

RETURN OF FEAR IN RATS DUE TO CONTEXT CHANGE, DELAY OF TESTING, AND THEIR COMBINATION.

*REGRESO DEL MIEDO EN RATAS DEBIDO AL CAMBIO
DE CONTEXTO, LA DEMORA DE PRUEBA Y SU COMBINACIÓN*

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Abstract

Conditioned and then extinguished fear recurs if the background context is changed after extinction (renewal effect) or if there is a temporal interval between extinction and testing (spontaneous recovery effect). The present study demonstrated summation of these effects in rats, and thus supports the claim that the spontaneous recovery effect is a special case of the renewal effect in that temporal contexts are changed after extinction. The demonstration of summation of renewal and spontaneous recovery effects has clinical implications for the return of fear (i.e., relapse) after exposure therapy for phobias, because the rats' fear conditioning has been considered to be a good animal model for understanding the neural and behavioral processes in human phobias.

Keywords: renewal, context, spontaneous recovery, extinction, conditioned fear, rats

Resumen

El miedo condicionado y posteriormente extinguido ocurre si se cambia el contexto después de la extinción (efecto de renovación) o si hay un intervalo del tiempo entre

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la extinción y la prueba (efecto de recuperación espontánea). El presente estudio mostró la sumación de estos efectos en ratas, y por lo tanto apoya la afirmación que el efecto de recuperación espontánea es un caso especial del efecto de renovación debido a que los contextos temporales se cambian después de la extinción. La demostración de sumación de los efectos de renovación y recuperación espontánea tiene implicaciones clínicas para el regreso del miedo (i.e., recaída) después de la terapia de exposición para las fobias, debido a que el miedo condicionado en ratas se ha considerado un buen modelo animal para entender los procesos neurales y conductuales de las fobias humanas.

Palabras clave: renovación, contexto, recuperación espontánea, extinción, miedo condicionado, ratas

Conditioned and then extinguished responding recurs if the background context is changed (renewal effect, Bouton, 2004) or if a retention interval is inserted between extinction and testing (spontaneous recovery effect: Rescorla, 2004). According to Bouton and colleagues (Bouton, 1993, 1994; Bouton, Nelson, & Rosas, 1999; Bouton, Westbrook, Corcoran, & Maren, 2006), spontaneous recovery is a special case of renewal in which the temporal rather than the physical context is changed after extinction. In supporting this theory, Rosas and Bouton (1998) demonstrated that these two effects are additive in rats' taste aversion learning. Specifically, they employed a 2 (context unchanged or changed) \times 2 (short or long retention interval) factorial design to show the weakest conditioned aversion in rats tested in the unchanged context after a short retention interval and the greatest return of aversion in rats tested in the changed context after a long retention interval. Notably, the summation of renewal and spontaneous recovery effects has been replicated in human causality judgement tasks (Rosas, Vila, Lugi, & López, 2001).

In the present study, we attempted to extend the generality of Rosas and Bouton's (1998) finding from a conditioned taste aversion preparation to a conditioned fear setting, where fear is measured by the suppression of ongoing behavior of rats. Such a demonstration has clinical implications, because rats' fear conditioning has been considered to be a good animal model for understanding the neural processes in human phobias (Delgado, Olsson, & Phelps, 2006) and their remission (Milad, Rauch, Pitman, & Quirk, 2006; Myers & Davis, 2007).

