing is a standard procedure for assessing ethanol-induced LMA in preweanling rats (Arias et al., 2008). In Experiment 3b ethanol-induced locomotor activity was examined after 4 h of maternal deprivation. The rationale for using the longer deprivation period was that, perhaps, MS animals would be more sensitive to this stressful treatment. Both experiments employed identical designs: a 2 (sex: male or female) × 2 (early rearing conditions: AFR or MS) × 4 (ethanol treatment: 0.0 [water vehicle], 0.5, 2.0 or 2.5 g/kg) factorial. Groups were composed of 6–9 animals.

Across designs no more than one animal per litter was assigned to any group condition. This procedure allowed controlling for potential litter effects.

2.2. General procedures

2.2.1. Subjects

Four-hundred and sixty-nine Sprague–Dawley rat pups born and reared in an AAALAC-accredited facility (vivarium of the Center for Development and Behavioral Neuroscience, Binghamton University, Binghamton, NY, USA) were employed. Number of animals and litter representation in each experiment was as follows: Experiment 1, 170 animals (20 litters, 10 experienced conventional animal facility rearing, 10 experienced daily episodes of maternal separation); Experiment 2, 45 animals (6 litters); Experiment 3, 254 animals [33 litters, 17 AFR, 16 MS]. Parturition was checked daily and labeled as postnatal day 0 (PD 0). Unless specified, pups were housed with the dam in standard cages with ad-libitum access to water and lab chow. Lighting conditions (12 h/12 h light/dark cycle, with lights on at 8:00 AM) and room temperature (22–24 °C) in the vivarium were automatically controlled. The Binghamton University Institutional Review Committee for the use of Animal Subjects approved the experimental protocol and all procedures complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals (National Research Council, 1985).

2.2.2. Ethanol and nor-BNI preparation and administration procedures

The ethanol doses of 0.5, 2.0 and 2.5 g/kg were achieved by intragastrically administering 0.015 ml of an ethanol solution at 4.2, 16.8 and 21% v/v (190-proof Ethanol, Pharmaco, Brookfield; vehicle: distilled water) per gram of body weight, respectively. The nor-BNI doses of 10 and 30 mg/kg (Sigma-Aldrich, St. Louis, MO) were derived from a 1 mg/ml solution (vehicle, 0.9% saline) and administered via IP injection. Injection volume was 0.01 ml/g for the lowest dose, and 0.03 ml/g for the highest dose and for the animals treated with

vehicle. Intragastric intubations were performed as described in Pautassi et al. (2007). Intraperitoneal administrations were performed with 1 cm^3 tuberculin syringes mounted with a 27 G ½ disposable needle (Becton Dickinson & Co., Rutherford, N.J), which was replaced after each injection. Intraperitoneal administrations took less than 10 s and were performed in the right side of the pup's body, in a region situated between the diaphragm and the genitalia. After the administration, the needle was kept in-site one second to allow adequate diffusion of the drug into the peritoneum and a small drop of Vaseline was applied to the site of the injection to prevent potential leakage, which was however quite rare.

The ethanol doses were chosen on the basis of previous second-order conditioning studies of ethanol reinforcement with preweanling rats (Molina et al., 2006, 2007). Specifically, the rationale for choosing 0.5 g/kg ethanol as unconditional stimulus was that infant rats given pairings of CS_1 and the pharmacological effects induced by this ethanol dose exhibit reliable appetitive second-order conditioned place preference. The 2.0 g/kg dose also results in appetitive reinforcement, although weaker than that induced by the lower dose (Molina et al., 2007). There is a scarcity of information on the effects of systemically-administered nor-BNI in infant rats, yet a recent study conducted in our lab (Nizhnikov et al., in press) revealed that 2.5 mg/kg nor-BNI given 24 h before training altered taste conditioning by ethanol in thirteen-day old rats. This result suggests that infants are sensitive to nor-BNI induced blockade of KOR receptors. Our aim was to induce long-term blockade of those receptors throughout the first two postnatal weeks. Nizhnikov et al. (in press), on the other hand, aimed at inducing only short-term KOR blockade. We thus decided to use higher doses of norbinaltorphimine. These higher doses were selected on the basis of previous studies in which 10 and 30 mg/kg nor-BNI blocked stress-induced potentiation of CPP by ethanol (Sperling et al., 2010), inhibited shock-induced place aversion (Land et al., 2008) and decreased operant responding for cocaine (Wee et al., 2009).

2.2.3. Rearing conditions across PDs 1–13 (Exp. 1, 2 and 3) and kappa antagonism treatment at PD 1 (Exp. 1) or PD 13 (Exp. 2)

On PD 1, at about 8:30 AM, the litters were culled to 10 animals (five males and five females). In Experiment 1 pups were randomly assigned to one of three nor-BNI treatments (0.0, 10 or 30 mg/kg, ip). Injections with this kappa antagonist were conducted at 9:00 AM on PD 1, 3 h before the first episode of maternal isolation. The rationale was that nor-BNI exhibits a slow onset of action and during the first hours after its administration is not selective for kappa antagonism over mu opioid antagonism (Metcalf and Coop, 2005). On the other hand, nor-BNI exhibits a long duration of action, on the order of several weeks (Metcalf and Coop, 2005). For instance, Broadbear et al. (1994) found that nor-BNI inhibited kappa agonism for at least 4 weeks. This indicates that, in the present experiment, pups should have experienced significant kappa antagonism throughout the first two weeks of life, when maternal isolation occurred.

After nor-BNI treatment the litters were randomly assigned to be housed under normal animal facility conditions or to experience 180 min of daily maternal separation. The separations occurred once daily during PDs 1 to 13 and began at 9:00 AM, except for those in Experiment 1 that were conducted on PD 1 and began 3 h after nor-BNI injections, at 12:00 AM. Maternal separation was conducted as follows. The pups were separated from the dam and placed, as a litter, in a clean maternity cage lined with fresh wood shavings. These cages were changed every day and kept warm (35 °C) through a heating pad placed underneath. The daily episodes of maternal isolation always took place in the same room. Home cages and beddings were changed twice a week for all litters (i.e., AFR or MS conditions). This procedure, which involved brief handling, was conducted between 7:30 and 8:30 AM by professionally-trained animal care personnel.

In Experiment 2 all animals experienced daily maternal separation during PDs 1–13, as described. In this experiment Norbinaltorphimine (0.0 or 10 mg/kg) was given on PD 13, following termination of the last episode of maternal deprivation (i.e., 24 h before the first phase of the second-order conditioning).

2.2.4. Second-order conditioning procedures (Exp. 1 and 2)

We closely followed a three-step, second-order conditioning procedure (Fig. 1) originally employed in infant rats by Molina et al. (2006, 2007) and later used in adolescents by Pautassi et al. (2008, 2011a).

