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Chronic tolerance to the locomotor stimulating effect of ethanol in preweanling rats as a function of social stress

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Abstract

During early stages of development rats are highly sensitive to the locomotor stimulating effect of relatively high ethanol doses, an effect strongly modulated by social stress. This ethanol effect can be modulated by pharmacological treatments that also can attenuate the development of ethanol-induced locomotor sensitization in mice. By the end of the preweanling period the mechanisms underlying sensitization induced by psychostimulants are functional. The aim of the present study was to analyze the locomotor response to ethanol in preweanling rats as a function of repeated exposure to the drug under two different social conditions. Subjects were treated with ethanol between postnatal days 15 and 18 after being isolated for four hours (Experiment 1a) or simply residing in their home-cage (Experiment 1b). After two days of withdrawal locomotor response to ethanol was assessed in both social conditions. In Experiment 2 naïve rats were tested in terms of ethanol-induced activation of the locomotor response in both social conditions. Results from the present study showed no evidence of locomotor sensitization in preweanling rats in any of the social conditions. Instead we observed behavioral tolerance to the stimulating effect of ethanol in animals trained in the home-cage condition, in which subjects trained with ethanol showed sedation in response to ethanol at testing. Overall these results contribute to the understanding of the sensitivity of rats to the acute and chronic locomotor response to ethanol in an ontogenetic period characterized by high sensitivity to ethanol.

Ethanol-induced locomotor stimulation has been frequently reported in adult mice. In contrast, adult rats normally show sedation after being treated with ethanol (Correa, Arizzi, Betz, Mingote and Salamone, 2003; Chuck, McLaughlin, Arizzi-LaFrance, Salamone and Correa, 2006). During early stages of development, however, rats are highly sensitive to the locomotor stimulating effect of ethanol. This effect can be easily observed when rats are given a relatively high ethanol dose (between 1.25 and 2.5 g/kg) and are tested during the rising phase of the blood ethanol curve (Arias, Mlewski, Miller, Molina and Spear, 2009;

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Arias, Mlewski, Molina and Spear, 2009a; Arias, Molina, Mlewski, Pautassi and Spear, 2008).

In a previous study we reported a synergism between ethanol and social stress in terms of locomotor stimulation in infant rats (Arias, Solari, et al., 2010). Ethanol-induced locomotor activity was strongly potentiated in pups isolated from the home-cage four hours before testing (Arias, Solari, et al., 2010). Additionally, we also reported that the stimulating effect of ethanol in social isolated pups was significantly reduced by blocking the CRH1 receptor with CP154,426 (Arias, Solari, et al., 2010). This receptor has been reported to have a critical role in the development of locomotor sensitization induced by ethanol in mice (Pastor, et al., 2008). Considering these antecedents, it is plausible to expect that response to repeated ethanol treatment would differ in isolated rats and non-isolated rats during the preweanling period. Following this rationale we designed the present study intending to assess the locomotor response to ethanol in preweanling rats as a joint function of repeated exposure to the drug and social condition (isolated vs. non-isolated).

In Experiment 1 subjects were treated with ethanol between postnatal days 15 and 18 (PDs 15-18) under two different social conditions. Half of the subjects received ethanol after being isolated for four hours (Experiment 1a) and the other half received ethanol while still in their home cage (Experiment 1b). During the preweanling period social isolation is considered a strong stressor (Levine, 2005). After two days of withdrawal (PD 21) locomotor response to ethanol was assessed in both social conditions. Considering the particular sensitivity to ethanol-induced locomotor activation in this phase of ontogeny (Arias, Mlewski, et al., 2009a) and the role of stress in the development of locomotor sensitization to ethanol (Phillips, Roberts and Lessov, 1997), we hypothesized that locomotor sensitization would develop only in the isolated subjects, but not in those that were not separated from the home-cage. In Experiment 2 we analyzed locomotor activity induced by ethanol on PD21 in naïve animals to test whether this effect is observable at this conventional age of weaning for laboratory rats without any prior manipulation.

