Pharmacology, Biochemistry and Behavior 150–151 (2016) 68–75

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Transition from ethanol-induced sensitization to tolerance across early and late infancy in the rat

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ARTICLE INFO

Article history:
Received 8 June 2016
Received in revised form 12 August 2016
Accepted 29 September 2016
Available online 30 September 2016

Keywords:
Ethanol
Locomotor activity
Tolerance
Sensitization
Infant rat

ABSTRACT

Drugs of abuse, as cocaine or amphetamine, induce locomotor sensitization during infancy and adulthood of the rat. This effect during the preweanling period is observed only after a short interval of time between training and testing. We recently reported short-term locomotor sensitization induced by ethanol in pups chronically exposed to the drug during the second postnatal week of life. The present series of experiments was designed to explore the persistence of the sensitization effect across the preweanling period. Pups were chronically exposed to ethanol in five consecutive days during the second or the third postnatal weeks, and their locomotor activity was evaluated in an open field 3, 8 or 15 days later. Our results showed that, contrarily to what has been observed with other drugs during infancy, sensitization to ethanol persisted at least 8 days in rats exposed to the drug during the second postnatal week. Surprisingly, in older pups, the same procedure induced tolerance instead sensitization. This ontogenetic model offers a potentially interesting tool for studying within the same species, how tolerance and sensitization are interrelated, and how these effects affect ethanol-mediated reinforcement and ethanol intake during ontogeny.

1. Introduction

Ethanol is a drug capable of inducing biphasic effects that can be determined through measuring various behaviors. In rodents, these opposing effects have mainly been studied through the analysis of locomotion. Mice have a tendency to increase locomotor activity in response to ethanol (Phillips et al., 1997), while rats tend to exhibit sedation even after the administration of low doses of the drug (Chuck et al., 2006). However, under particular conditions, ethanol can also stimulate this behavior in rats. Among these exceptions are: a) when the drug is locally (intraventricular) administered (Correa et al., 2003); b) when using rat strains selected for high ethanol consumption (Quintanilla, 1999); c) when specific subpopulation of adults are examined, such as high responders to novelty (Hoshaw and Lewis, 2001); and d) when using subjects from particular ontogenetic stages, including infancy (Arias, Mlewski, Molina, & Spear, 2009) or adolescence (Acevedo et al., 2010). The importance of studying this ethanol effect has recently been highlighted with the argument that, in humans, it is a good predictor of later ethanol abuse and future alcohol problems. For instance, King and collaborators found that an increased sensitivity to the stimulating effect of ethanol significantly predicts the number of alcohol use disorder symptoms (King et al., 2011; King et al., 2015; King et al., 2014).

A number of studies have consistently reported that rats at early ontogenetic stages of development are highly sensitive to a variety of ethanol effects. Furthermore, early exposure to the drug (during late gestation or infancy) can induce long-lasting changes in responsiveness to ethanol. Infant rats - particularly during the 2nd postnatal week of life - ingest high amounts of ethanol (Sanders and Spear, 2007; Truxell and Spear, 2004; Truxell et al., 2007). They are predisposed to appetitive learning induced by ethanol (Arias and Chotro, 2006; Chotro and Arias, 2007; Molina et al., 2007) and show strong locomotor stimulation in response to medium-to-high ethanol doses (1.25 to 2.5 g/kg, Arias et al., 2009a; Arias et al., 2009b). Recently, we have reported that repeated exposure to ethanol during the 2nd postnatal week induces both tolerance and sensitization to the stimulating motor effect, depending on a variety of procedural variables (Castello et al., 2015). Tolerance was found when infant rats (males or females) were tested in the same context in which they were previously exposed to ethanol. In contrast, sensitization was only observed in males when they were tested in a novel context, and when subjects were trained within the 2nd postnatal week of life. Sensitization induced by ethanol is infrequent in adult rats (Masur et al., 1986; Nestby et al., 1997), and although it is not clear why, some authors have proposed the possibility that the ethanol dosage required to promote locomotor sensitization produces strong sedation in the adult rat (Hoshaw and Lewis, 2001).

