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SHORT COMMUNICATION

Renewal of Formerly Conditioned Fear in Rats after Extensive Extinction Training

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We explored renewal of formerly acquired and then extinguished fear in rats. After 24 paired trials of a tone and an electric shock, acquired fear to the tone was extinguished in another context by repeated exposure to the tone alone. Conditioned fear was behaviorally extinguished by the 32nd trial of tone exposure; 40 or 80 additional extinction trials (i.e., a total of 72 or 112 extinction trials) were administered to independent groups of rats. Extinguished fear was renewed by testing the tone in the original context, independently of the amount of the preceding extinction training. The finding suggests that this type of fear renewal is difficult to prevent even after extensive extinction training prior to context change. Fear renewal also took place by shifting contexts after 72 extinction trials, when the fear had been extinguished in the context of acquisition. This kind of renewal was, however, prevented by extending extinction training to 112 trials.

Changing background contexts after extinction of conditioned fear causes renewal of the extinguished fear (Bouton & Bolles, 1979; Bouton & King, 1983; Bouton & Swartzentruber, 1989; Grahame, Hallam, Geier, & Miller, 1990; Hanford, Mulvaney, & Kelfer, 1980; Harris, Jones, Bailey, & Westbrook, 2000; Rauhut, Thomas, & Ayers, 2001; Tamai, Nakajima, Kitaguchi, & Imada, 2000). In a typical experimental paradigm of this phenomenon, laboratory rats (*Rattus norvegicus*) initially receive fear acquisition training in a chamber (Context X) with a signal (e.g., a tone) repeatedly paired with an electric shock. Next, the fear is extinguished in a second chamber (Context Y) by repeated exposure to the signal alone. Finally, when tested in the original acquisition chamber (Context X), the signal evokes fear again.

Bouton (1988) has claimed that XYX fear renewal is substantial even after extensive extinction training. For example, Bouton and Swartzentruber (1989) reported a renewal effect when 8 signal-shock conditioning trials in

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Context X had been followed by 84 signal exposures in Context Y. Although their experiment lacked a comparison group of a small number of extinction trials to assess any detrimental impact on renewal of the extensive extinction training, one of the experiments recently published by Rauhut et al. (2001, Experiment 1) strongly supported Bouton's claim. In this experiment, 12 conditioning trials in Context X were followed by either 20 or 100 extinction trials in Context Y, and the amount of fear renewal in testing upon return to Context X was equally evident, irrespective of the number of extinction trials.

The experiment reported below was designed to explore the same issue independent of Rauhut et al. (2001, Experiment 1). Although both Rauhut et al's experiment and ours employed a conditioned suppression preparation for measuring conditioned fear, the baseline responding was instrumental lever pressing for food reinforcement in Rauhut et al.'s case, while it was water licking in ours. Thus, our experiment would provide an assay of the generality of Rauhut et al.'s finding. In our procedure, 24 signal-shock pairings in Context X were followed by extinction training consisting of 32, 72, or 112 signal exposures in Context Y. As noted below, the shortest extinction training (i.e., the 32-trial training) was sufficient to erase behavioral signs of conditioned fear. Accordingly, the remaining two longer treatments were extinction overtraining conditions.

Bouton and Ricker (1994) and Tamai et al. (2000) also reported another type of fear renewal by contextual shifting. When a signal is conditioned and then extinguished in the same context (Context X), the extinguished fear reappears in a second context (Context Y). This XXY renewal, when compared with XYX renewal, is generally smaller in size and shorter-lived, and it is sometimes undetected (Bouton & King, 1983; Bouton & Swartzentruber, 1989; see also Goddard, 1999; Nakajima, Tanaka, Urushihara, & Imada, 2000, for failures to detect XXY renewal in other conditioning preparations). The second aim of our experiment was to assess the impact of extensive extinction training on XXY renewal effect with two levels of extinction training (72 and 112 trials). If XXY renewal is a relatively weak phenomenon, extensive extinction may prevent its occurrence.

Method

Subjects

Seventy-two experimentally naive male Wistar rats were used. The rats were approximately 90 days old at the beginning of the experiment. Free access to food was provided in the home cage, but access to water in the home cage was restricted to 10 min in duration immediately after daily training.