2.2.4.1. Phase 1 (first-order conditioning, PD 14). At 8:30 AM pups were removed from the maternal cage and placed in same-sex pairs in holding cages (30×20×20 cm) covered with pine shavings and maintained at 35 °C. Pups were then implanted in the cheek with a small section of polyethylene-10 tubing (Clay-Adams, Parsippany, NJ), as described in Abate et al. (2000). These devices served to deliver the conditional stimuli during conditioning. After cannulation, pups remained undisturbed for 120 min until commencement of the conditioning session. Pups were then individually placed into opaque, square-shaped Plexiglas chambers (10×10×12 cm) with cotton lining the floor. A 10-min habituation session was conducted to familiarize animals to the chambers. Pups were subsequently weighed to the nearest 0.01 g (Sartorius, Gottingen, Germany), intragastrically administered ethanol (0.0, 0.5 or 2.0 g/kg, Experiment 1; 0.0 or 2.0 g/kg, Experiment 2) and returned to their holding chambers. The rationale for employing pharmacological instead of unpaired controls was that previous studies – that incorporated unpaired controls and similar ethanol dosing and SOC procedures – in infant and adolescent rats indicated that ethanol-mediated SOC is unlikely to be explained by pseudoconditioning or non-specific changes caused by mere exposure to the US or the CS (Molina et al., 2006, 2007; Pautassi et al., 2008).

Conditioning began 5 min after ethanol intubation and took place in the square-shaped chambers. The infants received fifteen 5-s pulses (5 µl per pulse, interstimulus interval: 55 s) of distilled water (conditioned stimulus, CS_1). The conditioning trial took place during post-administration minutes 5–20 and CS_1 delivery was conducted by attaching the PE10 sized intraoral cannulae to a PE50 sized polyethylene tubing (Clay Adams, Parsippany, NJ). The latter was, in turn, connected to an infusion pump (KD Scientific, Holliston, Massachusetts). At termination of conditioning, the cannulae were removed and animals were placed into the warmed holding chambers. They were reunited with the dam 2 h after the administration of ethanol.

2.2.4.2. Phase 2 (second-order conditioning, PD 15). Animals were removed from the maternal cage, cannulated and placed with a same-sex littermate in a warmed holding chamber for 60 min. They were then individually placed into the square-shaped chambers used during phase 1. The floor of the chamber was now lined with a piece of sandpaper (50 grit, Gatorgrit, Fairborn, OH). The animals remained in contact with this surface, hereinafter referred to as CS_2, for 5 min and were stimulated with the CS_1. They were given pulsed distilled water every 55 s (volume: 5 µl, pulse duration: 5 s). Animals received a total of four water pulses while in contact with sandpaper. After these CS_1–CS_2 pairings animals were returned to the warmed holding chambers.

2.2.4.3. Phase 3 [test for conditioned place (defined by tactile stimulus) preference, PD 15]. Thirty minutes after termination of Phase 2, animals were tested in a two-way, five min tactile preference test. The test was conducted in a Plexiglas rectangular chamber (28×13×15.5 cm). Half of the floor of this chamber was lined with the sandpaper CS_2 (coarse: 50, Gatorgrit, USA) while the remaining

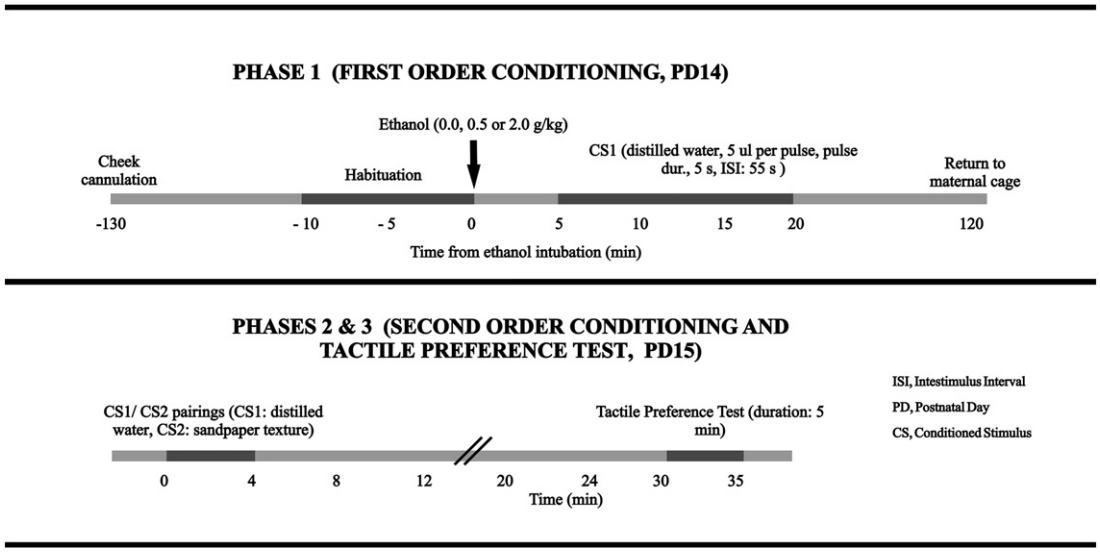

Fig. 1. Methods for analyzing the effects of early maternal separation on ethanol-mediated second-order conditioning. Phase 1, first-order conditioning, postnatal day (PD) 14: Pups were removed from the maternal cage, cannulated and then briefly (10 min) habituated to the experimental context. They were then given ethanol (0.0, 0.5 or 2.0 g/kg, Experiment 1; 0.0 or 2.0 g/kg, Experiment 2) and stimulated with a conditioned stimulus (CS_1) consisting of intraoral pulses of water. CS_1 delivery occurred 5–20 min after ethanol. Phase 2, second-order conditioning, postnatal day 15: animals were stimulated with water pulses while placed in a sandpaper-lined compartment (CS_2). Phase 3, tactile preference test, postnatal day 15: time spent on sandpaper was recorded during a 5 min preference test.
The figure and legend were adapted with permission from Pautassi et al. (2011a).

floor surface was lined with the smooth backside of a piece of sandpaper. Both textures were replaced in each new test, which was conducted under red light provided by an overhead 40-w bulb. Time spent over each section of the apparatus was recorded in a minute-by-minute basis by experimenters unaware of the treatment of each animal. The middle section (15% of the entire surface) was considered as a neutral area and not taken into account for data collection or analysis. Tactile preference scores were expressed as percent time (%) spent on sandpaper, which was calculated as follows: [(total time spent over sandpaper)/(total time spent over sandpaper + total time spent over smooth) × 100)].

2.2.5. Assessment of spontaneous motor activity and ethanol intake (Exp. 1)

On PD 15 (i.e., 24-h after the tactile preference test), at about 8:30 AM, the pups were removed from the maternal chamber and immediately assessed (test duration: 5 min) for spontaneous motor activity in a Plexiglas open field (42 × 42 × 30 cm; VersaMax Animal Activity Monitoring System, Accuscan Instruments, Columbus, OH, USA). The open field was dimly lighted inside a wooden, sound-attenuating chamber (53 × 58 × 43 cm) and surrounded by photocell beams. The VersaMax software registered distance traveled (cm) in a minute-by-minute basis.

