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Context-Dependent Latent Inhibition in Preweanling Rats

ABSTRACT: *Preexposure to a conditioned stimulus (CS) usually weakens conditioning, an effect known as latent inhibition. Similar to other learning interference effects, latent inhibition has been characterized as context-dependent, which means that the magnitude of this effect can be attenuated by changing the context between the different phases of the procedure (e.g., preexposure and conditioning). Latent inhibition has been found with a variety of procedures in infant rats, but the few studies that examined the context-dependency of this phenomenon during this ontogenetic period found no context-change effect. The present study explored the context-dependency of latent inhibition during infancy using a conditioned taste aversion preparation and employing contexts enriched with distinctive odors to increase the possible efficacy of the context manipulation. Experiment 1 showed that three preexposures to the CS (saccharin) were sufficient to retard conditioning to the same CS, although this effect was also observed in a control group preexposed to an alternative taste stimulus (saline), in comparison with a non-preexposed control group. In Experiment 2a, the CS-preexposure effect was found to be specific to the preexposed CS when the number of preexposures was increased. This effect was revealed as context-dependent in Experiment 2b, since it was attenuated by changing the context between preexposure and conditioning. The present result is consistent with recent studies showing the context-dependency of extinction in preweanling rats, thus demonstrating these animals' capacity to learn about context early on in their development.* *© 2014 Wiley Periodicals, Inc.* Dev Psychobiol

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INTRODUCTION

When an organism experiences a neutral stimulus (conditioned stimulus, CS) followed by a biologically relevant stimulus (unconditioned stimulus, US), the first one may acquire the property of inducing a response in the organism (conditioned response, CR) that is similar to the one triggered by the US (unconditioned response, UR). This is what happens in a typical Pavlovian conditioning experimental procedure (Pavlov, 1927), such as taste aversion learning. In this procedure, a rat consumes a novel taste (CS) before being injected with LiCl (US). As a consequence of this experience, the rat rejects the CS (CR), an effect that has been observed in numerous experiments with adult (Kalat & Rozin, 1973; Palmerino, Rusiniak, & Garcia, 1980) or infant rats (Arias, Pautassi, Molina, & Spear, 2010; Revillo, Spear, & Arias, 2011).

The CR can be interfered with by means of a variety of procedures, such as, for example, non-reinforced presentations of the CS before or after conditioning (i.e., latent inhibition or extinction) (Wasserman & Miller, 1997). In taste aversion learning, for instance, consumption of a sapid solution before conditioning

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attenuates the magnitude of the aversion (Best, 1975; Cannon et al., 1985). It is well known that interference procedures are highly sensitive to context change, at least in adult rats (Bouton, 1993). In other words, a context change between the different phases of the experiment attenuates the magnitude of the interference. However, this is not so clear during infancy. For example, in this ontogenetic stage, a context change was not found to attenuate the magnitude of some interference effects, such as the US-preexposure effect (Revillo, Arias, & Spear, 2012), extinction (Yap & Richardson, 2007), and latent inhibition (Yap & Richardson, 2005). These results are consistent with studies that failed to find long-term retention of contextual conditioning in infant rats under 21 days of age (Murawski & Stanton, 2011; Rudy & Morledge, 1994; Schiffino, Murawski, Rosen, & Stanton, 2011). In fact, according to some authors, long-term retention of context learning and context-modulation of interference learning (such as latent inhibition) are phenomena that may be causally linked in that they involve similar brain structures (such as the dorsal hippocampus) and both emerge after weaning, around postnatal day 23 (PD23) (Rudy, 1994; Yap & Richardson, 2005).

However, several studies have shown long-term context learning (Beane, Cole, Spencer, & Rudy, 2002; Brasser & Spear, 2004; Esmoris-Arranz, Mendez, & Spear, 2008; McKinzie, Lee, Bronfen, Spear, & Spear, 1994; McKinzie & Spear, 1995; Pisano, Ferreras, Krapacher, Paglini, & Arias, 2012; Pugh & Rudy, 1996) and context effects (Richardson, Riccio, & Axiotis, 1986; Solheim, Hensler, & Spear, 1980) in infant rats, which in some cases are equivalent to that displayed by adolescent or adult rats. There are important procedural differences between those studies that observed long-term context memory during infancy and those that did not, with one of the most important being the salience of the context used. Those studies which reported positive evidence of contextual memory in this ontogenetic stage have frequently employed sensory enriched contexts, using, for example, black walls instead of transparent ones (Pugh & Rudy, 1996) or including distinctive odor cues in the context (Brasser & Spear, 2004; McKinzie & Spear, 1995). Based on these latter antecedents, two recent studies reported evidence of the context-dependency of extinction in preweanling rats, using an ABA-renewal procedure in a conditioned taste aversion and fear conditioning paradigm (Revillo, Castello, Paglini, & Arias, 2014; Revillo, Molina, Paglini, & Arias, 2013). Specifically, in these studies, infant rats were trained in context A, and then extinction was carried out in context B. Finally, rats were tested in the presence of the CS in either context A or B. Results showed that the CR was higher in context A than in context B (indicating an ABA-renewal effect). Interestingly, this renewal effect was observed when contexts A and B differed in terms of visual and odor cues, although not when they different solely in their visual content (Revillo et al., 2013). A similar result was found in a study with adult rats, in which ABA-renewal was detected only when contexts A and B differed in three dimensions (size, odor, and position), and not when odors were excluded from contexts (Thomas, Larsen, & Ayres, 2003). These results highlight the importance of the sensory content of the context when studying context learning and context modulation of interference effects, regardless of subjects' age. In the ontogenetic analysis of this kind of learning phenomena, special attention should be paid to the sensory modality of the elements which make up the context, since the visual system of the infant rat is not yet fully mature during the preweanling period (infant rats open their eyes around PD14), and olfaction is the primary sense used for guiding behavior (Brasser & Spear, 2004).

