


Context-dependent extinction of an appetitive operant conditioned response in infant rats

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Abstract

The present study evaluated context-dependent learning under an operant conditioning procedure in infant rats. Preweanling rats were trained in context A during postnatal days (PDs) 16 and 17 to learn an appetitive operant conditioning task, employing milk chocolate as appetitive reinforcer. On PD18 the operant response was extinguished in context A, or in an alternative context B. The change from context A to B between acquisition and extinction did not affect the number of responses during extinction, but slightly modified the shape of the extinction curve. On PD19, a renewal test conducted in context A clearly showed ABA-renewal of the extinguished operant response. These results add to the body of evidence indicating that infants are able to acquire and retain contextual information, and support the notion that extinction during this ontogenetic period involves new learning.

KEYWORDS

context, extinction, infant rat, operant conditioning, renewal effect

1 | INTRODUCTION

It has been suggested that infant rats are less capable of forming long-term contextual memories (Kim & Richardson, 2010; Rudy, 1993; Rudy & Morledge, 1994). This inference rests on studies showing that a conditioned fear response to a conditioned context was higher in weaning rats around postnatal days (PDs 23–24) than in preweanlings (PDs 17–18) (Rudy, 1993; Rudy & Morledge, 1994; Schiffino, Murawski, Rosen, & Stanton, 2011). In contrast, the response to a tone-conditioned stimulus (CS) was similar in subjects from either age group (Rudy & Morledge, 1994). Recent studies promoted a more nuanced view of these results, by showing that the sensory content of the context and, importantly, the behavior measured as a memory index affect the ontogenetic differences observed in the response of preweanlings, weanlings, and adult rats (Revillo, Cotella, Paglini, & Arias, 2015). For example, it was shown that infant rats can effectively learn and retain contextual information when they are evaluated in contexts containing salient odors (Brasser & Spear, 1998; Revillo,

Trebucq, Paglini, & Arias, 2016). Furthermore, under certain specific experimental conditions, the magnitude of the infant's response to the context can even be greater than that of the adult rat (Brasser & Spear, 2004).

The ongoing debate about whether infant rats are able to retain contextual information has also encompassed the ontogeny of other learning phenomena, such as interference paradigms (Bouton, 1993), where context plays an important role. For instance, it is well-known that an extinguished conditioned response in adult animals can be partially recovered when it is assessed in a different context from that used for extinction, a phenomenon that has been termed "renewal" (Bouton, 1993, 2002, 2004; Bouton, Todd, Vurbic, & Winterbauer, 2011). Yap and Richardson (2007) observed renewal in weaning, but not in preweanling rats. It is important to note, however, that these authors used contexts without a salient odor component. Recently, the inclusion of salient odors in the context made it possible to observe the renewal effect in preweanlings (Revillo, Molina, Paglini, & Arias, 2013), as well as other recovery-from-extinction effects that can also be

considered context-dependent, such as reinstatement (Revillo, Trebucq, et al., 2016) or long-term spontaneous recovery (Revillo, Paglini, & Arias, 2014).

Bouton and colleagues have reported renewal by using operant conditioning settings, and showed that context can affect similarly the extinction of a pavlovian or an operant response (e.g., Bouton et al., 2011; Todd, Winterbauer, & Bouton, 2012a, 2012b). To date, no studies have addressed in infant rats the possibility of renewal of an extinguished operant response. In the present study, we assessed this question by adapting a self-administration procedure in which infants received an intraoral infusion of a highly reinforcing solution after a nose-poke response (Miranda-Morales, Molina, Spear, & Abate, 2012). We present the results of two experiments: in experiment 1, we set the parameters for acquisition and extinction of the operant response, and in experiment 2, we evaluated the possibility of renewal of this response after extinction.

2 | EXPERIMENT 1

2.1 | Materials and methods

2.1.1 | Subjects

Thirty-two Wistar-derived rats from eight litters (PD16 at the start of the experiment) were used, divided into four groups ($n = 8$ per group, four males and four females). In both, experiments 1 and 2, the subjects were quasi-randomly assigned to the treatment groups. Precautions were taken to prevent overrepresentation of any particular litter in any of the four groups (Holson & Pearce, 1992). Thus, no more than one male and one female from a given litter were assigned to the same treatment condition. The rats were born and reared at the vivarium of the Experimental Psychology Laboratory, Psychology Department, Córdoba National University, Argentina. The animal colony was kept at 22 ± 1 °C, under artificial lighting conditions (lights on: 08:00–20:00 hr). Animals had continuous access to rat chow (GEPISA, Pilar Group, Córdoba, Argentina) and tap water delivered through bottles. Births were monitored daily, and delivery day was labeled as postnatal day zero (P0). Pups were maintained undisturbed with their mother and counterparts until the beginning of the experimental phase.