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http://dx.doi.org/10.1016/j.pbb.2016.09.010
0091-3057/

McDougall and collaborators have systematically described, during early stages of development, locomotor sensitization induced by a variety of psychostimulant drugs, such as cocaine (McDougall et al., 2007; McDougall et al., 2009a; McDougall et al., 2009b; McDougall et al., 2011), D-amphetamine (McDougall et al., 2011; McDougall et al., 2013), methylphenidate (Crawford et al., 1998; McDougall et al., 1999), U-50, 488 (Collins et al., 1998) and methamphetamine (Crawford et al., 2003). A common characteristic of sensitization in subjects trained during infancy is that this effect usually persists for only 48-h after training (Collins et al., 1998; McDougall et al., 1999; McDougall et al., 1994), while in adult rodents sensitization has been described as a more long-lasting effect that can persist even months after drug exposure (Balda et al., 2009; Lessov and Phillips, 1998; Williams and Steketee, 2005). In our previous study, we observed sensitization induced by ethanol after a 72-hour interval between training and testing (Castello et al., 2015), but we did not evaluate the persistence of this effect. As mentioned, exposure to ethanol during early ontogeny can affect later reactivity to the drug even after a long time interval (Chotro et al., 2007; Spear and Molina, 2005). For instance, moderate exposure to ethanol for a few days at the end of the gestational period facilitates operant self-administration of the drug in newborns rats (March et al., 2009; Miranda-Morales et al., 2010; Miranda-Morales et al., 2014) and affects ethanol consumption during infancy (Arias and Chotro, 2005; Chotro and Arias, 2003) or even adolescence (Fabio et al., 2015; Chotro and Arias, 2003). Since the sensitization process has been associated with drug abuse (Robinson and Berridge, 2001, 2003), it is important to analyze whether sensitization induced by ethanol during infancy rapidly decays (similar to the effect induced by psychostimulants), or if it persists for a longer period of time.

The present series of experiments have two chief aims. Firstly, we explore whether sensitization induced by ethanol during the 2nd postnatal week of life persists over time by testing animals 1 or 2 weeks after training. The second goal is to explore whether sensitization induced by ethanol can be also observed in older infants. In our previous study, when training was carried out during the 3rd postnatal week, ethanol did not induce sensitization. It is likely that the dose of ethanol used at testing in that study (2.5 g/kg) was too high to explore stimulating effects in rats older than 2 weeks. Therefore, in the present study we employed lower ethanol doses at testing to avoid possible interfering sedative effects.

2. Experiment 1

In a previous study (Castello et al., 2015) we found that rats trained between postnatal days (PDs) 8 and 12 with a daily ethanol dose of 2.5 g/kg showed locomotor sensitization when tested on PD 15 in response to the same ethanol dose. This effect required that the testing context was different to that used in training (Castello et al., 2015). In the present experiment we analyzed sensitization induced by ethanol in preweanling rats using a wider range of ethanol doses on test. Rats were trained with 2.5 g/kg during PDs 8 to 12, and on PD 15 we tested their locomotor response after various ethanol doses (0.5, 1.5 or 2.5 g/kg).

2.1. Materials and methods

2.1.1. Subjects

For Experiment 1, we used a total of 57 male Wistar pups representative of 12 litters. In the present study we did not use females, since we found sensitization exclusively in males during the preweanling period. Animals used in the present experimental series were born and reared at the vivarium of the Instituto de Investigación Médica Mercedes y Martín Ferreyra, INIMEC-CONICET-UNC, under conditions of constant room temperature (22 ± 1.0 °C), on a 12 h light–12 h dark cycle. Births were examined daily and the day of parturition was termed PD 0. Litters were culled to 10 pups, 5 males and 5 females where possible. Subjects were 8 days old at the start of the experiment. All procedures were approved by the National Department of Animal Care and Health (SENASA–Argentina) and were in compliance with the National Institute of Health's general guidelines for the Care and Use of Laboratory Animals.

2.1.2. Apparatus

In this and subsequent experiments, all animals were tested in a circular open field (30 cm diameter for pups tested on PD 15; 38 cm diameter for rats evaluated after PD 15), with a white plastic wall and floor. A piece of cotton infused with almond odor (almond scent, 1 ml of a 0.1% solution v/v, Esencias del Boticario, Córdoba, Argentina) was placed on the top of the open field. The almond odor was included as a contextual cue since during this ontogenetic period infants can learn about contexts, including explicit odors (Revillo et al., 2015). In all experiments, locomotor activity was estimated through an index that was calculated by counting the number of quadrants that the subject crossed during the testing session. For this purpose, the floor of the open field was divided into four quadrants. Testing sessions were videotaped, and were later evaluated by a researcher blind to the treatments, who counted the number of quadrants crossed. Every time a pup passed its head and forepaws across one of the lines that divided the quadrant, the quadrant was considered to have been crossed.