Apparatus

Six drinking boxes, 20-cm long, 10-cm wide, and 15-cm high were located on a table in a sound-proof room. They were arranged on the table in a radial position (like the spokes of a wheel) with their front panels facing the center. Each box was made of white acrylic walls, a clear acrylic ceiling with small holes for ventilation, and a grid floor consisting of 5-mm metal rods spaced 13 mm center to center. One narrow wall was the front panel, and it had a hole (12 mm in diameter, 45 mm above the floor) through which the rat could access a drinking spout 3 mm behind a shutter. The rats' contact with the spout was detected with a low-current circuit between the spout and the floor. A speaker located 2 m above the center of the table presented a 10-s tone (1000-Hz, 85dB re Scale C) as the conditioned stimulus. Background noise level was 65 dB when measured in each box. The unconditioned stimulus was a 0.7-s scrambled electric shock (0.43 mA) delivered through the floor of the boxes. 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 center of the table. All events were controlled and recorded in computers located in an adjacent room.

Two contexts were made by modifying the illumination of the room and a few 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 each) were suspended from the ceiling of the individual boxes to provide for 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 (14.5 cm long, 9.5 cm wide, and 15 cm high) into the box. The inner wall of this frame was lined with black sandpaper. The ceiling of the box was intact (i.e., clear acrylic) and it had no chains.

Procedure

Each daily session started with the opening of the shutter for the drinking hole in each box, and it ended after 300 s with the closing of the shutter.

Acclimation and Baseline Training. For 16 sessions, rats could drink water from the spout; no other events were presented. To facilitate drinking from the spout, the spout was protruded into each box during the initial two sessions (10 mm on the first and 5 mm on the second). During these two initial sessions, training was administered in the white boxes without the chains and under ambient room illumination (i.e., a context different from both the bright and dark contexts). The remaining 14 sessions consisted of alternations of baseline lick training in the bright and dark contexts (7 days each). The spout was protruded 5 mm into the chamber on the first session of each context, but it was set at 3 mm behind the shutter from the second session onward.

Acquisition. For a half of the rats, the bright context was employed for acquisition of conditioned fear (Context X); the dark context played the same role for the other half. The rats were matched as closely as possible for body weight and number of licks measured during the last 6 baseline sessions. The acquisition phase consisted of 6 sessions. All rats received 4 trials per session. In each trial, a 10-s tone was immediately followed by a 0.7-s shock. Four different trial-spacing sequences were prepared; the average intertrial interval (ITI) was 68.2 s (range: 60.7-80.2 s). In order to equate familiarity with the two contexts, an additional session was administered in the alternate context (Context Y) approximately 90 min after each daily conditioning session. Neither the tone nor the shock were presented in Context Y and the rats were simply allowed access to water from the licking spout during 300 s.

Baseline Retraining. In order for licking rates to revert to their preconditioning level, baseline training in Contexts X and Y was reinstated for 6 days with no experimental events. Each day had two sessions, one in Context X and the other in Context Y, 90 min apart, with the order counterbalanced. This training would extinguish any direct context-shock, and also context-tone,

Table 1.
The contexts of experimental treatments.

Group	Alias	Conditioning	Extinction and testing
XXX-8	XXY-18	XXXXXX	XXXXXXXXX***X***XXXXXXXXXX***Y***
XXX-18	XXY-28	XXXXXX	XXXXXXXXXXXXXXXXXXXX***X***XXXXXXXXXX***Y***
XXX-28		XXXXXX	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX***X***
XYX-8		XXXXXX	YYYYYYYY***X***
XYX-18	XYY-8	XXXXXX	YYYYYYYYY***Y***YYYYYYYYYY***X***
XYX-28	XYY-18	XXXXXX	YYYYYYYYYYYYYYYYYYY***Y***YYYYYYYYYY***X***

Note. The group names indicate the context where conditioning, extinction, and test treatments were administered and the number of extinction sessions. Conditioned fear was established for 6 sessions by pairing a tone and a shock 4 times per session. As the test procedure was identical to the extinction procedure (4 exposures to the tone alone per session), four of the original groups (XXX-8, XXX-18, XYX-18, and XYX-28) also served as other groups of our interest (XXY-18, XXY-28, XYY-8, and XYY-18, respectively). Bold Italics mean the test contexts.

associations. The rats were, then, divided into 6 groups of equal size matched as closely as possible for the following variables: body weight, identity of the physical contexts, performance in the acquisition phase, and number of licks in baseline retraining sessions.