2.1.2 | Apparatus and solutions

We used two custom-made operant-conditioning chambers ($12 \times 12 \times 15$ cm³). Both lateral walls of each chamber had a hole (1 cm in diameter), with a peripheral metal ring in order to make it more salient. The ring on the right wall had a rough surface that served as a distractive cue. The ring on the left wall was smooth, and a touch-sensitive sensor was placed behind it (Model E11x Evaluation Board; Quantum Research Group, Pittsburgh, PA). Each time the snout of a paired subject touched this sensor, a signal went on, and activated an infusion pump. A 50 cm length of PE-50 polyethylene tubing was connected at one end to an oral cannula placed in the pup's cheek, and

at the other end to a 1 ml syringe placed in a pump. The pump was set to deliver 6 μ l of chocolate milk per pulse directly into the animal's oral cavity. Each pulse lasted two seconds, and the number of nose-pokings during this interval (operant response number) was recorded using custom-made software (ITTCOM, Argentina; sensitivity: 1 response/0.01 s).

Two contexts were used, both enriched with a salient odor to facilitate a possible renewal effect (Revillo, Molina, et al., 2013). The first context was built with black walls and scented with almond odor (a cotton swab containing a 0.5% v/v solution). The second context consisted of a white-walled box, scented with orange (a cotton swab soaked with a 0.3% v/v solution). Both solutions were prepared in distilled water and the scented cotton swab was placed on the top of the experimental chamber. These contexts were counterbalanced. The training and extinction phases took place in an experimental room, lit with white fluorescent light, with low constant background noise generated by an air extractor.

2.1.3 | Procedures

At the start of each experimental day, the pups were separated from the litter, and a polyethylene oral cannula was implanted into each pup's mouth, as previously described by Dominguez, Bocco, Chotro, Spear, and Molina (1993). Briefly, these devices were constructed from 5 cm lengths of polyethylene (PE) tubing (Clay Adams, PE 10). A small flange (external diameter 1.2 mm) was formed at one end of the cannula by applying heat. The non-flanged end was inserted into a tungsten pin. The pin was then pulled through the medial internal surface of the cheek. The flanged end of the cannula remained in the mouth while the remainder of this device exited from the oral cavity. As previously shown, this procedure seems minimally stressful to infant rats (Spear, Specht, Kirstein, & Kuhn, 1989). Pups were then housed in pairs for a period of 2 hr in a black-walled plexiglas box ($45 \times 20 \times 20$ cm³—holding cage-) lined with pine shavings. The temperature in the box was kept at 31–32 °C through the use of a heating pad. Immediately before starting each conditioning and extinction session, the anogenital region of the preweanlings was gently stroked with a cotton swab to stimulate defecation and voiding of the bladder. Afterwards, the animals were weighed (± 0.01 g, Ohaus Traveler TA302) and placed in their respective training chambers.

Conditioning

Training for acquisition of the operant response took place on PD16–17, in two daily sessions separated by 2 hr. The first session started 2 hr after maternal separation, when a subject assigned to the paired condition and its corresponding yoked control (of the same litter and sex) were placed in the conditioning chamber and the cannula was connected to the pump. The target behavior under training was nose-poking. Specifically, when a paired subject introduced its snout into the hole and contacted the touch-sensitive sensor, a pulse of reinforcer was delivered directly into its mouth. Yoked control pups received simultaneously an intraoral infusion when their paired counterpart displayed the operant behavior. Therefore, yoked controls received the same amount of the reinforcing solution as their paired pups, but experienced no contingency between the behavioral response and the

delivery of the reinforcer. In experiment 1, we used a fixed-ratio schedule of reinforcement equal to 1 (FR-1). Immediately after each conditioning and extinction session, the animals were weighed again to calculate the amount of reinforcer consumed, operationally defined by the body weight gained (BWG, in grams): (post-conditioning weight – pre-conditioning weight)/pre-conditioning weight). The total number of operant responses was recorded during the conditioning and extinction sessions. After each day's first training session, subjects were placed in the holding cage again until commencement of the second session of the day. After the last daily session, subjects were placed back in their homecage.

In a preliminary study, we observed that, in a fifteen minute session, some animals consumed high levels of chocolate milk (nearly 1 ml), and these subjects markedly reduced the number of operant responses in the following session. It has been reported that infant rats do not show activity of certain digestive enzymes in the intestine that metabolize several carbohydrates during the first two postnatal weeks (Henning, 1987; Henning & Guerin, 1981; Kojima et al., 1998). In addition, unpublished data from another laboratory indicate that infant rats acquire aversions after consumption of large quantities of sucrose (personal communication with Dr. Truxell, Binghamton University). For these reasons, we decided to limit the maximum number of pulses of the reinforcer that a given subject could receive within a session, a protocol that also helps reduce within-group variance. Based on our preliminary study, the maximum number of pulses was set at 20 for sessions during the first conditioning day, and at 30 for sessions on the second training day. Therefore, the duration of each conditioning session was variable for each pair of pups, depending on the amount of time required to reach the maximum number of reinforcing pulses. If a given paired animal did not reach this criterion in 15 min, the session was terminated and the subject was removed from the conditioning box.