To the best of our knowledge, the role of context in latent inhibition in preweanling rats has only been directly explored in a few studies, all of which consistently showed that context manipulation did not affect the interference effect in this developmental period (Hoffmann & Spear, 1989; Rudy, 1994; Yap & Richardson, 2005). In fact, in one of these studies, latent inhibition during infancy was only observed when subjects were trained after a short rather than a long period of time (Rudy, 1994). The specific question guiding the present study is whether a context change between preexposure and conditioning is effective in attenuating the CS-preexposure effect in preweanling rats. To answer this question we designed two experiments, using a conditioned taste aversion procedure. Experiment 1 analyzed the effect of three preexposures to the CS before conditioning. In Experiment 2, an additional preexposure trial was added in order to increase the magnitude and specificity of the effect observed in Experiment 1. In this experiment, subjects were preexposed to the CS either in the same context as that used for conditioning and testing (AAA—Experiment 2a) or in a different one (BAA—Experiment 2b). Given the importance of the odor component for context learning during infancy, in these experiments we employed contexts that included distinctive odors in order to increase the possible efficacy of the context manipulation. It is important to note that in previous studies which examined the context-dependency of latent inhibition during infancy, contexts varied in several characteristics (size, visual cues, background noise intensity, and illumination), but not in their odor content

(Hoffmann & Spear, 1989; Rudy, 1994; Yap & Richardson, 2005).

MATERIALS AND METHODS

Subjects

A total of 86 Wistar rat pups, representative of 13 litters, were used for the present study, including Experiments 1 ($n = 24$), 2a ($n = 27$), and 2b ($n = 35$). Table 1 shows the total number of subjects from each independent group. In all experiments, subjects were quasi-randomly assigned to the treatment groups. Precautions were taken to prevent overrepresentation of any one particular litter in any treatment (Holson & Pearce, 1992), and for this reason, no more than one male and one female from a given litter were assigned to the same treatment condition. Animals were born and reared at the Psychology Department vivarium under conditions of constant room temperature ($22 \pm 1.0^{\circ}$C), on a 12-hr light–12-hr dark cycle. Births were examined daily and the day of parturition was termed postnatal Day 0 (PD0). Litters were culled to 10 pups within 48 hr after birth. Subjects were 14 (PD14, Experiment 1) or 13 (PD13, Experiments 2a and 2b) days old at the start of the experiments. European regulations for the care and treatment of experimental animals were followed, and procedures were controlled and approved by the "Ethics and Animal Care Committee" at the University of the Basque Country UPV/EHU (CEBA) and the Diputacion Foral de Gipuzkoa, Spain, in compliance with the European Communities Council Directive (86/609/EEC).

Apparatus

Two different contexts were employed in all experiments (Experiments 1, 2a, and 2b). The first context consisted of an opaque Plexiglas chamber (15 cm × 15 cm × 20 cm) with white walls and a paper floor, located in a room illuminated by white light and scented with lemon odor by means of a piece of cotton wool impregnated with lemon odor (.1 ml of pure LorAnn oil scent, Lansing, MI) and positioned 30 cm away from the context. The second context was a transparent Plexiglas chamber (25 cm × 15 cm × 15 cm) located in a room illuminated by red light and scented with almond odor (.2 ml of pure LorAnn oil scent). A piece of cotton wool impregnated with the almond odor was also placed 30 cm away from the context. In all experiments the contexts were counterbalanced, so that for half of the subjects Context A was the first context (i.e., the one scented with lemon odor), while for the remaining half it was the second context (i.e., the one scented with almond odor). Furthermore, in Experiment 2b, context B was always the alternative context to the one used during the preexposure phase.

Procedures

Preexposure. Preexposure was carried out between PDs 14 and 16 in Experiment 1, and between PDs 13 and 16 in Experiments 2a and 2b. In all experiments, subjects were preexposed to the CS once a day. Therefore, in Experiment 1, subjects were preexposed to the CS three times, between PDs 14 and 16 (once a day), and in Experiment 2, subjects were preexposed four times, between PDs 13 and 16 (once a day). On preexposure days, subjects were removed from their home cage and assigned to one of the three independent groups [Non Preexposed (N-P), Preexposed to saline (P-Sal), or Preexposed to saccharin (P-Sac)]. The names of the groups allude to the solution that these groups received during the preexposure phase. Immediately afterwards, an intraoral cannula (PE 10 polyethylene tubing, length: 5 cm, Clay Adams, Parsippany, NJ) was implanted into the right cheek of each pup from groups P-Sac and P-Sal, as described previously (Arias, Molina, & Spear, 2009; Arias et al., 2010; Revillo, Fernandez, Castello, Paglini, & Arias, 2012). Briefly, the 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

