Ethanol-mediated aversive learning as a function of locomotor activity in a novel environment in infant Sprague-Dawley rats

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ABSTRACT

Unlike adult heterogeneous rats, infant rats are sensitive to ethanol's locomotor stimulating effects. Susceptibility to this ethanol effect varies as a function of baseline locomotor activity levels. Infant rats with higher baseline activity levels are more sensitive to ethanol's stimulating effects than those with lower baseline activity levels. The present study was designed to analyze susceptibility to ethanol-induced motivational learning in subpopulations of infant heterogeneous rats that differ in baseline activity in a novel environment. On postnatal day 11 (PD 11) baseline locomotor activity was registered and infants were divided into high and low responders (HR, LR). In Experiment 1, pups were trained in a procedure of conditioned taste aversion employing ethanol (0.0, 0.5 or 2.5 g/kg) as unconditioned stimulus (US) and saccharin as conditioned stimulus. In Experiment 2 the same procedure was employed with LiCl (0.0, 0.25 or 0.5% of body weight of a 0.3 M LiCl solution) as US. HR were more resistant to the aversive effects of ethanol than LR while magnitude of LiCl-induced conditioned taste aversion was similar in HR and LR. These results suggest the possibility of early detection of subpopulations of rats with differential sensitivity to ethanol's effects.

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During the first two postnatal weeks of life, preweanling rats are highly sensitive to ethanol's effects (we consider preweanling the period before the young animal is weaned, usually beginning at birth and including the neonatal period). Voluntary ethanol consumption is higher in 8- and 12-day-old infant rats than in later stages of development (Sanders and Spear, 2007; Truxell and Spear, 2004; Truxell et al., 2007). In addition, during the first and second postnatal weeks infant rats are predisposed to acquire appetitive reinforcement mediated by ethanol (Arias and Chotro, 2006a; Chotro and Arias, 2007; Molina et al., 2007; Petrov et al., 2003) and seem more resistant than older rats to aversive consequences of the drug (Arias and Chotro, 2006a; Chotro and Arias, 2007; Hunt et al., 1991). Acute tolerance to motor impairment (sedation) effects of ethanol is more marked in infant than adult heterogeneous rats (Arias et al., 2008; Silveri and Spear, 2001). Finally, unlike adult rats (Chuck et al., 2006; Erickson and Kochhar, 1985), at least during the second postnatal week of postnatal life heterogeneous rats are sensitive to ethanol's locomotor stimulating effects (Arias et al., 2009a,b, 2008). This effect was clearly observed when infant rats received a relatively high ethanol dose (1.25 or 2.5 g/kg) and were tested in a novel environment during the rising phase of the blood ethanol curve. The

post-administration interval in which preweanling rats display ethanol-induced locomotor activation coincides with that in which ethanol induced conditioned appetitive responses (Molina et al., 2007). This temporal coincidence suggests a possible association between ethanol's activating effects and positive motivational effects of the drug during early ontogeny in the rat.

In several animal models, increased susceptibility to ethanol's reinforcing or locomotor stimulating effects is accompanied by lower sensitivity to ethanol-induced aversive effects. For example, inbred rat strains genetically selected for increased ethanol consumption, such as Alcohol-preferring (P), University of Chile B (UChB), Alko-alcohol (AA), Marchigian Sardinian (MsP) and Sardinian alcohol-preferring (sP) rats, are less sensitive to ethanol's aversive effects and more sensitive to ethanol-induced locomotor stimulation than strains selected for low ethanol intake (Bell et al., 2006; Ciccocioppo et al., 2006; Colombo et al., 1998; Colombo et al., 2006; Quintanilla et al., 2006; Sommer et al., 2006). Mice selectively bred for sensitivity to ethanol-induced locomotor stimulation (FAST and SLOW mice) also differ in susceptibility to ethanol-induced conditioned taste aversion, with FAST mice more resistant than SLOW mice to the aversive effects of ethanol (Risinger et al., 1994). Finally, adult rats with high baseline locomotor activity levels (high responders, HR) are also more sensitive to ethanol's activating effects than those with low baseline activity (low responders, LR: Cools and Gingras, 1998; Gingras and Cools, 1996; Hoshaw and Lewis, 2001). We didn't find studies

analyzing sensitivity to ethanol-induced taste aversion as a function of locomotor activity in a novel environment, but it has been found that HR are more resistant to the aversive effects of amphetamine than LR (Kunin et al., 2001). Studies that have examined ethanol self-administration in these subpopulations of heterogeneous rats (HR and LR) have not agreed about their conclusions. Although a positive relationship between ethanol self-administration and response to novelty was reported in a few studies (Nadal et al., 2002; Nowak et al., 2000), other studies failed to find this relationship (for example, Bienkowski et al., 2001; Fahlke et al., 1995; Samson and Chappelle, 1995), while another reported a negative association (Cools and Gingras, 1998).