Extinction

On PD18, the subjects were divided into two groups of equal size. One group received an extinction session in the same context as that used at training (Group: AA), and the other group was placed in an alternative new context (Group: AB). The experimental procedures were similar to those employed during conditioning, with the exception that this phase lasted 10 min and the reinforcer was not delivered.

2.1.4 | Experimental design and data analysis

Mixed ANOVAs were conducted to analyze body weights, reinforcer consumption (BWG) and the number of operant responses during both conditioning and extinction phases. The learning condition (paired vs. yoked) and the context (AA vs. AB) were always included as between-group factors.

For body weights, the day (PDs 16–18) was included as a within-group variable. During conditioning, consumption (BWG) and the total number of operant responses were analyzed by two separate mixed ANOVAs, one for each training day (PD16 and 17), in view of the different parameters used in each day. In these ANOVAs, the session (with two levels, session 1 and 2) was included as a within-group factor. Because each training session had a different duration for each

subject, only the total number of responses was analyzed. The number of responses during the extinction session was analyzed by a mixed ANOVA, including 2 min blocks (blocks 1 to 5) as the within-group variable. When interactions from mixed ANOVAs reached significance, additional two-way ANOVAs were conducted, followed by Duncan's post hoc tests to establish the *loci* of significant effects. A rejection criterion of $p < .05$ was adopted for all statistical analysis in the present study. Preliminary analyses of the data included sex and type of context (context A or context B) as between-group factors. These factors consistently failed to exert any significant main effect or to interact with any other factor under analysis. For this reason, further statistical comparisons were performed by collapsing across sex and context type in all conditions.

2.2 | Results

2.2.1 | Body weights and reinforcer consumption (BWG)

The mean body weight increased daily ($F(4, 112) = 38.32, p < 0.01$) and did not differ between groups. On PD16 and 17, consumption was almost significantly different between sessions [PD16: ($F(1, 28) = 3.52, p = .07$), Mean = .06 g, SEM \pm .008 on session 1 and Mean = .08 g, SEM \pm .006 on session 2, PD17: ($F(1, 28) = 3.77, p = .06$), Mean = 0.14 g, SEM \pm .007 on session 1 and Mean = 0.16 g, SEM \pm .004 on session 2], and did not differ between groups.

2.2.2 | Proportion of subjects reaching the criterion of maximum number of pulses at conditioning

At training, subjects responded until they reached a certain number of responses, 20 for the first training session, and 30 for the last training session. The percentage of the paired subjects that reached this criterion across the four training sessions was as follows: S1: 11/16: 69%, S2: 12/16: 75%, S3: 12/16: 75% and S4: 15/16: 94%. In general, the criterion was reached after approximately 9–11 min during S1 and S2, and after 6–7 min at S3 and S4.

2.2.3 | Operant responses

Figure 1 shows the number of operant responses at conditioning (Figure 1A) and extinction (Figure 1B) as a function of learning condition (paired and yoked) and context (AA and AB). On the first training session (PD16), the total number of operant responses by the paired group was close to being significantly higher than those from yoked controls ($F(1, 28) = 4.05, p = .054$). The ANOVA with scores from the second training day (PD17) revealed a significant interaction between learning condition and session ($F(1, 28) = 5.44, p < .05$). There was a significant increase in the number of operant responses in session 2, compared to session 1, but only in subjects from the paired group ($F(1, 15) = 5.03, p < .05$), and not among the yoked controls ($F(1, 15) = 0.56, p = 0.47$). As expected, context did not exert significant effects or interact with the remaining factors.

At extinction, only the main learning condition factor achieved significance ($F(1, 28) = 9.40, p < .05$), showing that responses from the paired group were significantly higher than those from yoked controls. No other significant main effect or interaction was observed.

