Published in final edited form as:

Dev Psychobiol. 2013 March ; 55(2): 193–204. doi:10.1002/dev.21011.

The unconditioned stimulus preexposure effect in preweanling rats in taste aversion learning: role of the training context and injection cues

D.A Revillo^{#b}, C. Arias^{#a,b}, and N.E Spear^c

^aInstituto de Investigación Médica M. y M. Ferreyra (INIMEC – CONICET), Córdoba, C.P 5000, Argentina

^bUniversidad Nacional de Córdoba, Córdoba, CP 5000 Argentina

^cCenter for Developmental Psychobiology, Binghamton University, Binghamton, NY 13902-6000, USA

These authors contributed equally to this work.

The unconditioned stimulus preexposure effect (US-PE) refers to the interference paradigm in which acquisition of the conditioned response is retarded due to prior experience with the US. Most studies analyzing the psychological mechanisms underlying this effect have been conducted with adult rats. The most widely accepted hypothesis explains this effect as a contextual blocking effect. Contextual cues associated with the US block the conditioned stimulus (CS)-US association during conditioning. The modulatory role of a context devoid of distinctive olfactory attributes is not observable until approximately PD23 in rats, including modulation of interference paradigms such as latent inhibition or extinction. In this study we analyzed US-PE in preweanling rats along with the role of the training context in this effect in terms of conditioned taste aversion preparation. Preexposure to LiCl before conditioning retarded the acquisition of taste aversion. The US-PE was observed in preweanling rats when, during preexposure, subjects were exposed to the conditioning context, and this effect was not attenuated either by the administration of the US in a familiar environment (Experiment 1a), or by the presence of an alternative, more salient context during preexposure (Experiment 1b). Additionally, the US-PE was still observed when the route by which the US was administered was changed between the preexposure and conditioning phases (Experiment 2a) as well as when the injection cues were removed during conditioning (Experiment 2b). These experiments show a strong US-PE in preweanling rats and fail to support the contextual blocking hypothesis, at least in this stage of ontogeny.

Proactive interference refers to a phenomenon in which learning in one phase of the experiment interferes with learning or performance in a later phase (Bouton, 1993). An example of this phenomenon is the unconditioned stimulus preexposure effect (US-PE), which can be empirically defined as retardation in acquisition of the CR due to prior

* Corresponding author. Instituto de Investigación Médica M. y M. Ferreyra (INIMEC – CONICET), Córdoba, C.P 5000, Argentina. afelicidade@yahoo.es.

experience with the US (Randich & LoLordo, 1979). The US-PE has been described in laboratory rodents using a wide range of procedures and experimental conditions (A. L. Riley & Simpson, 2001).

Two major non-exclusive hypotheses have been raised to explain the US-PE. The first understands this phenomenon as habituation to the US effects, which interferes with the effectiveness of the US to act as a reinforcer and support conditioning (Randich & LoLordo, 1979; A. L. Riley & Simpson, 2001). The alternative hypothesis interprets the US-PE in terms of blocking. During the preexposure phase, the US is associated with contextual cues which block the CS-US association in a later conditioning phase (Randich & LoLordo, 1979; A. L. Riley & Simpson, 2001). The latter hypothesis has received more empirical support. For example, in conditioned taste aversion preparations, competing external (Rudy, Iwens, & Best, 1977) or internal (injection-related) cues (de Brugada, Gonzalez, & Candido, 2003; de Brugada, Hall, & Symonds, 2004) can interfere with taste-US association. Associative theories that emphasize the importance of context-US to explain this interference paradigm are supported, for example, by the fact that a shift in the context between the preexposure and conditioning phases significantly attenuates LI or the US-PE (Wasserman & Miller, 1997).

Interestingly, according to some authors (for example, Rudy & Morledge, 1994; Foster & Burman, 2010), contextual conditioning in preweanling rats is weak unless the context contains salient cues for the infant rat, such as explicit odors (for example, Brasser & Spear, 1998). Evidence of contextual conditioning has been consistently found around PD23, when, according to some authors, the hippocampus becomes integrated into other brain structures, thus supporting contextual conditioning in an adult-like way (Foster & Burman, 2010). Furthermore, the role of context in different interference paradigms, such as extinction (Yap & Richardson, 2007) or latent inhibition (LI, Yap & Richardson, 2005), varies considerably during the ontogeny of the rat. For example, a shift in context between preexposure and conditioning does not effectively reduce latent inhibition in preweanling rats (Yap & Richardson, 2005). This result conflicts with those explanations which use CS-context associations to explain LI, at least for immature animals. LI becomes context-dependent only after the weaning period, around PD23 (Yap & Richardson, 2005).