Ethanol-induced locomotor sensitization has rarely been reported in adult rats and only under very specific experimental conditions (Conroy, Rodd and Chambers, 2007; Hoshaw and Lewis, 2001). Hoshaw and Lewis suggested that the lack of sensitization to ethanol in adult rats may be because the relatively high ethanol doses apparently required to induce sensitization generates locomotor sedation in this rodent (Hoshaw and Lewis, 2001). If this hypothesis is correct, we shouldn't have this limitation in preweanling rats, since the ethanol dose that promotes locomotor activation at this age is relatively high (2.5 g/kg) and coincides with the dose normally used in mice to induce sensitization (for example, Pastor and Aragon, 2006). For the present study we selected the third postnatal week of life because at this age the mechanisms supporting short- and long-term sensitization to stimulant drugs such as cocaine or amphetamine are already developed (Duke, O'Neal and McDougall, 1997; McDougall, Duke, Bolanos and Crawford, 1994; Sibole, Matea, Krall and McDougall, 2003). Moreover, by this age the stress hyporesponsive period is already overcome and the stress response has begun to work in an adult-like way (Levine, 2001). The stress response is an important modulator of the acquisition of locomotor sensitization to ethanol in rodents, whether preweanlings or adults (Phillips, et al., 1997; Roberts, Lessov and Phillips, 1995).

Several studies from our research group have consistently shown during the last few years that infant rats are highly sensitive to ethanol's motivational and locomotor effects (Arias and Chotro, 2006; Arias et al., 2009a; Molina, Pautassi, Truxell and Spear, 2007; Nizhnikov, Pautassi, Truxell and Spear, 2009). Additionally, some authors have proposed that alcohol dependence may be conceptualized as a developmental disorder (Tarter and Vanyukov,

1994), not only because of the interaction between genes and social environment that influences temperament and behavioral development, but also because early exposure to ethanol can affect later responsiveness to the drug (Abate, Pueta, Spear and Molina, 2008; Chotro, Arias and Laviola, 2007; Spear and Molina, 2005). From this perspective, the infant rat model can represent a valuable tool for understanding alcohol dependence. Within this framework, the present study may add new and complementary information about the interaction between social stress and repeated experience with ethanol during this critical ontogenetic period.

Material and Methods

Subjects

Sixty-four Sprague-Dawley pups, representative of 18 litters, were utilized for each of Experiments 1a and 1b. For Experiment 2 we utilized 35 Sprague-Dawley pups derived from 18 litters. Animals were born and reared at the vivarium of the Center for Development and Behavioral Neuroscience (Binghamton University, NY) under conditions of constant room temperature (22 ± 1.0 °C), on a 12-hour light 12-hour dark cycle. Births were examined daily and the day of parturition was considered as postnatal day 0 (PD0). All litters were culled to 10 pups (5 females and 5 males, whenever possible) within 48 hours after birth. All procedures were in accordance with the guidelines for animal care and use established by the Institute of Laboratory Animal Resources (1996).

Procedures

Experiment 1

Training phase: In this phase rats were given ethanol (0 or 2.5 g/kg) each day from postnatal day 15 (PD15) to PD18. This ethanol dose was selected because we have consistently shown that it stimulates locomotor activity in preweanling rats during the rising phase of the blood ethanol curve, approximately between 5 and 20 minutes after ethanol administration (Arias et al., 2009a). Furthermore, as we discussed in the introduction, Hoshaw and Lewis suggested that the lack of sensitization to ethanol in rats may be because the relatively high ethanol doses apparently required to induce sensitization generates locomotor sedation in this rodent (Hoshaw and Lewis, 2001). Consecutive drug injections may not be the better option to induce sensitization. Protocols to induce this process commonly included drug treatments every other day. However, as mentioned, the aim of this study was to analyze the interaction between social stress and ethanol in the development and expression of sensitization in the infancy of the rat. Because stress hormones seem to be critical for the induction of sensitization to ethanol (Phillips, Roberts and Lessov, 1997), and the stress response starts to respond in an adult-like way by the end of the second postnatal week of life (Levine, 2005), we have limited time-interval to carry out a sensitization protocol during the third postnatal week of life until weaning. For these reasons, for this first study we selected the mentioned protocol.

In Experiment 1a, during the training phase, rats were separated from the home-cage four hours before ethanol administration and placed in a holding maternity cage ($45 \times 20 \times 20$ cm) partially filled with clean wood shavings. During this time each subject was effectively isolated, not in contact with their dam or littermates. The floor of the cage was maintained at 35 C (± 1 °C) through the use of a heating pad. In Experiment 1b subjects remained with their mother in the home-cage until the moment at which ethanol was administered. We will refer to these social conditions as “isolation” (Experiment 1a) or “home-cage” (Experiment 1b) conditions throughout the manuscript.

