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**Long-term contextual memory in infant rats as evidenced by an ethanol conditioned tolerance procedure**

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Highlights

- The context was able to modulate ethanol-induced tolerance in 2- and 3-week-old rats.
- Contextual conditioned tolerance was stronger during the third than during the second postnatal week.
- After a long interval of time, when the context presumably lost its influence over tolerance, ethanol pre-exposure resulted in sensitization instead of tolerance.

**ABSTRACT**

Conditioned tolerance can be conceptualized as a particular case of Pavlovian conditioning in which contextual cues play the role of the conditioned stimulus. Although the evidence is contradictory, it is frequently assumed that long-term contextual conditioning in pre-weanling rats is weak or even absent. This hypothesis comes from and is sustained mainly by behavioral studies that explored different contextual effects in 16-18 day-old rats using a fear-conditioning paradigm, but their conclusions are stated in terms of an immature (hippocampal-dependent) declarative memory system. The main goal of the present manuscript was based on a recent antecedent from our laboratory, to analyze whether context-dependent tolerance induced by ethanol during the pre-weanling period persists over time. Results showed that the context was able to modulate ethanol-induced tolerance in 2- and 3-week-old rats. Interestingly, contextual conditioned tolerance was stronger (in terms of persistence) during the third than during the second postnatal week. When subjects were tested 8 days after training, when the context presumably lost its influence over tolerance, the opposite effect emerged (sensitization). These results are important for the ethanol literature, adding new evidence of long-term retention of ethanol effects acquired during infancy, whilst also showing striking ontogenetic differences in the sensitivity to ethanol between the 2nd and 3rd postnatal weeks. Importantly, contextual information modulates the expression of these ethanol effects even eight days after training, a result that is particularly relevant to the discussion of the ontogeny of contextual memory.

**Key words**: context – infant rat – conditioned tolerance – locomotor activity – ethanol

## INTRODUCTION

Drug effects are highly influenced by context. A history of contingency between some drug effects and specific environmental cues can lead to these cues exerting an influence on the behavioral responses to the drug. A typical case is conditioned tolerance (Poulos et al., 1981, Siegel, 1987, Siegel et al., 2000), which can be defined as a reduced effectiveness of the drug in the presence of environmental cues that were repeatedly paired with this drug. Conditioned tolerance can be conceptualized as a particular case of Pavlovian conditioning (Siegel, 1987, Ramsay and Woods, 1997, Millin and Riccio, 2002), in which contextual cues play the role of the conditioned stimulus (CS), eliciting in some cases a compensatory response that counteracts the effect of the drug. In this way, the presence of the context CS attenuates the drug effect (Siegel et al., 2000).

Results concerning contextual conditioning during infancy are contradictory. Some studies support the hypothesis that the retention of contextual information is poor during infancy, presumably because of an immature declarative memory system in which the hippocampus is a key brain structure for mediating the memory of contextual cues (for example, Rudy and Morledge, 1994, Poulos et al., 2014, Robinson-Drummer and Stanton, 2015). It has been reported that contextual conditioning acquired during the pre-weanling period cannot be retained for a long period of time (24 hours), and that at some point between postnatal day (PD) 17 and PD 23 the capability for long-term hippocampus-dependent contextual memory emerges (Rudy and Morledge, 1994, Jablonski et al., 2012). In agreement with these results, some studies have found context-dependent interference learning in 23-day-old rats, but not in younger pre-weanlings (around PD 17), leading to the conclusion that the context does not influence interference learning during

infancy (Kim and Richardson, 2010). However, other studies have recently reported clear evidences of context-dependent interference effects (for example, Revillo et al., 2013, Revillo et al., 2014), context learning (Ramsaran, Westbrook and Stanton, 2016) and Pavlovian contextual conditioning in pre-weanling rats (for example, Pugh and Rudy, 1996, Brasser and Spear, 1998, Park, Ganella and Kim, 2017; Pisano et al., 2012). In fact, under particular conditions, contextual memory is even greater in infants than in adult rats (Brasser and Spear, 2004). The discrepancy between studies may be related to details of the procedures and/or with the experimental paradigm used (Revillo et al., 2015). It is worth noting that in the majority (if not all) of the studies with rats, a fear-conditioning paradigm was used to analyze the early ontogeny of contextual memory, and the behavior most frequently used as an index of memory was the freezing response (see Revillo et al., 2015 for a detailed discussion). This experimental paradigm may be adding some noise to the discussion, because the behavioral repertoire of defensive reactions, including the freezing response, emerges during the pre-weanling period, between PD10 and PD20, and there are qualitative and quantitative differences in the expression of these kinds of responses between infants and weaning rats (Collier and Bolles, 1980, Takahashi, 1992). Although the ontogeny of contextual memory in rats has been almost exclusively examined with this procedure, conclusions are drawn alluding to the declarative memory system (for example, Poulos et al., 2014).