Similar to what has been observed in adult rats (Cools and Gingras, 1998; Hoshaw and Lewis, 2001), in 12-day-old rats susceptibility to ethanol's activating effects also varies as a function of baseline locomotor activity levels. Infant rats with higher baseline activity levels are more sensitive to ethanol's stimulating effects than those with lower baseline activity levels (Arias et al., 2009c). In the study by Arias et al. (2009c) baseline activity did not predict other ethanol effects, such as hypothermia, motor impairment or motor suppressive effects of the drug. This result opened the possibility of early detection of subpopulations of heterogeneous rats with differential sensitivity to ethanol's effects.

The present study was designed to analyze susceptibility to ethanol-induced aversive learning among infant heterogeneous rats in accord with their baseline activity in a novel environment. If an association between response to a novel environment and susceptibility to ethanol-mediated aversive learning is observed, the present study could lead to early detection of traits that help predict differential response to ethanol. On postnatal day 11 (PD 11) baseline locomotor activity in a novel location was registered and infants were divided into HR and LR. In Experiment 1, pups were trained in a procedure of conditioned taste aversion employing ethanol as unconditioned stimulus (US) and saccharin as conditioned stimulus (CS, Experiment 1). Conditioned taste aversion is a paradigm commonly employed for assessment of motivational properties of drugs of abuse, including ethanol (Hunt and Amit, 1987; Parker, 1995). In this experimental paradigm animals learn to avoid a tastant (conditioned stimulus, CS) previously paired with ethanol's post-absorptive effects (US). Ethanol-mediated taste aversion is encountered in adult mice (Broadbent et al., 2002) and adult rats (Cailhol and Mormede, 2002; Eckardt, 1976) as well as infant rats (Arias and Chotro, 2006a,b; Hunt et al., 1990, 1991). In Experiment 2 the same procedure was employed with LiCl as US to test whether differences between infant HR and LR in the acquisition of conditioned taste aversion depend on the US employed. Adult HR and LR do not differ in terms of sensitivity to the aversive effects of LiCl (Kunin et al., 2001).

1. Experiment 1 and 2

1.1. Material and methods

1.1.1. Subjects

Sixty-five Sprague-Dawley pups (34 females and 31 males), representative of 11 litters, were utilized for Experiment 1, and sixty-six (33 males and 33 females) derived from another 11 litters were employed for Experiment 2. Animals were born and reared at the vivarium of the Center for Developmental Psychobiology (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 h after birth. All procedures were in accordance with the guidelines for animal care and use established by the National Institutes of Health (1986) and the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources,

1996) as indicated by the Binghamton University's institutional animal care and use committee.

1.1.2. Procedures

1.1.2.1. Phase 1: baseline activity.
On PD 11, pups from a given litter were separated from their mothers and placed in couples in a holding maternity cage (45×20×20 cm) partially filled with clean wood shavings. The floor of the cage was maintained at 33 °C (±1 °C) through the use of a heating pad. One hour later, locomotor activity was evaluated in a novel Plexiglas container (10×10×12 cm). The floor of this environment was lined with absorbent paper. A fresh piece of paper was employed for each animal. A circuit board (2-cm in width) surrounded the four sides of each chamber. This board had six infrared photo emitters and six infrared photoreceptors. The photo beams crossed the chamber generating a matrix of nine cells that allowed measurement of overall activity. Custom-made software developed by W. Kashinsky served to analyze the number of beams crossed by each subject every 10th of a second. Each activity test continued for 5 min and data were divided into 1-min bins. The dependent variable analyzed (number of beams broken) may reflect locomotion and other kind of behaviors as well, such us stereotypy. Stereotypy was not measured in this study. In a prior pilot study, the number of beams broken was highly and positively correlated with time walking and wall climbing during the second postnatal week of life, holding experimental conditions constant (PD8: rxy = 0.89, $n = 15$; PD12: rxy = 0.85, $n = 15$; PD15: rxy = 0.86, $n = 15$, all ps < 0.0001; rxy represents Pearson's product-moment correlation coefficient). At these ages we rarely observe stereotypy, particularly in baseline conditions.

For the second phase of the experiment, from each litter we selected three pups with the highest and three pups with the lowest locomotor activity scores, to be composed toward detection of differences in response to ethanol as a function of baseline activity.

1.1.2.2. Phase 2: conditioned taste aversion training.
On PDs 12 and 13, those six pups (with the highest and lowest baseline activity scores) from a given litter selected in the previous phase were separated from their mothers and placed in a holding maternity cage under the same conditions as those described for Phase 1. From a given litter, the three subjects that showed highest baseline activity scores were randomly assigned to one specific ethanol (Experiment 1: 0.0, 0.5 or 2.5 g/kg) or LiCl (Experiment 2: 0.0, 0.5 or 1.0% of body weight) condition. The same criterion of distribution was employed to assign pups that displayed lower activity levels during baseline.