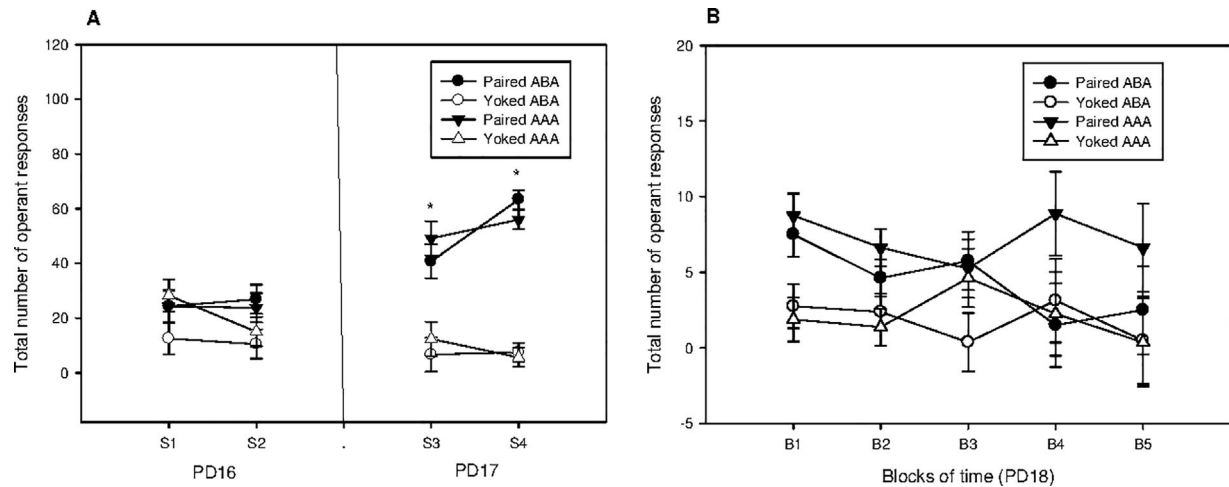


FIGURE 1 Number of operant responses recorded during conditioning sessions on PD16 (S1; S2) and on PD17 (S3; S4) (A), and extinction on PD18 (B) as a function of the learning condition (paired or yoked) and the context change presented at extinction (AA vs. AB). Vertical bars represent the standard error of the means (SEM)

In summary, data from experiment 1 showed a clear and rapid acquisition of the operant response, with the majority of subjects reaching the maximum number of allowed responses for a training session. At extinction, the paired group responded significantly more than the yoked control. However, under these procedural conditions, a clear extinction curve of the operant response was not evident, although the paired subjects showed more responses than the yoked pups during the extinction session. In the present experiment, a context-effect was not observed during extinction.

3 | EXPERIMENT 2

The goal of experiment 2 was to analyze the possibility of renewal of the operant response after extinction. Several procedural modifications were made. First, the reinforcement schedule during the second training day was changed (from a fixed ratio FR-1 to FR-3) with the aim of increasing the number of responses during the extinction session (Hochman & Erev, 2013; Humphreys, 1939), thereby facilitating the observation of possible between-group differences at testing. In addition, the extinction phase lasted 5 min longer than in experiment 1. With these modifications, we expected to observe a clearer extinction curve than in the previous experiment. Finally, we added a renewal phase 24 hr after extinction (PD19).

3.1 | Materials and methods

3.1.1 | Subjects

Forty-eight infant rats derived from 12 litters were used, with 12 animals per group (six males and six females), reared under identical conditions to those described for experiment 1.

3.1.2 | Procedure

Apparatus and solutions were the same as those used in experiment 1.

Conditioning

Operant conditioning sessions were similar to those of experiment 1, with the exception that the reinforcement schedule was increased during the second training day (FR-3 instead of FR-1).

Extinction

The extinction session lasted 15 min instead of 10 min. The remaining parameters were the same as those of the previous experiment.

Renewal

On PD19 a renewal test was performed consisting of a session identical to the extinction session. This was carried out in the same context as that used at training (A).

The dependent variables considered for the present experiment were as follows: (i) body weights throughout all experimental days; (ii) reinforcer consumption (at training); (iii) operationalized by BWG) number of operant responses (at training, extinction and renewal); and (iv) latency to the first operant response (at training, extinction, and renewal).

3.1.3 | Experimental design and data analysis

As in experiment 1, mixed ANOVAs were conducted to analyze body weights, reinforcer consumption (BWG) and the number of operant responses during conditioning, extinction and renewal. Additionally, ANOVAs were run to analyze the latency to the first operant response at training, extinction and renewal. Learning condition (paired vs. yoked) and context (AAA vs. ABA) were always included as between-group factors. For the analysis of body weights, the day (PDs 16–19) was included as a within-group variable.

At training, reinforcer consumption (BWG), the number of operant responses and latency to the first operant response were analyzed by separate mixed ANOVAs, one for each training day (PD16 and 17). In these analyses, session (with two levels, session 1 and 2) was the within-group factor. During both the extinction and renewal sessions, the number of operant responses were grouped in 3 min blocks (blocks 1–5), and block was considered the within-group variable. Follow-up

ANOVAs and Duncan's post hoc tests were used to establish the *loci* of significant effects. As in Experiment 1, sex and context type (context A or context B) consistently failed to exert any significant main effect, or to interact with any other factor under analysis. For this reason, further statistical analyses were performed collapsing across sex and type of context. Finally, latency to the first operant response was analyzed using a non-parametric Kruskal–Wallis test because this measure was not normally distributed. Significant effects were analyzed using Mann–Whitney *U*-tests.