We have recently reported results that constitute clear evidence of contextual memory in 2-week-old rats (Castello et al., 2015). This evidence was found in a study in which we examined the effect of repeated experience with ethanol upon the ethanol locomotor stimulating effect. It is important to note that infant rats, contrarily to what has been observed in adult animals (Chuck, McLaughlin, Arizzi-LaFrance, Salamone, & Correa, 2006), are particularly sensitive to the locomotor stimulating effect of ethanol (Arias, Mlewski, Molina, & Spear, 2009). In the mentioned

study we observed that, when subjects were chronically exposed to ethanol in a different context than the one used at testing, ethanol induced locomotor sensitization. However, when the training and testing contexts coincided, the outcome was quite the opposite, i.e. tolerance. Furthermore, tolerance was evaluated 72 hours after training, indicating that infantile contextual memory can persist for a considerable amount of time, at least when compared with those studies that used a contextual fear conditioning paradigm with older pre-weanling rats, which rarely exceeded an interval of 24 hours (Revillo et al., 2015).

The main goal of the present manuscript was to analyze whether context-dependent tolerance induced by ethanol during the pre-weanling period persists for a longer period of time than 72 hours. In the first experiment we explored retention of context-dependent tolerance induced by ethanol during the second postnatal week. In the second experiment we repeated the procedure but with older infants, trained during the third postnatal week. It is well known that retention of learning acquired during infancy increases with age (Miller and Spear, 1989), so we expected longer retention in the older than in the younger infant rats. Few published studies have explicitly explored retention of context learning acquired during the pre-weanling period for a longer period of time than 24 hours, and always use a contextual fear-conditioning paradigm (Foster and Burman, 2010, Robinson-Drummer and Stanton, 2015). The present study is also relevant for the ethanol literature, since many studies during the last three decades have repeatedly shown how early experience with ethanol can impact on ethanol intake patterns later in life (Spear and Molina, 2005, Chotro et al., 2007). Since ethanol tolerance influences ethanol consumption, it is informative to know whether this ethanol effect persists over time, as well as the circumstances contributing to its retention.

## MATERIALS AND METHODS

### *Subjects*

For Experiment 1a, we used a total of 29 female Wistar rats representative of 16 litters for the 3-day interval, and 33 rats from 9 litters for the 8-day interval. In Experiment 1b, 46 female rats belonging to 15 litters were used. For Experiment 2a, 32 rats from 16 litters were included for the 3-day interval, while 24 female rats derived from 6 litters were used for the 8-day interval. In Experiment 2b we used 29 rats derived from 16 litters for the 3-day interval, and 29 female rats from 8 litters for the longer interval. In all these experiments we only used female rats. In our previous study we found a sex effect on sensitization induced by ethanol in preweanling rats, but not in ethanol-induced tolerance (Castello et al., 2015). All animals of this 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 postnatal day 0 (PD0). Litters were culled to 10 pups (5 males and 5 females when possible). Subjects were 8 days old at the start of Experiments 1a and 1b, and 14 days old when Experiments 2a and 2b began. 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.

### *Apparatus*

In all experiments, animals were tested in a circular open field (30 cm diameter for pups trained during the second postnatal week; 38 cm diameter for rats trained during the third postnatal week), 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 the preweanling period context learning and context effects are more robust when the context contains explicit odors (Revillo et al., 2015). The open field also served as the training context in Experiments 1a and 2a, while a holding cage (25 cm x 23 cm x 23 cm) partially filled with clean wood shavings, was used as an alternative context during the training phase of Experiments 1b and 2b. In all of the 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 they were later evaluated by a researcher blind to the treatments, who counted the number of quadrants crossed.

***General procedures***

*Training phase*. This phase was conducted between PDs 8 and 12 in Experiments 1a and 1b, or between PDs 14 and 18 in Experiments 2a and 2b (one session per day). On these days rats were separated from their mothers and placed in pairs in the holding cage. The floor of the cage was maintained at 36° C (± 1°C) through the use of a heating pad. An hour later, the body weights of the pups were individually recorded and they immediately 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 and, more recently, we reported that the same chronic exposure to ethanol at this age induced tolerance (Castello et al., 2015). The volume administered was equivalent to 0.015 ml per gram of body weight 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 with rats until PD 21, while a 50-cm length of polyethylene tubing (PE-50 Clay Adams, Parsippany, New Jersey) connected to a 3 ml syringe with a 25 G x 1 needle was used for i.g. administration of PD 26 rats. This tubing was gently inserted through the mouth and slowly guided into the stomach. The entire procedure took less than 20 seconds per rat. After the i.g. administration, pups were placed in the open field (see *Apparatus* section) for a 5-minute period (Experiment 1a and 2a), or remained in the holding cage for the same period of time (Experiments 1b and 2b). They were then returned to their home cages.

*Testing phase*. Rats were evaluated in response to water or ethanol in terms of locomotor activity in the open field. Testing was carried out 3 or 8 days after training (on PDs 15 or 20 for Experiment 1a; on PDs 21 or 26 for Experiments 2a and 2b), or only 3 days after training (on PD 15 in Experiment 1b). Half of the subjects trained with water received the same treatment on the test session, while the other half was administered with ethanol (2.5 g/kg). 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. In all the present experiments, the ethanol dose used at testing was 2.5 g/kg, and we additionally used 1.5 g/kg ethanol only in Experiment 1b.

As in the training phase, after an hour of maternal separation, subjects were given the i.g. administration of water or ethanol, and five min later they were placed in the open field (see *Apparatus* section), and their behavior was videotaped for further analysis of locomotor activity.