2.1.3. Procedures

2.1.3.1. Training phase. This phase was conducted between PDs 8 and 12 (one session per day). On PD8, the pups were separated from their mothers and placed in pairs in a holding cage (25 cm × 23 cm × 23 cm) partially filled with clean wood shavings. The floor of the cage was maintained at 36 °C (±1 °C) through the use of a heating pad. Four hours later, pups were randomly assigned to one experimental group and their body weights were individually recorded. Immediately after they received an intragastric (i.g.) administration of water or ethanol (2.5 g/kg). This ethanol dose was selected because it consistently induces locomotor stimulation in preweanling rats (Arias et al., 2009b) and, more recently, we reported that the same chronic exposure to ethanol at this age induced locomotor sensitization in male rats (Castello et al., 2015). The volume administered was equivalent to 0.015 ml per gram of bodyweight of a 21% (v/v) ethanol solution. Pups assigned to the vehicle control group received the same volume of tap water. 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 × 1/2 needle. This tubing was gently inserted through the mouth and slowly guided into the stomach. The entire procedure took <20 s per pup. After the i.g. administration, pups remained in pairs in the holding cage for 10 min until being returned to their home cages.

2.1.3.2. Testing phase. After two days of withdrawal (on PD 15) pups were evaluated in response to water or ethanol in terms of locomotor activity after 4-h of maternal separation. In this case, three different ethanol doses were used (0.5, 1.5 or 2.5 g/kg), in order to evaluate whether animals tested with a lower ethanol dose than the one used at training were also capable of displaying locomotor sensitization. Half of the subjects trained with water received the same treatment on the test session, while the other half were administrated with one of the ethanol doses. In the case of the animals trained with ethanol, half of them were tested with water, while the other half was evaluated in response to ethanol. Locomotor scores from water-tested animals are not strictly necessary to show sensitization. However, we included it to illustrate the acute stimulating effect of the drug, similarly to other studies in the field (McDougall et al., 1994; McDougall et al., 2009a, 2009b; Zavala et al., 2000).

Five min after the i.g. administration, male pups were placed in the open field (see Section 2.1.2), where their behavior was videotaped

for further analysis of locomotor activity. Locomotion was estimated through the total number of quadrants crossed during testing. With this measure system, we have reproduced all the effects previously reported in previous research (Arias et al., 2009a, 2009b), including sensitization (Castello et al., 2015). In a previous study we used a 5-min test period, although the stimulating effect was only observed during the first minutes of the test (Castello et al., 2015). For this reason, the length of the testing session in the present study was 3 min.

2.1.4. Experimental design and statistics

A preliminary ANOVA was conducted to explore whether animals trained with water or ethanol and evaluated with water differed in their locomotor scores on test. This analysis did not reveal any significant difference between the W-W and E-W groups (see Table 1), and therefore, as in our previous work (Castello et al., 2015), all of the subjects evaluated with water were included in the same control condition (Control). Thus, the analysis for this study comprised one between-group variable named Group, composed of 7 independent experimental conditions: Control, W-0.5, W-1.5, W-2.5, E-0.5, E-1.5, and E-2.5. The letter of the names of the experimental groups indicates training treatment with water (W) or ethanol (E), while the number indicates the testing treatment (0.5, 1.5 or 2.5 g/kg ethanol).

The dependent variable analyzed was locomotor activity. In this, as well as in the following experiments, the loci of the significant main effects were further explored using post-hoc tests (Newman-Keuls) with an alpha level set at 0.05.

2.2. Results

Fig. 1 represents locomotor activity scores as a function of Group (Control, W-0.5, W-1.5, W-2.5, E-05, E-1.5 or E-2.5). The ANOVA revealed a significant main effect of Group [$F(6, 50) = 6.36$, $p < 0.05$]. According to the post-hoc tests, only the higher ethanol dose promoted acute locomotor stimulation (locomotor scores from the W-2.5 group were significantly higher than those from the Control group). Interestingly, the ethanol treatment at training promoted sensitization, an effect that was expressed when subjects were tested with the two higher doses, 1.5 or 2.5 g/kg. Locomotor activity scores from these subjects were significantly higher than those from their respective control conditions trained with water (W-1.5 and W-2.5, respectively) and from the Control group. Also, the analysis showed that the lowest dose used (0.5 g/kg) did not exert any effect on locomotion (see Table 2). These results show that administration of ethanol during the 2nd postnatal week can induce locomotor sensitization, an effect that was expressed in response to moderate-to-high ethanol doses.