The remaining procedures were exactly the same for both experiments. Ethanol was intragastrically delivered (0 or 2.5 g/kg ethanol) in a volume equivalent to 0.015 ml per gram of body weight of a 21 % ethanol solution. Intragastric administrations were performed using a 10-cm length of polyethylene tubing (PE-10 Clay Adams, Parsippany, New Jersey) attached to a 1 ml syringe with a 27 G $\times$ 1/2 needle. This tubing was gently introduced through the mouth and slowly pushed into the stomach. The entire procedure took less than 20 seconds per pup. Five minutes after ethanol administration locomotor activity was evaluated for 10 minutes. This evaluation period was selected on the basis of prior studies showing ethanol-induced stimulation at a similar post-administration testing interval (Arias, Mlewski, et al., 2009a). The testing environment consisted of a Plexiglas open field (42 $\times$ 42 $\times$ 30 cm; VersaMax Animal Activity Monitoring System, Accuscan Instruments, Columbus, OH, USA). In this apparatus, locomotion was detected by interruption of eight pairs of intersecting photocell beams evenly spaced along the walls of the testing environment. This equipment was situated in sound-attenuating box chambers (53 $\times$ 58 $\times$ 43 cm) equipped with a light and fan for ventilation and background noise. Consecutive photocell beam interruptions were translated to distance traveled in cm by the VersaMax. This dependent variable takes into account the path of the animal during locomotion, and is an accurate indicator of ambulatory activity. Immediately after the locomotor activity test pups were returned to their home-cage.

Testing phase: In a second phase of the experiment subjects were tested for ten minutes in response to water or to the same ethanol dose (2.5 g/kg) employed during the training phase. Half of the subjects from Experiment 1a were tested in the same social condition in which they were trained (isolation) while the remaining subjects were tested in the alternative condition (home-cage). Subjects from Experiment 1b were also tested in one of the two social conditions (isolation or home-cage). For this phase rats were tested on PD21, after two days of withdrawal from the ethanol treatment given PD15-18.

Determination of the blood ethanol concentrations (BECs): Immediately after the testing phase pups were sacrificed. Trunk blood was obtained following decapitation. Blood samples were collected using a heparinized capillary tube. The blood samples were immediately centrifuged (6,000 rpm; Micro-Haematocrit Centrifuge, Hawksley and Sons LTD, Sussex, England) and stored at -70°C . BECs were determined using an AM1 Alcohol Analyzer (Analox Instruments, Lunenburg, MA). Calculation of BECs was made by oxidating ethanol to acetaldehyde in the presence of ethanol oxidase. The apparatus measures the rate of oxygen required by this process, which is proportional to ethanol concentration. BECs were expressed as milligrams of ethanol per deciliter of body fluid (mg/dl = mg%). The analysis of the BECs provided information about whether differences in the locomotor response to ethanol may be explained by changes in ethanol's pharmacokinetic characteristics induced by the prior experience with the drug or the social condition to which the animals were assigned.

Experiment 2—In Experiment 2—21-day-old naïve rats were tested for 10 min in terms of locomotor activity in response to ethanol (0 or 2.5 g/kg) in both social conditions (home-cage or isolation). Procedures employed in this experiment were exactly the same as those described for the testing phase of Experiment 1.

Experimental design and data analyses—The experimental design of Experiments 1a and 1b was defined by the factorial combination of the following between-subject factors: ethanol treatment at training (0 or 2.5 g/kg), ethanol treatment at testing (0 or 2.5 g/kg), and social condition at testing (isolation or home-cage). Day was considered as a within-subject factor. In any case no more than one pup of a given litter was assigned to the same

experimental condition. The experimental design of Experiment 2 included two between-subject factors: ethanol treatment at testing (0 or 2.5 g/kg), and social condition at testing (isolation or home-cage).