### ***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 at testing. This analysis did not

reveal any significant between-group difference (see Table 1) in any of the present experiments, which indicates that when rats did not receive ethanol at testing, the locomotor response was not affected by familiarity with the context or by ethanol pre-exposure. Therefore, as in previous studies (Castello et al., 2015, Castello et al., 2016) all of the subjects evaluated in response to water were grouped in the same control condition (Control), one for each testing interval and for each experiment. As a consequence, the experimental design for this study comprised two between-group variables named Group, with 3 independent experimental conditions (Control, W-E, and E-E), and Interval, which contains two levels (3 or 8 days). Letters of the experimental groups indicate training and testing treatments, water (W) or ethanol (E). Due to the fact that in Experiment 1b we used two different ethanol doses at testing, in this case Group included 5 independent levels and the name of the groups referred to the drug used at training (W or E) and to the ethanol dose used at testing (1.5 or 2.5 g/kg) (Control, W-1.5, W-2.5, E-1.5 and E-2.5). Besides, in Experiment 1b Interval was not included as a factor because animals were only tested after a 3-day interval.

The dependent variable analyzed was locomotor activity, which was estimated through the total number of quadrants crossed during the first 3-min of testing. This variable was analyzed by means of a one way ANOVA, one for each testing interval (3 or 8 days). In all 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.

**EXPERIMENT 1a**

The aim of the first experiment was to evaluate whether context-dependent tolerance induced by ethanol during the second postnatal week could still be observed after a long period of

time (8 days). Rats were trained following the protocol that we used in previous studies (Castello et al., 2015). Subjects received a daily ethanol dose (2.5 g/kg) for 5 consecutive days (between PD 8 and 12). Five minutes later they were placed in a context (open field) in which they spent 5 minutes. Subjects were tested 3 or 8 days after the last training day for ethanol-induced locomotor activity in the same context (PDs 15 and 20). As in our previous study (Castello et al., 2015), tolerance was operationalized as a reduction or absence of the stimulating effect of ethanol.

RESULTS

Figure 1a displays locomotor activity levels from pups trained during the second postnatal week (PDs 8-12) and tested 3 or 8 days after training (on PDs 15 and 20) in response to water or ethanol. Subjects given water at testing were assigned to the Control condition (see *General procedures* and Table 1). The ANOVA revealed significant main effects of Group [$F$ (2, 57) = 13.77, $p < 0.05$] and Interval [$F$ (1, 57) = 9.89, $p < 0.05$], and a significant interaction between them [$F$ (2, 57) = 6.21, $p < 0.05$].

Post-hoc analysis with scores from rats tested after a 3-day interval indicated that ethanol-induced locomotor stimulation was only observed in pups receiving the drug for the first time at testing (W-E group). Scores from these subjects were significantly higher than those from the remaining groups (W-E vs Control: $p = 0.005$; W-E vs E-E: $p = 0.02$). Furthermore, locomotor activity level from the E-E group was statistically similar to the one from the Control group, a result compatible with a tolerance effect.

**PLEASE, INSERT FIGURE 1a and TABLE 1 HERE**

The post-hoc analysis of scores from the longest interval (8 days) also showed a significant stimulating effect in the W-E group when compared to the Control condition ($p = 0.02$). Interestingly, the E-E group showed significantly higher locomotor activity scores than both Control ($p = 0.0001$) and W-E groups ($p = 0.005$), which indicates that after an interval of 8 days, tolerance had disappeared and the rats instead demonstrated locomotor sensitization.

Thus, the same ethanol treatment resulted in two opposite effects, tolerance and sensitization, that were expressed depending on the interval between training and testing. Tolerance was observed 3 days after training and sensitization after the longer interval of 8 days. In order to show that (as reported before) at this age, tolerance to ethanol depends on the context, we ran Experiment 1b in which the subjects did not experience the testing context during training.

**EXPERIMENT 1b**

In previous studies from our laboratory (Castello et al., 2015, Castello et al., 2016), we observed that when training and testing phases were conducted in different contexts, 3 days after training, subjects expressed locomotor sensitization, instead of tolerance. However, sensitization was only observed in males, not in females. Surprisingly, in Experiment 1a, in which we only used females, rats also expressed sensitization, but in this case when they were tested 8 days after training in response to a high ethanol dose (2.5 g/kg). It is then possible that female subjects, in our previous study, acquired sensitization, but they did not express it because they were differentially sensitive to the ethanol dose used for testing, in comparison with males. Therefore, in Experiment 1b we used two different doses at testing (1.5 or 2.5 g/kg). The training procedure was similar to that employed in the previous experiment, but in this case subjects did not go to the testing context during the training phase (see *General procedures* section). This experimental design is important

for showing that the tolerance reported in Experiment 1a, three days after training, was context-dependent.