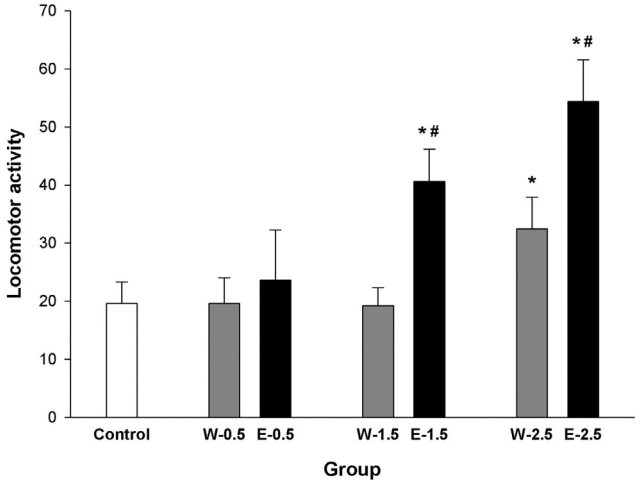

Fig. 1. Locomotor activity scores at testing from pups trained during the 2nd postnatal week of life and tested after a 3-days interval with one of three ethanol doses. * indicates significant differences from Control group, $p < 0.05$. # represents significant differences from the specific ethanol control group, $p < 0.05$. Vertical bars illustrate standard errors of the mean.

3. Experiment 2

The goal of the second Experiment was to explore whether sensitization induced by ethanol in preweanling rats persists after a longer interval between phases. Previous studies have characterized sensitization during this ontogenetic period as a short-term effect, which lasts for 2 days, but not for a longer period of time such as 8 days (Collins et al., 1998; McDougall et al., 1999; McDougall et al., 1994). These studies, however, used only psychostimulants such as amphetamine or cocaine. We evaluated whether this feature of sensitization observed particularly during infancy in response to some psychostimulants also characterizes sensitization induced by ethanol. For this purpose, rats were trained between PDs 8 and 12 following the procedure used in Experiment 1, and they were tested in response to water or ethanol 8 or 15 days after training.

3.1. Materials and methods

3.1.1. Subjects

In this experiment we used 35 male pups representative of 9 litters for the 8 withdrawal days, and 23 male pups from 6 litters for the longer interval (15 days). The training phase started when pups were 8-days-old.

3.1.2. Procedures

Training was carried out between PDs 8 and 12, and testing occurred after an interval of 8 or 15 days, on PD 20 or 27. For training and testing we followed the same procedures as those described for Experiment 1. The ethanol dose used for training was 2.5 g/kg, and the ethanol dose selected for testing was 1.5 g/kg, because in a previous study we did not find evidences of sensitization using at testing a higher ethanol dose (2.5 g/kg) in rats tested on PD 21 (Castello et al., 2015).

Table 1
Locomotor activity scores at testing carried out 3, 8 or 15 days after training, depending on the experiment. Rats were trained with water (W) or ethanol (E), while all of them received water at testing. Values represent mean and standard error of the mean.

Experiment	Interval	Group	Mean	SE	N
1	3-days	W-W	15.33	4.89	6
		E-W	23.28	5.34	7
2	8-days	W-W	28.33	3.33	9
		E-W	29.62	2.65	8
	15-days	W-W	19.80	1.15	6
		E-W	21.5	1.54	5
3	3-days	W-W	15.80	1.49	6
		E-W	18.87	3.58	9
	8-days	W-W	22.60	1.39	11
		E-W	25.66	1.87	11

Table 2
Locomotor activity scores from rats trained during the second postnatal week with water (W) or ethanol (E), and tested in PD 15 in response to one of three ethanol doses (0.5, 1.5 or 2.5 g/kg). Values represent mean and standard error of the mean (SE).

Experiment 1			
Group	Mean	SE	N
Control	19,61	3,68	13
W-0.5	19,60	4,41	6
W-1.5	19,22	3,10	9
W-2.5	32,50	5,41	8
E-0.5	23,66	8,56	6
E-1.5	40,62	5,54	8
E-2.5	54,37	7,22	8

3.1.3. Experimental design and statistics

The experimental design for this study included Group, consisted of 3 independent experimental conditions (Control, W-1.5 and E-1.5), as a between-group variable. A one-way ANOVA was conducted with scores from each testing interval (8 or 15 days) to explore significant between-group differences. As in Experiment 1, a preliminary ANOVA was conducted to explore whether animals trained with water or ethanol and evaluated with water differed in their locomotor scores during the test session. This analysis did not reveal any significant difference between W-W or E-W groups at any interval (see Table 1). Hence, activity levels corresponding to these two groups were collapsed across one group, referred to as Control.