The aim of the present study was to analyze some aspects of the US-PE in preweanling rats using a conditioned taste aversion preparation. The specific question under analysis is whether this learning process can be detected during this ontogenetic period when capacity to acquire context conditioning seems to be limited. Few studies have empirically tested this effect in this particular developmental stage, and only one of these used a conditioned taste aversion preparation (Kraemer, Kraemer, Smoller, & Spear, 1989). In this study, Kraemer and collaborators found that preexposure to one non-reinforced LiCl injection did not affect subsequent acquisition of taste aversion learning induced by the same LiCl dose, but the authors did not test longer preexposure treatments. Rudy and Cheattle (1983) have reported evidence of the US-PE, but employing an odor conditioning preparation.

The first specific aim of the present study was to test whether the US-PE depends on the number of US preexposure presentations given to preweanling rats in a conditioned taste

aversion preparation. In adult rats, the magnitude of the US-PE positively correlates with the number of preexposures to the US (for example, Canon, Berman, Baker, Atkinson, 1984). The second aim was to assess whether, in preweanling rats, the US-PE is mediated by the context-US association, or by the association between the US and the injection cues, as observed in adults (de Brugada, et al., 2003; de Brugada, Gonzalez, Gil, & Hall, 2005). The results of this study should further our understanding of how preexposure to drugs affects later learning in which the same drugs are involved as USs (Chotro, Arias, & Laviola, 2007; N. E. Spear & Molina, 2005).

Experiment 1a

In Experiment 1a we tested acquisition of conditioned taste aversion induced by LiCl in preweanling rats, as a function of the number of exposures to the US (0, 1, 2 or 4) prior to conditioning. Additionally, we also explored whether the presence of the conditioning context during the preexposure phase mediates the US-PE at this age. Some studies with adult rats which aimed to analyze the US-PE in taste aversion learning employed a procedure in which the US was injected in the home cage (see for example, Canon et al., 1975), or before rats were placed in the novel context (Cole, VanTilburg, Burch-Vernon, & Riccio, 1996). However, other authors have shown strong context conditioning induced by LiCl using a procedure in which LiCl was injected after subjects were removed from the target context, thus ensuring that they experienced the LiCl effects in a familiar environment (for example, Symonds & Hall, 1997; Symonds, Hall, Lopez, Loy, Ramos & Rodriguez, 1998). By using this specific procedure, these authors showed that prior pairings of a novel context with LiCl reduced the expression of the conditioned response to a novel tastant CS. Following this rationale, in our study we employed a procedure in which, during preexposure, LiCl was injected once pups had been removed from the training context. In this first experiment, during the preexposure phase, some animals received LiCl after being exposed to the same context in which they were going to receive the gustatory CS during conditioning (context A), while the remaining subjects received LiCl in a familiar environment (context B, see procedures for a detailed description of the contexts). Subsequently, all subjects were trained in context A in a conditioned taste aversion preparation in which they were injected with LiCl after being exposed to saccharin in this context. Using this procedure, subjects preexposed to context A were administered LiCl in both phases (preexposure and conditioning) at the same time, once they had been removed from the context. If contextual blocking is indeed responsible for the US-PE, this effect should not be observed, or should at least be attenuated, in those subjects receiving LiCl in context B (the familiar environment) during preexposure. For example, in adult rats it has been shown that the US-PE is attenuated when subjects are preexposed to LiCl in a familiar environment (Cole et al., 1996).

Materials and methods

Subjects—Two-hundred and five Sprague-Dawley pups, representative of 25 litters, were utilized for the present study (including experiments 1a, 1b, 2a and 2b). Table 1 indicates the total number of subjects included in each independent group in each experiment. The animals used in the study were born and reared at the vivarium of the Center for

Development and Behavioral Neuroscience (Binghamton University, NY) under conditions of constant room temperature (22 ± 1.0 °C), on a 12-hour light 12-hour dark cycle. Births were examined daily and the day of parturition was termed postnatal day 0 (PD0). All litters were culled to 10 pups (5 females and 5 males, whenever possible) within 48 hours after birth. All procedures were in accordance with the guidelines for animal care and use established by the National Institute of Health (1996).

Procedures—Preexposure: In all experiments, the preexposure phase was carried out between PDs 13 and 16, one session per day. On preexposure days, pups from a given litter were assigned to experimental groups defined by the number of preexposures to the US (0, 1, 2 or 4) and the context (A or B). Context A consisted of an individual Plexiglas chamber ($10 \times 10 \times 12$ cm) with white plastic walls. The floor of the cage was covered with absorbent white paper towel. The context was located in a lighted room and placed upon a plastic floor maintained at 37 °C by means of a heating pad. In all the experiments, during conditioning pups were exposed to the CS (saccharin) in this context. Context B was the familiar environment. It consisted of a cage identical to those utilized in the laboratory as home-cages. This context was used as a holding chamber for the pups throughout all the time for which they were separated from their home-cage. This context was also situated in a lighted room, and its floor was also covered by pan shavings and maintained to 37 °C by means of a heating pad. It is considered a familiar environment because of its similarity to the home-cage. This context was divided into 8 squares ($7 \times 7 \times 12$) in order to allow pups to be held in couples with a littermate from the same experimental condition during the experiment.