Table 1. Experimental Design and Number of Subjects for Each Experimental Group

Group	Preexposure Trials	Preexposure	Context	Conditioning	Context	Testing	Context	*n*
Experiment 1								
N-P	3	Context-only	A	Saccharin-LiCl	A	Saccharin	A	8
P Sal	3	Context + Saline	A	Saccharin-LiCl	A	Saccharin	A	8
P Sac	3	Context + Saccharin	A	Saccharin-LiCl	A	Saccharin	A	8
Experiment 2a								
N-P	4	Context-only	A	Saccharin-LiCl	A	Saccharin	A	9
P Sal	4	Context + Saline	A	Saccharin-LiCl	A	Saccharin	A	9
P Sac	4	Context + Saccharin	A	Saccharin-LiCl	A	Saccharin	A	9
Experiment 2b								
N-P	4	Context-only	B	Saccharin-LiCl	A	Saccharin	A	12
P Sal	4	Context + Saline	B	Saccharin-LiCl	A	Saccharin	A	12
P Sac	4	Context + Saccharin	B	Saccharin-LiCl	A	Saccharin	A	11

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 flanged end rested on the mouth's mucosa. A new cannula was implanted each experimental day. This procedure requires no more than 20 s per subject and does not induce major stress to infant rats (Spear, Specht, Kirstein, & Kuhn, 1989). Subsequently, the pups' bladders were voided by gentle brushing of the anogenital area, since consumption was estimated on the basis of body weight gained during the intake session. By using this procedure, we prevented subjects from losing weight during the intake session through urination. Next, body weights were recorded and then subjects were immediately placed in the preexposure context, where they received an intraoral infusion of sodium saccharin (.15% w/v, group P-Sac), saline (.9% w/v, group P-Sal) or nothing (group N-P). The total volume administered was equivalent to 1 ml and was delivered over the course of 10 min at a constant rate (.1 ml/min) by means of an infusion pump (KD Scientific, Holliston, MA) connected to each pup's oral cannula by a polyethylene catheter (PE 50, Clay Adams). With similar parameters, pups are capable of either consuming or rejecting the infused solution (Arias et al., 2010; Revillo et al., 2011). Immediately after this procedure, pups were reunited with their mother in their corresponding home-cage. In Experiments 1 and 2a, the preexposure, conditioning and testing phases were all carried out in the same context (AAA), while in Experiment 2b preexposure took place in a different context from that used for conditioning and testing (BAA) (see the *apparatus* section).

Conditioning. In all experiments conditioning was carried out 24 hr after the last preexposure session, on PD 17. Thus, in all experiments latency between preexposure to the CS and conditioning was equivalent. On the conditioning day, all subjects consumed saccharin for 10 min using the procedures described for the preexposure phase, and were then immediately administered an intraperitoneal injection of LiCl (.15 M, 1% of body weight). This LiCl dose was selected from previous studies with preweanling rats, and produces consistent conditioned taste aversion with only one conditioning trial during the third postnatal week (Revillo et al., 2014; Revillo, Fernandez, et al., 2012). After conditioning, pups were returned to their home-cage.

Testing. On PD 18 rats again consumed saccharin for 10 min, but in this case they did not receive an injection after the intake session. The procedures used for consumption were the same as those described for the preexposure phase.

Data Analysis

Saccharin intake scores during conditioning and testing were calculated by subtracting preinfusion body weight from postinfusion body weight. A preliminary ANOVA revealed that consumption at conditioning or testing was statistically equivalent in males and females, and that this factor did not interact with the preexposure treatment. Hence, conditioning and testing scores were analyzed by means of one-way ANOVAs, including preexposure treatment (N-P, P-Sal, or P-Sac) as the only between-group factor. An additional analysis was performed with a differential score calculated by subtracting scores at testing from scores at conditioning. This dependent variable reflects the magnitude of conditioning. In this variable, higher scores indicate stronger aversion. Significant effects were analyzed using post hoc tests (Newman–Keuls), with p level set at .05.

RESULTS

Experiment 1

Figure 1a shows saccharin intake at conditioning (left) and testing (right) as a function of preexposure treatment. The ANOVA with conditioning scores found no significant differences between groups. The analysis with testing data revealed a significant main effect of preexposure [$F(2,\ 21) = 3.83,\ p < .05$], indicating that subjects preexposed to saccharin or saline (groups P-Sal or P-Sac) consumed more than those from the N-P group. Figure 1b shows the differential score (intake at conditioning—intake at testing). No between-group differences were detected by the ANOVA for this variable. These results indicate that the conditioning treatment was less effective in those subjects that had the opportunity of consuming during the preexposure phase (groups P-Sal and P-Sac), than in those exposed exclusively to the context. This weak preexposure effect was independent of the solution ingested at preexposure. In the following experiment we increased the number of preexposures to see if the preexposure effect was specific to the CS (i.e., latent inhibition effect).

Experiment 2a

Saccharin intake scores from Experiment 2a are given in Figure 2a. According to the ANOVA, consumption at conditioning was statistically equivalent in all groups. However, intake at testing was significantly influenced by the preexposure treatment [$F(2,\ 24) = 4.97,\ p < .05$]. Post hoc analyses revealed that subjects preexposed to saccharin (group P-sac) consumed more of this solution than the other groups. In this experiment, the preexposure effect was found to be specific to the CS, since no statistical differences were observed between the scores of subjects preexposed to saline and those not preexposed. The analysis of the differential score was also sensitive to differences between groups [$F(2, 24) = 6.90,\ p < .05$], and post hoc tests indicated that the magnitude of conditioning was weaker in subjects preexposed to saccharin than in the other conditions (Fig. 2b).