To our knowledge, there have been only two previous studies demonstrating the summation of renewal and spontaneous recovery of conditioned fear in rats. First, Laborda and Miller (2013) recently have shown it in an experiment with rats. In their study, renewal of conditioned fear was demonstrated in the ABC paradigm, where acquisition, extinction, and testing were administered in different contexts. By contrast, in the present study, we have attempted to show the summation of ABA renewal

and spontaneous recovery as did Rosas and Bouton (1998) and Rosas et al. (2001). This type of renewal is the recovery of an extinguished responses when animals are tested in the acquisition context (A) after extinction in the second context (B). Second, Woods and Bouton (2008, Experiment 1) showed both ABA renewal and spontaneous recovery of fear in rats, but their demonstration of spontaneous recovery was based on a comparison between the final day of extinction training and the test day. Such a within-subjects between-day design, however, is susceptible to age, motivational, and other day-to-day fluctuation factors. In the present experiment, we thus attempted to show both ABA renewal and spontaneous recovery on the same test day by using a between-group design in order to control these factors as did others studying such summation using taste aversion (Rosas & Bouton, 1998; Rosas et al., 2001) or fear and an ABC renewal procedure (Laborda & Miller, 2013).

Method

Subjects

Forty-eight experimentally naive male Wistar rats housed in individual hanging home cages were used. The rats were approximately 90 days old at the beginning of the experiment. Throughout the experiment, the animals were maintained on an ad-lib food schedule and given 10-min water access per day in the home cages immediately after the last treatment of each day. The vivarium was on a 12–12 h light–dark cycle (lights on at 0800 hr) with controlled temperature (23 °C) and humidity (60%).

Apparatus

The apparatus was identical to that used in our previous report on renewal of conditioned fear (Tamai & Nakajima, 2000). Specifically, 6 drinking boxes (10 × 20 × 15 cm, w × l × h) were located on a table in a sound-proof room maintained at 23°C. Each box was constructed of white acrylic walls, a clear acrylic ceiling with small holes for ventilation, and a grid floor consisting of 0.5-cm metal rods spaced 1.3 cm center to center. Scrambled electric shock (0.43 mA) was delivered through the floor. There was a 1.2-cm hole with a shutter in the left-right center of one narrow wall (4.5 cm above the floor). The rat could access a drinking spout through this hole, and the rat's contact with the spout was detected using a low-current circuit between the spout and the floor. A speaker located 2 m above the table presented a tone (1000 Hz, 85 dB re Scale C) against a background noise level of 65 dB made by an exhaust fan under the table. The boxes could be illuminated by two light sources: indirect illumination (10 lx) by four 100-W bulbs located near the ceiling of the experimental

room, and direct illumination (137 lx) by a 100-W bulb fixed in a lampshade suspended 1.6 m above the boxes. All events were controlled and recorded by computers located in an adjacent room.

Two contexts were created by changing both the room illumination and some features of the boxes. In one context (bright), the ambient room lamps were continuously on and the direct lamp was flashed at intervals of 2 s (0.5-s on, 1.5-s off) throughout the session. In addition, 13 metal beaded chains (10 cm long) were suspended from the ceiling of the individual boxes to provide tactile cues. In the other context (dark), there was no illumination and the inner size of each box was reduced by inserting a four-sided acrylic frame (9.5 × 14.5 × 15 cm, w × l × h) into the box. The inner wall of this frame was lined with black sandpaper, and the ceiling of the box had no chains. These two physical contexts were counterbalanced within each group of rats (see below) with respect to their functional roles as Contexts A and B.

Procedure

All experimental sessions were conducted in 8 squads of 6 rats each in the initial two hours (2000–2200 hr) of the dark period of the vivarium on consecutive days. Each session started with the opening of the shutter for the drinking hole in each box, and it ended after 5 min with the closing of the shutter.

Lick Training. For 10 sessions, each rat was trained to drink water by licking the spout in the boxes. To facilitate drinking from the spout, it was protruded into each box during the initial two sessions (1 cm in the first and 0.5 cm in the second) in the white boxes without the chains and under ambient room illumination (i.e., a context different from Contexts A and B). The remaining 8 sessions consisted of alternations of lick training in Contexts A and B (4 days each). The spout was protruded 0.5 cm into the chamber on the first session of each context, but it was set 0.3 cm behind the shutter from the second session onward.