Immediately after assignment to their experimental condition, pups from a given litter were separated from the mother and placed for 20 minutes with a single littermate in the holding cage (context B). During this period, pups were marked and body weights were recorded. Immediately afterwards, one half of the litter was moved to context A for 15 minutes while the other half remained in context B. During this 15-min period, the pups that remained in context B were placed individually in one of the squares into which the context was divided in order to equate groups as regards the amount of time for which they were isolated. After this period, pups received the corresponding vehicle or LiCl (0.3 M, 0.5% of body weight). This LiCl dose was employed in all the phases and experiments of the present study, and was selected from previous studies using a similar treatment in preweanling rats (Arias, Molina, & Spear, 2009; Arias, Pautassi, Molina, & Spear, 2010). Rats preexposed 4 times to LiCl received a LiCl injection every day (from PD13 to PD16); pups exposed twice received saline injections on PDs 13 and 14 and LiCl on PDs 15 and 16, while pups exposed to LiCl only once received saline from PDs 13 to 15, and LiCl on the last preexposure day. A separate group of rats were treated with vehicle (NaCl 0.9 %) on all preexposure days. After LiCl injection, pups were then placed again in couples in the holding cage (context B) for another 15 minutes to reduce the possible influence of the presence of the mother on the magnitude of the aversion. During this period, subjects experienced the peak effect of LiCl (Parker et al., 1984) without the interference of maternal care. After this period, pups returned to their home cage, where they remained undisturbed until the following day.

Conditioning and testing: Conditioning took place on PDs 17 and 18, and testing on PD 19. On PD 17, subjects were separated from the home cage and an intraoral cannula (PE 10 polyethylene tubing, length: 5 cm, Clay Adams, Parsippany, NJ) was implanted into the right cheek of each pup, as described previously (Arias, et al., 2009). 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 procedure requires no more than 20 s per subject and does not induce major stress to infant rats (L. P. Spear, Specht, Kirstein, & Kuhn, 1989). Pups then remained in the holding chamber for an hour to enable them to become habituated to the cannula. Immediately afterwards, the pups' bladders were voided by gentle brushing of the anogenital area and body weights were recorded. Then subjects were placed into the conditioning context (context A for all subjects). In this environment, pups received an intraoral infusion of saccharin (CS, 0.15% w/v, duration: 15 min). The total administration volume was equivalent to 1.5 ml. Saccharin solution was delivered at a constant rate (0.1 ml/min) by means of an infusion pump (KD Scientific, Holliston, MA) connected to each pup's oral cannula by a polyethylene catheter (Clay Adams, PE 50 Parsippany, NJ). With these infusion parameters, pups are capable of either consuming or rejecting the infused solution (Arias, et al., 2010; Diaz-Cenzano & Chotro, 2010). After the infusion procedure, subjects were weighed to estimate saccharin consumption scores by means of the following formula: $[(\text{Post-infusion body weight} - \text{Pre-infusion body weight}) / \text{Pre-infusion body weight}] \times 100$. Immediately after CS exposure, pups were intraperitoneally injected with LiCl. After drug treatment, pups were placed in the holding chamber for 15 minutes before being reunited with their mother. The second conditioning trial was conducted the following day (PD18), applying the same procedures as those described for the first conditioning trial. The procedures used for testing (on PD19) were identical to those described for conditioning, with the only exception being that the US (LiCl) was not injected.

Data analysis—Both in this and in all subsequent experiments, no more than one male and one female from a given litter were assigned to the same treatment condition, in order to avoid overrepresentation of any given litter in any particular treatment (Holson & Pearce, 1992). Male and female subjects were equally balanced across experimental groups. No significant effect of sex or interaction with the remaining factors was found in any of the experiments included in the present study. Hence, for the inferential analysis and descriptive representation of the results, data were collapsed across sex. Intake scores from Experiment 1a were analyzed by means of a mixed ANOVA including number of preexposures (0, 1, 2 or 4) and context (A or B) as between-group factors and day as a within-group variable with 3 levels, corresponding to two conditioning and one testing trial. In both this and subsequent experiments, significant main effects and/or interactions were further analyzed by means of follow-up ANOVAs and post-hoc analyses (Newman-Keuls). All inferential analyses conducted in the present study employed an α level equal to 0.05.