RESULTS

Figure 1b displays the locomotor scores from female pups tested with water or ethanol on PD 15, three days after training. As mentioned previously, they had received no experience with the testing context until testing. The ANOVA revealed a significant main effect of Group, [$F$ (4, 41) = 8.15, $p < 0.05$]. When compared with Control animals, pups given ethanol for the first time at testing displayed locomotor stimulation only with the highest dose (W-2.5 vs Control: p = 0.002), while the lower ethanol dose did not affect the locomotion index. Interestingly, subjects from the E-1.5 group showed locomotor sensitization, that is, their scores were significantly higher than those from Control (p = 0.03), while the difference between the E-1.5 and W-1.5 groups was almost significative (p = 0.06). Scores from the E-2.5 condition were statistically similar to those from the W-2.5 group, and significantly higher than those from the Control group (p = 0.002).

**PLEASE, INSERT FIGURE 1b HERE**

These results reproduce previous data from our laboratory supporting the notion that at this young age sex may have an influence on the sensitivity to ethanol (Castello et al., 2015). In order to detect sensitization in female rats 3 days after training it seems necessary to use a lower ethanol dose at testing than the one that we have used previously. Results from Experiment 1b are relevant for the present study in that they confirm that when rats are tested three days after training, but in a different context than that used for training, preexposure to ethanol does not result in tolerance, and,

depending on the dose, it can instead induce sensitization. We can therefore conclude that tolerance observed in Experiment 1a is context-dependent, and that contextual memory induced by our experimental protocol during the 2nd postnatal week of life lasts for at least 72 hours.

**EXPERIMENT 2**

In Experiment 2 we repeated the procedure with 3-week-old rats to explore the persistence of tolerance and the influence of the context. Rats were trained between PDs 14-18 and tested 3 or 8 days later. In Experiment 2a subjects were trained in the testing context, and in Experiment 2b outside of the testing context, in order to evaluate the influence of contextual cues on the effects of ethanol.

RESULTS

Experiment 2a

Figure 2a shows locomotor activity from rats tested in response to ethanol 3 or 8 days after training (PD 21 and 26, respectively). These subjects were trained and tested in the same context. The ANOVA only revealed a significant main effect of Group [$F$ (2, 50) = 11.44, $p < 0.05$]. According to post-hoc tests subjects from the W-E group displayed significantly more locomotion than the remaining groups (W-E vs Control: $p = 0.0003$; W-E vs E-E: $p = 0.01$), which did not differ between them (i.e. tolerance). Neither the Interval factor nor its interaction with Group reached statistical significance, which would lead us to conclude that tolerance was not dependent on the testing interval.

**PLEASE, INSERT FIGURE 2a HERE**

Experiment 2b

Figure 2b depicts activity levels from females tested 3 or 8 days after training, but in this case all of them were trained in a different context to that used for training. The ANOVA indicated a significant main effect of Group [$F$ (2, 52) = 13.37, $p < 0.05$] and Interval [$F$ (1, 52) = 4.31, $p < 0.05$]. The interaction between both factors also reached statistical significance, [$F$ (2, 52) = 5.32, $p < 0.05$]. Subsequent post-hoc tests considering scores from the short interval (3 days) showed the typical acute locomotor stimulating effect only in W-E group (W-E vs Control: $p = 0.002$). In contrast, locomotor scores from the E-E group were significantly lower than those from the W-E group ($p = 0.01$), and statistically similar to those from the Control group, which indicates that ethanol preexposure resulted in tolerance, but in this case, in subjects without prior experience with the testing context.

**PLEASE, INSERT FIGURE 2b HERE**

Post-hoc analysis with scores from the 8-day interval revealed that W-E subjects displayed higher scores than Control animals ($p = 0.02$). Subjects trained and tested with ethanol (E-E group) displayed significantly higher scores than those from the Control ($p = 0.004$) and W-E ($p = 0.03$) groups. Therefore, tolerance observed eight days after training in Experiment 2a was no longer observed when subjects were trained in a context different to that used for the test.

## DISCUSSION

The present data indicate that infant rats can show long-term contextual memory even during the second postnatal week of life. In these experiments contextual memory was operationalized through the influence of the training context (the open field) on ethanol locomotor effects in subjects preexposed to ethanol. That is, when these subjects were assessed in a context that was present during training, ethanol lost its capacity to induce locomotor stimulation, i.e. conditioned tolerance. Subjects trained during the second postnatal week showed contextual conditioned tolerance three days after training (Experiment 1a), while contextual modulation of tolerance was clear even eight days after training in subjects trained during the third postnatal week of life (Experiment 2a).

In Experiment 1b rats not preexposed to the testing context showed locomotor sensitization in response to ethanol, but only in response to the lower ethanol dose (1.5 g/kg). Although the highest ethanol dose did not promote sensitization in this experiment, it stimulated locomotion to the same level than those subjects not preexposed to ethanol. These results replicated previous findings from our laboratory (Castello et al., 2015, Castello et al., 2016), and they show that tolerance observed in Experiment 1a requires the presence of the training context (the open field) at testing.

The results from Experiment 2 reveal important ontogenetic differences between subjects from the second and third postnatal week in response to our experimental protocol, which were expressed in terms of sensitivity to ethanol, and in the duration of contextual memory. The first main difference is that ethanol-induced tolerance was found in subjects not given preexposure to the testing context when subjects were tested three days after training (Experiment 2b). This effect was only found in subjects trained during the third, but not the second postnatal week, and it is coherent

with previous reports (Arias et al., 2012), suggesting that by this age this ethanol effect is either context-independent or it is mediated by cues other than the open field, such as handling or those related to the injection that were present at training and testing. This would not be surprising since drug effects can be effectively conditioned to injection cues (de Brugada et al., 2003, Davis et al., 2010) and various (proximal vs distant) contextual cues can exert differential levels of control over tolerance, at least in adult animals (Costanzo et al., 1995).