3.2. Results

Fig. 2 shows locomotor activity levels as a function of Group (Control, W-1.5 and E-1.5) and Interval (8 or 15 days). The ANOVA revealed significant main effect of Group for each testing interval [8 days: $F(2, 23) = 3.93$, $p < 0.05$; 15 days: $F(2, 15) = 10.83$, $p < 0.05$]. Post-hoc analysis indicated that 8 days after training the lower ethanol dose (1.5 g/kg) did not induce acute locomotor stimulation (W-1.5 vs Control group). Interestingly, the locomotor sensitization effect persisted after an 8-days interval, which was reflected in the increased locomotor activity levels from pups trained and tested with ethanol (E-1.5 group) when compared with the W-1.5 and Control groups. However, after a 15-days interval no further evidence of sensitization was detected. Subjects from W-1.5 and E-1.5 groups showed higher locomotor activity scores than those from the Control group (see Table 3). These results show that sensitization induced by ethanol in preweanling rats persists at least for 8 days.

4. Experiment 3

Experiment 3 was aimed at studying the effectiveness of the ethanol treatment used in Experiments 1 and 2 to promote sensitization in older infant rats, during the 3rd postnatal week of life. In a previous study we did not find evidence of sensitization at this age (Castello et al., 2015), but we only tested subjects in response to one ethanol dose (2.5 g/kg) and after a 72-hours interval. In the present experiment, rats were trained between days 14 and 18 with ethanol (2.5 g/kg) and they were tested in response to ethanol (1.5 or 2.5 g/kg) 3 or 8 days later, since after these intervals we observed sensitization in the previous experiments.

4.1. Materials and methods

4.1.1. Subjects

In this experiment, we used 39 male pups from 10 litters for the 3-days interval, and 49 rats from 12 litters for the 8-days interval. The first day of training phase took place on PD 14.

4.1.2. Procedures

Procedures corresponding to the training phase were similar to those described in Experiment 1, with the only difference that this phase was carried out between PDs 14 and 18. Testing took place after a 3 or 8-days interval, on PD 21 or 26, and two ethanol doses were used: 1.5 or 2.5 g/kg.

4.1.3. Experimental design and statistics

As in Experiment 2, the experimental design of this study included Group composed of 5 independent experimental conditions (Control, W-1.5, W-2.5, E-1.5 and E-2.5), as the only between-group factor. A

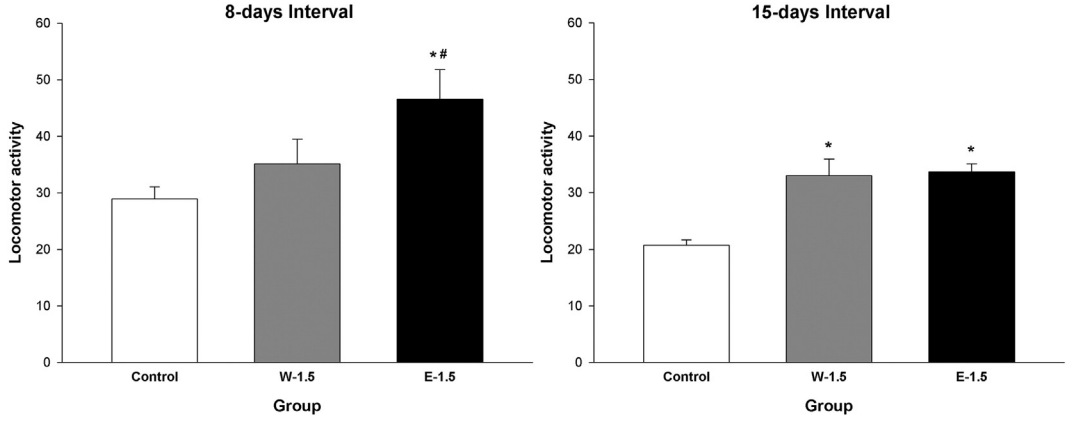

Fig. 2. Locomotor activity levels at testing from rats trained during the 2nd postnatal week, as a function of Group and Interval. * indicates significant differences from Control group, $p < 0.05$. # represents significant differences from the specific ethanol control group, $p < 0.05$. Vertical bars illustrate standard errors of the mean.