Results

Figure 1a shows intake scores during conditioning and testing days as a function of the number of LiCl preexposures (0, 1, 2 or 4). On the left-hand side are intake data from subjects exposed to context A during preexposure, while on the right-hand side are scores from subjects preexposed to LiCl in the holding cage (context B). The ANOVA revealed significant main effects of preexposure and day [$F(3,75) = 5.42$, $p < 0.05$ and $F(2,150) = 170.00$, $p < 0.05$, respectively], and a significant interaction between preexposure and day [$F(6,150) = 4.75$, $p < 0.05$]. To determine the locus of this interaction, follow-up one-way ANOVAs were performed with preexposure condition as the only between-group variable. The dependent variable in these analyses was the intake score from each day. These ANOVAs revealed a significant effect of preexposure on conditioning day 2 and testing day [$F(3,79) = 8.66$; $p < 0.05$ and $F(3,79) = 5.61$; $p < 0.05$, respectively]. According to the post-hoc analyses, rats preexposed 4 times to LiCl consumed more saccharin than the other groups on conditioning day 2. Additionally, on testing day, rats preexposed 4 times ingested more saccharin than those preexposed once to LiCl or not at all, while those preexposed twice also consumed more saccharin than non-preexposed controls.

Experiment 1b

Results from Experiment 1a indicate that the strength of the preexposure effect was dependent on the number of LiCl preexposures, a finding which is congruent with that observed by studies with adult rats. Interestingly, the US-PE was observed regardless of whether or not subjects had had prior experience with the training context (context A) during the preexposure phase. This result contrasts with evidence found in adult rats, which shows that when subjects are preexposed to LiCl in a familiar environment, the US-PE is significantly attenuated (for example, Cole et al., 1994). In the following experiment, subjects were preexposed to an alternative and more distinctive environment (context C) during the preexposure phase. Context C was completely different (see procedures) than the one employed for conditioning (context A). If the US-PE is indeed mediated by contextual blocking, then the US-PE should be attenuated in pups preexposed to context C in comparison with those preexposed to context A, since context C was not present during conditioning.

Procedures

The procedures were identical to those described for Experiment 1a, with two exceptions. Firstly, during the preexposure phase, subjects were placed in context A or in context C prior to LiCl administration. Context C consisted of a Plexiglas chamber ($10 \times 10 \times 12$ cm) resting on a grid with 3 mm diameter bars spaced 0.5 cm apart and placed in a dark room. In this case, the walls and the top of the cage were completely covered by a smooth black carpet. The grid was elevated approximately 2 cm, and below it a heating pad was placed to keep the chamber heated to 37 °C. And secondly, only two preexposure conditions were included in the experimental design, 4 preexposures to LiCl (since this condition had been found to induce the strongest US-PE in Experiment 1a) and the non-preexposure treatment, which was included as a control condition.

Data analysis

Intake data from Experiment 1b were analyzed by means of a mixed ANOVA including number of preexposures (0 or 4) and context (A or C) as between-group factors and day as a within-group variable with 3 levels, corresponding to two conditioning and one testing trial.

Results

Experiment 1b—Figure 1b represents intake scores during conditioning and testing days as a function of the number of preexposures (0 or 4) and the context in which pups were preexposed. The left-hand side of Figure 1b shows intake scores of subjects preexposed to context A before LiCl, whereas intake data from subjects preexposed to context C are shown on the right-hand side. The ANOVA revealed significant main effects of preexposure and day [$F(1,36) = 44.08$, $p < 0.05$] and [$F(2,72) = 394.79$, $p < 0.05$, respectively]. The following interactions also reached statistical significance: preexposure by context [$F(1,36) = 5.61$, $p < 0.05$], and preexposure by day [$F(2,72) = 25.71$, $p < 0.05$]. According to the post-hoc analyses, subjects preexposed to context C and given LiCl consumed more saccharin than those preexposed to context A and given LiCl. Saccharin intake did not differ between subjects preexposed to context A and C and treated with saline during the preexposure phase. The interaction between preexposure treatment and day was explored by follow-up one-way ANOVAs with preexposure treatment as the only between-group variable. The dependent variable for these analyses was saccharin consumption. These ANOVAs revealed a significant main effect of preexposure on conditioning day 2 and testing day [$F(1,38) = 39.19$, $p < 0.05$] and [$F(1,38) = 38.18$, $p < 0.05$, respectively], indicating that saccharin consumption was significantly higher in pups preexposed to LiCl than in their non-preexposed counterparts. The three-way interaction was close to significance [$F(2,72) = 2.79$, $p = 0.06$], a borderline effect driven by the preexposure by context interaction during the second conditioning trial [$F(1,36) = 7.57$, $p < 0.05$]. Post-hoc analyses revealed that, during the second conditioning trial, pups exposed to context C during preexposure consumed more saccharin than the other groups, indicating that, contrary to the working hypothesis, the magnitude of the US-PE was enhanced when pups were exposed to the more salient context, even when this context was not present during conditioning.