Immediately after, an intraoral cannula (PE 10 polyethylene tubing, length: 5 cm, Clay Adams, Parsippany, NJ) was implanted in the right cheek of each pup, as previously described (Chotro and Alonso, 2003; Chotro and Arias, 2003). Briefly, a flanged end of the cannula was shaped by exposure to a heat source (external diameter: 1.2 mm). A dental needle (30-gauge Monoject, Sherwood Medical, Munchen, Germany) was attached to the non-flanged end of the cannula and positioned in the middle portion of the intraoral mucosa. The needle was inserted through the cheek and the cannula was pulled through the tissue until the flange end rested on the mouth's mucosa. This cannulation procedure requires no more than 20 s per subject and does not induce major stress in infant rats (Spear et al., 1989). Ninety minutes after cannulation, pup's bladders were voided by gentle brushing of the anogenital area. Following this procedure body weights were recorded and subjects were placed into individual Plexiglas chambers (10×10×12 cm). Then pups received an intraoral infusion of saccharin (CS, 0.05% w/v, duration: 10 min). Total administration volume was equivalent to 3.66% of the subject's pre-infusion body weight. Saccharin was delivered at a constant rate by means of a infusion pump (KD Scientific, Holliston, MA) connected to the oral cannula of each pup by a polyethylene catheter (Clay Adams, PE 50 Parsippany, NJ). When

Table 1
Descriptive statistics and sample size corresponding to baseline locomotor activity from subjects included in Experiment 1 and 2.

	Experiment 1	Experiment 2
Mean	130.94	146.80
Standard deviation	60.14	62.33
Minimum	17	36
Maximum	237	291
Median	140	141
N	65	66

employing these infusion parameters, pups are capable of either consuming or rejecting the infused solution (Arias and Chotro, 2006a; Chotro and Alonso, 1999, 2003; Dominguez et al., 1998). After the infusion procedure, subjects were weighed to estimate saccharin consumption scores. Percentage body weight gain (% BWG) was calculated as follows: $100 \times [(\text{Post-infusion weight} - \text{Pre-infusion weight}) / \text{Pre-infusion weight}]$. This dependent variable has been previously employed to estimate saccharin consumption in infant rats (for example, Hunt et al., 1991).

Immediately following CS exposure, pups received the corresponding ethanol (Experiment 1) or LiCl (Experiment 2) administration. Pups from Experiment 1 received an intragastric (i.g.) administration of 0.0, 0.5 or 2.5 g/kg ethanol. Ethanol was administered in a volume equivalent to 0.015 ml per gram of body weight of a 4.1% or 21% v/v ethanol solution. Pups given 0.0 g/kg received an equivalent volume of water. Subjects received the same dose of ethanol or LiCl on both PD12 and PD13. Within this range of ethanol dosage reliable conditioned taste aversion have been obtained during the infancy in rats (Arias et al., 2007; Hunt et al., 1990; Hunt et al., 1991). Pups from Experiment 2 were administered an intraperitoneal, (i.p.) injection of LiCl. Dosage was 0.0, 0.25 or 0.5% of body weight of a 0.3 M LiCl solution (Sigma Aldrich, St Louis, MO, USA). Pups from the control condition received an equivalent volume of vehicle (saline). As can be observed, we used different routes of administration for ethanol (i.g.) and LiCl (i.p.). In a recent study we observed that CTA induced by ethanol (2.5 g/kg intragastrically) or LiCl (0.5% of body weight of a 0.3 M solution, intraperitoneally) induced similar levels of taste avoidance and disgust reactions (measured through a taste reactivity

test) in preweanling rats (Arias et al., 2007). In addition, the present study is based on a recent investigation in which we observed a significant correlation between baseline locomotor activity and locomotor activity induced by ethanol (2.5 g/kg ethanol; Arias et al., 2009c). In this study ethanol was administered intragastrically, so we chosew this route of administration for ethanol in Experiment 1 as well.

After drug treatment, pups were returned to their holding cage, where they remained undisturbed for three hours until being reunited with their mother.

1.1.2.3. Phase 3: testing. On PD 14, pups were separated from their mothers, intraorally cannulated, and placed in pairs for 90 min in a heated holding cage. Then pups were tested in terms of saccharin intake for 10 min. The apparatus, parameters, and behavioral recordings of this test were similar to those described for the saccharin infusion procedure conducted on PDs 12 and 13. The intake score from one subject assigned to the 0.5 g/kg ethanol condition in Experiment 1 was not registered due to a technical problem with the infusion pump. Hence, this subject was excluded from the study.

1.1.3. Data analysis

Subjects from Experiments 1 and 2 came from independent litters. Hence, before the inferential analysis of the data we corroborated that baseline activity data were normally distributed and that means and variances did not differ across experiments. Descriptive statistics for baseline locomotor activity in Experiments 1 and 2 are presented in Table 1. Baseline data from each experiment were normally distributed (evaluated through the Kolmogorov–Smirnov test: Experiment 1: $d = 0.11$, and Experiment 2: $d = 0.06$, all $ps > 0.20$). Levene's test revealed that variances from samples assigned to each experiment were homogeneous [$F(1,129) = 0.02$, $p > 0.2$]. Finally, an ANOVA also revealed that baseline activity means did not differ across experiments [$F(1,29) = 2.20$, $p > 0.1$].