Extinction and Test. Extinction of conditioned fear was administered in Context X for three of the six groups (Groups XXX-8, XXX-18, and XXX-28), but in Context Y for the remaining three groups (Groups XYX-8, XYX-18, and XYX-28). The procedure was the same as that used in the acquisition phase, except that the shock was never presented: the tone was presented alone 4 times per session with the mean ITI of 68.2 s. As in the acquisition phase, rats were also allowed access to water in the alternate context with no experimental events. The extinction phase continued for 8 days in Groups XXX-8 and XYX-8, 18 days in Groups XXX-18 and XYX-18, and 28 days in Groups XXX-28 and XYX-28. Finally, all groups were tested in Context X where the tone was presented 4 times without reinforcement. The alternate context session was administered 90 min later.

As the test procedure was identical to that of the extinction phase, we exploited the same rats for examination of XXY and XYY treatments. Table 1 shows the design. Groups XXX-8 and XXX-18 received 9 additional sessions in Context X and then one test session in Context Y, forming Groups XXY-18 and XXY-28, respectively. The XYY procedure, on the other hand, was assessed with the data of the 9th extinction day of Groups XYX-18 (Group XYY-8) and with the data of the 19th extinction day of Group XYX-28 (Group XYY-18). It was also possible to assess the XXX-8 performance with the data of Groups XXX-18 and XXX-28, the XXX-18 performance with the data of Group XXX-28, and the XYY-8 performance with the data of Group XYX-28. We will not report these data in the present article for the simplicity of data presentation and statistical analysis. However, the analyses including these data sets resulted in the same conclusion reported below. Rats tested in the context of extinction (XXX and XYY) were control animals against which XYX or XXY renewal of extinguished fear was contrasted.

Results

Because there were no statistically reliable differences in the baseline lick rate in any critical comparisons, conditioned fear to the tone was indexed in terms of a suppression ratio. The ratio was calculated by dividing

the total number of responses made in all trials of a session by the sum of it plus the total number of responses in the corresponding pretrial periods of the same session. That is, a single ratio was computed for each session in each rat. The session-based, rather than trial-based, suppression ratios were targeted for analysis because the short sampling time (10 s for individual trial and pretrial periods) sometimes provided zero values for the denominator of the trial-based suppression ratio. A suppression ratio of 0.5 indicates no suppression of licking, and of 0 indicates complete suppression, suggesting strong conditioned fear. Although the significance level was set at $p < 0.05$, marginally significant effects ($p < 0.10$) are also reported.

Acquisition and Extinction

Pairing a tone and a shock rapidly yielded suppression of licking behavior: the suppression ratios averaged across all rats were 0.39, 0.10, 0.11, 0.09, 0.09, and 0.13 from the first to the final (sixth) sessions of acquisition. Not surprisingly, there were no reliable group differences in the acquisition phase.

Figure 1 illustrates average performance of each group in the extinction phase. All groups gradually ceased to show conditioned fear to the tone, although fear extinction was slightly slower in the original context than in the new context. To verify these impressions, we focused on the initial 8 sessions of all groups and assessed the data with a 2 (context: X vs. Y) x 3 (length of total extinction training: 8, 18, or 28) x 8 (session) analysis of variance. There were significant main effects of context, $F(1, 66) = 5.00$, and session, $F(7, 462) = 80.71$, and a marginally significant interaction between these factors, $F(7, 462) = 1.82$, $p = 0.08$. All other main or interactive effects were far from significance, Fs < 1. Hence, the impression from Figure 1 was statistically supported.

Figure 1 also shows that conditioned fear was extinguished by the 8th session and that there were no group differences in performance on the day before testing. These impressions were statistically supported by an analysis of the final extinction session of the 6 groups (the 8th session for Groups XXX-8 and XYX-8, the 18th session for Groups XXX-18 and XYX-18, and the 28th session for Groups XXX-28 and XYX-28). A 2 (context: X vs. Y) x 3 (length of total extinction training: 8, 18, or 28) analysis yielded nonsignificant main, Fs < 1, and interactive, $F(2, 66) = 1.74$, effects.