Locomotor activity was operationalized as distance traveled (cm) by a given pup during the 10-minute test. Locomotor activity was statistically analyzed using analysis of variance (ANOVA). In Experiment 1a and 1b, locomotor activity collected during the training phase was analyzed by means of a 2 [ethanol treatment at training (0 or 2.5 g/kg)] by 2 [ethanol treatment at testing (0 or 2.5 g/kg)] by 2 [social condition at testing (isolation or home-cage)] by 4 (day) mixed ANOVA. In these analyses day was treated as a within-subject variable, while the remaining ones were considered between-subject variables. Locomotor activity at testing in both experiments was analyzed by a 2 [ethanol treatment at training (0 or 2.5 g/kg)] by 2 [ethanol treatment at testing (0 or 2.5 g/kg)] by 2 [social condition at testing (isolation or home-cage)] between-factor ANOVA. Locomotor activity data from experiment 2 were analyzed by means of a 2 [ethanol treatment (0 or 2.5 g/kg)] by 2 [social condition (isolation or home-cage)] ANOVA. Sex was counterbalanced across the experimental conditions, and ANOVAs indicated that this variable did not exert a significant effect or interact with the remaining factors in any of the phases of the experiment. Significant main effects or interactions indicated by the ANOVAs were further analyzed through post-hoc tests (Newman-Keuls post-hoc test with a Type I error set at 0.05). Due to the probability of type I error because of the number of variables included in the design, we also reported the effect size (Cohen's *f*). This value indicates the magnitude of the difference between groups (in standard deviation units).

Results

Experiment 1a

Training phase—Figure 1a represents distance traveled (cm) at training (days 1, 2, 3 and 4) as a function of ethanol treatment (0 or 2.5 g/kg). The ANOVA used to analyze these data indicated a significant main effect of the following factors: Ethanol treatment at training [$F(1,56)=27.88$, $p<0.05$], day [$F(3,168)=15.98$, $p<0.05$] and their interaction, [$F(3,168)=4.66$, $p<0.05$]. Follow-up ANOVAs were performed to analyze this interaction. In these analyses ethanol dose at training was considered as the only between-factor variable, and independent ANOVAs were conducted specifically with the locomotor activity data from each day. The four ANOVAs revealed a significant effect of ethanol treatment at training [Day 1: $F(1,56)=14.99$, Cohen's $f = 0.48$; Day 2: $F(1,56)=26.65$, Cohen's $f = 0.65$; Day 3: $F(1,56)= 10.71$, Cohen's $f = 0.42$; Day 4: $F(1,56)=5.61$, Cohen's $f = 0.29$; all $p<0.05$]. In all cases rats given ethanol showed a significant increase in locomotor activity when compared to water controls. Since this initial strategy did not allow us to detect the locus of the interaction, additional ANOVAs were performed in which locomotor activity data from pups given ethanol or water at training were analyzed separately, and day was considered the within-subject factor. In the case of pups treated with water the ANOVA revealed a significant main effect of day [$F(3,168)=4.66$, $p<0.05$; Cohen's $f = 0.44$]. Post-hoc analyses revealed that locomotor activity was significantly higher on day 2 than on days 1 and 4. The ANOVA conducted with data collected from pups given ethanol also revealed a significant effect of day [$F(3,168)=11.67$, $p<0.05$; Cohen's $f = 0.61$]. Post-hoc analyses revealed that locomotor activity on day 1 was significantly lower than on day 2. Locomotor activity on day 4 was also significantly lower than in the remaining days.

Testing phase—The ANOVA conducted with the activity data collected at testing (Figure 1b) revealed a significant effect of ethanol treatment at testing [$F(1,56)=18.73$, $p<0.05$; Cohen's $f = 0.58$] and social condition at testing [$F(1,56)=7.15$, $p<0.05$; Cohen's $f = 0.35$]. According to the post hoc analyses, subjects given ethanol at testing (groups water-ethanol

and ethanol-ethanol) showed more locomotor activity than subjects given water (groups water-water and ethanol-water), an effect that was observed regardless of the prior experience with the drug and the social condition in which subjects were evaluated. Additionally, subjects that were isolated before testing (isolation condition) showed more activity than those non-isolated (home-cage condition).

Blood ethanol concentrations—The ANOVA employed to analyze the BECs did not reveal any significant effect. BECs (mean and standard error of the mean) and the number of subjects included in each group are represented in Table 1.

Experiment 1b

Training phase—Locomotor activity scores from Experiment 1b are depicted in Figure 2a. The ANOVA revealed a significant main effect of ethanol treatment at training [$F(1,56)=15.90$, $p<0.05$], day [$F(3,168)=3.07$, $p<0.05$], and the interaction between both factors, [$F(3,168)=7.64$, $p<0.05$]. To analyze the focus of this interaction we utilized follow-up ANOVAs in which ethanol treatment at training was treated as the only between-factor variable. This analysis was repeated with the locomotor activity data from each day. These ANOVAs revealed a significant effect of ethanol treatment at training on day 1, [$F(1,62)=17.37$, $p<0.05$; Cohen's $f = 0.53$] and day 2 [$F(1,62)=18.85$, $p<0.05$; Cohen's $f = 0.54$]. In both days distance traveled was significantly higher in subjects given ethanol than in those given water. Following the statistical strategy employed for experiment 1a, we also performed repeated measures ANOVAs separately with locomotor activity scores from pups given ethanol or water at training. In the case of pups treated with water the ANOVA revealed a significant effect of day [$F(3,93)=9.96$, $p<0.05$; Cohen's $f = 0.56$], indicating that distance traveled by subjects were significantly higher on days 3 or 4 than on days 1 or 2. The analysis performed with ethanol-treated subjects also revealed a significant effect of day [$F(3,93)=3.46$, $p<0.05$; Cohen's $f = 0.33$], indicating that locomotor activity was significantly lower on day 4 than on the remaining days.