3.2 | Results

3.2.1 | Body weights and reinforcer consumption (BWG)

BWG progressively increased across sessions ($F(5, 220) = 240.52$, $p < .001$), and this variable did not differ between groups. On PD16, consumption was significantly higher during the second session [PD16 ($F(1, 44) = 5.57$, $p < .05$); Mean = .06 g, SEM \pm .004 on session 1, and Mean = .08 g, SEM \pm .004 on session 2]. On PD 17, intake levels were similar across sessions and experimental groups [Mean = .07 g, SEM \pm .007 on session 1 and Mean = .08 g, SEM \pm .007 on session 2].

3.2.2 | Percentage of subjects reaching criterion of maximum number of pulses at conditioning

The percentage of subjects that reached the maximum number of responses allowed per session was as follows: S1: 12/24: 50%, S2: 17/24: 71%, S3: 6/24: 25%, and S4: 5/24: 21%. Under these conditions, the majority of pups were exposed to the context during the entire period of 15 min in the last two training sessions.

3.2.3 | Operant responses

At training, the total number of operant responses from the paired group was significantly higher than that exhibited by yoked controls. Figure 2A depicts the number of operant responses at conditioning as a function of the learning condition (paired and yoked) and context (AAA or ABA). This effect reached statistical significance on both conditioning days [PD16: ($F(1, 44) = 14.83$, $p < .05$); PD17: ($F(1, 44) = 56.47$, $p < .05$)].

The ANOVA run for extinction scores revealed a significant main effect of learning condition ($F(1,44) = 7.5$, $p < .01$), indicating that the number of responses displayed by the groups of paired subjects during the entire extinction session was significantly higher than those of the groups of yoked subjects. Interestingly, the amount of responses displayed by the ABA and AAA paired groups during the entire extinction session was statistically similar (ABA condition: Mean = 19.75, SEM \pm 3.91, and AAA condition: Mean = 24.67, SEM \pm 3.91). Distribution of these responses across extinction was slightly different in the ABA and AAA paired groups as evidenced by the triple significant interaction between learning condition, context and block ($F(4, 176) = 2.89$, $p < .05$). Follow-up ANOVAs across context were run to determine between-group and within-group differences. Two ANOVAs were run, one for each context condition (AAA and ABA), in which learning condition was the between-group factor and block the within-group variable. The ANOVA with scores from the AAA condition showed a significant interaction between

learning condition and block ($F(4, 88) = 3.04$, $p < .05$). Post hoc tests indicated that on the first block the number of responses displayed by paired rats was higher than those from their control yoked group. Furthermore, operant responses showed by paired animals in block 1 were higher than those from the remaining blocks. This pattern of response, which is indicative of extinction of the operant behavior, was not observed in the yoked group, which responded similarly throughout the extinction session.

In the ABA condition, the ANOVA revealed that learning condition and block also significantly interacted ($F(4, 88) = 3.14$, $p < .05$). Post-hoc tests showed that paired animals responded more than yoked controls in block 2. Additionally, operant responses of paired pups in block 2 were also significantly higher than those displayed in blocks 3 and 5. These results also indicate extinction of the operant response in the ABA condition, but in this case, the maximum number of responses was reached in block 2. In fact, the number of responses in block 1 for the AAA group did not statistically differ from those observed in block 2 for the ABA group ($F(1, 22) = 1.12$, $p = .30$). Extinction scores are depicted in Figure 2B, representing the number of operant responses at extinction, as a function of learning condition (paired and yoked) and context (AAA and ABA).

A similar ANOVA was run to explore a possible renewal effect on PD19. The ANOVA also revealed a significant interaction between learning condition, context and block ($F(4, 176) = 2.80$, $p < .05$). Follow-up ANOVAs run for the ABA context group indicated a significant interaction between learning condition and block ($F(4, 88) = 2.6$, $p < .05$). In post-hoc tests, subjects from the paired group responded significantly more than yoked control pups in block 1. Additionally, the number of responses exhibited by paired pups during this first renewal block was significantly higher than that displayed in the remaining blocks. No significant differences between blocks were observed in yoked controls. The ANOVA run for the AAA group did not show significant differences between groups or interactions. These results indicate recovery of the extinguished response in the ABA, but not in the AAA condition. Figure 2C summarizes the number of operant responses during the renewal session, as a function of learning condition (paired and yoked) and context (AAA and ABA).

3.2.4 | Latency to the first operant response

The analysis of latencies to the first operant response on the first training day revealed significant between-group differences in the second session [$H(3, N = 48) = 8.80$, $p < .05$], and also in the first training session on the second training day [$H(3, N = 48) = 8.03$, $p < .05$]. Nevertheless, further comparisons employing Mann–Whitney tests showed that only the latency was significantly lower in the paired ABA group than in the corresponding yoked control ($U = 24.5$, $p < 0.01$). During extinction, a Kruskal–Wallis test also showed a statistically significant difference in the latency to the first operant response [$H(3, N = 48) = 16.70$, $p < .01$]. Mann–Whitney tests revealed that AAA paired animals differed from their corresponding control groups ($U = 12$, $p < .01$), and from ABA paired subjects ($U = 22.5$, $p < .01$). In contrast, no significant differences were observed between paired and yoked subjects from the ABA condition. No significant differences were observed in the renewal test. The latency values