Pups were divided into two groups by the median of baseline locomotor activity registered on PD 11, high responders (HR) and low responders (LR). Locomotor activity during baseline (operationalized through the number of photo beams broken during the 5-minute test) was analyzed with a 3 [Conditioning treatment (Experiment 1: 0.0, 0.5
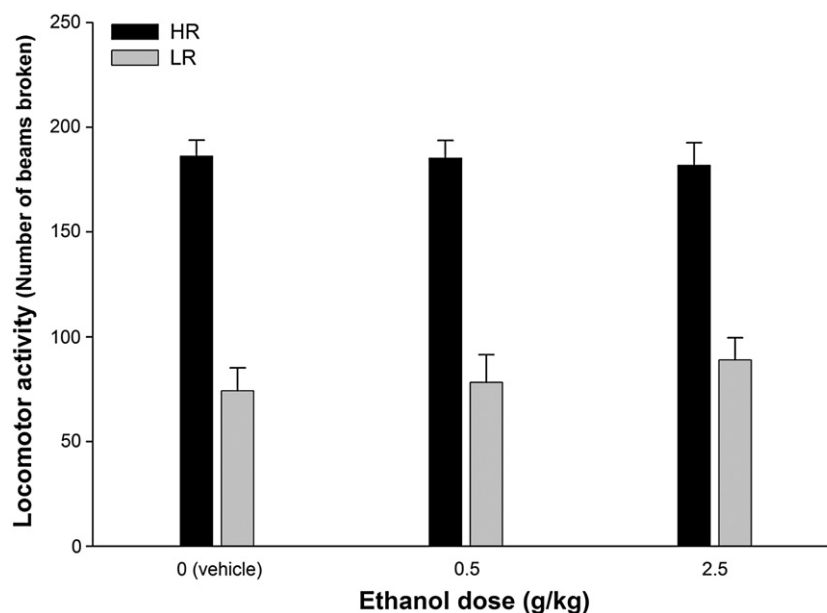

Fig. 1. Baseline locomotor activity (operationalized through the number of beams broken) registered on PD 11. Subjects are separated as a function of: a) Ethanol treatment (0, 0.5 or 2.5 g/kg) administered during conditioning; and b) locomotor activity level during baseline (high responders, HR, and low responders, LR). Vertical lines illustrate standard errors of the means. Group HR-0 ($n = 11$), Group HR-0.5 ($n = 12$), Group HR-2.5 ($n = 9$), Group LR-0 ($n = 11$), Gropu LR-0.5 ($n = 9$), Group LR-2.5 ($n = 13$).
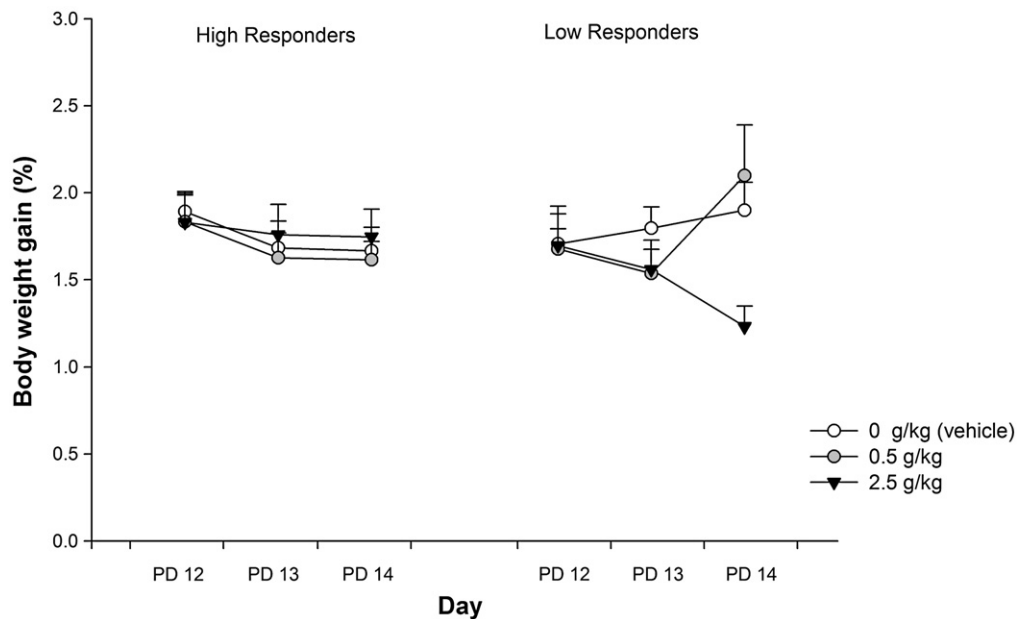
Fig. 2. Intake of saccharin (operationalized through the percentage of body weight gained) as a function of ethanol treatment (0.0, 0.5 and 2.5 g/kg ethanol) and baseline activity level (high responders Vs low responders) during conditioning (postnatal days 12 and 13) or testing (PD14). Vertical lines illustrate standard errors of the means. Group HR-0 ($n = 11$), Group HR-0.5 ($n = 12$), Group HR-2.5 ($n = 9$), Group LR-0 ($n = 11$), Gropu LR-0.5 ($n = 9$), Group LR-2.5 ($n = 13$).