Acquisition. The acquisition phase consisted of 6 drinking sessions of 4 trials each in Context A with an average intertrial interval (ITI) of 68.2 s (range: 60.7–80.2 s). In each trial, a 10-s tone was immediately followed by a 0.7-s shock. To equate familiarity with the two contexts, an additional session was administered in the alternate context (Context B) approximately 50 min after each daily conditioning session in Context A. Neither the tone nor the shock was presented in Context B, and the rats were simply allowed to drink water from the spout for 5 min and the number of licks were recorded.

Lick retraining. For baseline licking rates to revert to their preconditioning levels, lick training was reinstated for 6 days with no experimental events. Each day had two 5-min sessions, first in Context A and then in Context B, with a 50-min interval between sessions.

Extinction and testing. The conditioned fear then was extinguished under the drinking situation by presenting a 10-s tone with no shock in Context A for half of the rats (Groups AAA–Imm and AAA–Delay) or in Context B for the remaining rats (Groups ABA–Imm and ABA–Delay). This condition continued for 8 days, and the number of trials per daily session was doubled to 8 with an average ITI of 34.1 s (range: 30.3–40.1 s) in an attempt to obtain reliable renewal and spontaneous recovery after extinction (Urcelay, Wheeler, & Miller, 2009). Parenthetically, a pilot experiment without these modifications had yielded a similar, but statistically weak, pattern of test results. The rats also were allowed to drink water for 5 min in the alternate context with no experimental event 50 min after each daily extinction session.

Testing was administered on the next day for Groups AAA–Imm and ABA–Imm (Imm = immediate), while it started 7 days after the extinction phase for Groups AAA–Delay and ABA–Delay. The tone was presented 4 times per session without shocks. The average ITI was the same as in the acquisition phase. During the interval days, the rats in the latter groups were simply allowed to drink water for 5 min in Context A and then in Context B with an interval of 50 min between exposure to the two contexts.

Group assignment and treatment on interval days. The rats were assigned to the four groups noted above ($n = 12$ each) so that the groups were equated as closely as possible for mean bodyweight and baseline licking rate. Because the daily sessions were executed in 8 squads of 6 rats each, each experimental group consisted of 2 squads of rats exposed to the conditions in a counterbalanced order.

To control motivational and age factors in test performance, all rats were tested on the same day. As shown in Figure 1, the acquisition training started on the day following the first lick training for Groups AAA–Delay and ABA–Delay, while it started 7 days later for Groups AAA–Imm and ABA–Imm. During the interval days, rats in the latter groups were simply allowed to drink water in Context A and then Context B with an interval between exposure to the two contexts of 50 min.

Group	Days																																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
AAA–Imm	lick	lick in A or B										lick in A and B					acquisition in A lick in B					lick in A and B					extinction in A lick in B							test in A			
ABA–Imm												lick in A and B					acquisition in A lick in B					lick in A and B					extinction in B lick in A										
AAA–Delay												acquisition in A lick in B					lick in A and B					extinction in A lick in B							lick in A and B								
ABA–Delay												acquisition in A lick in B					lick in A and B					extinction in B lick in A							lick in A and B								

Figure 1. The experimental design. A and B represent the background contexts of training. The licking training on Days 1 and 2 were administered in a third context. Critical treatments (acquisition, extinction, and testing) are not shaded.

Results

Because there were no statistically significant differences in the baseline lick rate in any critical group comparisons, conditioned fear to the tone was indexed in terms of a suppression ratio (Annau & Kamin, 1961). The ratio was calculated by dividing the total number of licks made in all trials of a session by that sum plus the total number of licks in the corresponding pretrial periods of the same session. That is, a single ratio was computed for each session in each rat. A suppression ratio of .50 therefore indicates no suppression of licking, while a suppression ratio of .00 indicates complete suppression, suggesting strong conditioned fear. For simplicity, *F* values from analyses of variance (ANOVA) are reported only when they were statistically significant with the alpha level set at .050.