Secondly, contextual memory lasted longer in rats trained during the third than in those trained during the second postnatal week. In the older subjects contextual conditioned tolerance was observed even 8 day after training, while in the younger rats only after the short interval. Contextual conditioned tolerance in our study may depend not only on the external physical context, but also on the internal context because, at testing, subjects that expressed tolerance (E-E group), in addition to the external features of the context, they were also re-exposed to the internal context induced by ethanol, and therefore more cues were available to retrieve the memory of the training experience.

It is striking that in two cases from the present study (Experiment 1a and 2b) when tolerance was not observed at the longest testing interval, ethanol induced sensitization. In a previous study we observed sensitization only in males, not in females (Castello et al., 2015). Results from Experiments 1a and 1b show that infant female rats can also display sensitization induced by ethanol, although the conditions that favor the expression of this ethanol effect, for example, the testing dose of ethanol, vary slightly between males and females. The mechanisms underlying both ethanol effects — tolerance and sensitization — have been widely studied and they appear to be independent processes (Phillips et al., 1997, Siegel et al., 2000, Pietrzykowski and Treistman, 2008, Phillips, 2011, Tran and Gerlai, 2014). With our experimental setting both effects resulted from application of the same training protocol, but their expression was dependent on when

and where testing was carried out and on the age of the subjects. If novelty enhances the stimulating effect of ethanol (Arias et al., 2009) and is of critical importance for sensitization to ethanol to occur in young rats (Castello et al., 2015), the expression of sensitization at 8 days may reflect forgetting of stimulus attributes of the context (Riccio & Joynes, 2007). In the younger rats (Experiment 1), the reverse of the ethanol effect (from tolerance to sensitization) was observed in spite of the fact that subjects were trained in the same physical context (open field) as that used for testing, while in the older rats (Experiment 2) this switch appeared in subjects trained and tested in different contexts. This ontogenetic difference may be influenced by differences in the relevance of some developmental events happening between the final training and the testing day. Although in both experiments the same amount of days had passed (from PD 12 to PD 20 in Experiment 1; from PD 18 to PD 26 in Experiment 2), during this period of time in the younger rat some dramatic developmental changes took place which could have critically affected their perception (for example, rats open their eyes on PD 14-15). In addition there are also qualitative differences in response to the ethanol dose that we used in our protocol between PDs 8-12 to PDs 15-21. These differences may contribute towards perceiving the testing situation as novel in spite of the presence of the physical context. We acknowledge that this explanation is speculative in the absence of new data and we have raise it here only as a possible hypothesis for future research.

We have repeatedly reported sensitization in response to ethanol in rats trained during the preweanling period, an effect that is very infrequent in adult laboratory rats (Masur et al., 1986). Moreover, we observed that this effect lasts for at least eight days in the infant rat (Castello et al., 2016), which also contrasts with the literature employing different psychostimulants, showing that sensitization acquired during the second postnatal week of life does not last for more than three days (McDougall et al., 1994, Collins et al., 1998, McDougall et al., 1999). Considering the long-

term persistence of tolerance and sensitization, the present results also have relevance for the ethanol literature. In particular, there is an entire body of research over recent decades showing that different ethanol experiences during early stages of development result in changes in ethanol intake patterns days or even weeks later (Spear and Molina, 2005, Chotro et al., 2007). Both tolerance and sensitization may have an impact on ethanol consumption, and as we have shown, these effects appear to last for a long period of time in the infant rat. Contextual cues have not received enough attention in ontogenetic studies, but the present results encourage the inclusion of context effects among the factors that may influence retention of early experiences with ethanol.

The present findings are partially reminiscent of those from some studies previously reported with adult rats that found contextual modulation of drug-induced tolerance (Feinberg and Riccio, 1990, Kissinger and Riccio, 1995, Larson and Siegel, 1998). In some of these studies, a context-change effect resulted in attenuation of tolerance after a short training-to-testing interval, and, interestingly, this context effect was lost or diminished when subjects were tested after a long retention interval (for example, 7 days) (Kissinger and Riccio, 1995). The attenuation of the context-change effect may reflect the possibility that subjects forget specific features of the environment that was originally paired with drug administration (Feinberg and Riccio, 1990). In our experiments the results were not identical to those just described, possibly due to the nature of drug and the effect that we were studying. In Experiment 1 we observed a clear context-change effect with a short testing interval, but we were unable to test the context-change effect on tolerance 8 days after training because tolerance did not persist for this period of time. In Experiment 2, we did see a context-change effect in subjects from the E-E groups, regardless the testing interval. These results appear to support the possibility that forgetting of stimulus attributes is faster in younger than in older infant rats (Anderson and Riccio, 2005, Riccio and Joynes, 2007), and that contextual

memory is important for persistence of tolerance in infant rats. Furthermore, it is important to note that tolerance was always observed in the absence of compensatory conditioned responses, i.e. a significantly higher locomotor response at testing in the E-W group when compared to that from the W-W subjects (see Table 1).