Experiments 2a and 2b

The results from the previous experiments show that the US-PE can be observed in preweanling rats using a taste aversion preparation. As mentioned earlier, this ontogenetic period is characterized by weak context conditioning. The US-PE was found regardless of the presence of the conditioning context during preexposure, and the magnitude of this effect was not attenuated when pups were exposed to LiCl in a familiar environment, or when they were injected with LiCl after being exposed to a salient context. Some authors have presented empirical evidence showing that adult rats can associate injection cues with LiCl effects, an association that may be responsible for the US-PE, at least in the taste aversion learning preparation (de Brugada, et al., 2003; de Brugada, et al., 2005; de Brugada, et al., 2004). Experiments 2a and 2b were designed to analyze whether or not injection cues play a role in the US-PE in preweanling rats.

Procedures

In both experiments the preexposure procedures were identical to those used in Experiment 1b: 0 or 4 LiCl preexposures after being exposed to context A or B. As mentioned above, since context B was a familiar environment, the injection cues may be more salient in this context than in context A. Therefore, the injection cues may predict the US better in this environment than in context A, which has more novel features that may compete with the injection cues. For this reason we included both contexts in these experiments. During the preexposure phase of both experiments, LiCl was administered intraperitoneally. For conditioning (always in context A), however, LiCl was administered through different routes. In Experiment 2a, the same LiCl dose (0.3 M, 0.5% of body weight) was intragastrically delivered, whereas for conditioning in Experiment 2b, LiCl administration was intraoral. For the LiCl-saccharin solution in Experiment 2b, the saccharin concentration (0.15%) was the same as that employed in the previous experiments, while the LiCl concentration (0.06 M) was chosen to allow the pups to ingest an amount of the drug similar to that injected in the previous experiments. On the first conditioning day (PD 17) pups usually weigh about 40 grams, and during the injection procedures, the volume administered was 0.5% of body weight of a 0.3 M LiCl solution, which is equivalent to 0.20 ml. On the first conditioning day (on PD 17), pups ingest around 1 ml of saccharin solution (0.15%). Hence, a 0.06M LiCl solution should result in a dose similar to that injected in Experiments 1a, 1b and 2a. In Experiment 2b the LiCl-saccharin solution was delivered over 15-minute period on both PDs 17 and 18. A significant reduction in acceptance of the solution was expected on the second testing day due to the aversive properties of the LiCl ingested on the first day. For this reason, only two intake tests were carried out. Even if the US-PE were still observable on the second day, it would not be comparable across preexposure conditions. Our working hypothesis was that, to the extent that the injection-related cues blocked the taste-LiCl association in the previous experiments, the US-PE should be eliminated by either changing the injection procedures (Experiment 2a) or completely removing the injection cues (Experiment 2b) for conditioning.

Data analysis

Intake data from Experiment 2a were analyzed by means of a mixed ANOVA including number of preexposures (0 or 4) and context (A or B) as between-group factors and day as a within-group variable with 3 levels, corresponding to two conditioning and one testing trial. Data from Experiment 2b were analyzed by means of a mixed ANOVA [number of preexposures (0 or 4)] $\times$ context (A or B) $\times$ day (one conditioning and one testing trial)].

Results

Experiment 2a—Figure 2a represents saccharin consumption during conditioning and testing days as a function of preexposure condition. Intake scores from rats preexposed to context A are shown on the left, while data from subjects preexposed to context B are shown on the right. The ANOVA revealed significant main effects of preexposure treatment and day [$F(1,27) = 22.44$; $p < 0.05$ and $F(2,54) = 15.58$; $p < 0.05$, respectively], and a significant interaction between both factors [$F(2,54) = 13.33$; $p < 0.05$]. To further analyze this interaction, separate follow-up one-way ANOVAs were performed with preexposure

condition as the only between-group factor, with the intake scores collected each conditioning or testing day. According to these ANOVAs, pups preexposed to LiCl consumed more saccharin than those given saline during the preexposure phase on conditioning day 2 and testing day [$F(1,30) = 54.38$; $p < 0.05$ and $F(1,30) = 16.93$; $p < 0.05$, respectively].