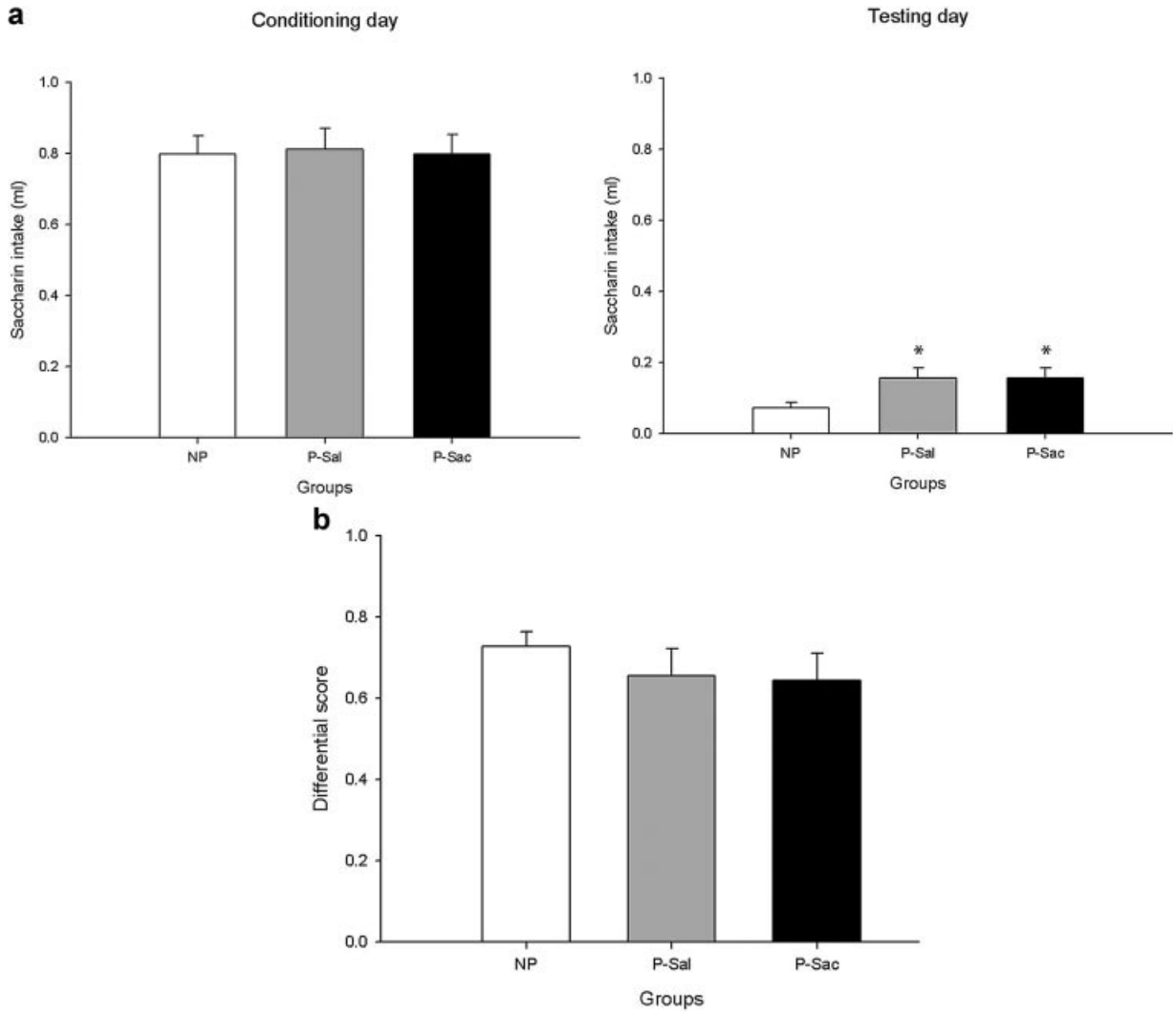

FIGURE 1 (a) Corresponds to Experiment 1, and represents saccharin intake values as a function of preexposure treatment (NP, P-Sal, or P-Sac). Intake values at conditioning day are shown in the left panel, while right panel shows the amount of saccharin consumed at testing. Subjects were preexposed, conditioned and evaluated in the same context (AAA). Vertical bars represent the standard error of the means (SEM); $^{*}p < .05$ versus the NP group. (b) Corresponds to Experiment 1, and represents the differential score (intake values at conditioning—intake values at test) as a function of preexposure treatment (NP, P-Sal, or P-Sac). Vertical bars represent the standard error of the means (SEM).