The parameters for the ethanol intake test closely followed those described by Arias et al. (2010a). Briefly, the pups were implanted with PE10 cannulae immediately after the LMA test and placed in pairs in a warmed holding chamber. Three hours after cannulation, pup's bladders were voided by gentle stroking of the anogenital area and body weights were registered to the nearest 0.01 g. Animals were then placed into individual Plexiglas chambers (10 × 10 × 12 cm) and received intraoral ethanol (6% w/v, duration: 15 min), delivered at a constant rate through a syringe infusion pump (KD Scientific, Holliston, MA) connected to the oral cannula of each pup by a polyethylene catheter (Clay Adams, PE 50 Parsippany, NJ). Total administration volume was equivalent to 5.5% of the subject's pre-infusion body weight. After the infusion, subjects were disconnected from the pup and weighed to estimate percentage body weight gain (% BWG: Post-infusion weight − Pre-infusion weight/Pre-infusion weight × 100].

2.2.6. Assessment of ethanol-induced motor activity and blood ethanol levels (Exp.3)

On PD 14 the preweanlings were removed from their home cage and placed either in pairs or individually (Experiments 3a and 3b, respectively) in a warmed holding chamber (30 × 20 × 20 cm). Sixty or 240 min later (Experiments 3a and 3b, respectively) they received one of four ethanol doses (0.0, 0.5, 2.0 or 2.5 g/kg). The 0.5 and 2.0 doses were selected because they had been employed in the SOC procedure of Experiments 1 and 2. The 2.5 g/kg dose had not been evaluated in the second-order procedure because it was not expected to differ in reinforcing capability from 2.0 g/kg. It was, however, employed in this experiment to facilitate the comparison with previous studies. Specifically, 2.5 g/kg has been the standard dose for assessing motor activating effects of ethanol during the rising limb of the blood ethanol curve in preweanling (Arias et al., 2008, 2009, 2010a) and adolescent (Acevedo et al., 2010) rats. The temporal gap between removal of pups from the dam and testing was selected on the basis of previous studies (Pautassi et al., 2011b; Arias et al., 2010b).

Ethanol-induced motor activity (i.e., distance traveled in cm) was registered at ethanol post-administration time 5–20 min using a Plexiglas open field (42 × 42 × 30 cm; VersaMax Activity Monitoring System, Accuscan Instruments, Columbus, OH, USA).

Blood trunk samples were obtained from AFR and MS pups given 0.5 and 2.0 g/kg ethanol in Experiment 3a. The aim was to assess if maternal separation on PDs 1–13 altered metabolism and hence levels of blood ethanol, induced by the doses employed in Experiments 1 and 2. The samples were obtained immediately following the motor activity test, a time period that corresponds with the termination of phase 1 of the SOC procedure. The samples were analyzed with an AM5 Alcohol Analyzer (Analox Instruments, Lunenburg, MA) (see Nizhnikov et al., 2009). BELs were expressed as milligrams of ethanol per deciliter of body fluid (mg/dl = mg %).

2.3. Data analyses

Body weights registered on PD 14 (Exp. 1 and 2) were analyzed through a two or three-way Analysis of Variance (ANOVA; between factors: sex, nor-BNI and – for Experiment 1 – early rearing condition). In these analyses there were some instances in which MS or

AFR litters provided more than one male or female. These values were thus collapsed so as not to assign more than one male and one female per litter to any cell.

Percent (%) time in contact with the sandpaper CS (Experiments 1 and 2) during the 5-min tactile preference test was analyzed through a four or three-way ANOVA (between factors: early rearing condition, sex, nor-BNI treatment and ethanol treatment [Experiment 1]; sex, nor-BNI treatment and ethanol treatment [Experiment 2]).

Distance traveled on the open field (Experiment 1) was divided into five 1-min bins and was processed through a five-way mixed ANOVA (between factors: sex, early rearing condition, nor-BNI treatment and ethanol treatment; within factor: bin of evaluation). Ethanol intake (Experiment 1) was processed through a four-way ANOVA (between factors: sex, early rearing condition, nor-BNI treatment and ethanol treatment). We also analyzed the correlation (Pearson's r product-moment) between distance traveled in the open field and ethanol intake. The question was to what extent spontaneous LMA activity in the open field predicted ethanol intake scores. Separate correlations were made for the overall population of subjects as well as for the groups derived from the factorial design, after collapsing for sex to reduce the number of associations and therefore decrease the possibility of type I error.

In Experiments 3a and 3b, ethanol-induced locomotor activity (total distance traveled on the open field, [cm]) recorded during the 15 min assessment was divided into three 5 min bins and analyzed through a four-way mixed ANOVA. Ethanol dose (0.0, 0.5, 2.0 or 2.5 g/kg), sex and early rearing condition were the between-group factors, whereas bin of evaluation (bin 1, 2, or 3; i.e., post-administration intervals of 5–9 min, 10–14 min, and 15–19 min) served as the repeated measure factor. Blood ethanol levels (BELs, Experiment 4a) were analyzed using a two-way ANOVA (comparative factors between groups: early rearing condition and sex).

The loci of significant main effects or interactions were further examined through follow-up ANOVAS or post-hoc comparisons (Fisher's Least Mean Significant tests, alpha level set at 0.05).

3. Results

3.1. Experiment 1

3.1.1. Body weights

Pups that experienced repeated maternal deprivation exhibited a slight, yet significant, reduction in body weight at PD 14 (significant main effect of early rearing condition: $F_{1,102}=9.00$, $p<0.05$). Mean and SEM (g) in control rats given conventional rearing and in MS pups was 35.84 +/−.34 and 34.79 +/−.55, respectively.

3.1.2. Second-order conditioning

The ANOVA for percent preference for sandpaper revealed a significant main effect of ethanol treatment and a significant interaction between ethanol treatment and early rearing condition ($F_{2,134}=4.87$, $p<0.01$ and $F_{2,134}=4.36$, $p<0.05$, respectively). There was no significant main effect of nor-BNI treatment or sex, and no significant interaction involving these factors was found. For animals given vehicle at conditioning, a planned comparison ($p<0.05$) revealed that level of sandpaper preference did not depend on early rearing condition (AFR or MS). In other words, baseline level of sandpaper preference was not altered by maternal separation. Another planned comparison, however, revealed a significant difference between AFR and MS animals given 2 g/kg ethanol dose ($p<0.05$), indicating emergence of an effect of early maternal separation when ethanol was introduced.