or 2.5 g/kg ethanol; Experiment 2: 0.0, 0.25 or 0.5% LiCl)] by 2 (Baseline activity: HR vs LR) between-factor ANOVA. Intake scores (operationalized through the percentage of body weight gained during the intake test) were analyzed by means of a mixed ANOVA. In this case, conditioning treatment (Experiment 1: 0.0, 0.5 or 2.5 g/kg ethanol; Experiment 2: 0.0, 0.25 or 0.5% LiCl) and baseline activity (HR or LR) served as between-group factors, while day (conditioning day 1, conditioning day 2 and testing) was considered the within-group variable. Significant main effects and/or interactions were further analyzed by means of follow-up ANOVAs and post-hoc analysis [Duncan post-hoc tests]. All inferential analyses conducted in the present study employed an α level equal to 0.05. No significant effect of sex or

interaction with the remaining factors was found in any of the analysis performed in the present study. Hence, for the inferential analysis and descriptive presentation of the results, data were collapsed across sex.

2. Results

2.1. Experiment 1

Fig. 1 represents baseline activity scores as a function of subsequent ethanol treatment (0.0, 0.5 or 2.5 g/kg) and baseline activity level (HR vs LR). This analysis was conducted to corroborate that pups assigned to the different ethanol conditions before conditioning did not differ
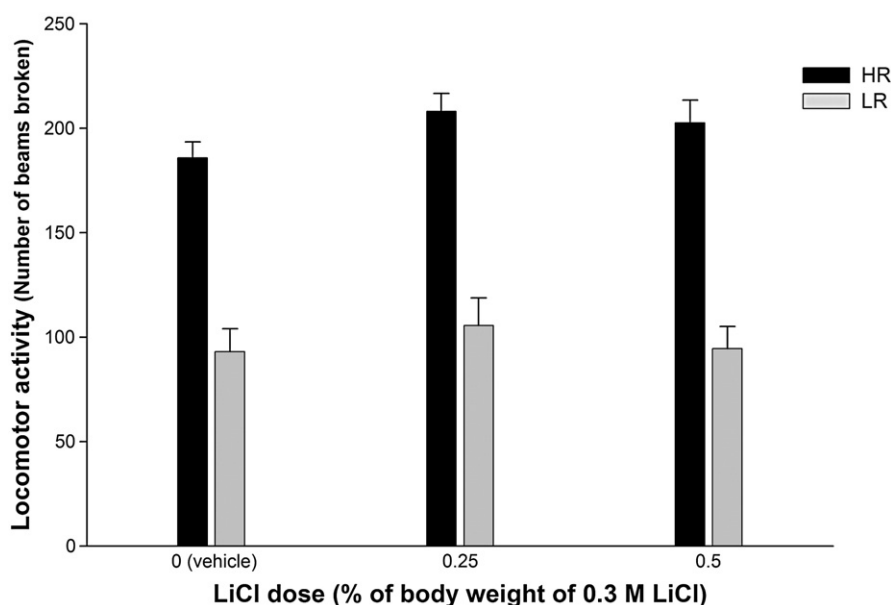

Fig. 3. Locomotor activity scores on PD11 (operationalized through the number of beams broken) as a function of LiCl dose (0, 0.25 or 0.50% % of body weight of 0.3 M LiCl). Subjects were divided as a function of baseline activity level in high or low responders (HR or LR). Vertical lines illustrate standard errors of the means. Group HR-0 ($n = 11$), Group HR-0.25 ($n = 9$), Group HR-0.5 ($n = 12$), Group LR-0 ($n = 11$), Gropu LR-0.25 ($n = 13$), Group LR-0.5 ($n = 10$).

Fig. 4. Intake of saccharin (operationalized through the percentage of body weight gained) as a function of ethanol treatment (0.0, 0.5 and 2.5 g/kg ethanol) and baseline activity level (high responders vs low responders) during conditioning (postnatal days 12 and 13) or testing (PD14). Vertical lines illustrate standard errors of the means. Group HR-0 ($n = 11$), Group HR-0.25 ($n = 9$), Group HR-0.5 ($n = 12$), Group LR-0 ($n = 11$), Gropu LR-0.25 ($n = 13$), Group LR-0.5 ($n = 10$).
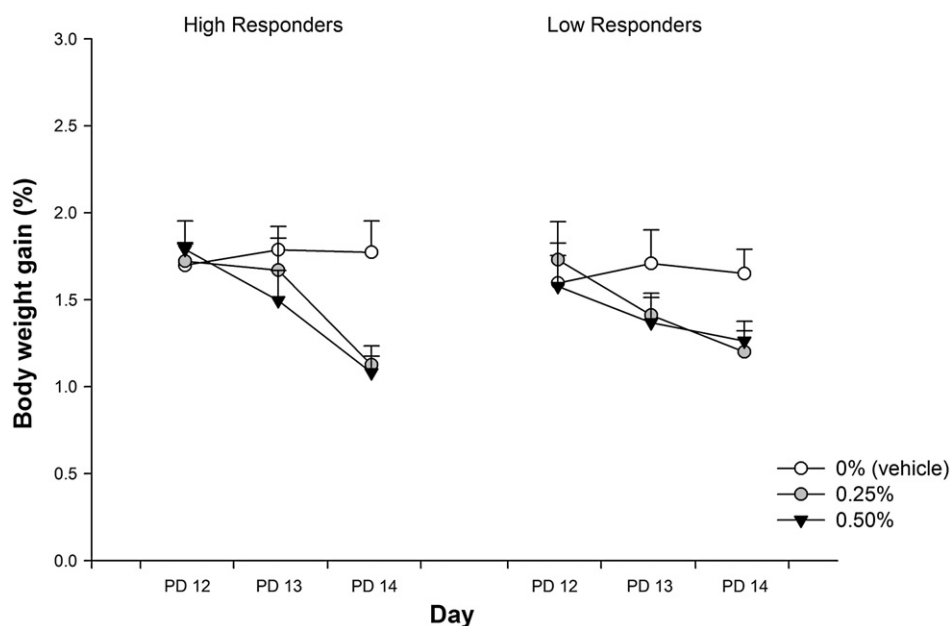
between them in terms of baseline locomotor activity. Baseline levels did not differ across conditions. This analysis also substantiated that baseline locomotor activity scores from HR were significantly higher than those from LR, $F(1,59) = 153.48$, $p < 0.001$.