Results from this experiment indicate that changing the procedure of LiCl administration between preexposure and conditioning does not eliminate the US-PE in preweanling rats. However, it is likely that the magnitude of the effect would be attenuated, although not necessarily eliminated. To rule out this possibility, we explicitly compared saccharin intake from Experiments 1a and 2a, but only from those subjects that received the same amount of US preexposure. The ANOVA utilized for this analysis included the variables number of preexposures (0 or 4), context (A or B) and administration route during conditioning (ip or ig) as between-group factors, and day as the within-group variable with 3 levels. This analysis revealed significant main effects of preexposure [$F(1,64) = 31.59$; $p < 0.05$], administration route [$F(1,54) = 12.07$; $p < 0.05$], and day [$F(2,128) = 91.65$; $p < 0.05$], and the interactions preexposure by day [$F(2,128) = 26.63$; $p < 0.05$] and administration route by day [$F(2,128) = 13.74$; $p < 0.05$]. Follow-up one-way ANOVAs were performed to explore these interactions. According to these analyses, subjects preexposed to LiCl consumed more saccharin on conditioning day 2 [$F(1,70) = 34.14$; $p < 0.05$] and testing day [$F(1,70) = 24.04$; $p < 0.05$]. Additionally, subjects given LiCl intragastrically consumed more than those given LiCl intraperitoneally during the second conditioning trial [$F(1,70) = 8.97$; $p < 0.05$] and testing [$F(1,70) = 10.74$; $p < 0.05$]. This result shows that the magnitude of the aversion induced through the intragastric administration of LiCl was not as strong as the one induced when LiCl was injected intraperitoneally. This difference could be due to the fact that, although in both experiments (1a and 2a) we employed the same LiCl dose, in Experiment 1a we employed a hypertonic (0.3 M) solution. It is well known that hypertonic injections induce pain (Giesler & Liebeskind, 1976) which may contribute to the magnitude of the taste aversion. However, preexposure treatment did not interact with administration route, indicating that the magnitude of the US-PE was not affected by the change in administration procedure.

Experiment 2b—Figure 2b shows saccharin intake data collected on intake days 1 and 2, on which preweanling rats were allowed to ingest the saccharin and LiCl solution. On the left are the scores from subjects for which the context for the preexposure and conditioning phases was the same, and on the right are the intake scores from subjects given US preexposure after being placed in context B and trained in context A. The ANOVA revealed significant main effects of preexposure and day [$F(1,36) = 60.84$; $p < 0.05$ and $F(1,36) = 56.83$; $p < 0.05$, respectively], and a significant interaction between both factors [$F(1,36) = 36.08$; $p < 0.05$]. This interaction was explored by means of follow-up one-way ANOVAs, in which the independent variable was the preexposure treatment, and the dependent variable was the consumption scores from each day. These ANOVAs revealed a significant main effect of preexposure treatment on the second day [$F(1,38) = 48.60$; $p < 0.05$], indicating higher intake scores from pups given LiCl than from those treated with saline

during the preexposure phase. Removing the injection cues from conditioning does not affect the US-PE in preweanling rats.

Discussion

Two main conclusions can be drawn from the present study: a) Prior exposure to the US before conditioning results in a significant attenuation of the conditioned response in preweanling rats; and b) this effect is observed regardless of the presence of the training context during preexposure, and regardless of whether the US is administered in a familiar or non-familiar and salient environment. In none of the experiments did preexposure to the US affect response to the CS (saccharin intake) on the first conditioning day, indicating that the greater saccharin intake by US-preexposed rats on conditioning day 2 or during testing was due to impaired learning rather than the nonspecific effects of LiCl on saccharin consumption. The US-PE has been reported using a variety of preparations in a variety of animal species (Abramson & Bitterman, 1986; A. L. Riley & Simpson, 2001), but most of the studies seeking to analyze this effect have been carried out with adult rats. To our knowledge, this is the first systematic test of the US preexposure effect in preweanling rats with conditioned taste aversion. Using a similar paradigm, Kraemer and collaborators failed to find the US-PE in preweanling rats (Kraemer, et al., 1989). However, in that study the authors preexposed infant rats only once to LiCl, a treatment that was also ineffective in Experiment 1 to reduce the CR. Rudy and Cheattle also reported evidence of the US-PE in infant rats using a completely different preparation. In their study a single LiCl exposure reduced odor aversion induced by LiCl (Rudy & Cheattle, 1983). Our results extend this observation and demonstrate that, similarly to that reported in adult rats, preexposure to LiCl retards the acquisition of subsequent conditioned taste aversion with the same US.

According to the present experiments, the presence of the conditioning context during preexposure does not mediate the US-PE in preweanling rats, because this effect was observed both when rats were injected with LiCl in a familiar environment and when administration took place in an alternative and salient context different from the one employed during conditioning. This result is consistent with prior reports analyzing the role of context in alternative interference paradigms, such as latent inhibition, during the same ontogenetic period (Hoffmann & Spear, 1989; Yap & Richardson, 2005). Preweanling rats preexposed to a CS in a given context and trained in an alternative context still show attenuation of the CR (Yap & Richardson, 2005). The lack of context-dependence makes it difficult to interpret the US-PE by alluding to a contextual blocking effect. Some recent studies have indicated that the US-PE in the conditioned taste aversion paradigm can be explained by an association formed between injection cues and the aversive effects of the US during the preexposure phase. This association is said to block the taste-illness association during conditioning (de Brugada, et al., 2003; de Brugada, et al., 2005; de Brugada, et al., 2004). This explanation also fails to fit well with our results, since varying the injection procedures (Experiment 2a) or eliminating the injection cues (Experiment 2b) during conditioning had no effect on the US-PE.