Testing phase—The ANOVA conducted with locomotor activity data collected on the testing day indicated a significant main effect of ethanol treatment at training [$F(1,56)=9.21$, $p<0.05$], and a significant interaction between ethanol treatment at training and ethanol treatment at testing, [$F(1,56)=14.07$, $p<0.05$; Cohen's $f = 0.50$]. Post-hoc analysis indicated that subjects treated with water during the training phase and given ethanol for the first time at testing (group water-ethanol) showed more activity than the corresponding controls treated with water at testing (group water-water). In contrast, locomotor activity from subjects given ethanol at training and treated with ethanol at testing (group ethanol-ethanol) was significantly lower than that of controls trained with ethanol and given water at testing (group ethanol-water). Furthermore, rats given ethanol at testing for the first time (group water-ethanol) also showed more activity than those given ethanol at testing after training with ethanol in the first phase of the experiment (group ethanol-ethanol) (see figure 2b).

Experiment 2

In Figure 3 we tested locomotor activity by naïve rats treated with ethanol or water on PD21 after being in isolation or with littermates in their home cage for the previous 4 hr. The ANOVA revealed a significant effect of ethanol treatment [$F(1,31)=6.94$, $p<0.05$; Cohen's $f = 0.46$] but no interaction with housing condition, indicating that rats given ethanol showed more locomotor activity than those given water, regardless of whether or not they had just been isolated socially.

Data from experiment 2 indicates that the stimulant effect of ethanol is also observable on PD21. We explicitly compared ethanol-induced locomotor activity on PD21 (Experiment 2)

with the ethanol effect on PD15 (data from the first testing day from Experiments 1a and 1b) to determine whether the magnitude of the stimulant effect varies during the third postnatal week of life. The ontogenetic difference cannot be analyzed with data exclusively from Experiment 1, because animals that received ethanol for the first time on PD21 had prior experience with the testing environment, intragastric intubation and handling that could affect their response to ethanol. We previously reported differences in the sensitivity to ethanol-induced locomotor stimulation during the first 2 postnatal weeks of life (Arias, Mlewski, et al., 2009a). The present analysis is justified because the lack of sensitization expressed on PD21 in Experiment 1 may be attributed simply to less ethanol-induced stimulation at this age (Hoshaw and Lewis, 2001). For these analyses we utilized standardized scores, that were calculated for each ethanol group tested on PD15 (Experiment 1) or 21 (Experiment 2) as a function of their social condition (isolation or home-cage). Standardized scores (z-scores) were calculated by subtracting the mean of the corresponding water-control group (same social condition) from the individual score of ethanol-treated pups, and dividing by the standard deviation (of the corresponding control group). Standardized scores allow direct comparison between ages. Negative z scores indicate that pups treated with ethanol were less active than their respective water-treated control. Positive z scores reflect more locomotion than controls. We utilized a two-way between-factor ANOVA in which age (PD15 vs. PD21) and social condition (isolation vs. home-cage) were treated as between-subject variables. Figure 4 represents z-scores corresponding to these analyses. The ANOVA did not reveal any significant effect of interaction, a result indicating that magnitude of the ethanol stimulating effect did not vary as a function of age.