Experiment 2b

Experiment 2b analyzed whether or not a context-change between the preexposure and conditioning phases affected the CS-preexposure effect observed in Experiment 2a. In this case, saccharin consumption at conditioning or testing was unaffected by the preexposure treatment (Fig. 3a). The ANOVA found no differences between groups in the analysis of the differential score (Fig. 3b). This result supports the context-dependency of the CS-preexposure effect from the previous experiment. In order to confirm this conclusion, differential scores from Experiments 2a and 2b were compared by means of a factorial ANOVA, including preexposure treatment and context change as between-group factors. The ANOVA indicated a significant main effect of preexposure [$F(2, 56) = 5.73$, $p < .05$], and a significant interaction between preexposure and context change [$F(2, 56) = 3.33$, $p < .05$]. Post hoc analyses revealed that the magnitude of the conditioned taste aversion was lower in subjects preexposed to saccharin in the same context than in those from the other conditions (Fig. 4). Differential

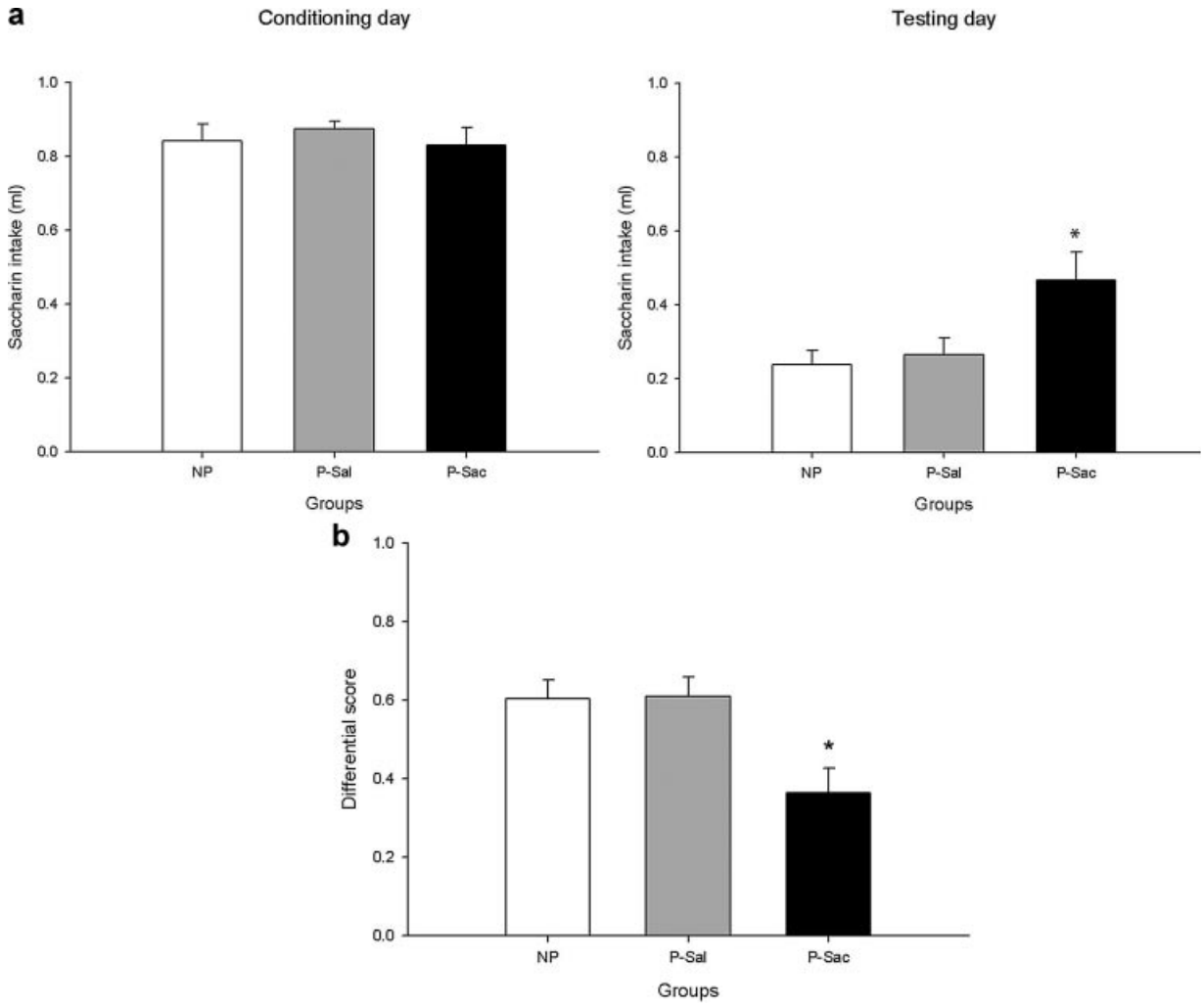

FIGURE 2 (a) Corresponds to Experiment 2a, and represents saccharin intake scores as a function of preexposure treatment (NP, P-Sal, or P-Sac). Intake values at conditioning day are shown in the left panel, while scores from the right panel represent the amount of saccharin consumed at testing. Subjects were preexposed, conditioned and evaluated in the same context (AAA). Vertical bars represent the standard error of the means (SEM); $^{*}p < .05$ versus the remaining groups. (b) Corresponds to Experiment 2a, and represents the differential score (intake values at conditioning—intake values at test) as a function of preexposure treatment. Vertical bars represent the standard error of the means (SEM); $^{*}p < .05$ versus the remaining groups.

scores for rats from groups N-P and P-Sal were not significantly affected by the context change.