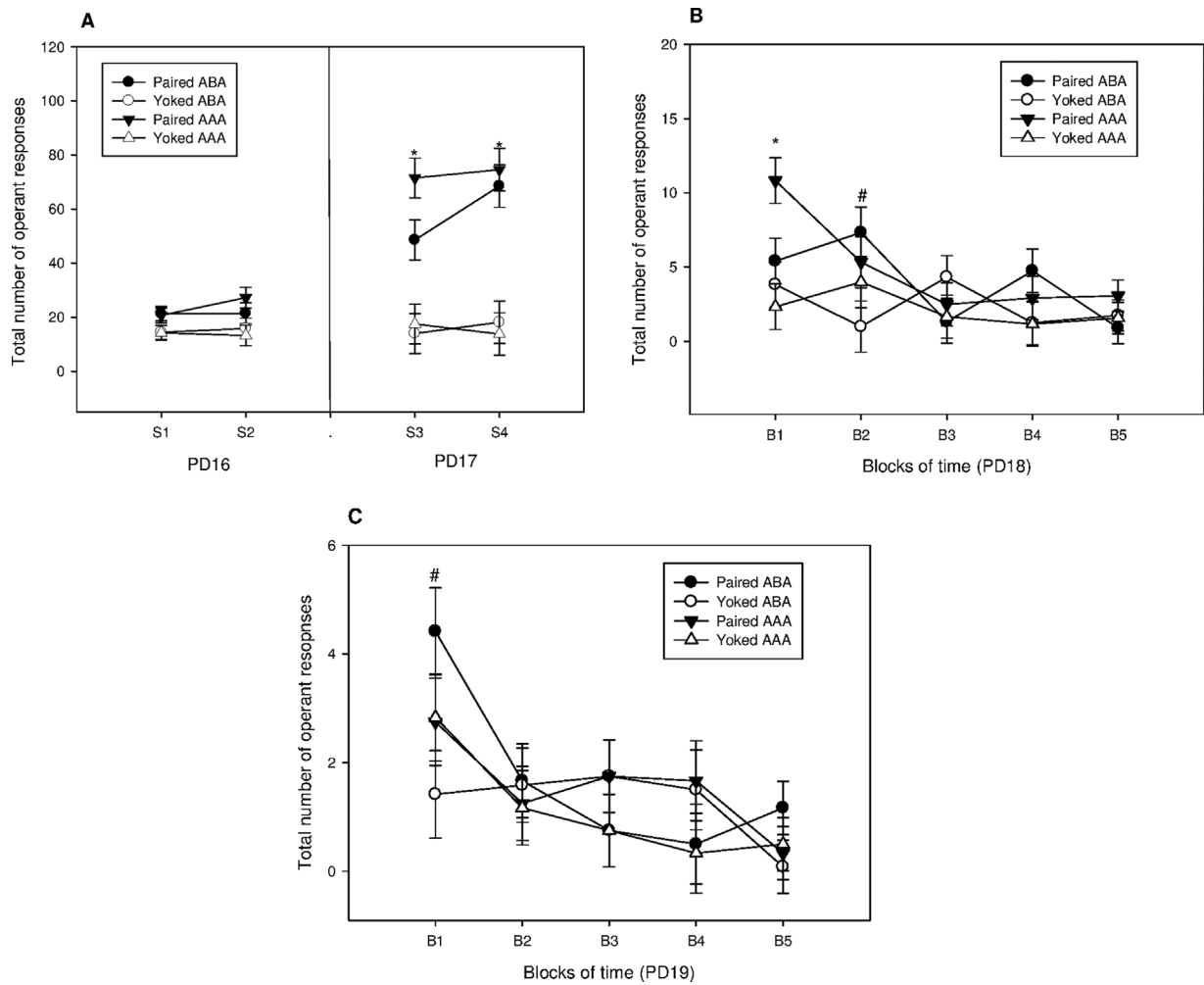


FIGURE 2 (A) Number of operant responses recorded during the conditioning sessions on PD16 (S1; S2), and on PD17 (S1; S2), as a function of the learning condition (paired or yoked) and the context change presented at extinction (AAA vs. ABA). Vertical bars represent the standard error of the means (SEM). (B) Number of operant responses recorded during the extinction session on PD18, as a function of the learning condition (paired or yoked), context change (AAA vs. ABA) and block of time (B1–B5). Vertical bars represent the standard error of the means (SEM). (C) Number of operant responses recorded during the renewal session on PD19, as a function of the learning condition (paired or yoked), context change (AAA vs. ABA) and block of time (B1–B5). Vertical bars represent the standard error of the means (SEM)

observed at training, extinction, and renewal sessions are depicted in Figure 3A–C, respectively.