Discussion

Our working hypothesis for the present study was that repeated ethanol treatment would induce locomotor sensitization in preweanling rats trained with ethanol in the isolated condition. Even though locomotor sensitization has been rarely reported in rats (Hoshaw and Lewis, 2001), we raised this hypothesis based on relevant antecedents. First, the ethanol dose that induced stimulation in this ontogenetic period was high (2.5 g/kg) and comparable to doses employed in adult mice to promote locomotor sensitization (Arias, Mlewski, et al., 2009a). Additionally, the locomotor activating effect induced by ethanol in preweanling rats is attenuated by a variety of pharmacological treatments that not only reduce the acute locomotor response to ethanol but also interfere with the development of locomotor sensitization in mice. For example, blocking mu opioid receptors (Arias, Mlewski, et al., 2009b; Arias, Molina, et al., 2010), dopamine receptors (Arias, Mlewski, et al., 2010) or CRH1 (Arias, Solari, et al., 2010) significantly attenuates both the stimulating effect of ethanol in preweanling rats and development of locomotor sensitization in mice. Finally, during the ontogenetic period in which subjects were trained with ethanol the mechanisms necessary for the development of locomotor sensitization are functional, at least those that mediate sensitization to psychoactive drugs such as amphetamine or cocaine (McDougall, et al., 1994). Although the present data did not support our working hypothesis, several important findings were derived from the present study that we will discuss in the following sections.

Response to ethanol during this ontogenetic period varied dramatically depending on the social condition in which the drug was experienced

The first evidence of the interaction between ethanol and social condition emerged during the training phase. Animals trained in the home-cage condition (Experiment 1b) showed stimulation in response to ethanol only during the first two training sessions, but in the isolation condition the stimulating effect of ethanol was observed throughout the entire training phase and became even more evident on the testing day. Also in the isolation condition, ethanol induced the locomotor stimulating effect regardless of the prior

experience with the drug, whereas for rats trained in the home-cage condition, prior experience with ethanol from PD15 to PD18 eliminated the stimulating effect of ethanol at testing.

These results are in agreement with prior observations from our laboratory indicating that the acute stimulating effect of ethanol in preweanling rats is strongly modulated by the social condition in which the animal experienced the drug. The results of Arias, Solari, et al. (2010) indicate that the interaction between social stress and ethanol is mediated by activation of the CRH1 receptor. Activation of these receptors seems to be necessary for development of locomotor sensitization in mice (Pastor, et al., 2008). For this reason we expected that the isolated rats, but not those from the home-cage condition, would develop locomotor sensitization. However, on the testing day the effect of ethanol in isolated rats was not affected by prior experience with the drug.

Lack of ethanol-induced sensitization in preweanling rats

The stimulating effect induced by the present ethanol dose is expected to be mediated by the homologous receptors that critically participate in the development of locomotor sensitization in mice. In mice significant attenuation of locomotor sensitization has been observed by blocking D1 (Camarini, et al., 2010), mu (Pastor and Aragon, 2006) or CRH1 (Pastor, et al., 2008) receptors, or by agonizing GABA B receptors (Broadbent and Harless, 1999). These pharmacological manipulations have also disrupted the stimulating effect of ethanol in preweanling rats (Arias, Mlewski, et al., 2010; Arias, Mlewski, et al., 2009b; Arias, Molina, et al., 2010; Arias, Solari, et al., 2010), suggesting that similar neurochemical systems are activated in the acute response to ethanol in preweanling rats and during the development of locomotor sensitization to ethanol in mice. However mice readily develop locomotor sensitization, which is in contrast to the absence of the effect in adult (Masur, Oliveira de Souza and Zwicker, 1986) or developing rats. Differences between adult mice and adult rats in their response to ethanol have been discussed extensively previously (for example, Masur, et al., 1986; Pohorecky, 1977). Rats do not develop sensitization to ethanol's activating effects even after sufficient exposure to ethanol to induce tolerance to its sedative effects (Masur, et al., 1986). This result suggests that the lack of sensitization in adult rats is not due to the masking of ethanol's depressant effect. Interestingly, Hunt and collaborators (P. S. Hunt, Molina, Rajachandran, Spear and Spear, 1993) also reported that preweanling rats developed tolerance to the sedative effect induced by the same ethanol dose (2.5 g/kg) that we employed (measured through the latency to perform the righting reflex). This result also suggests that the lack of sensitization in preweanling rats also is not due to masking of the sedative effect of the drug.

Hoshaw and Lewis (2001) found sensitization in rats employing relatively low ethanol doses at testing. Similarly, Netsby et al. (1997) trained rats with 1 g/kg ethanol, then evaluated them in terms of locomotor response to morphine and reported cross-sensitization to the stimulating effects of the drugs. These studies suggested that the lack of sensitization to ethanol in rats may be due to sedating effects at ethanol doses required to induce sensitization (Hoshaw and Lewis, 2001). This explanation does not directly account for the present results, since the ethanol dose we employed induced stimulation during both training and testing. According to our analysis, the magnitude of the activating effect of ethanol was similar in naïve animals from PD15 to PD21. The present study illustrates the low probability of locomotor sensitization to ethanol in rats, even under circumstances designed to be especially favorable.