Figure 2 shows the group averages in the acquisition, extinction, and test phases. Conditioned fear was acquired quickly in all groups, and the acquisition was somewhat stronger for the rats to be tested without delay (Groups AAA–Imm and ABA–Imm). These impressions were statistically supported by a 2 (AAA or ABA) \times 2 (Imm or Delay) \times 6 (session) ANOVA, which yielded significant main effects of delay ($F [1, 44] = 12.25, p = .001$) and session ($F [5, 220] = 23.53, p < .001$). The main effect of context and all interactions were nonsignificant.

The conditioned fear was extinguished over sessions as shown in the middle portion of Fig. 2. A 2 (AAA or ABA) \times 2 (Imm or Delay) \times 8 (session) ANOVA yielded

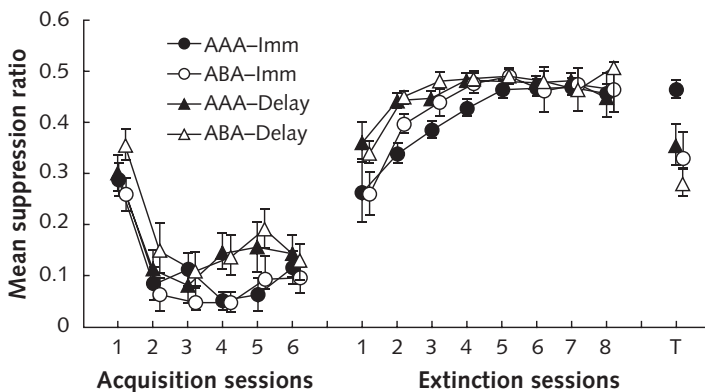


Figure 2. Average suppression ratios of four groups in the acquisition, extinction, and test (T) phases. The groups differed in two respects: the context of extinction (A or B) and in the delay between the end of extinction and the test (1 day for the immediate groups or 7 days for the delayed groups). The error bars indicate standard errors. The data points of Group ABA-Imm and ABA-Delay were slightly offset for clarity.

significant main effects of delay ($F [1, 44] = 8.83, p = .005$) and session ($F [7, 308] = 27.54, p < .001$), and their interaction ($F [7, 308] = 2.14, p = .039$). The main effect of context and the other interactions were nonsignificant. Subsequent analyses of the delay \times session interaction revealed a significant delay effect in the first, second, and third sessions ($F_s [1, 44] > 4.54, p_s < .039$). The rats to be tested with and without delay showed statistically equivalent performance in the remaining sessions.

The data of major interest are depicted in the rightmost portion of Fig. 2. Changing the context as well as the delay of testing both resulted in a recurrence of fear in the rats as indexed by the low suppression ratios. Furthermore, these effects were additive: Group ABA–Delay showed the strongest fear recurrence among all the groups. These visual analyses were supported by a 2 (AAA or ABA) \times 2 (Imm or Delay) ANOVA, which yielded significant main effects of context ($F [1, 44] = 9.02, p = .004$) and delay ($F [1, 44] = 5.23, p = .027$). The nonsignificant interaction of these factors implies a linear summation of these effects.

It is noteworthy that similar results were found in the suppression ratios calculated for the first test trial: the mean (\pm standard error) scores were $.47 \pm .02, .13 \pm .05, .20 \pm .06$, and $.03 \pm .01$ for Groups AAA–Imm, ABA–Imm, AAA–Delay, and ABA–Delay, respectively. A similar ANOVA yielded significant main effects of context ($F [1, 44] = 138.47, p < .001$) and delay ($F [1, 44] = 19.34, p < .001$). The context \times delay interaction was significant ($F [1, 44] = 4.07, p = .050$); this apparent nonlinear summation is explainable by the almost-lowest possible score for Group ABA–Delay (a floor effect).

Discussion

The present study demonstrated summation of ABA renewal and spontaneous recovery effects in rats' fear conditioning preparation using a between-group design. This effect systematically replicates a similar one found using taste aversion learning by rats (Rosas & Bouton, 1998) and in human causality judgement tasks (Rosas et al., 2001).