Fig. 2 depicts intake of saccharin as a function of baseline activity, conditioning treatment and day. At testing, LR given 2.5 g/kg consumed less saccharin than LR given water, an effect that was not evident in HR. The ANOVA revealed a significant interaction involving ethanol treatment, baseline activity and day, $F(4, 118) = 2.82$, $p < 0.05$. To determine the loci of this significant interaction, follow-up two-way ANOVAs were performed considering conditioning treatment and baseline activity as independent factors, and analyzing separately intake data from each conditioning or testing day. No significant differences between groups were detected at conditioning (PDs 12 and 13). The ANOVA for testing revealed a significant interaction between conditioning treatment and baseline activity [$F(2, 60) = 5.29$, $p < 0.01$]. Post-hoc analyses indicated no differences as a function of ethanol treatment in the HR condition, but in the LR condition, pups given 2.5 g/kg ethanol as the US ingested less saccharin than pups given either 0 or 0.5 g/kg ethanol as US. Post-hoc analyses also revealed that HR given 2.5 g/kg ethanol consumed more saccharin than LR given the same ethanol dose and LR administered with 2.5 g/kg ethanol consumed less ethanol than LR treated with water.

2.2. Experiment 2

According to the corresponding ANOVA, in Experiment 2 HR also had higher locomotor activity scores than LR at baseline, $F(1,60 = 120.76$, $p < 0.0001$. As expected, pups that subsequently differed in LiCl condition did not differ in baseline activity (see Fig. 3).

Fig. 4 represents intake of saccharin (CS) as a function of LiCl treatment, baseline activity level and day. The ANOVA revealed a significant main effect of day, [$F(2,120) = 10.77$, $p < 0.001$], and interaction between LiCl treatment and day, [$F(4,120) = 3.83$, $p < 0.01$]. To determine the loci of this significant interaction, follow-up one-way ANOVAs were performed considering conditioning treatment as the only independent factor, and analyzing intake data from each day separately. No significant differences between groups were detected at conditioning. The ANOVA for testing day revealed a significant

effect of conditioning treatment, [$F(2,60) = 11.54$, $p < 0.0001$]. Regardless of LiCl dose, pups ingested less saccharin than controls, and unlike the results with ethanol as US, this was so whether they were HR or LR.

3. Conclusion

Recent findings from our laboratory suggest that baseline activity is a valuable predictor of ethanol's stimulating effects in infant rats: pups with high baseline activity levels are more sensitive to ethanol's locomotor stimulating effects than those with low baseline activity levels (Arias et al., 2009c). According to the present study, high responders (HR) are more resistant to the aversive effects of ethanol than are low responders (LR). In Experiment 1, 2.5 g/kg ethanol induced clear conditioned taste aversion in LR, but not in HR. However this differential sensitivity seems to be specific to ethanol, since in Experiment 2, LiCl-induced conditioned taste aversion was similar in HR and LR. This result also indicates that differences in ethanol-mediated conditioned taste aversion between HR and LR are not due to differences in their learning capabilities.

Infant HR apparently are more sensitive to ethanol's activating effects than LR, although less sensitive to ethanol-induced conditioned taste aversion. This suggests that similar neurochemical pathways underlie ethanol-induced conditioned taste aversion and locomotor stimulation. For example, Dopamine D1 and D2 receptor antagonists attenuate ethanol's activating effects in infant and adult (Pastor et al., 2005) rodents. Similarly, conditioned taste aversion induced by ethanol can be attenuated by antagonists of these dopamine receptors (for example, Risinger et al., 1999). Furthermore, inhibition of NMDA receptors attenuates both ethanol-induced locomotor stimulation (Liljequist, 1991) and conditioned taste aversion (Bienkowski et al., 1998).