To better understand the significant ethanol treatment and early rearing interaction, follow-up one-way ANOVAs (comparative factor between groups: ethanol treatment at SOC) were performed for each early rearing condition. In the case of animals given normal rearing (AFR group) the ANOVA revealed a significant main effect of ethanol treatment, $F_{2,84}=4.18$, $p<0.05$. The post-hoc indicated that pups given 0.5 g/kg ethanol spent significantly more time on the sandpaper CS_2 than vehicle-treated counterparts, thus exhibiting appetitive, ethanol-mediated second-order conditioning. There was a trend for appetitive conditioning in pups given 2.0 g/kg ethanol, yet the post-hoc comparison between this group and the vehicle-treated control failed to achieve significance ($p=0.06$). These results can be observed in Fig. 2, top panel.

When considering pups that experienced repeated, daily episodes of maternal deprivation during PDs 1–13 the ANOVA indicated a significant effect of ethanol treatment: $F_{2,80}=6.48$, $p<0.005$. The post-hocs revealed that animals given the highest ethanol dose spent significantly less time on the ethanol-paired sandpaper CS than control animals ($p<0.01$), thus exhibiting conditioned tactile avoidance.

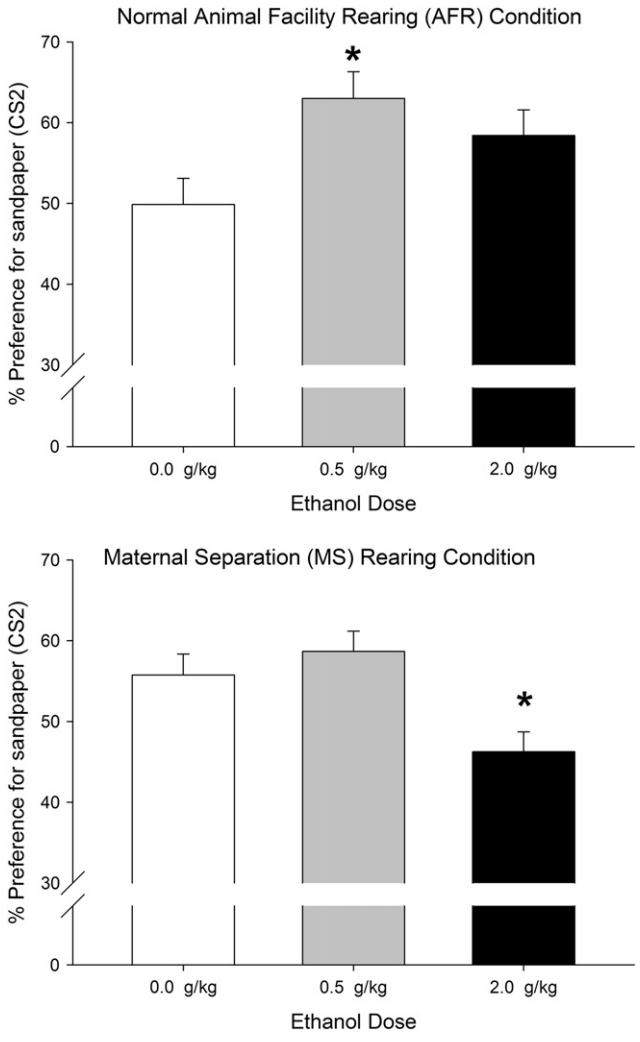

Fig. 2. Ethanol-induced second-order conditioning in infant rats that experienced either normal animal facility rearing (AFR, upper panel) or daily episodes of maternal separation (MS, lower panel) during postnatal days (PD) 1 to 13, with percent time spent in the rough CS_2 texture (sandpaper) during the test as a function of ethanol treatment. On PD 1 animals were administered nor-BNI (0.0, 10 or 30 mg/kg, ip). During conditioning on PD 14 animals were given ethanol administration (0.5, 2.0 or 0.0 g/kg, i.g.), and were then stimulated with intraoral pulses of water (CS_1). The following day, animals experienced CS_1–CS_2 pairings and were then tested for CS_2 preference. To facilitate data visualization, data have been collapsed nor-BNI treatment. The latter factor did not affect tactile preference scores nor significantly interacted with the remaining factors. Asterisks (*) indicate significant differences between an ethanol-treated group and its corresponding vehicle-treated control ($p<0.05$).Vertical bars indicate the SEM.

Pups given 0.5 g/kg ethanol did not differ from controls. These results have been depicted in Fig. 2, lower panel.

3.1.3. Spontaneous LMA and ethanol intake scores

Spontaneous motor activity in the open field was greater in females than in males and locomotion gradually decreased across testing (significant main effects of sex and bin of evaluation, $F_{1,134}=11.22$ and $F_{4,536}=8.06$, respectively; both p's<0.0001). Mean and SEM (cm) for males and females were 287.94 +/− 30.55 and 456.98 +/− 43.89, respectively. There was no significant main effect of nor-BNI dosing at PD 1 or ethanol treatment at PD 14. None of the interactions between these factors achieved significance. On the other hand, the interaction between early rearing condition and bin of evaluation achieved significance, $F_{4,536}=3.15$, $p<0.05$. Post-hoc tests indicated that initial (i.e., bin 1) spontaneous locomotion was significantly lower in MS pups than in control rats given conventional rearing. This difference was no longer observed in the subsequent bins. Mean and SEM (cm) for AFR and MS subjects were as follows: bin 1, 111.55 +/− 9.14 and 79.95 +/− 7.33; bin 2, 83.89 +/− 10.22 and 75.34 +/− 11.21; bin 3, 70.32 +/− 11.48 and 81.05 +/− 13.24; bin 4, 57.90 +/− 9.54 and 65.28 +/− 9.99; bin 5, 65.15 +/− 9.91 and 53.71 +/− 9.50; respectively.

Ethanol intake was unaffected by the factors under analysis. The ANOVA revealed the lack of significant main effects or significant interactions. Mean and SEM for AFR and MS pups as a function of ethanol dose at SOC were as follows: 0.0 g/kg, 2.28 +/− 0.21 and 1.18 +/− 0.15; 0.5 g/kg, 1.89 +/− 0.17 and 1.82 +/− 0.20; 2.0 g/kg, 1.82 +/− 0.15 and 1.80 +/− 0.14; respectively.

The correlation between spontaneous LMA scores and ethanol intake yielded a positive, significant association in animals reared under normal conditions and later treated with vehicle on both PD 1 and PD 13. In this basic control condition greater motor activity in the open field predicted greater avidity for ethanol ($r=.86$; $p<.05$). None of the other correlations achieved significance.

3.2. Experiment 2

3.2.1. Body weights

Body weights were not affected by sex or nor-BNI treatment. Mean and SEM (g) in pups given nor-BNI or its vehicle were 36.03 +/− .70 and 37.17 +/− .65, respectively.

3.2.2. Second-order conditioning

Consistent with the results found in Experiment 1, a relatively high ethanol dose (2.0 g/kg) readily induced aversive second-order conditioning in preweanlings that had experienced maternal deprivation. Pretreatment with nor-BNI – 10 mg/kg given 24 h before conditioning – did not alter the expression of this conditioned response. These impressions were confirmed by the ANOVAs, which revealed an independent significant main effect of ethanol treatment ($F_{1,37}=8.13$, $p<0.01$), that did not interact with sex or nor-BNI treatment. These results have been depicted in Fig. 3.