An unexpected result of the present study was that the rats in Groups AAA–Imm and ABA–Imm showed stronger fear than those in Groups AAA–Delay and ABA–Delay in the acquisition and early extinction sessions. It is not obvious why this occurred, but this tendency was small in size and in the opposite direction of responding observed in testing. We are confident that this unexpected tendency is not critical for yielding our test results, because the same pattern of results (i.e., summation of ABA renewal and spontaneous recovery effects) was observed in the aforementioned pilot experiment, where Groups AAA–Imm and ABA–Imm showed slightly weaker, not stronger, fear than Groups AAA–Delay and ABA–Delay in the acquisition and early extinction sessions.

One feature of the experimental procedure that merits a comment is that we shortened the ITI by doubling the number of trials per day in the extinction phase and reinstated the original ITI (and the trial number) in testing. Because ITIs can function as contextual cues (Bouton & García-Gutiérrez, 2006; see also Beeman, Hartman, & Grant, 1960), changing the ITIs may have increased the sensitivity of return of fear, though this factor should have contributed equally to the performance of all groups including Group AAA–Imm (i.e., the control rats). Furthermore, the summation of renewal and spontaneous recovery occurred on the first test trial, which was relatively free from the effect of the changed ITI.

Although the present study demonstrated the summation of renewal and spontaneous recovery effects as have other investigators (Laborda & Miller, 2013; Rosas & Bouton, 1998; Rosas et al., 2001; Woods & Bouton, 2008), these results do not necessarily support the claim that the two effects have a common underlying process. One may argue that the two independent and noninteractive mechanisms of response restoration work together to produce response summation. Although this possibility cannot be rejected unequivocally, we prefer the common-process view because of its simplicity. Other lines of research also support this view. For example, renewal and spontaneous recovery are both attenuated by presenting a retrieval cue for extinction (Brooks & Bouton, 1993, 1994), by spacing extinction trials (Urcelay et al., 2009), and by administration of gamma-aminobutyric acid A receptor inverse agonist FG7142 (Delamater, Campese, & Westbrook, 2009).

As repeatedly discussed by Bouton and colleagues (Bouton, 1988, 2000, 2002, 2014; Bouton & Nelson, 1998; Bouton & Swartzentruber, 1991; Bouton, Woods, Moody, Sunsay, & García–Gutiérrez, 2006), an understanding of renewal and spontaneous recovery may contribute to the clinical treatment of human maladaptive behavior, especially for the prevention of a return of fear after exposure therapy (Rachman, 1989). Following this view, in recent years studies of fear renewal have increased considerably with both healthy participants (see Vervliet, Baeyens, Van den Berg, & Hermans, 2013, for a review) and those exhibiting phobic behavior (Mineka, Mystkowski, Hladek, & Rodriguez, 1999; Mystkowski, Craske, & Echiverri, 2002; Mystkowski, Craske, Echiverri, & Labus, 2006; Rodriguez, Craske, Mineka, & Hladek, 1999; Vansteenwegen et al., 2007). We hope that our demonstration of summation of renewal and spontaneous recovery effects in the fear conditioning of rats further promotes this view.

Spontaneous recovery and renewal effects are not specific to Pavlovian (i.e., respondent) preparations. They also occur after extinction of instrumental (i.e., operant) responses (Bouton, Winterbauer, & Todd, 2012; Todd, Vurbic, & Bouton, 2014; Vurbic & Bouton, 2014). Thus, their summation in instrumental conditioning preparations is what remains to be shown. In this regard, it is suggestive that Nakajima (2014, Experiment 5) recently has reported a between–group demonstration of ABA and ABC renewal of negatively reinforced instrumental behavior superimposed on spontaneous

recovery, which was observed on a within–subject basis. Confirmation of the summative effects of renewal and spontaneous recovery with a between–group design seems an appropriate goal for future research of negatively, as well as positively, reinforced instrumental behavior.

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