DISCUSSION

The present study provides further evidence of latent inhibition in preweanling rats in a conditioned taste aversion paradigm (Experiment 2a) (Chotro & Alonso, 1999; Kraemer, Hoffmann, & Spear, 1988). In accordance with previous results, detection of the CS-preexposure effect was facilitated by increasing the number of preexposures to the CS (Chotro & Alonso, 1999). Interestingly, the results show that, similar to the observations reported with adult rats (Quintero et al., 2011) this effect was context-dependent, because when preexposure was carried out in a different context from conditioning, the magnitude of conditioning was not affected by preexposure to the CS (Experiment 2b).

Non-reinforced exposure to the CS before conditioning can retard or facilitate acquisition of the CR, depending on a variety of circumstances. One important factor in modulating the preexposure effect seems to be developmental stage. While prior experiences with the

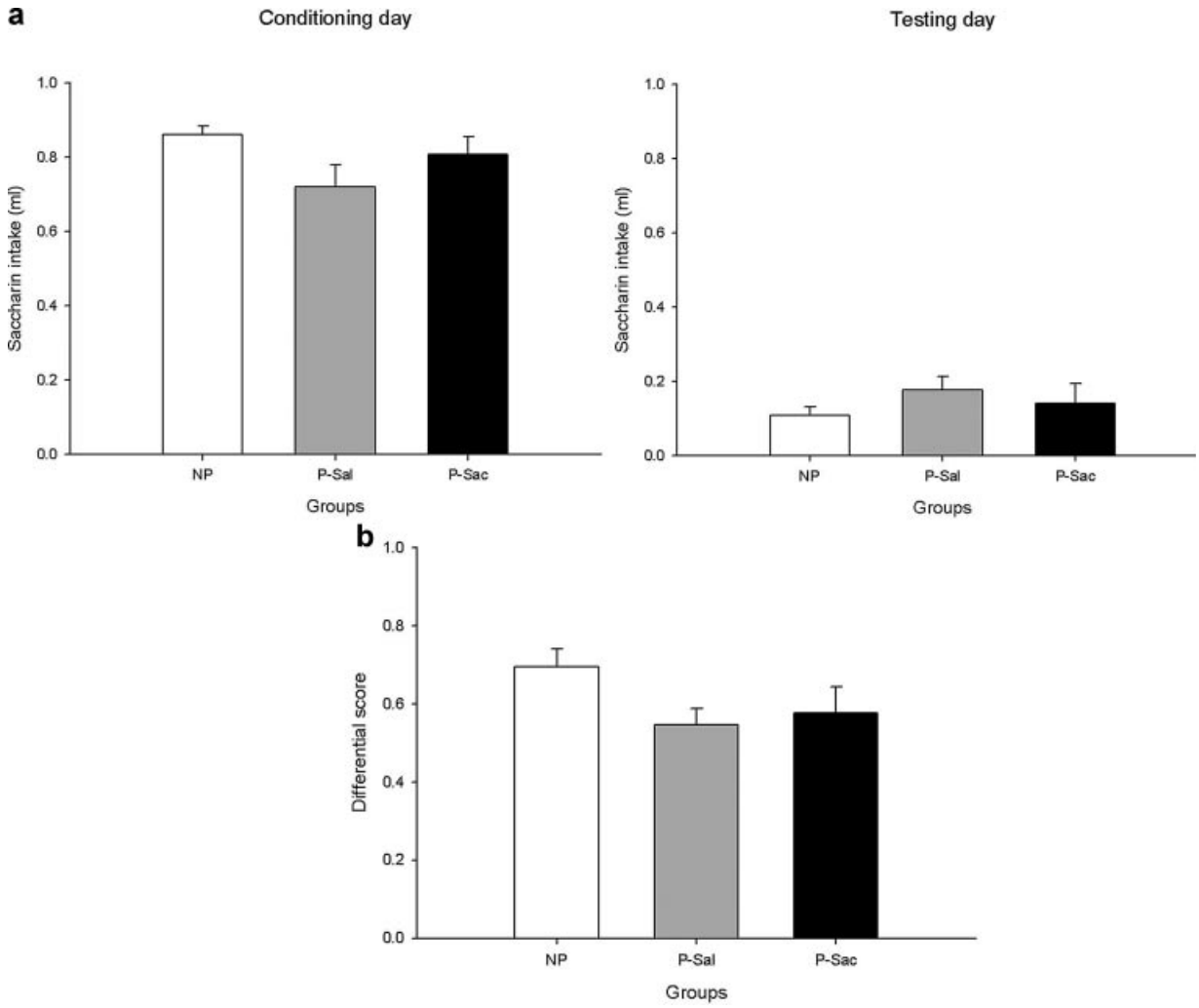

FIGURE 3 (a) Corresponds to Experiment 2b, and represents saccharin intake scores as a function of treatment preexposure (NP, P-Sal, or P-Sac). Intake values at conditioning day are shown in the left panel, while right panel shows the amount of saccharin consumed at testing. Preexposure was carried out in a different context than the one employed at conditioning and testing phases (BAA). Vertical bars represent the standard error of the means (SEM). (b) Corresponds to Experiment 2b, and represents the differential score (intake values at conditioning—intake values at test) as a function of treatment. Vertical bars represent the standard error of the means (SEM).