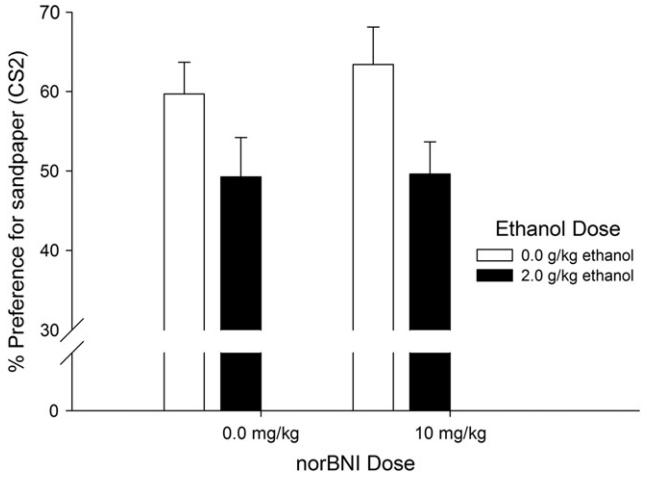

Fig. 3. Ethanol-induced second-order conditioning in infant rats that experienced daily episodes of maternal separation during postnatal days (PD) 1 to 13, with percent time spent in the rough CS_2 texture (sandpaper) during the test as a function of nor-BNI treatment (0.0 or 10 mg/kg, ip) and ethanol treatment (0.0 or 2.0 g/kg) On PD 13, after termination of the last episode of maternal deprivation, animals were administered with nor-BNI. During conditioning on PD 14 animals were given ethanol administration and were then stimulated with intraoral pulses of water (CS_1). The following day animals experienced CS_1–CS_2 pairings and were then tested for CS_2 preference. Ethanol (2.0 g/kg) readily induced aversive second-order conditioning and nor-BNI treatment did not alter this result. The ANOVA indicated a main effect of ethanol treatment ($F_{1,37}=8.13$, $p<0.01$), that did not interact with sex or nor-BNI treatment. Vertical bars indicate the SEM.

3.3. Experiment 3

3.3.1. Ethanol-induced motor activity in AFR or MS pups

In Experiment 3a pups were removed from the dam and placed in couples in a warmed holding chamber. Sixty-minutes later they were assessed for ethanol-induced motor activity. This temporal gap between home cage removal and testing is a standard procedure for assessing ethanol-induced LMA in preweanling rats (see Arias et al., 2008; Pautassi et al., 2011b). The ANOVA for distance traveled revealed independent significant main effects of early rearing conditions and bin of evaluation as well as a significant interaction between bin of evaluation and ethanol treatment, $F_{1,119}=3.99$, $p<0.05$; $F_{2,238}=76.23$, $p<0.001$ and $F_{6,238}=13.14$, $p<0.001$, respectively. As depicted in Fig. 4, overall locomotion scores across groups were highest on the first evaluation bin (postadministration time 5–9) and then significantly decreased during bins 2 and 3 (postadministration time 10–14 and 15–19 min, respectively). Perhaps more important, 2.0 and 2.5 – but not 0.5-g/kg ethanol induced clear behavioral activation during the first evaluation bin. This drug-induced activation subsided during the second and third bin. Pups that experienced repeated episodes of maternal deprivation exhibited slight, yet significant increased overall levels of motor activity, an effect that was statistically similar across ethanol- and vehicle-treated animals. Visual inspection of Fig. 4 may suggest that dosing with 2.0 and 2.5 g/kg ethanol induced higher motor scores in MS than AFR pups. This impression, however, was not supported by the ANOVA, which revealed the lack of significant two- or three-way interactions.

Fig. 5 depicts motor activity levels in the open field after varying doses of ethanol or vehicle in AFR or MS pups that experienced 4 h of maternal deprivation immediately before testing (Experiment 3b). The highest ethanol doses (2.0 and 2.5 g/kg) induced motor activation during the first evaluation bin. During postadministration time 15–19 min (third bin) pups given 2.5 g/kg ethanol exhibited significantly less distance traveled than any other group. These activating and depressing effects of ethanol were similar across control rats given conventional rearing or MS pups. The ANOVA indicated a significant main effect of bin testing and a significant ethanol × bin interaction, $F_{2,206}=14.09$; $F_{6,206}=6.55$, respectively; both p's<0.0001.

3.3.2. Blood ethanol levels in AFR or MS pups (Experiment 3a)

Blood samples were obtained from AFR and MS animals, 20 min after intubation with 0.5 or 2.0 g/kg ethanol. This time point coincides with the end of conditioning phase 1 in Experiments 1 and 2. The ANOVA indicated that early maternal isolation did not affect BELs. As expected, pups given 2.0 g/kg had higher BELs than pups given 0.5 g/kg (significant main effect of ethanol treatment: $F_{1,54}=1623.0$, $p<0.0001$). Mean and SEM (mg/dl) in pups given 0.5 or 2.0 g/kg ethanol were 41.33 +/− 3.57 and 176.80 +/− 3.36 (AFR pups), and 42.97 +/− 3.46 and 181.34 +/− 3.70 (MS pups); respectively.