To sum up, the experimental parameters used in experiment 2 were more effective in promoting a clear extinction curve of the operant response than the procedures used in experiment 1. At training, the number of operant responses from paired animals was significantly higher than those from yoked controls at the last training session that took place in the maximum amount of time allowed (15 min). Extinction curves were clear in both the ABA and AAA paired groups, although these groups reached the maximum number of responses in different blocks. Finally, ABA renewal was observed in the last experimental session.

4 | GENERAL DISCUSSION

The present study explored whether a context shift influences the expression of an acquired operant response and its extinction in

preweaning rats. For this purpose, we adapted a self-administration technique that has been mainly used for ethanol self-administration studies in infant rats (Miranda-Morales, Molina, Spear, & Abate, 2010; Miranda-Morales et al., 2012). This experimental protocol proved effective for the purpose of the study, as it allowed acquisition and extinction of the operant response.

The parameters in experiment 1 were appropriate for observing clear acquisition curves of the operant response. The limit that we put to the number of responses during each training session was effective for reducing the within-group variance and, most importantly, for preventing the possibility of aversion to the reinforcer. Because the subjects from all groups consumed equally, regardless of the learning and context condition, the differences in the operant response during extinction or renewal testing cannot be explained by differences in exposure to the reinforcer. In experiment 2, in contrast to experiment 1, we found that only a small percentage of subjects reached the criterion of maximum number of pulses allowed by the end of the training session. For example, in the last training session on PD17, only

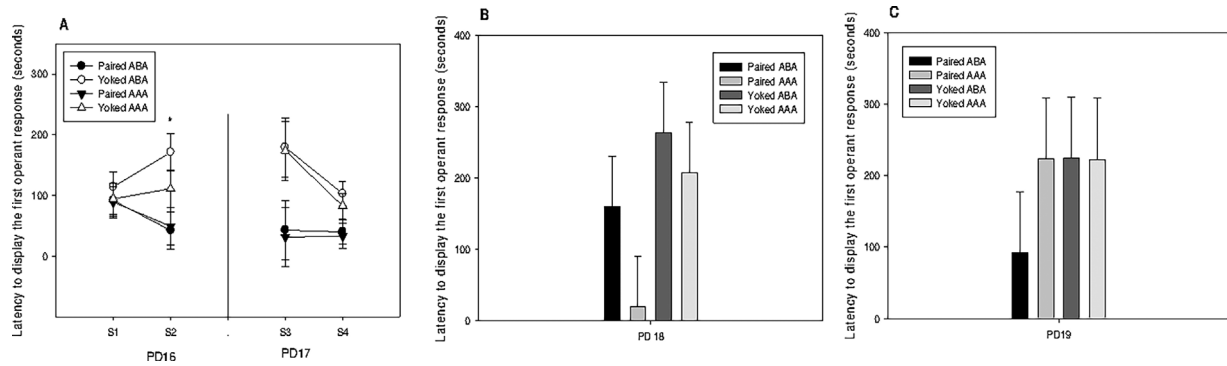


FIGURE 3 Latency to the first operant response (in seconds) at the training sessions during PD16 (S1 and S2) and PD17 (S3 and S4) (A); extinction session on PD18 (B) and renewal session on PD19 (C) as a function of the learning condition (paired or yoked) and the context change (AAA vs. ABA). Vertical bars represent the standard error of the means (SEM)

25% of the subjects from the paired condition received the maximum number of pulses allowed (30) before 15 min. Therefore, most of the subjects (75% in the case of the last session from experiment 2) spent the full 15 min in the tested context in each training session.

In experiment 1, the number of responses during extinction was low, and no clear extinction curves could be observed. In experiment 2, during the second training day, we increased the ratio (FR-3 instead of FR-1), and this resulted in clearer extinction curves, which reached a peak in the first block for the AAA condition, and in the second block for the ABA group, despite the fact that the number of responses did not increase. The low number of responses during extinction is striking, when compared with those from the second training day in both experiments, a finding that highlighted the importance of the reinforcer in maintaining this response. In adult rats, the magnitude of operant responding with appetitive reinforcers is likewise markedly reduced during extinction, compared to responses during an acquisition phase (e.g., Bouton et al., 2011; Todd et al., 2012b), suggesting that this outcome may be a pattern inherent to certain types of procedures involving appetitive operant conditioning, not necessarily related to the young age of the subjects in the present study.

In experiment 2, the context switch between training and extinction (PD18) affected the time course of expression of the operant response, but not its magnitude. In the first block, paired animals from the AAA condition were significantly more responsive than their yoked controls. The maximum number of nose-poke responses displayed by paired pups from the ABA condition occurred in the second extinction block, where they responded more than their respective yoked controls. These results indicate that preweaning rats, similarly to adults (Bouton & Todd, 2014), discriminated the contexts A and B, and retained the context where they learned the operant response, which affected performance during extinction. This evidence of long-term context memory is compatible with previous findings in infant rats (Anderson & Riccio, 2005; Revillo, Trebucq, et al., 2016). In the present work, not only was the peak number of responses achieved at a different time-point for paired subjects from the AAA and ABA conditions, but also the first operant responses were emitted faster in paired subjects from the AAA condition than in the remaining groups.