In sum, the present results are pertinent for the ethanol literature, adding new evidence for the long-term retention of ethanol effects acquired during infancy, whilst also showing important ontogenetic differences in response to the experimental protocol. Interestingly, contextual information can modulate the expression of ethanol effects even eight days after training, which is relevant for the discussion of the ontogeny of contextual memory. This evidence of long-term contextual memory is also in accordance with previous studies showing the importance of contextual memory in preverbal human infants (Rovee-Collier and Cuevas, 2009, Rovee-Collier and Giles, 2010). According to these evidences, it may be more appropriate to analyze the ontogeny of contextual memory in terms of which variables could differentially modulate the retention of contextual information across the different stages of ontogeny. In answering this question it is important to consider that infants can respond quantitatively or qualitatively different to particular experimental conditions than adult organisms (McKinzie and Spear, 1995, Brasser and Spear, 1998).

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| Experiment | Interval | Training treatment | Mean | SE | N |
|---|---|---|---|---|---|
| **Experiment 1a** | 3 days | Water | 33,43 | 4,85 | 7 |
| | | Ethanol | 32,00 | 3,61 | 8 |
| | 8 days | Water | 18,87 | 2,25 | 8 |
| | | Ethanol | 20,62 | 2,98 | 8 |
| **Experiment 1b** | 3 days | Water | 27,14 | 2,97 | 7 |
| | | Ethanol | 29,55 | 2,70 | 9 |
| **Experiment 2a** | 3 days | Water | 12,18 | 2,05 | 11 |
| | | Ethanol | 15,20 | 1,35 | 5 |
| | 8 days | Water | 12,00 | 1,59 | 6 |
| | | Ethanol | 14,16 | 3,15 | 6 |
| **Experiment 2b** | 3 days | Water | 19,87 | 1,76 | 8 |
| | | Ethanol | 20,80 | 5,82 | 5 |
| | 8 days | Water | 25,14 | 1,86 | 7 |
| | | Ethanol | 26,00 | 3,47 | 6 |

**Table 1**. Locomotor activity scores from rats trained during the second (Experiments 1a and 1b) or the third postnatal week (Experiment 2a and 2b) with water (W) or ethanol (E). All of them were tested with water. Values represent mean and standard error of the mean (SE).

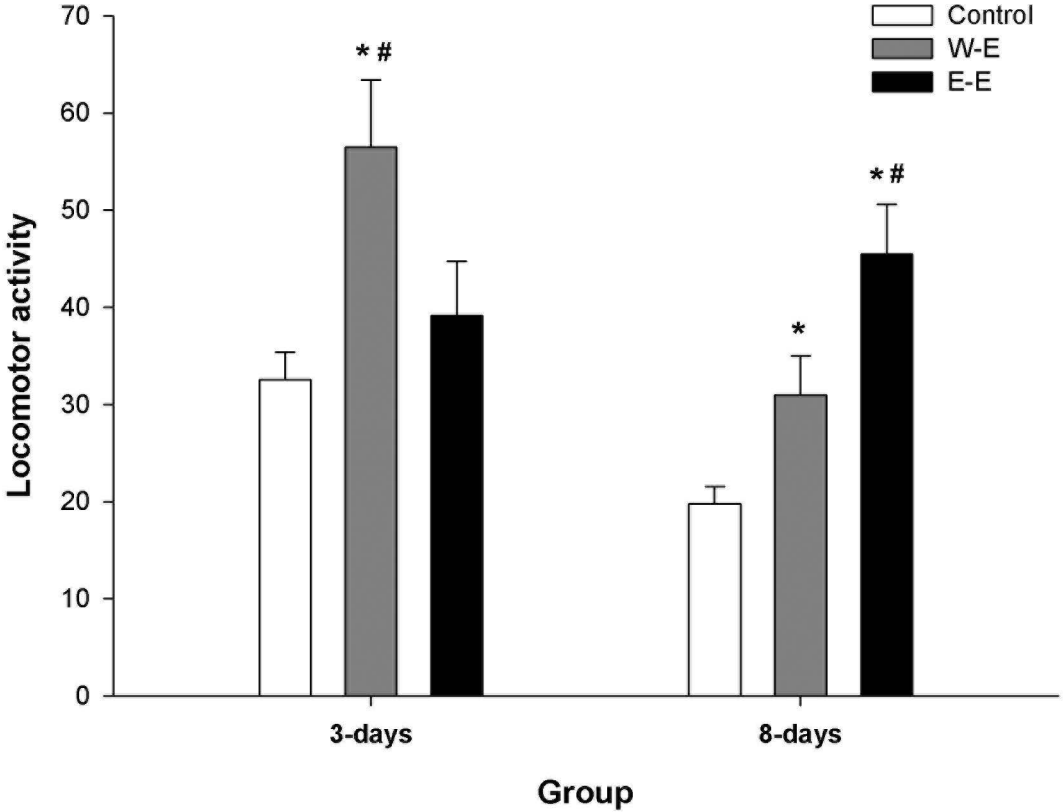
**Figure captions**

**Figure 1a**. Locomotor activity scores from rats trained during the second postnatal week, and tested 3 or 8 days after. * indicates significant differences from Control group, and # represents significant differences from the specific ethanol control group (W-E), $p < 0.05$. Vertical bars denote standard errors of the mean.