An association between ethanol-induced locomotor stimulating effects and appetitive reinforcing properties of the drug has been hypothesized (Robinson and Berridge, 1993; Wise and Bozarth, 1987). Recent results from our laboratory also suggest this association during the infantile period of the rat, since the post-administration interval in which infants rats display ethanol-induced locomotor activation coincides with that in which ethanol induces conditioned appetitive

responses (Molina et al., 2007). Since infant HR are more sensitive than LR to the stimulating effects induced by ethanol (Arias et al., 2009c), it is plausible that pups with high baseline locomotor activity levels are also more sensitive to ethanol-induced appetitive learning. We have not yet tested this hypothesis directly, but recently found that infant HR ingest more ethanol than LR (Miller, Arias, and Spear, 2008). However this result is not conclusive, because ethanol consumption can be modulated either by sensitivity to the rewarding or aversive effects of the drug (Broadbent et al., 2002; Green and Grahame, 2008). Greater sensitivity to the rewarding properties of ethanol among HR pups may be why HR pups are less sensitive than LR to ethanol's aversive effects. This might also explain why there were no differences between infant HR and LR in LiCl-induced taste aversion learning, since LiCl is not a drug that induces appetitive learning. Studies with adult rats have observed that HR are also less sensitive than LR to amphetamine-induced taste aversion learning but do not differ in acquisition of LiCl-mediated aversive conditioning (Kunin et al., 2001). According to Kunin et al. (2001), this result may explain why adult HR self-administer more amphetamine than LR (Kabbaj, 2004, 2006; Piazza et al., 1989). We also consider relevant the fact that outbred mice sensitized to ethanol's locomotor activating effects were more resistant than non-sensitized mice to ethanol-induced, (2 g/kg), but not LiCl-induced, conditioned taste aversion (Lessov, Risinger and Phillips, 2001).

In several animal models, sensitivity to ethanol's aversive effects has been negatively related to ethanol consumption and ethanol's activating and rewarding effects. For example, mouse strains showing stronger taste aversion had lower ethanol preference (Broadbent et al., 2002). Mice selectively bred for sensitivity to ethanol-induced locomotor stimulation are more resistant to ethanol's aversive effects and also ingest more ethanol than those bred for low sensitivity to ethanol's activating effects (Risinger et al., 1994). Similarly, rat strains genetically selected for increased ethanol intake, such as P, UChB, AA, MsP and sP alcohol-preferring rats, are more resistant to ethanol-induced conditioned taste aversion and more sensitive to ethanol's activating effects than strains selected for low ethanol intake (Bell et al., 2006; Ciccocioppo et al., 2006; Colombo et al., 2006; Quintanilla et al., 2006; Sommer et al., 2006). Overall, these results suggest that these traits may be related, although there are also studies that do not support this hypothesis. For example, STDRHI mice, which were genetically selected for high ethanol consumption, showed less ethanol-induced activity and greater sensitivity to ethanol's reward-ing properties than STDRLO, selected for low ethanol intake. However, these strains did not differ in terms of ethanol-induced conditioned taste aversion (Phillips et al., 2005). Mice genetically selected for high sensitivity to ethanol-induced conditioned taste aversion consumed less ethanol than mice selected for resistance to ethanol's aversive consequences, but these strains did not differ in terms of ethanol-induced locomotor stimulation (Phillips et al., 2005). The widely used strain of inbred mouse, C57BL/6J (B6), voluntarily consumes larger amounts of ethanol than DBA/2J (D2) mice (Yoneyama, Crabbe, Ford, Murillo and Finn, 2008). But the C57BL/6J (B6) strain do not typically show ethanol-induced locomotion activation (or only under very restrictive conditions, e.g., Middaugh et al., 1987, while DBA/2J (D2) mice are highly sensitive to ethanol's activating effects (e.g., Chester and Cunningham, 1999). Finally, in a recent study ethanol-induced locomotor stimulation in Long-Evans rats failed to correlate with ethanol preference or ethanol intake (Chappell, and Weiner, 2008).

It is also possible that although greater sensitivity to ethanol-induced locomotor stimulation and greater resistance to ethanol-mediated conditioned taste aversion coexist in the same subpopula-tion of infant rats, these responses are not related functionally. In other words, HR may be more resistant than LR to ethanol-induced aversive learning independently of differences that might exist in their sensitivity to ethanol's rewarding properties. This hypothesis would also require that ethanol- and LiCl-mediated aversive learning are

modulated by mechanisms that are at least partially different, since HR and LR do not differ in terms of sensitivity to LiCl-induced aversive learning.

Taste avoidance induced by emetic drugs (e.g., LiCl) and psychoac-tive drugs (e.g., amphetamine or cocaine) seems to be regulated by different mechanisms. Parker (1995; 2003) proposed that the conditioned taste aversion induced by psychoactive drugs may be qualitative and mechanistically distinct from that mediated by aversive emetic agents, such as LiCl. This hypothesis is supported, for example, by the fact that treatments that alleviate nausea do not affect the establishment of taste avoidance induced by psychoactive drugs, but they interfere with the establishment and expression of taste aversion induced by emetic agents (Parker, 1995, 2003). According to Parker taste avoidance induced by psychoactive drugs is mediated by fear conditioning, likely promoted by drug-related changes in homeostatic states. Parker based this hypothesis in the fact that rats cannot vomit, and, because of that, any novel homeostatic change signals danger to this rodent. Hence, a flavor associated with homeostatic change would indicate a potential risk for the rat and would result in subsequent avoidance of that taste (see Parker, 2003). An alternative hypothesis to explain taste avoidance generated by psychoactive drugs was provided by Grigson (1997). Grigson suggested that intake suppression (taste avoidance) induced by psychoactive drugs reflects the appetitive, reinforcing effects of these substances. Specifically, rats may avoid drinking the drug-paired CS because they are anticipating a more rewarding stimulus, the appetitive properties of the drug. In other words, the value of the taste CS is outweighed by the much preferred drug state, a process leading to the animal avoiding the taste CS on the basis of incentive contrast (Flaherty et al., 1996; Grigson, 1997).