Table 3
Locomotor activity levels from rats trained during the second postnatal week of life with water (W) or ethanol (E), and tested in response to ethanol (1.5 g/kg) 8 or 15 days after training. Values represent means and standard error of the mean (SE).

Experiment 2				
Interval	Group	Mean	SE	N
8 days	Control	28,94	2,1	17
	W-1.5	35,11	4,35	9
	E-1.5	46,55	5,26	9
15 days	Control	20,93	1,01	11
	W-1.5	39,37	4,95	6
	E-1.5	37,62	3,78	6

one-way ANOVA was conducted to explore significant main effects with scores from each testing interval (3 or 8 days after training). As in previous experiments, differences in the locomotor activity during the testing of rats trained with water or ethanol and tested with water were explored by conducting a preliminary ANOVA. As was the case in the preceding experiments, this analysis did not reveal any significant difference between groups at any interval (see Table 1), and a single Control group was formed for the subsequent analysis.

4.2. Results

Fig. 3 displays locomotor activity scores as a function of Group (Control, W-1.5, W-2.5, E-1.5 and E-2.5) and Interval (3 or 8 days). Sensitization to the locomotor stimulating effect of ethanol was no longer observed during the 3rd week of life. Instead, rats expressed tolerance when the higher ethanol dose (2.5 g/kg) was used on test. The ANOVA conducted using scores collected 3 days after training revealed a significant main effect of Group [$F(4, 34) = 4.21$, $p < 0.05$]. Post-hoc test revealed that scores from the W-2.5 group were significantly higher than those from the Control group, while scores from the E-2.5 group did not differ from those from Control animals. In addition, we did not observe locomotor stimulation in subjects treated with 1.5 g/kg regardless the prior experience with ethanol. The analysis conducted with scores collected 8 days after training revealed a significant effect of Group [$F(4, 44) = 5.89$, $p < 0.05$]. Post-hoc tests indicated higher levels of activity for the W-2.5 group in comparison with Control and E-2.5 groups. Further, locomotion scores from the E-2.5 group did not differ from those from the Control group, and the lower ethanol dose (1.5 g/kg), similarly to what we observed after the short-interval, did not exert any effect over locomotion (see Table 4).

5. Discussion

The results from the present series of experiments demonstrate locomotor sensitization induced by ethanol in the infant rat, and that this atypical effect of ethanol in the adult rat can be observed during the 2nd postnatal week of life. Interestingly, when compared with sensitization induced by different psychostimulant drugs during the preweanling period (Collins et al., 1998; Crawford et al., 1998; McDougall et al., 2011), the one generated by ethanol exposure persisted over a longer time interval, at least 8-days. It is also worth noting that the same ethanol treatment administered during the 3rd postnatal week of life not only failed to generate sensitization, but it produced the opposite effect, i.e. tolerance.

In Experiment 1, daily administration with a high dose of ethanol (2.5 g/kg) across 5 consecutive days within the 2nd postnatal week resulted in an increased locomotor response to two different ethanol doses (1.5 and 2.5 g/kg), thus replicating previous findings from our laboratory (Arias et al., 2009b). This result is particularly striking when considering that this ethanol effect can hardly be observed in later stages of the ontogeny of the rat (Chuck et al., 2006) and that the few cases in the literature in which it has been reported were mainly those conducted with subpopulations of subjects that differentially respond to ethanol or to novelty (Quintanilla, 1999; Hoshaw and Lewis, 2001).

In addition, we found marked differences between the 2nd and 3rd postnatal week of life regarding sensitization induced by ethanol. We were unable to observe any evidence of such an effect during the 3rd postnatal week in spite of using a two ethanol doses at testing. This marked ontogenetic difference may be related with some important and well-described metabolic changes that take place between the 2nd and 3rd postnatal week. In particular, the balance between the central and peripheral ethanol metabolism suffers dramatic changes in a few days. While during the 2nd postnatal week the central metabolism is higher and the peripheral metabolism lower (in comparison with the 3rd one), this metabolic pattern is reversed by the 3rd postnatal week of life. The central metabolism of ethanol depends on the catalase system, whose activity is particularly high during the first postnatal weeks of life when comparing to adult rats (Gill et al., 1992; Hamby-Mason et al., 1997). Around 50% of catalase system activity tends to decay during