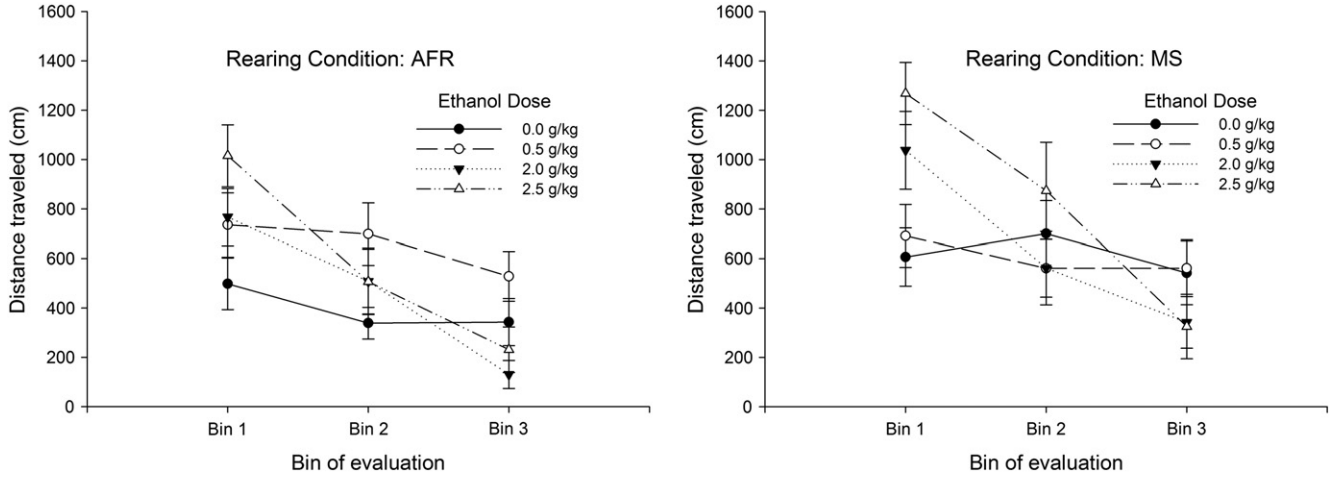

Fig. 4. Ethanol-induced locomotor activity (distance traveled, cm) 5–9, 10–14, and 15–19 min after ethanol administration (bins 1, 2, and 3, respectively) in infant rats that experienced either normal animal facility rearing (AFR, left panel) or daily episodes of maternal separation (MS, right panel) during postnatal days (PD) 1 to 13. Animals experienced 60 min of maternal deprivation before the onset of testing, which was conducted at PD 14. Vertical bars indicate the SEM.

4. Discussion

The most striking new finding of the present study was that repeated maternal separation during the preweanling period had a powerful and reliable main effect on the reinforcing effect of ethanol, as measured through second-order conditioning. Pups that experienced normal rearing conditions exhibited conditioned preference for a stimulus that had been associated with 0.5 g/kg ethanol and a non-significant trend towards conditioned preference when given the highest ethanol dose. In sharp contrast, MS infants failed to express any SOC when given 0.5 g/kg ethanol and spent less time than controls in the CS associated with 2.0 g/kg ethanol. That is, MS pups showed ethanol-induced conditioned aversion to the higher ethanol dose.

These results indicate that MS can alter ethanol-induced reinforcement and suggest that the net effect of ethanol was appetitive for control pups but aversive for MS pups. Although the directional hypothesis was that MS pups would find ethanol more appetitive, we could not discard the possibility that maternal separation might make pups more sensitive to ethanol-induced stress or aversion. As many other psychoactive drugs (including anxiolytics such as buspirone, Neisewander et al., 1990), ethanol induces both appetitive and aversive effects, and it is possible that the MS treatment employed in the present study selectively enhanced the aversive effects of the drug. To our knowledge, there is only one study that assessed sensitivity to the aversive properties of psychoactive drugs after MS. Roma et al. (2008) found reliable but similar amphetamine-induced conditioned taste aversion (CTA) in maternally-isolated and control animals. Taste aversion memories, however, are known for their potency and biological preparedness (Dellarosa Cummins and Cummins, 1999). Therefore, they are perhaps less likely to be affected by early maternal isolation than conditioned place preference, perhaps especially when induced by a higher-order conditioning preparation such as SOC.

The present result, however, fits well with prior evidence indicating that adolescent rats undergoing MS during infancy decreased subsequent ethanol intake during puberty (Daoura et al., 2011). Studies conducted with adult rats or mice, however, revealed heightened ethanol intake after early maternal deprivation (Huot et al., 2001; Cruz et al., 2008) and previous work (Michaels and Holtzman, 2008; Song et al., 2007) indicated that maternal separation or chronic stress exposure facilitated, rather than inhibited, drug-induced conditioned place preference at adolescence and adulthood. One possible explanation for this apparent discrepancy is that the initial effects of maternal separation can interact with the subsequent social environment to produce the behavioral phenotypes found at adulthood. Indeed, it has been observed that environmental factors can reverse the effects of early maternal separation (Francis et al., 2002).

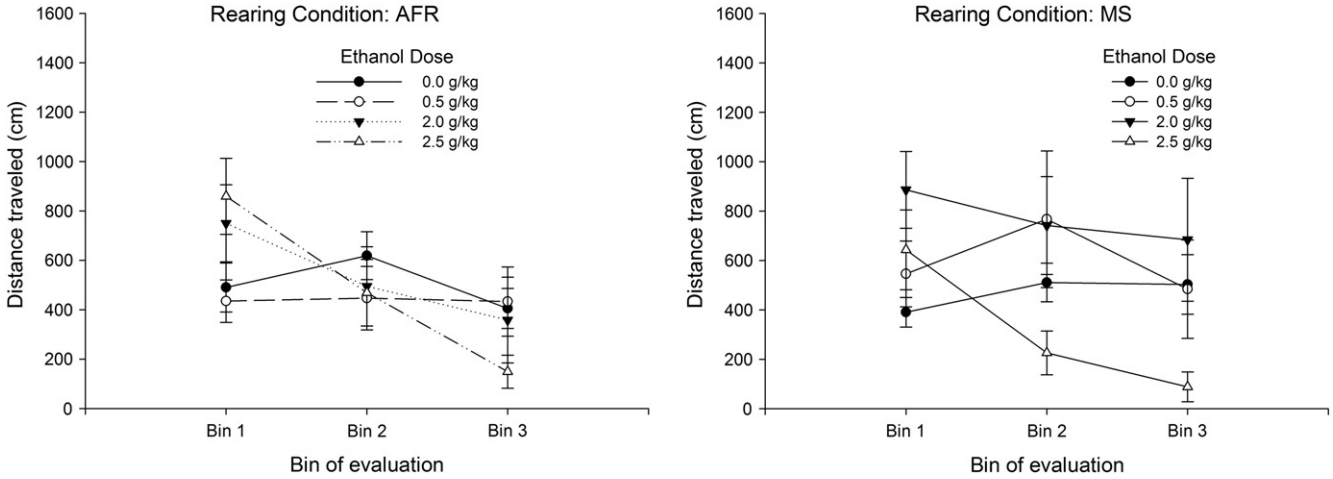

Fig. 5. Ethanol-induced locomotor activity (distance traveled, cm) 5–9, 10–14, and 15–19 min after ethanol administration (bins 1, 2, and 3, respectively) in infant rats that experienced either normal animal facility rearing (AFR, left panel) or daily episodes of maternal separation (MS, right panel) during postnatal days (PD) 1 to 13. Animals experienced 240 min of maternal deprivation before the onset of testing, which was conducted PD 14. Vertical bars indicate the SEM.

Differences in spontaneous exploration of a novel environment are thought to be the most reliable index of behavioral changes resulting from early stress (Meaney et al., 2002). Consistent with this claim, in the present study early maternal separation was associated with a transient decrease in spontaneous exploration of the open field (Experiment 1, test duration: 5 min) and overall higher motor activity across the 15-min testing trial of Experiment 3a. These results confirm previous literature on the effects of maternal separation. As reviewed by Meaney et al. (2002), during the first minutes of an open field test control animals explore the arena whereas MS animals usually freeze, a behavior likely reflecting anxiety enhancement by the MS experience. After about 5 min of testing, however, MS animals become significantly more active than control counterparts. Our findings of overall hyperactivity and a slight, yet significant, reduction in body weight in MS than in control animals are also consistent with those reported in Shalev and Kafkafi (2002) and Arnold and Siviy (2002). Furthermore, the decreased spontaneous exploration of the open field in MS animals is in agreement with studies that showed a decrease in exploration after stress (Arnsten et al., 1985; Berridge and Dunn, 1986).