CS tend to weaken the CR in adult rats (Rodriguez & Alonso, 2002), the same procedure usually facilitates conditioning in infant rats (Chotro & Alonso, 1999; Hoffmann & Spear, 1989). Nevertheless, there is some evidence of latent inhibition during infancy (Chotro & Alonso, 1999; Hoffmann & Spear, 1989; Kraemer et al., 1988; Rudy, 1994; Yap & Richardson, 2005), and even in the fetal rat (Mickley et al., 2013), and this effect seems to emerge earlier for taste aversion conditioning than for fear learning. The direction of the CS-preexposure outcome (facilitation or retardation) depends, among other factors, on the number of preexposures to the CS (Chotro & Alonso, 1999). Short preexposure to the CS prior to conditioning facilitates conditioning, while long preexposure treatments retard learning, a result that is consistent with that observed in adult rats. In our study, we did not observe latent facilitation, but the specificity of the preexposure treatment did depend on the number of preexposure trials. Four preexposures were necessary to observe latent inhibition. In Experiment 1, three preexposures to the saccharin infusion interfered with aversive conditioning to saccharin, but this effect was also observed in subjects preexposed to saline. The only

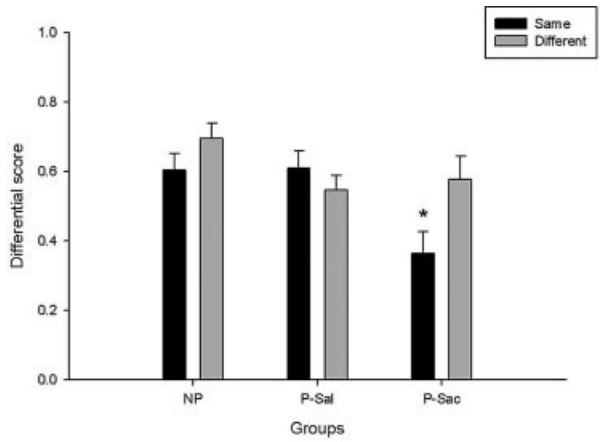

FIGURE 4 Differential score (intake values at conditioning —intake values at test) as a function of treatment (NP, P-Sal, or P-Sac) and context. This figure compares differential scores from Experiment 2a and 2b. Black bars represent the differential score of subjects preexposed, conditioned, and evaluated in the same context (AAA), while gray bars show the differentia score of subjects preexposed in a different context than the one employed at conditioning and testing (BAA). Vertical bars represent the standard error of the means (SEM); $^{*}p < .05$ versus the remaining groups.

difference between subjects from N-P and those from P-Sal or P-Sac in this experiment was that, during the preexposure phase, subjects from the two latter conditions had the opportunity of consuming, while those from the N-P condition were only exposed to the context. Consumption entails exposure to a variety of stimuli in addition to the taste of the solution, such as the presence of the cannula, the characteristics of the fluids, such as temperature, or the intraoral stimulation produced by the infusion itself. These elements can also be considered part of the CS, and therefore, attenuation of the aversion to saccharin in subjects exposed to saline in Experiment 1 may be explained in terms of the generalization of latent inhibition. Previous studies have found that the effect of latent inhibition can be generalized between stimuli that share common elements (Rodriguez & Alonso, 2011). In fact, shorter exposures increase the generalization of latent inhibition, whereas longer exposures increase discrimination between the stimuli, rendering the effect specific to the preexposed stimulus (Rodriguez & Alonso, 2011). This is consistent with what we observed in the present series of experiments, since the preexposure effect was specific to the preexposed CS when the number of preexposures was increased. These results support the importance of including a control group preexposed to an alternative CS when studying latent inhibition.

Experiment 2 provided evidence of the contextual modulation of the CS-preexposure effect in preweanling rats. The lack of context-dependency in learning interference during infancy has been linked to a theoretically weak capacity of the infant rat to retain contextual conditioning (Rudy, 1994; Rudy & Morledge, 1994; Yap & Richardson, 2005), since some explanations of latent inhibition postulated that this effect is mediated by the context-CS association. This analysis assumes that contextual learning capacity is not completely absent during infancy, because in some cases contextual freezing has been observed at this age, although to a lesser extent than in weaning rats (Rudy & Morledge, 1994). However, some evidence fails to support this ontogenetic limitation and suggests that ontogenetic differences may be related to procedural issues, rather than to memory capacities (e.g., Brasser & Spear, 2004; McKinzie & Spear, 1995; Pisano et al., 2012). In fact, the procedures usually used with adult rats must sometimes be adapted in order to detect retention of this kind of learning. For instance, in a fear conditioning preparation, enriching the salience of the context by adding explicit odors potentiates contextual learning during infancy (Brasser & Spear, 2004; McKinzie & Spear, 1995). Other studies have found equivalent contextual fear learning between preweanling (PD18) and post-weaning (PD23) rats using contexts with black walls, instead of white ones (Pugh & Rudy, 1996). Moreover, in another study that failed to find differences between preweanling and adolescent rats in contextual fear learning, the lack of ontogenetic differences was attributed to the number of foot-shocks administered (Beane et al., 2002), although other authors failed to find evidence of context fear learning in preweanling rats even when the number of footshocks was increased (Schiffino et al., 2011). This latter study used a paradigm known as the contextual preexposure facilitation effect, which theoretically enables the acquisition of the configurational representation of the context to be separated from shock conditioning (Rudy, 2009; Schiffino et al., 2011). Recently, this paradigm has also proved successful in revealing infantile contextual learning when scores from preweanling rats were analyzed separately from those from weaning rats (with appropriate control groups for each ontogenetic stage), and several measures were taken to infer the existence of contextual memory (Pisano et al., 2012). These results regarding context learning during infancy are consistent with a variety of findings which show that infantile behavior (Arias, Mlewski, Miller, Molina, & Spear, 2009) and learning (Revillo et al., 2013, 2014) can be modulated by the context in which they occur, similarly to during adulthood. The present results provide additional evidence for this.