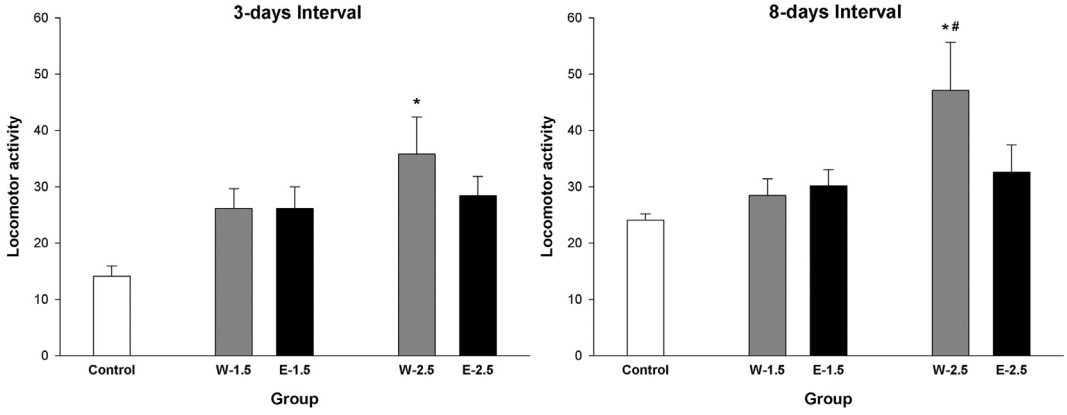

Fig. 3. Locomotor activity scores at testing, from rats trained during the 3rd postnatal week of life, and tested 3 or 8 days later with water or one of three ethanol doses. * indicates significant differences from Control group, $p < 0.05$. # represents significant differences from the specific ethanol control group, $p < 0.05$. Vertical bars illustrate standard errors of the mean.

Table 4
Locomotion levels from rats trained during the third postnatal week of life with water (W) or ethanol (E), and tested 3 or 8 days after training with one of two ethanol doses (1.5 or 2.5 g/kg). Values represent mean and standard error of the mean (SE).

Experiment 3				
Interval	Group	Mean	SE	N
3 days	Control	17,20	2,03	15
	W-1.5	26,14	3,53	7
	W-2.5	35,80	6,58	5
	E-1.5	26,14	3,85	7
	E-2.5	28,40	3,45	5
8 days	Control	24,00	1,14	22
	W-1.5	28,50	2,91	6
	W-2.5	47,14	8,51	7
	E-1.5	30,16	2,89	6
	E-2.5	32,62	4,81	8

the first two weeks of life (Del Maestro and McDonald, 1987). Furthermore, peripheral ethanol metabolism is higher during the 3rd than during the 2nd postnatal week (Hollstedt et al., 1980; Kelly et al., 1987), reaching a level of activity comparable to adulthood between PDs 20 and 40 (Zamatkin and Lis, 1990). Interestingly, ethanol-induced sensitization in mice has been functionally linked to catalase system activity (Correa et al., 2004), while acetate - a metabolite that results from peripheral metabolism of ethanol - mediates the sedative effects of ethanol (Carmichael et al., 1991; McLaughlin et al., 2008). According to our observations, sensitization was restricted to the 2nd postnatal week, when the catalase system is more active and the peripheral metabolism of ethanol is reduced. By the 3rd week, when this metabolic profile is reversed, no signs of sensitization were detected and the ethanol treatment resulted in tolerance.

Hence, it is possible that changes in metabolism can affect the sensitivity of the organism to specific behavioral ethanol effects. The early ontogeny of the rat can be thought as an ontogenetic stage in which this association (between metabolic profile and sensitivity to ethanol) is expressed. However, we need to be cautious and do not attribute the whole responsibility of the ontogenetic difference in the way subjects respond to ethanol to a single cause. As we have shown, other factors such as sex or contextual variables modulate critically the expression of sensitization, and sensitivity seems to arise from a complex interaction of these (and probably many other) factors. For instance, the way in which subjects from each age group respond to novelty seems to be involved in the ontogenetic transition from ethanol-induced sensitization to tolerance during infancy. Both the acute stimulation and the sensitized locomotor response induced by ethanol are highly dependent on novelty (Arias et al., 2009a; Didone et al., 2015; Hoshaw and Lewis, 2001; Meyer et al., 2005). Although we did not manipulate novelty in our study, other authors have reported that on PD 15 of the rat there is a peak in the locomotor response to a novel open-field (Campbell and Raskin, 1978), precisely the day on which we tested our subjects trained during the 2nd week, which could influence the expression of sensitization. This increased response to novelty decays during the following days (Campbell and Raskin, 1978) and sensitization induced not only by ethanol (Castello et al., 2015) but also by different psychostimulants, is attenuated around the weaning period (Snyder et al., 1998; Zavala et al., 2000).