We hypothesized that kappa antagonism would block alterations in ethanol reinforcement after early maternal separation, considering MS a potential source of stress. This hypothesis, however, was not confirmed. An injection of nor-BNI – a long-lasting kappa antagonist – immediately before the onset or just after termination of maternal deprivation treatment (Experiments 1 and 2, respectively) did not affect the ethanol-induced conditioned aversion in MS pups. It could be argued that an effect would have been observed if additional nor-BNI injections had occurred or if the antagonist had been given at another time. During the first two postnatal weeks rats exhibit a reduced adrenal response to stress and low levels of hypothalamic corticotrophin-releasing hormone (Sapolsky and Meaney, 1986). Also, the doses of nor-BNI were chosen on the basis of studies in which this compound blocked stress-induced potentiation of ethanol-mediated CPP (Sperling et al., 2010) and inhibited shock-induced place aversion (Land et al., 2008). We cannot discard the possibility, however, that the most appropriate doses of this kappa antagonist were not used in the present study. Future experiments are needed to elucidate these issues.

Ethanol induced, as observed by Arias et al. (2008), reliable motor activation during the initial, rising limb of the blood ethanol curve and, in Experiment 3b, motor depression during the last testing bin. These biphasic effects were unaffected by early maternal separation stress. Across experiments, sex only exerted a significant main effect in terms of spontaneous open field exploration but it was not involved in any significant interaction with ethanol, nor-BNI treatment or early rearing. This independence was not unexpected. Several reports (e.g. Nizhnikov et al., 2009) indicated that ethanol-induced conditioning or ethanol-induced activation (Arias et al., 2008) do not differ in male and female preweanling rats. There are, however, several studies showing that females are much less sensitive to the effects of MS than males (e.g., Gustafsson et al., 2005).

In Experiment 1, rat pups that experienced normal animal facility rearing, received vehicle injections at PD 1 and were infused with 2.0 g/kg ethanol did not exhibit a conditioned preference for sandpaper. This result is different from that found in Molina et al. (2007). Procedural differences between these studies – that may explain the discrepancy – are the stress associated with the vehicle injections at PD 1 and that pups in the present study socially interacted with peers experiencing kappa antagonism.

The effects of early maternal separation during second-order conditioning did not appear to be related to alterations in ethanol metabolism. At doses and post-administration intervals that coincided with those employed during conditioning, BELs were similar in MS and AFR pups. Likewise, ethanol intake in the intraoral intake test did not differ as a function of rearing experience (Experiment 1). This lack of difference is intriguing, since previous studies have revealed greater initiation in ethanol self-administration in adult animals that experienced early maternal separation (Huot et al., 2001). Aside from the obvious difference in age of testing, the apparent discrepancy could be explained by procedural differences, the most obvious being the mode of testing (a short intraoral administration test vs. a two-bottle, 24-hour long free-choice intake test). The intraoral intake test did provide important information, however. Ethanol intake in the basic control condition (i.e., animals reared under normal facility conditions and then treated with vehicle) was positively and significantly associated with the previously recorded spontaneous open field exploration. This outcome adds to other studies indicating that ambulation predicts ethanol intake (Bisaga and Kostowski, 1993).

In summary, the results of the present study suggest that early environmental stress, modeled by maternal separation, reliably alters ethanol-mediated reinforcement during infancy. EES, however, has no significant effect on the motor activating properties of the drug, ethanol metabolism or ethanol intake measured at this early stage of development. MS pups perceived ethanol as significantly more aversive than pups that experienced normal rearing – an effect that was not sensitive to KOR antagonism – and exhibited an altered pattern of open field exploration. Future research should examine the neurobiological basis of these effects and their persistence during later stages of development, and should further scrutinize the association between early ethanol-induced reinforcement and ethanol intake.

Acknowledgments

This work, a collaborative project between the Research Foundation of SUNY Binghamton and Instituto Ferreyra (Argentina), was supported by NIAAA grants AA011960, AA01309 and AA017823 to NES, AA018164 and AA017823 to MN and grants PIP CONICET 2010–2012, PICT-PRH3 and SECYT-UNC to RMP; as well by a fellowship provided by Fulbright Commission to RMP. We thank Teri Tanenhaus and Dustin Waters for their technical assistance.

References

Abate P, Pepino M, Dominguez H, Spear N, Molina J. Fetal associative learning mediated through maternal alcohol intoxication. Alcohol Clin Exp Res 2000;24:39–47.
Acevedo MB, Molina JC, Nizhnikov ME, Spear NE, Pautassi RM. High ethanol dose during early adolescence induces locomotor activation and increases subsequent ethanol intake during late adolescence. Dev Psychobiol 2010;52:424–40.
Arias C, Molina JC, Mlewski EC, Pautassi RM, Spear N. Acute sensitivity and acute tolerance to ethanol in preweanling rats with or without prenatal experience with the drug. Pharmacol Biochem Behav 2008;89:608–22.
Arias C, Mlewski EC, Molina JC, Spear NE. Naloxone and baclofen attenuate ethanol's locomotor-activating effects in preweanling Sprague–Dawley rats. Behav Neurosci 2009;123:172–80.
Arias C, Molina JC, Spear NE. Differential role of micro, delta and kappa opioid receptors in ethanol-mediated locomotor activation and ethanol intake in preweanling rats. Physiol Behav 2010a;99:348–54.
Arias C, Solari AC, Mlewski EC, Miller S, Haymal B, Spear NE, et al. Social isolation and stress related hormones modulate the stimulating effect of ethanol in preweanling rats. Behav Brain Res 2010b;211:64–70.
Arnold JL, Siviy SM. Effects of neonatal handling and maternal separation on rough-and-tumble play in the rat. Dev Psychol 2002;41:202–15.
Arnsten AF, Berridge C, Segal DS. Stress produces opioid-like effects on investigatory behavior. Pharmacol Biochem Behav 1985;22:803–9.
Berridge CW, Dunn AJ. Corticotropin-releasing factor elicits naloxone sensitive stress-like alterations in exploratory behavior in mice. Regul Pept 1986;16:83–93.
Bisaga A, Kostowski W. Individual behavioral differences and ethanol consumption in Wistar rats. Physiol Behav 1993;54:1125–31.
Broadbear JH, Negus SS, Butelman ER, de Costa BR, Woods JH. Differential effects of systemically administered nor-binaltorphimine (nor-BNI) on kappa-opioid agonists in the mouse writhing assay. Psychopharmacology 1994;115:311–9.
Caldji C, Francis D, Sharma S, Plotsky PM, Meaney MJ. The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. Neuropsychopharmacology 2000;22:219–29.
Cruz FC, Quadros IM, Planeta CDS, Miczek KA. Maternal separation stress in male mice: long-term increases in alcohol intake. Psychopharmacology 2008;201:459–68.