Previous studies analyzing the context-dependency of latent inhibition during infancy (Chotro & Alonso, 1999; Hoffmann & Spear, 1989; Kraemer et al., 1988; Rudy, 1994; Yap & Richardson, 2005) or even during early adolescence (Manrique, Gamiz, Moron, Ballesteros, & Gallo, 2009) failed to find differences in the CR between subjects preexposed and conditioned in the same or in different contexts. The discrepancy between our results and these studies may be linked to a variety of procedural differences. First, we used a conditioned taste aversion paradigm instead of fear conditioning (Hoffmann & Spear, 1989; Rudy, 1994; Yap & Richardson, 2005), and the context-modulation of this CS-preexposure effect may depend on the kind of learning tested, which in turn may be related to the different ontogenetic maturation rates of the different sensory systems (Stanton, 2000). The second possibility has to do with the salience of the contexts. Although previous studies used contexts that differed in a variety of features, the contexts that we used were the only ones that, in addition to size, illumination and texture, also differed in their odor component. This is important because sensitivity for detecting the contextual modulation of behavior critically depends on the number of physical dimensions in which the contexts differ in infant (Revillo et al., 2013) and in adult (Thomas et al., 2003) rats. As discussed above, in preweanling rats, the inclusion of explicit odors in contexts A and B was required to observe ABA-renewal of a fear CR (Revillo et al., 2013). Finally, as observed in Experiment 1, preexposure to saccharin also attenuated aversion to saccharin when intake scores from subjects preexposed and not preexposed to saccharin were compared. However, preexposure to an alternative taste (saline) also produced this attenuation in saccharin aversion. This outcome cannot be considered latent inhibition, since it is not specific to the CS. We do not know whether or not this non-specific preexposure effect is sensitive to context manipulations. Latent inhibition depends on the context-CS association (Escobar, Oberling, & Miller, 2002), but it is unlikely that this mechanism mediates the non-specific preexposure effect observed in Experiment 1, because in the case of the P-Sal group, the CS was not presented at conditioning. Hence, it is likely that this effect is non-context-dependent, although this possibility was not tested in our study. This may be important, since in previous ontogenetic studies, the performance of non-preexposed subjects was directly compared with that of those preexposed to the CS, but not with that of a different group exposed to an alternative stimulus (Hoffmann & Spear, 1989; Rudy, 1994; Yap & Richardson, 2005). All these methodological differences may contribute to explaining why, in our study, latent inhibition was sensitive to context manipulation.

Contextual conditioning has been used as a marker of the hippocampal function (Maren, 2008). Following this line of argument, some authors have suggested that the lack of retention of contextual learning and the lack of context effects during infancy reported in some studies may be linked to a functional immaturity of the hippocampus (Rudy & Morledge, 1994). It is striking that a lesion in this structure in the adult rat results in some interference effects (such as latent inhibition or extinction) becoming insensitive to a context change (Grecksch, Bernstein, Becker, Hollt, & Bogerts, 1999; Schmajuk, Lam, & Christiansen, 1994; Zelikowsky, Pham, & Fanselow, 2012). This is similar to that found in some studies with infant rats (Rudy, 1994; Yap & Richardson, 2005) and is consistent with the idea that the lack of context effect during infancy may be related to a functionally immature hippocampus. However, as mentioned above, there is a considerable amount of evidence supporting the claim that preweanling rats are indeed able to acquire and retain direct associations between context and USs (Brasser & Spear, 2004; McKinzie et al., 1994; McKinzie & Spear, 1995; Pisano et al., 2012), and that context can also have a modulatory effect on interference learning during infancy, as demonstrated in both the present and previous studies (Revillo et al., 2013, 2014). Furthermore, a lesion study also showed that the ability to acquire and retain contextual learning during infancy requires an intact hippocampus (Foster & Burman, 2010). Overall, these results raise the question of whether the lack of context effects in interference paradigms during infancy reported in some studies actually reflects the existence of ontogenetic differences in memory capacities, or rather is more related to procedural issues (as discussed above), including the salience or sensory content of the contexts used. Indeed, in adult rats it has been observed that similarity between contexts attenuates or even eliminates the effects of a context change in an interference paradigm such as extinction (Thomas et al., 2003), thus supporting the idea that discrimination between contexts is critical at any developmental stage for modulating interference effects. Since the hippocampus, through its connection with structures such as the amygdala or the medial prefrontal cortex, has been implicated in the contextual modulation of conditioned responses rendered ambiguous during interference learning paradigms (Bouton, 2002), it would be interesting to test in future studies whether the brain circuit constituted by these brain structures is engaged during interference paradigms when contexts are salient and discriminable, regardless of the ontogenetic stage.

NOTES

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