Our results may be explained through non-associative mechanisms, such as tolerance to the US effects (A. L. Riley, Jacobs, & LoLordo, 1976). This explanation is difficult to confirm

because even when tolerance to one of the US effects is detected (for example, tolerance to LiCl-induced hypothermia or hypo-locomotion), it is necessary to prove that this measure is causally linked to the conditioned taste aversion. In some cases, tolerance to the hypothermic or locomotor effects of LiCl has been reported (Batson, 1983), but no convincing evidence has been found to support a causal link between this tolerance and the US-PE (Hall, 2009). For example, tolerance to LiCl-induced hypothermia and hypoactivity is not evident even after LiCl treatments that are more extensive than both those required to induce the US-PE and the one employed in our study (Batson, 1983). Yet, this hypothesis cannot be completely ruled out, and further research will be required to explore whether tolerance to the US can explain the US-PE in preweanling rats.

Contextual and US information converge in the amygdala. For example, Radulovic and collaborators (1998) showed that, in the context of fear conditioning, the US (footshock) by itself induces fos production in the central nucleus of the amygdala, and the fos level induced by a non-preexposed US presented in a novel environment is significantly enhanced in this area of the amygdala (Radulovic, et al., 1998). Some authors have suggested that, during the preweanling period, the hippocampus is not sufficiently functionally integrated with other structures, such as the amygdala, to support contextual fear conditioning (Foster & Burman, 2010). This may help explain why some interference paradigms, such as latent inhibition (Yap & Richardson, 2005) and extinction (Yap & Richardson, 2007) are not context-dependent during the preweanling period. Foster and Burman (2010) provided evidence that the hippocampus is sufficiently functional during the preweanling period to generate contextual learning, but context information seems not to be integrated by the amygdala to support contextual fear conditioning until PD23. Yap and Richardson (Yap & Richardson, 2005) showed that although preweanling rats (PD18) seem to associate the CS with the context, this association does not modulate latent inhibition unless CS-US conditioning takes place on PD23. A similar result was reported by the same authors when analyzing the role of context in the renewal effect after extinction of fear memories during this ontogenetic period (Yap & Richardson, 2007). Manrique and collaborators (2009) also reported that the temporal context-dependency of latent inhibition, a hippocampal-dependent phenomenon (Molero, Moron, Angeles Ballesteros, Manrique, Fenton & Gallo, 2005), emerges late in adolescence. What is happening in this developmental stage in response to experimental preparations assessing the role of context in these interference paradigms is reminiscent of similar phenomena in rats with hippocampal inactivation or lesions. Selective hippocampal inactivation or lesion impairs the context dependence of latent inhibition (Honey & Good, 1993; Maren & Holt, 2000) or extinction (Corcoran, Desmond, Frey, & Maren, 2005; Corcoran & Maren, 2004). Considering these antecedents, we can postulate that the lack of context-dependence of the US-PE in our study may be related to the lack of association between the US and the context during the preexposure phase, since the amygdala and hippocampus seem not to be functionally integrated.

There is, however, an important caveat that arises in interpreting the effect of age on conditioning to context: the animal's processing of features that define context are subject to the sensory and perceptual constraints of developing rats that have only recently opened their eyes and ears. The defining features of the context conventionally used for tests of fear conditioning in adult rats and mice lack the olfactory distinctiveness used by preweanling

rats to negotiate their world. When olfactory distinctiveness is added to contextual variance, conditioning to context is as effective in preweanling rats (e.g., P16-18) as in adults, and even more effective than in adults in some circumstances (Brasser & Spear, 1998; Esmoris-Arranz, Mendez & Spear, 2008). Considering this, it is possible that the contexts employed in our study are not salient enough for subjects, thus resulting in weak contextual conditioning. If this is indeed the case, and we assume that, in our study, preweanling rats fail to generate an association between environmental cues and the occurrence of the US during the preexposure phase, it is difficult to explain using associative theories why the CS (saccharin) is not associated with the US (LiCl) during conditioning. For example, the Rescorla and Wagner model (Rescorla & Wagner, 1972) predicts that, in the event of the US not being associated with other specific events during the preexposure phase, the associative potential of the US should remain intact and susceptible, in the present study, to association with saccharin. This hypothesis is congruent with results obtained by Cole and collaborators showing a strong attenuation of the US-PE when subjects were preexposed to the US in a familiar environment (Cole et al., 1996). In our study, an attenuation of the US-PE should similarly be expected when, during the preexposure phase, preweanling rats fail to use any cues to predict the occurrence of the US. However, the effect was observed in all the experiments, even when the injection cues were eliminated during conditioning or when the contextual cues were explicitly changed between the preexposure and conditioning phases, conditions that seem to maximize the use of these cues as predictors of the US. Additionally, in Experiment 1b, in which context C, a more salient context that should facilitate context conditioning (Brasser & Spear, 1998; Pugh & Rudy, 1996), was used during preexposure, the US-PE was also observed and it was even stronger.