Tolerance to the locomotor stimulating effect of ethanol may be the result of compensatory conditioned responses counteracting the stimulating effect of ethanol (Siegel et al., 2000). A reason to doubt this possibility, however, is the fact that during the 2nd and 3rd postnatal weeks we did not observe any evidence of conditioned motor responses in subjects treated with ethanol when trained and tested with water (Castello et al., 2015 and the present results, see Table 1).

The sensitization effect generated by the ethanol treatment during the 2nd postnatal week persists for at least 8 days after training (Experiment 2). As mentioned previously, there are results showing that short exposures to ethanol during early stages of development, including the gestational period or infancy, can result in long-term modulation of ethanol intake (Spear and Molina, 2005). Our results open up the possibility to explore whether sensitization can play a role in the long-term responding to ethanol after early experiences with the drug. This result is also important when compared with those obtained with different drugs during the same ontogenetic period. McDougall and collaborators consistently found that sensitization induced by a variety of psychostimulants such as cocaine, amphetamine, methamphetamine, NPA (a direct DA receptor agonist), U50,488 (a kappa opioid receptor agonist) or methylphenidate has a short-term life, being detectable only after one or two days of interval (Collins et al., 1998; McDougall et al., 1999; McDougall et al., 1994). However, in some cases, long-term sensitization was also observed in this period of the rat, and apparently the long-term expression of this effect depends on the amount of pre-testing exposures to the drug. For example, when a higher number of cocaine administrations were employed at training (7 to 10 injections), preweanling rats were capable of displaying locomotor sensitization after a 7-day interval (Herbert et al., 2010) or even after a period of three weeks (Snyder et al., 1998). Therefore, it is also possible that longer treatments with ethanol can prolong the duration of sensitization.

In the present study we only used males, since in a previous study we did not find sensitization in females (Castello et al., 2015). Although during infancy some results indicate that female pups display more consumption of a solution paired with ethanol (Kozlov et al., 2009; Varlinskaya et al., 1999), during the weaning period sex-differences are more pronounced, and in some cases it has been reported that females are more sensitive to ethanol than males, including the predisposition to show sensitization (Didone et al., 2015; Quoilin et al., 2014). Therefore the lack of sensitization in weaning rats is a finding that should be treated with caution until more data are available.

6. Conclusions

Taken together, our results are also consistent with the idea that, under the specific conditions of this animal model, it is possible to establish associations between “age” and sensitivity to particular ethanol effects. During the 2nd postnatal week of life, infant rats have a strong predisposition for showing long-term locomotor sensitization after repeated ethanol exposure, supporting previous findings that have highlighted this ontogenetic period as being particularly sensitive to some positive, rewarding, and stimulating effects of ethanol (Arias and Chotro, 2006; Arias et al., 2009b; Molina et al., 2007; Sanders and Spear, 2007). Once sensitivity to the aversive effects of ethanol begin to predominate (Hunt et al., 1991; Arias and Chotro, 2006), and infants consume less amounts of this substance, sensitization is no longer observed and gives way to the opposite effect, tolerance. This ontogenetic model offers a potentially interesting tool for studying within the same species, how these effects are interrelated, and which (internal and external) variables influence the expression of tolerance or sensitization. In addition, due to the fact that sensitization and tolerance induced by ethanol during infancy appear to be relatively long-term effects that persist for at least a week, the present results highlight the need to consider these effects as possible modulators of the increased ethanol affinity resulting from early experiences with the drug.

Acknowledgments

This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (FONCyT, PICT 2011-0999 and PICT 2014-1606), SECyT and CONICET PIP 11220110100763 awarded to Juan C. Molina, and grant PICT-2015-2416 awarded to Carlos Arias. We would like to specially thank to the lab members and to the technicians of the vivarium for their assistance, support and suggestions. SC holds a doctoral fellowship from CONICET, and is a PhD student in Neuroscience of the Universidad Nacional de Córdoba (UNC).

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