The most important finding from our study was the observation of a renewal effect, shown as differences in the number of responses emitted during the renewal session, without changes in the latency to the first operant response. The differential sensitivity of these parameters may relate to the fact that the variance of the latency scores is much larger than that of the operant response. Consistent with our observations during extinction, the number of operant responses during the renewal sessions was very low, and between-group differences emerged during the first testing block, followed by rapid extinction. Interestingly, the measure was still sensitive enough to capture the renewal effect. It is worth noting that the renewal effect reported by Bouton and colleagues in adult rats showed, like in the present work, a very low number of responses, compared to those emitted at training (Bouton et al., 2011; Todd et al., 2012a). This leads us to reaffirm the view that this appetitive operant response is highly sensitive to the availability of the reinforcer.

Renewal is one of the recovery-from-extinction effects that puts in evidence that extinction does not imply memory erasure. Many lines of evidence indicate that extinction can be understood as new context-dependent learning, although this conclusion was drawn only from studies in adult subjects (Bouton, 2004). Two previous studies in infant rats found renewal in weaning, but not in preweaning rats (Kim & Richardson, 2007; Yap & Richardson, 2007). These latter authors interpreted those results as indicating that extinction was qualitatively different in infants and older animals, as a result of the presumably still incomplete functional development of the hippocampus in infants (Kim & Richardson, 2010). However, the present results are inconsistent with this hypothesis. Moreover, renewal effects in infant rats were previously reported in three pavlovian conditioning studies, using protocols of taste aversion (Revillo, Castelló, Paglini, & Arias, 2014) and fear conditioning (Revillo, Molina, et al., 2013; Revillo, Cotella, et al., 2015).

An alternative view that may help reconcile these apparently contradictory findings has been proposed by Spear and Rovee-Collier, and referred to as the “ecological model” (Rovee-Collier & Giles, 2010; Spear, 1984). This theoretical framework proposes that infantile learning and retention capacities cannot be considered poorer or weaker than those of the adult organisms; rather, such capacities allow

infants to respond adaptively to their environment at each ontogenetic stage (Rovee-Collier & Giles, 2010; Spear, 1984). This model is relevant for the present discussion because it emphasizes the importance of adapting learning tasks to the motivational, sensory and motor capacities of the subjects in order to accurately explore their cognitive capacities. Subtle changes in task parameters can lead to important differences in performance, particularly at early stages of development during which rapid maturational changes occur. For instance, the sensory content of the context seems to be critical for some context effects during infancy, facilitating not only direct context conditioning (Brasser & Spear, 2004; Pugh & Rudy, 1996), but also interference effects (Revilla, Gaztanaga, et al., 2014, Revillo, Trebucq, et al., 2016). Interestingly, this and other studies demonstrating ABA-renewal in preweaning rats included salient odors in the testing context (Revilla, Molina, et al., 2013, Revillo, Castelló et al., 2014, Revillo, Cotella, et al., 2015). Conversely, the studies that failed to observe this effect during infancy did not enrich contexts with salient odors (Kim & Richardson, 2007; Yap & Richardson, 2007). Furthermore, recovery from extinction can be observed in weaning rats without salient odors in the context (Revilla, Trebucq, et al., 2016, Yap & Richardson, 2007). Therefore, comparing preweaning and weaning rats in context-dependent learning tasks using contexts without odor cues may lead to interpreting ontogenetic differences in terms of memory mechanisms, when it may actually reflect ontogenetic differences in sensory capacities (Brasser & Spear, 2004). Following this rationale, it is important to address the question of which conditions may favor specific learning effects when analyzing them ontogenetically.

From the data suggesting that extinction is not context-dependent and involves unlearning in infants, it has been inferred that exposure therapies for anxiety disorders would be more effective in young children because they may be less susceptible to relapse (Kim & Richardson, 2010). However, our results, as well as those from work conducted in infant rats (Revilla, Molina, et al., 2013, Revillo, Paglini et al., 2014, Revillo, Trebucq, et al., 2016) or in human babies (Cuevas, Learmonth, & Rovee-Collier, 2016) do not agree with this hypothesis. Thus, the evidence that extinction is context-specific even in early infancy underscores the significant role of context as a potential factor in the elimination of undesirable behaviors (Cuevas et al., 2016), and prompts a reassessment of the assumption that infants are unable to retain context learning because of functional immaturity of the hippocampus. As a whole, the experiments presented in this work, together with other reports in the literature, argue that context learning and retention may occur among preweaning rats and that, during infancy, the context modulates interference learning in a similar manner as in adulthood. Our results provide more evidence for this case by showing the involvement of context in the regulation of operant learning (Bouton & Todd, 2014).

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