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Research report

A sensory-enhanced context allows renewal of an extinguished fear response in the infant rat

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HIGHLIGHTS

- ABA-renewal is not observed in infant rats when using standard contexts (without explicit odors).
- The ABA-renewal procedure was effective in reinstating the extinguished CR when contexts (A and B) included an explicit odor.
- This is the first evidence of renewal of an extinguished fear conditioning response in the infant rat.

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ABSTRACT

Studies of extinction in preweanling rats have failed to find ABA-renewal in a fear conditioning paradigm. This result supports the hypothesis postulating ontogenetic qualitative differences in experimental extinction. A similar result in adult rats led to the conclusion that ABA-renewal requires contexts A and B to differ in several types of features, including odor cues. Recently we reported experimental evidence of the renewal of an extinguished taste aversion response in infant rats employing contexts which differ in their odor content. The present study examines the possibility of renewing an extinguished fear response in infant rats when contexts A and B do not include (Experiment 1) or include (Experiment 2) an explicit odor. Results showed absence of renewal when using standard contexts (without explicit odors, Experiment 1). However, when contexts A and B varied also in their odor content, the ABA-renewal procedure was effective in reinstating the extinguished CR (Experiment 2). Thus, it can be concluded that the sensory content of the context determines the observation of renewal in the infant rat, a result that is coherent with previous observations in the adult rat. As a whole, these results challenge our understanding of extinction as a learning process that is qualitatively different in preweanling rats than in later stages of ontogeny.

1. Introduction

Repeated experience with a relatively neutral stimulus (conditioned stimulus, CS) followed by a relevant stimulus (unconditioned stimulus, US) can result in the emission of a conditioned response (CR). Within the associative framework, it is assumed that the CR reflects the learned association between both stimuli (CS and US). For example, in a fear conditioning procedure, rats may respond with a specific type of behavior (i.e. freezing) to a tone that was previously paired with a foot-shock. After this procedure, repeated experience with the CS in absence of the US progressively weakens the CR. Since its discovery in 1927 [1], this procedure, called experimental extinction, has been the subject of a large amount of scientific research.

Extinction has been explained in terms of the weakening of the CS-US association [2,3] or alternatively, in terms of a new inhibitory learning (CS-noUS) which competes with the CS-US association [1]. Evidence supporting the latter hypothesis comes from studies which show that, after extinction, the CR can be recovered through a variety of procedures, such as reinstatement, spontaneous recovery or renewal [4,5]. In a typical renewal procedure, for example, conditioning takes place in a given context (A), while extinction is carried out in a different one (B). After this procedure, the CR is stronger in context A than in context B. This procedure illustrates the context-dependent nature of the extinction phenomenon. The most critical manipulation for observing the recovery of the CR is to change the context between extinction and testing [4,6]. However, such a procedure does not always result in recovery of the CR. Detection of the renewal effect can in some cases require that contexts A and B differ in terms of a variety of attributes. For example, Thomas et al. [7] found ABA-renewal when contexts A and B were different in location and physical characteristics,

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including odor, but not when they differed in only one of these attributes.

The role of context in learning interference effects, such as the US-preexposure effect, latent inhibition or extinction, seems to vary across ontogeny. While in the adult rat these effects are highly sensitive to context change [8,9], in the infant rat they seem to be context-independent [10–12]. For example, a context change between extinction and testing in an ABA-renewal procedure did not produce recovery of a conditioned fear response [10], and similarly, a context change between preexposure and conditioning did not affect the magnitude of latent inhibition [12,13]. The ineffectiveness of context change in these interference paradigms with infant rats has been related to their hypothetical incapacity to acquire context learning [13]. However, results from several studies call this idea into question because they show evidence of context learning in infant rats under a variety of experimental conditions. For example, contextual fear conditioning was detected when the salience of the context was enhanced by using black walls [14] instead of transparent ones [14,15], including odor cues in the context [16], or using a standard context but appropriate age-matched control conditions and analyzing a variety of behavioral indexes [17]. Given these empirical antecedents, the question which logically arises is whether or not interference effects such as renewal would be observed in the infant rat if an odor cue were to be included as a contextual feature. A recent study from our laboratory, using a conditioned taste aversion preparation, supports this possibility [18]. Specifically, in this study, on postnatal days (PDs) 14 and 15, rats consumed saccharin in context A and were then injected with LiCl. After conditioning, extinction training was carried out on PDs 16 and 17 in context B. Finally, rats were tested in terms of saccharin acceptance in either context A or B. Results showed that rats consumed less saccharin when they were evaluated in context A than when they were evaluated in context B (i.e. ABA-renewal effect). Interestingly, in this study, contexts A and B differed not only in terms of tactile and visual cues, but also in its odor content [18].

The specific question guiding the present study is whether ABA-renewal of a conditioned fear response can be detected in the infant rat when contexts A and B differ in their odor content. In Experiment 1, an ABA-renewal procedure was applied to infant rats, using two contexts (A and B) that differed only in terms of visual cues. In Experiment 2, the same procedure was repeated, but in this case an explicit odor was added to each of these contexts. Results from this study may contribute to our understanding of the ontogeny of both contextual learning and the contextual modulation of extinction learning, as well as other interference paradigms, emphasizing the importance of using different sensory cues to constitute the context.