**Figure 1b**. Locomotion levels from rats trained during the second postnatal week of life, and tested 3 days after in response to 2 ethanol doses (1.5 or 2.5 g/kg). Training and testing contexts were different. * denotes significant differences from Control group, $p < 0.05$. Vertical bars indicates standard errors of the mean.

**Figure 2a**. Locomotor activity levels from rats trained during the third postnatal week, and tested 3 or 8 days after in the training context. * represents significant differences from Control rats, and # indicates significant differences from the E-E group, $p < 0.05$. Vertical bars denote standard errors of the mean.

**Figure 2b**. Locomotor activity scores from rats trained during the third postnatal week of life, and tested after 3 or 8 days. Training and testing phases were carried out in different contexts. * denotes significant differences from Control animals, and # represents significant differences from the other ethanol-tested group (W-E or E-E), $p < 0.05$. Vertical bars indicates standard errors of the mean.


Control
W-E
E-E
Locomotor activity
70
60
50
40
30
20
10
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* #
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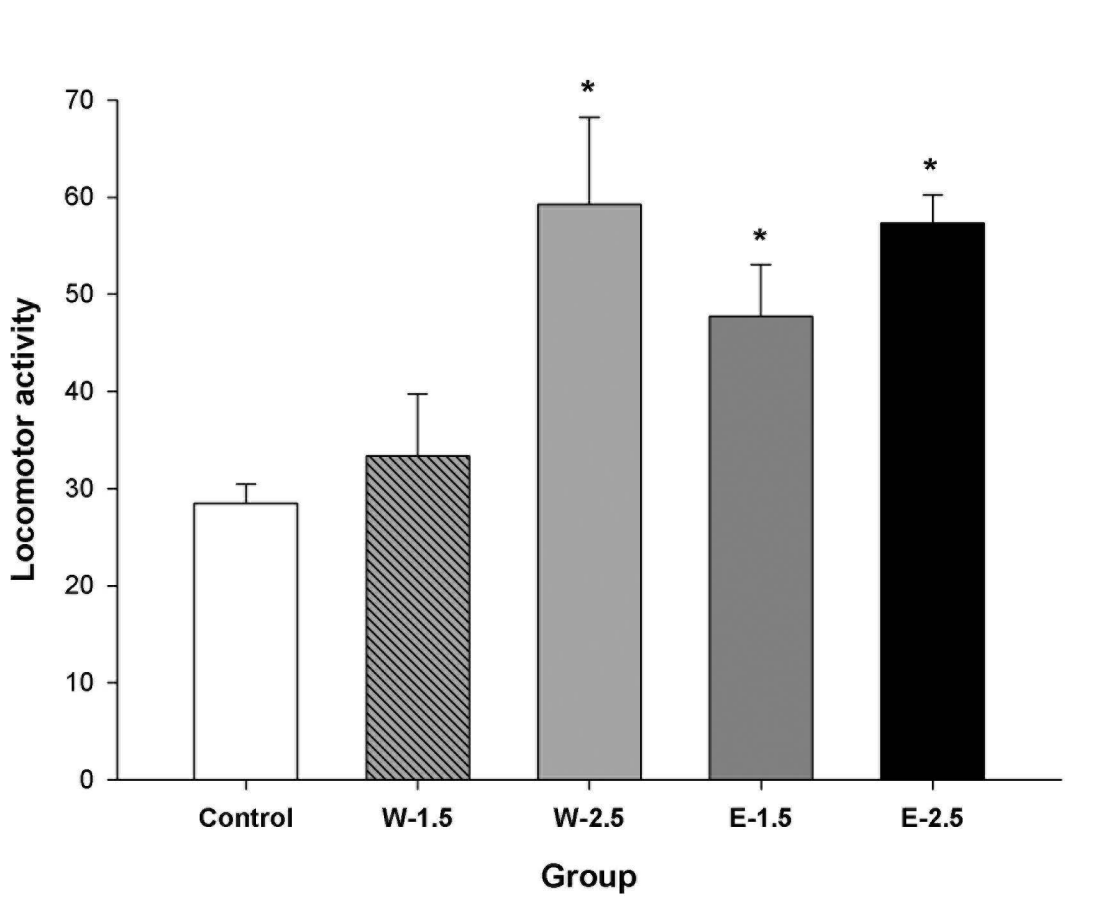
*
3-days
8-days
Group