Daoura L, Haaker J, Nylander I. Early environmental factors differentially affect voluntary ethanol consumption in adolescent and adult male rats. Alcohol Clin Exp Res 2011;35:506–15.
Dellarosa Cummins D, Cummins R. Biological preparedness and evolutionary explanation. Cognition 1999;73:B37–53.
Dewit DJ, Adlaf EM, Offord DR, Ogborne AC. Age at first alcohol use: a risk factor for the development of alcohol disorders. Am J Psychiatry 2000;157:745–50.
Francis DD, Diorio J, Plotsky PM, Meaney MJ. Environmental enrichment reverses the effects of maternal separation on stress reactivity. J Neurosci 2002;22:7840–3.
Gustafsson L, Ploj K, Nylander I. Effects of maternal separation on voluntary ethanol intake and brain peptide systems in female Wistar rats. Pharmacol Biochem Behav 2005;81:506–16.
Huot RL, Thrivikraman KV, Meaney MJ, Plotsky PM. Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. Psychopharmacology (Berl) 2001;158(4):366–73.
Kawakami SE, Quadros IMH, Takahashi S, Suchecki D. Long maternal separation accelerates behavioural sensitization to ethanol in female, but not in male mice. Behav Brain Res 2007;184:109–16.
Land BB, Bruchas MR, Lemos JC, Xu M, Melief EJ, Chavkin C. The dysphoric component of stress is encoded by activation of the dynorphin kappa-opioid system. J Neurosci 2008;28:407–14.
Land BB, Bruchas MR, Schattauer S, Giardino WJ, Aita M, Messinger D, et al. Activation of the kappa opioid receptor in the dorsal raphe nucleus mediates the aversive effects of stress and reinstates drug seeking. Proc Natl Acad Sci U S A 2009;106:19168–73.
Meaney MJ, Brake W, Gratton A. Environmental regulation of the development of mesolimbic dopamine systems: a neurobiological mechanism for vulnerability to drug abuse? Psychoneuroendocrinology 2002;27:127–38.
Metcalf MD, Coop A. Kappa opioid antagonists: past successes and future prospects. AAPS J 2005;7:E704–22.
Michaels CC, Holtzman SG. Early postnatal stress alters place conditioning to both mu- and kappa-opioid agonists. J Pharmacol Exp Ther 2008;325:313–8.
Molina JC, Ponce LF, Truxell E, Spear NE. Infantile sensitivity to ethanol's motivational effects: ethanol reinforcement during the third postnatal week. Alcohol Clin Exp Res 2006;30:1506–19.
Molina JC, Pautassi RM, Truxell E, Spear N. Differential motivational properties of ethanol during early ontogeny as a function of dose and postadministration time. Alcohol 2007;41:41–55.
National Research Council. Guide for the care and use of laboratory animals [NIH publication no. 85-23]. Washington DC: National Academy Press; 1985.
Neisewander JL, McDougall SA, Bowling SL, Bardo MT. Conditioned taste aversion and place preference with buspirone and gepirone. Psychopharmacology 1990;100:485–90.
Nizhnikov ME, Pautassi RM, Truxell E, Spear NE. Opioid antagonists block the acquisition of ethanol-mediated conditioned tactile preference in infant rats. Alcohol 2009;43:347–58.
Nizhnikov ME, Pautassi RM, Varlinskaya E, Rahmani P, Spear NE. Ontogenetic differences in ethanol's motivational properties during infancy. Alcohol; in press.
Pautassi RM, Nizhnikov M, Molina JC, Boehm SL, Spear NE. Differential effects of ethanol and midazolam upon the devaluation of an aversive memory in infant rats. Alcohol 2007;41:421–31.
Pautassi RM, Myers M, Spear LP, Molina JC, Spear NE. Adolescent but not adult rats exhibit ethanol-mediated appetitive second-order conditioning. Alcohol Clin Exp Res 2008;32:2016–27.
Pautassi RM, Nizhnikov ME, Spear NE. Assessing appetitive, aversive, and negative ethanol-mediated reinforcement through an immature rat model. Neurosci Biobehav Rev 2009;33:953–74.
Pautassi RM, Nizhnikov ME, Acevedo MB, Spear NE. Naloxone blocks ethanol-mediated appetitive conditioning and locomotor activation in adolescent rats. Behav Brain Res 2011a;216:262–9.
Pautassi RM, Nizhnikov ME, Fabio MC, Spear NE. An acetaldehyde-sequestering agent inhibits appetitive reinforcement and behavioral stimulation induced by ethanol in preweanling rats. Pharmacol Biochem Behav 2011b;97(3):462–9.
Plotsky PM, Meaney MJ. Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. Brain Res Mol Brain Res 1993;18:195–200.
Ponce LF, Pautassi RM, Spear NE, Molina JC. Maternal care alterations induced by repeated ethanol leads to heightened consumption of the drug and motor impairment during adolescence: a dose-response analysis. Physiol Behav 2011;103:477–86.
Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 1993;18:247–91.
Roma PG, Davis CM, Kohut SJ, Huntsberry ME, Riley AL. Early maternal separation and sex differences in the aversive effects of amphetamine in adult rats. Physiol Behav 2008;93:897–904.
Sapolsky RM, Meaney MJ. Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. Brain Res 1986;396:64–76.
Shalev U, Kafkafi N. Repeated maternal separation does not alter sucrose-reinforced and open-field behaviors. Pharmacol Biochem Behav 2002;73:115–22.
Song M, Wang XY, Zhao M, Wang XY, Zhai HF, Lu L. Role of stress in acquisition of alcohol-conditioned place preference in adolescent and adult mice. Alcohol Clin Exp Res 2007;31:2001–5.
Spear NE, Molina JC. Fetal or infantile exposure to ethanol promotes ethanol ingestion in adolescence and adulthood: a theoretical review. Alcohol Clin Exp Res 2005;29:909–29.
Sperling RE, Gomes SM, Sypek EI, Carey AN, McLaughlin JP. Endogenous kappa-opioid mediation of stress-induced potentiation of ethanol-conditioned place preference and self-administration. Psychopharmacology 2010;210:199–209.
Spivey J, Barrett D, Padilla E. Mother–infant separation leads to hypoactive behavior in adolescent Holtzman rats. Behav Processes 2008;79:59–65.
Vazquez V, Penit-Soria J, Durand C, Besson MJ, Giros B, Daugé V. Maternal deprivation increases vulnerability to morphine dependence and disturbs the enkephalinergic system in adulthood. J Neurosci 2005;25:4453–62.
Wee S, Orio L, Ghirmai S, Cashman JR, Koob GF. Inhibition of kappa opioid receptors attenuated increased cocaine intake in rats with extended access to cocaine. Psychopharmacology 2009;205:565–75.