2. Materials and methods

2.1. Animals

A total of 41 Wistar pups, representative of 12 litters, were utilized for the present study, including experiments 1 ($n = 18$) and 2 ($n = 23$). Only females were employed in these experiments, and in any case no more than one subject from a given litter was assigned to the same treatment condition to avoid overrepresentation of a particular litter in any treatment [19]. Animals were born and reared at the vivarium of the Instituto de Investigación Médica Mercedes y Martín Ferreyra, INIMEC–CONICET, under conditions of constant room temperature ($22 \pm 1.0\,^{\circ}$C), on a 12 h light–12 h dark cycle. Births were examined daily and the day of parturition was termed postnatal day 0 (PD0). Subjects were 17 days of age at the start of the experiments. All procedures were approved by the National Department of Animal Care and Health (SENASA–Argentina) and were in compliance with the National Institute of Health's general guidelines for the Care and Use of Laboratory Animals.

2.2. Apparatus

Contexts A and B were counterbalanced in both Experiments (1 and 2). For Experiment 1, the first context consisted of a Plexiglas chamber (29 cm × 17 cm × 20 cm) with white opaque walls, except for the front one that was transparent to enable the testing sessions to be videotaped. The grid floor (30 cm × 18 cm) consisted of stainless steel rods (0.2 cm in diameter) and separated by a distance of 1 cm. The grid was wired to a scrambling electrical stimulation generator (L.I.A.D.E. – FCEFyN, UNC, Cordoba, Argentina) that delivered, 0.6 mA foot-shocks (duration: 2 s). In Experiment 1, the second context was similar to the first one, but with vertical black lines (2 cm wide), 5 cm apart, all along the white walls. Both contexts were placed in the same room during conditioning, extinction and testing. This room was illuminated with dim light and with a constant low noise generated by an air extractor.

For Experiment 2 the contexts were identical to those used in Experiment 1 with the only exception being that an explicit odor was added to each of them. The odor was placed in a small piece of cotton located at the top of the chamber. The odor used for the context with the white walls was orange (0.5 ml of pure orange scent, Esencias Bangladesh, Buenos Aires, Argentina), and for the context with the black lines it was almond (Esencias del Boticario, Cordoba, Argentina). Contexts A and B had a speaker located at the top of the chamber, near the back wall.

2.3. Procedures

The procedures employed in Experiments 1 and 2 were identical, with the only exception being that for Experiment 2 an explicit odor was added to each of the contexts (A or B) as described in the apparatus section.

Conditioning: Conditioning was carried out on PD 17. On this day, subjects from each litter were randomly assigned to the experimental groups (ABA or ABB), removed from their home-cage and individually placed in an experimental chamber (context A) for conditioning. After one minute of contextual adaptation, the CS (a 90 dB tone) was presented for 10 s and immediately after it, the US shock (0.6 mA, 2 s) was delivered. Rats received a total of eight conditioning trials with an intertrial interval (ITI) of 50 s. Thirty seconds after the last trial, subjects were returned to their home-cages.

Extinction: Twenty-four hours after conditioning (on PD 18), pups were placed in the experimental chamber (context B) and after a minute of contextual adaptation, they were exposed to 30 extinction trials, in which the tone-CS (10 s tone) was presented in the absence of the US, with an ITI of 10 s. Immediately after the last extinction trial, rats were returned to their home-cages.

Testing: On the third day of the experiment (PD 19), pups were placed in the corresponding experimental chamber and after one minute, the CS was presented for 2 min. Subjects from the ABB condition were evaluated in context B, while subjects from the ABA group were evaluated in context A. The parameters used for conditioning, extinction and testing were extracted and adapted from previous studies with infant rats [20,21].

The extinction and testing sessions were videotaped. The conditioning index was the time that the subjects spent freezing, behavior that was defined as the absence of all movement other than that required for respiration. During extinction, the time for which each subject remained frozen was registered during each CS presentation. For the statistical analysis, 5 blocks were calculated, each corresponding to the sum of six consecutive extinction trials. Each of these blocks represents the freezing time in response to 60 s of CS exposure. At testing, freezing was measured during the first minute in the absence of the CS (baseline) and then for 2 min in response to the CS. The scores analyzed corresponded to the total freezing time during these time periods.

2.4. Data analysis

No subjects were excluded from the statistical analysis in this study. We took into account the exclusion criterion selected for previous studies analyzing extinction of a conditioned fear response with preweanling rats [20], according to which subjects that remained frozen for more than 50% of the baseline period were not included in the inferential analysis. In our study, no subject met this criterion. Freezing scores from extinction were analyzed by means of a mixed ANOVA, including Context as the only between-group factor, with two levels (ABA or ABB). Block was the only within-group variable, with five levels, (block1 to block5). Freezing baseline scores and freezing scores in response to the CS collected at testing were analyzed with between-group ANOVAs, including Context (ABA or ABB) as the only factor. Significant main effects and/or interactions were further analyzed by means of post hoc analyses (Newman-Keuls). All inferential analyses conducted in the present study employed an α level equal to 0.05.

3. Results

3.1. Experiment 1

Fig. 1a shows freezing scores registered during the five extinction blocks (see Section 2). The ANOVA revealed a significant main effect of Block [$F(4,64) = 3.77$; $p < 0.05$]. Post hoc tests indicated that freezing scores in response to the tone-CS were significantly