Tolerance to the stimulating effect of ethanol as a function of the social condition

In Experiment 1b (animals trained in the home-cage condition) the stimulating effect of ethanol was reduced by repeated experience with ethanol and by the end of the training session was no longer observed. Also, animals trained with water and given ethanol for the first time at testing had ethanol-induced locomotor activation, whereas animals trained with ethanol and given ethanol at testing showed locomotor sedation. This suggests tolerance to the stimulating effect of ethanol, an effect otherwise observed only in animals assigned to the home-cage condition. Tolerance to the locomotor stimulating effect of ethanol has previously been reported in adult rats (G. P. Hunt and Overstreet, 1977). These authors observed that rats chronically treated with ethanol simultaneously developed tolerance to the stimulating and sedation effects of ethanol.

Few studies have tested tolerance to the effects of ethanol in preweanlings. Two of these studies reported acute tolerance to the sedative effects of ethanol (Arias, et al., 2008; Silveri and Spear, 2001), and another found chronic tolerance to the sedative effect of ethanol, measured in terms of latency to perform the righting reflex (P. S. Hunt, et al., 1993). In the latter study, tolerance developed during an ontogenetic period similar to that of the present experiments and with a similar ethanol dose (2.5 g/kg). These studies collectively suggest that in preweanling rats, tolerance to the sedative and stimulating effects of ethanol can be developed simultaneously, as suggested for adults by Hunt and Overstreet (1977). Although no index of sedation was used in our study, if tolerance to the sedative effects of ethanol also developed in our animals, it did not facilitate the expression of ethanol-induced activation since the locomotor response to ethanol in animals trained with the drug was similar (Experiment 1a) or even lower (Experiment 2b) than was displayed by animals given ethanol at testing after training with water.

Analysis of the BECs suggested that tolerance was not due to differences in ethanol absorption or metabolism. BECs were unaffected by prior experience with the drug and whether subjects were trained or tested in the isolation or the home-cage condition. The BECs also are in agreement with prior data in confirming that ethanol pharmacokinetics in infant rats are not affected by social condition (Arias, Solari, et al., 2010).

Locomotor stimulation or sedation was induced by differential ethanol and social treatment

It is interesting to observe that in the home-cage condition subjects trained with ethanol not only did not show stimulation, but instead showed marked sedation, an effect not observed in animals trained in the isolation condition. This finding is important for the understanding of the mechanisms that regulate effects of ethanol in rats. Our results suggest that mechanisms underlying both ethanol locomotor effects (activation and sedation) start functioning soon after ethanol administration and that tolerance to the stimulating effect of ethanol may lead the expression of the sedative effects of the drug. The high sensitivity of infant rats to the locomotor stimulating effect of ethanol may be a result of competition between mechanisms that regulate the excitatory and depressive effects of the drug. Ethanol-induced locomotor sedation seems to be related to the peripheral metabolism of the drug (Carmichael et al., 1991; McLaughlin et al., 2008), which is known to be relatively low in the infant rat (Hollstedt et al., 1980; Kelly et al., 1987).

It is plausible that the sedative effects of ethanol are more marked in adult rats than in greenlings and these effects could mask the stimulating effects of ethanol (Masur et al., 1986). This hypothesis is congruent with the fact that central ethanol administration can induce stimulating effects in adult heterogeneous rats (Correa et al., 2003). Ethanol-induced

locomotor sedation has been observed in adult rats at post-administration times similar to those of the present study (Chuck, et al., 2006).

Beyond the ontogenetic differences in ethanol metabolism, there are important features that distinguish our protocol from that typically employed in adult rats. First, we delivered ethanol through intragastric intubation while in adults, ethanol normally is administered intraperitoneally (Chuck, et al., 2006). Second, our subjects were not familiarized with the environment before the experiment. This is a common procedure utilized in most studies with adult rats, and previous studies indicate that prior familiarization with the testing chamber attenuates the stimulating effect of ethanol in preweanling rats (Arias, Mlewski, Miller, et al., 2009). These procedural differences seem to be important for the analysis of the locomotor effects induced by ethanol and have to be considered for the ontogenetic analysis of ethanol locomotor effects. Unpublished data from our laboratory also indicate that adult rats show ethanol-induced locomotor stimulation when ethanol is administered by means of an intragastric intubation and subjects are tested in a novel environment (Miller, Arias and Spear, 2009). In the present study locomotor sedation was observed under very specific conditions: only when animals were trained with ethanol in the home-cage condition, a treatment that probably minimizes the stress of the animal (Arias, Solari, et al., 2010), and had substantial experience with the testing environment and with the drug, which probably reduced novelty and led to development of tolerance to ethanol-induced activity.