70
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10
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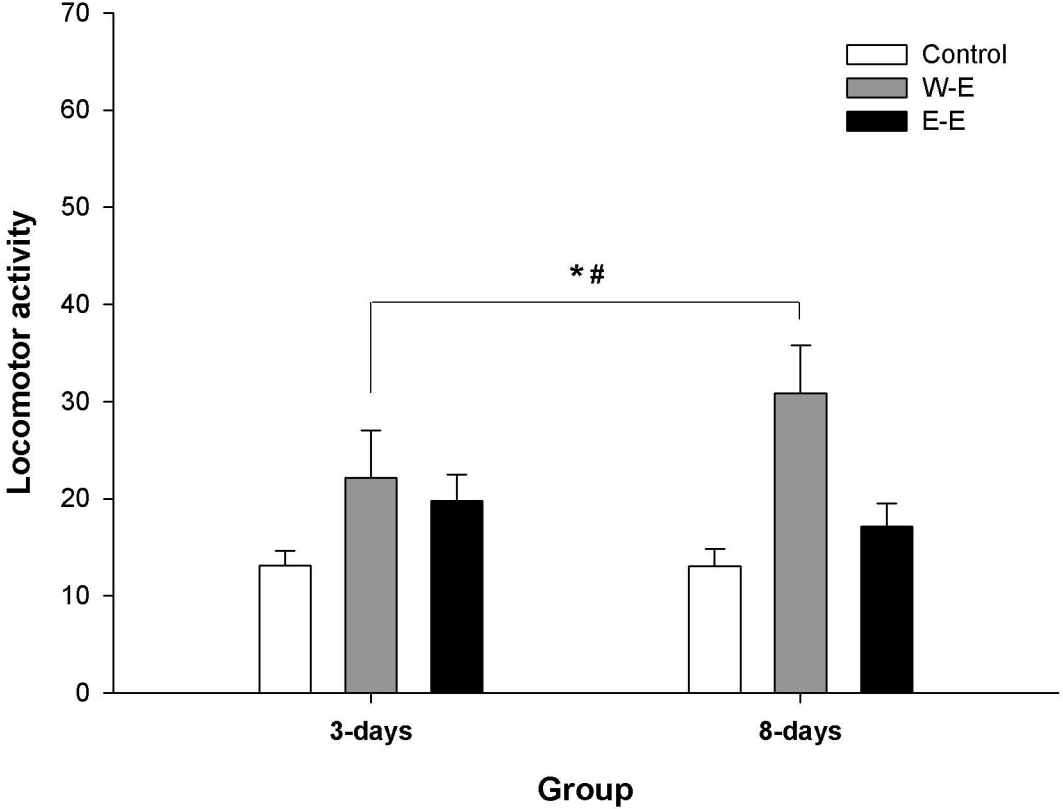
Locomotor activity
*
*
*
Control
W-1.5
W-2.5
E-1.5
E-2.5
Group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Locomotor activity
Control
W-E
E-E
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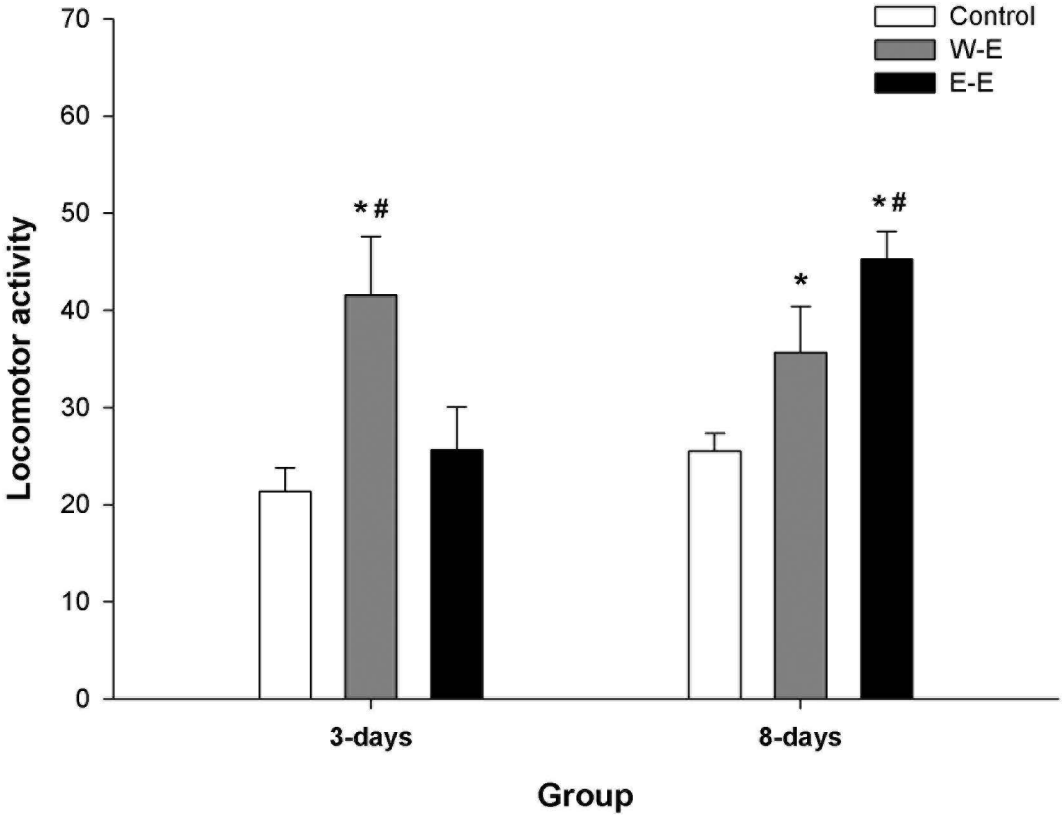
3-days
8-days
Group


Control
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Locomotor activity
70
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Group