Considering these results, it will be interesting in future studies to analyze the ontogeny of the US-PE. The taste aversion paradigm is probably not the most appropriate paradigm for this analysis, because procedural requirements for this paradigm vary considerably from infancy to adulthood. For example, adult rats require a water deprivation schedule for several days before training while infants do not. Additionally, consumption in infant rats requires forced techniques, such as the intraoral infusion, while in adult rats intake can be easily estimated through the bottle test. Even so, our procedure shares some similarities with the one employed by Symonds et al. (1998). In both studies LiCl was injected after subjects were exposed to a distinctive environment, and subjects experienced the effects of LiCl in a familiar environment. Symonds and collaborators reported that, under their experimental conditions, the context in which subjects were placed before LiCl administration during preexposure blocked the taste-illness association when, during conditioning, a novel taste was exposed in this context (Symonds et al., 1997; Symonds et al., 1998). As these authors discussed (see Symonds et al., 1997, discussion section), this blocking effect provides strong empirical evidence to support the contextual blocking hypothesis for the US-PE. Our results suggest that, in infant rats, the US-PE seems not to be mediated by the same learning mechanism. However, as mentioned earlier, the question about the ontogenetic profile of this learning phenomenon should be approached in future studies using an alternative procedure, such as fear conditioning, that requires more similar conditions across age.

There may be alternative explanations related to procedural manipulations that may have influenced the results reported here. It is likely that infants given LiCl during preexposure

received different attention from the mother once they returned to their home cage. We did not explore whether the mother treated pups differently depending on whether or not they were sick. However, according to a recent unpublished study from our laboratory, the magnitude of the US-PE in the infant rats is similar in both pups treated with LiCl in the home cage and in those that experienced LiCl effects out of the home cage. Hence, the US-PE cannot be completely explained by alluding to differences in the maternal care provided.

In short, the present study confirms the US-PE in preweanling rats. Our results seem not to support the contextual blocking hypothesis, at least for this specific stage of ontogeny.

Acknowledgments

Contract grant sponsor: NIAAA

Contract grant numbers: AA11960, AA013098, AA015992

Contract grant sponsor: NIMH

Contract grant number: MH035219

Contract grant sponsor:

Contract grant sponsor: Ministerio de Educacion y Ciencia, Spain, Subprograma Ramon y Cajal to CA

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Figure 1.

(a) Mean saccharin intake at conditioning (1 and 2) and testing days, as a function of the number of US preexposures (0, 1, 2, or 4) and the context in which subject were preexposed (context A or B). Vertical lines illustrate the standard error of the mean. (b) Mean saccharin intake at conditioning (1 and 2) and testing days, as a function of the number of US preexposures (0 or 4) and the context in which subject were preexposed (context A or C). Vertical lines illustrate the standard error of the mean.

Figure 2.

(a) Mean saccharin intake at conditioning (1 and 2) and testing days, as a function of the number of US preexposures (0 or 4) and the context in which subject were preexposed (context A or B). In this experiment, the US was delivered intraperitoneally administered during the preexposure phase, and intragastrically at conditioning. Vertical lines illustrate the standard error of the mean. (b) Mean consumption of the LiCl and saccharin solution (days 1 and 2) as a function of the number of US preexposures (0 or 4) and the context in which subject were preexposed (context A or B). Vertical lines illustrate the standard error of the mean.

Table 1

Number of subjects (n) per group included in each experiment. Pre-exposure treatment refers to the number of times that LiCl was presented during the pre-exposure phase. Context during preexposure and context during conditioning indicates in which context subjects were placed in the preexposure or conditioning phase. Route of administration indicates which route (intraperitoneal, ip; intragastric, ig; intraoral, io) was employed to deliver LiCl to subjects in each experiment at conditioning.

	Preexposure treatment	Context during preexposure	Context during conditioning	Route of LiCl administration during conditioning	n
Experiment 1a	0 LiCl	A	A	ip	7
	1 LiCl	A	A	ip	8
	2 LiCl	A	A	ip	9
	4 LiCl	A	A	ip	8
	0 LiCl	B	A	ip	11
	1 LiCl	B	A	ip	13
	2 LiCl	B	A	ip	13
	4 LiCl	B	A	ip	14
Experiment 1b	0 LiCl	A	A	ip	10
	4 LiCl	A	A	ip	10
	0 LiCl	C	A	ip	10
	4 LiCl	C	A	ip	10
Experiment 2a	0 LiCl	A	A	ig	8
	4 LiCl	A	A	ig	8
	0 LiCl	B	A	ig	8
	4 LiCl	B	A	ig	7
Experiment 2b	0 LiCl	A	A	io	10
	4 LiCl	A	A	io	10
	0 LiCl	B	A	io	10
	4 LiCl	B	A	io	10