In sum, repeated exposure to a relatively high ethanol dose did not induce locomotor sensitization in preweanling rats. Instead we observed, in rats under low-stress conditions, tolerance to this effect. Overall these results contribute to understand the sensitivity of rats to acute and repeated exposure to ethanol during an ontogenetic period characterized by high sensitivity to ethanol.

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Figure 1.

a: Distance traveled (cm) at training (days 1, 2, 3 and 4) as a function of ethanol treatment (0 or 2.5 g/kg) in pups from the isolation condition (Experiment 1a). * $p < 0.05$ vs water control. Rats given ethanol showed a significant increase in locomotor activity when compared to water controls every training day. Figure 1b: Distance traveled (cm) as a function of ethanol treatment at training (0 or 2.5 g/kg), ethanol treatment at testing (0 or 2.5 g/kg) and social condition at testing (* $p < 0.05$ vs water controls). Subjects given ethanol at testing (groups water-ethanol and ethanol-ethanol) showed more locomotor activity than subjects given water (groups water-water and ethanol-water). Vertical lines illustrate standard errors of the means.

Figure 2.

a: Distance traveled (cm) at training (days 1, 2, 3 and 4) as a function of ethanol treatment (0 or 2.5 g/kg) in pups from the home-cage condition (Experiment 1b). * $p < 0.05$ vs water control. Distance traveled was significantly higher in subjects given ethanol than in those given water during the first two training days. Figure 2b: Distance traveled (cm) at testing as a function of ethanol treatment at training (0 or 2.5 g/kg), ethanol treatment at testing (0 or 2.5 g/kg) and social condition at testing. Subjects treated with water during the training phase and given ethanol for the first time at testing (group water-ethanol) showed more activity than the corresponding controls (groups water-water or ethanol-ethanol; * $p < 0.05$). Locomotor activity scores from rats given ethanol at training and treated with ethanol at testing (group ethanol-ethanol) were significantly lower than those from the corresponding control condition (group ethanol-water; # $p < 0.05$). Vertical lines illustrate standard errors of the means.

Figure 3. Locomotor activity displayed by naïve rats treated with ethanol (2.5 g/kg) or water on PD21 as a function of the social condition (home-cage or isolation). Rats given ethanol showed more locomotor activity than those given water, regardless the social condition in which they were tested (* $p < 0.05$). Vertical lines illustrate standard errors of the means.

Figure 4. Standardized scores (z-scores) as a function of age (PD15 or PD21) and social condition (Isolation or home-cage). Positive z scores reflect more locomotion after ethanol administration (2.5 g/kg) than the respective water-controls. The ANOVA did not revealed any significant effect of interaction, a result indicating the magnitude of the stimulating effect induced by ethanol did not vary as a function of the age. Vertical lines illustrate standard errors of the means.

Table 1

Blood ethanol concentrations (BECs) as a function of ethanol treatment at training [water (0 g/kg) or ethanol (2.5 g/kg)] and social condition at testing (isolation or home-cage). BECs from experiment 1a belong to subjects trained in isolation, while BECs from experiment 1b are from subjects trained in the home-cage condition. Values represent mean and standard error of the mean (SE).

	Ethanol treatment at training	Social condition at testing	Blood ethanol concentration (mean $\pm$ SE)	n
Experiment 1a (isolation at training)	Water (0 g/kg)	Isolation	148.27 $\pm$ 12.05	8
	Ethanol (2.5 g/kg)		168.46 $\pm$ 13.34	8
	Water (0 g/kg)	Home Cage	178.81 $\pm$ 13.11	8
	Ethanol (2.5 g/kg)		166.82 $\pm$ 27.29	8
Experiment 1b (Home-cage at training)	Water (0 g/kg)	Isolation	170.37 $\pm$ 15.01	8
	Ethanol (2.5 g/kg)		157.37 $\pm$ 13.90	8
	Water (0 g/kg)	Home Cage	155.86 $\pm$ 16.30	8
	Ethanol (2.5 g/kg)		168.68 $\pm$ 16.30	8