On the other hand, it seems to be clearer that taste aversion reflects conditioned nausea (Parker, 1995, 2003). Ethanol is a complex psychoactive drug that shares with other drugs of abuse the capacity for activating the mesocorticolimbic dopaminergic system (Boehm et al., 2002; Di Chiara and Imperato, 1985; Tupala and Tiihonen, 2004; Xiao et al., 2007). Adding to its complexity, there is evidence that at least high ethanol doses also can induce nausea. For example, CSs paired with high ethanol doses (3.5 g/kg) or LiCl induce similar c-Fos activity in the area postrema, the lateral parabraquial nucleus and the nucleus of the solitary tract (Thiele et al., 1996). These structures are located in the brainstem and participate in nausea and emesis (Yamamoto et al., 1994). In fact, there is evidence supporting the hypothesis that emetic-like effects underlie ethanol's capability to produce taste aversion (Hunt and Amit, 1987; Orr, Whitford-Stoddard, & Elkins, 2004). In addition 13-day-old rats exhibit similar patterns of conditioned disgust reactions when stimulated with a taste previously paired with either LiCl or ethanol, even when these drugs were titrated to induce similar levels of taste avoidance (Arias et al., 2007). Hence, taste aversion mediated by this drug may be associated with conditioned nausea.

Although suppression of tastant CSs previously paired with ethanol intoxication may be modulated by mechanisms that underlie taste avoidance induced by any psychoactive drugs, there are relatively unique effects of ethanol that can contribute to ethanol-induced conditioned taste aversion, such as motor impairment or hypothermia, which occur may act also during the infancy of the rat (Hunt et al., 1991). In a recent study, however, we did not find differences in ethanol-induced hypothermia or motor impairment as a function of baseline locomotor activity in 12-day-old rats (Arias et al., 2009c).

Toward identification of ontogenetic change in ethanol's effects, it will be interesting to resolve in future studies whether neurochemical differences observed in adult HR and LR are also present in infant HR and LR, and also to analyze whether baseline activity levels during infancy are predictive of response to the stimulatory effects of ethanol in later stages of development. In adult rats, differences in response to

a novel environment seem be associated with differences in sensitivity of the mesocorticolimbic dopaminergic system and HPA axis (Kabbaj, 2006; Piazza and Le Moal, 1997). There is not much information about these differences during early development. In one study it was reported that differences between high responders and low responders in dopaminergic and HPA-axis activity are not present in 10-day-old preweanling rats (see Cools and Gingras, 1998). These authors also indicate that by the end of the preweanling period (PD 18) ACTH plasma levels under baseline conditions are greater in high responders than in low responders, and that differences in dopaminergic activity emerge later in development. These conclusions are based on expression of m-RNA of D1 and tyrosine hydroxylase (see Cools and Gingras, 1998). However, it has been found recently that activity of the dopamine transporter, rather than tyrosine hydroxylase, may represent the critical factor in the enhanced susceptibility of high responders to locomotor activating effects of amphetamine during adulthood (Dietz et al., 2005). More research will be required to specifically determine the nature of the neurochemical differences between high and low responders. We recently reported that infant HRs are more sensitive to ethanol's locomotor stimulating effects than LR (Arias et al., 2009c), an ethanol effect that seems to be mediated by the dopaminergic system even during infancy (see Arias et al., 2009a). These data suggest that differences between infants HR and LR may be associated with the dopaminergic system.

With respect to how the two major classes of ethanol reinforcement, appetitive or aversive, fit together generally in relation to ethanol's activation and sedation effects, our guess is that neither ethanol's reinforcement effect (i.e., appetitive vs aversive) nor its activating effect (i.e., increase or decrease) is absolute, even for a particular dose. Instead, the expression of ethanol reinforcement for any given dose of ethanol can be more or less appetitive (or aversive) depending on genetic (alcohol preferring or nonpreferring) or ontogenetic disposition, the CS, BEC and its previous course(increasing or decreasing) and arousal, as well as cognitive or environmental features that determine memory retrieval at the time of the test. And the same conditions, probably except those influencing memory retrieval, are likely to determine whether a given dose induces ethanol-induced increase or decrease in general activity.

In summary, the present evidence in conjunction with recent findings from our laboratory suggests that differential sensitivity to ethanol's effects may be detected in subpopulations of preweanling rats that differ in baseline activity levels in a mildly novel context. These results may help to investigate early detection of traits that could predict differential responses to drugs of abuse, as well as developmental origins of behavioral traits that predict differential response to drugs of abuse.

Acknowledgements

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