Testing

Figure 2 presents the suppression ratio observed during the test session, the data of primary interest. As noted in the *Procedure* section, Group

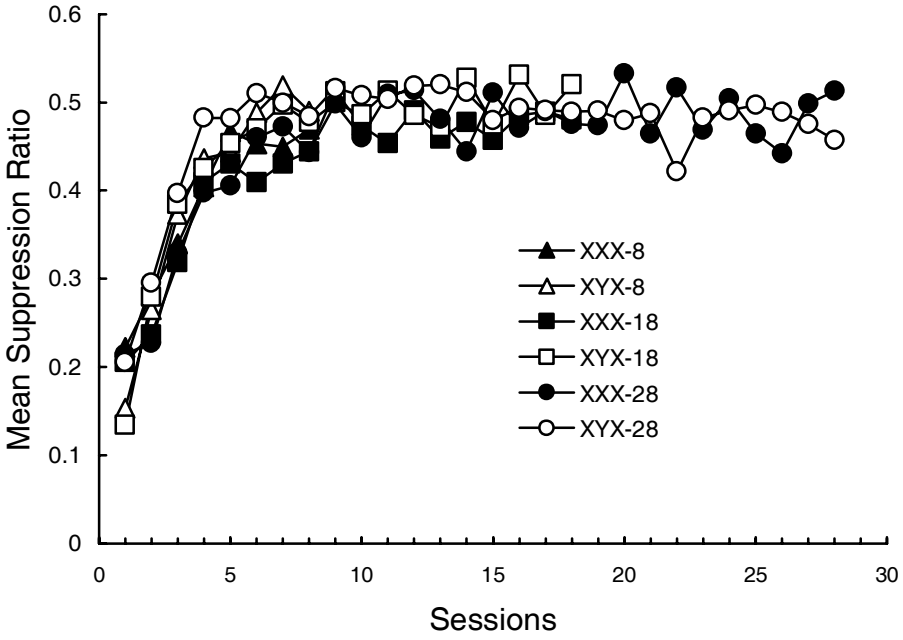

Figure 1. Average suppression ratios of six groups during the extinction phase. The groups differed in two respects: the context of extinction (A or B) and in the length of extinction training (8, 18, or 28 sessions).

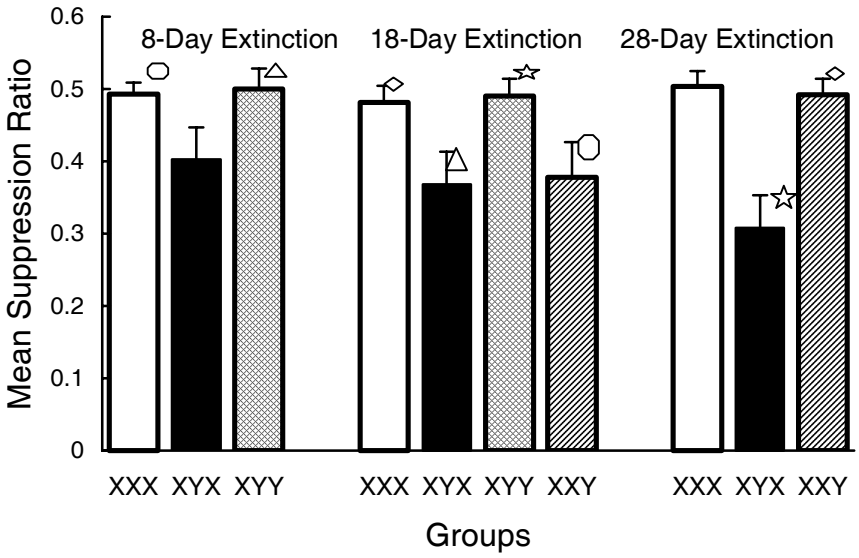

Figure 2. Average suppression ratios of all groups in the test. Group XXX-8 was also tested under the condition of XXY-18 (circles), and Groups XXX-18 under the condition of XXY-28 (diamonds). The XYY-8 data were from the early session of Group XYX-18 (triangles), while the XYY-18 data were from the early session of Group XYX-28 (stars). See Table 1 for the group treatments. Error bars indicate standard errors.

XXX-8 was also tested under the condition of XXY-18 (see circles in Figure 2), and Groups XXX-18 under the condition of XXY-28 (see diamonds). The XYY-8 data were from the early session of Group XYX-18 (see triangles), while the XYY-18 data were from the early session of Group XYX-28 (see stars).

The tone evoked little suppression in all conditions of XXX and XYY, in which the tone was tested in the context of extinction. Shifting the context after the extinction phase in both XYX and XXY fashions, however, renewed the extinguished fear when the extinction training was modest. The extensive extinction training thwarted the XXY renewal effect, but the XYX renewal effect was resistant.

These impressions from Figure 2 were supported statistically by two series of analyses. First, we compared performance of rats that had received the same number of extinction sessions before the critical test. For the groups being tested after 8 sessions of extinction, a one-way analysis yielded a marginally significant group effect, $F(2, 33) = 2.94$, $p = 0.07$. As the ratios of the two control groups (XXX-8 and XYY-8) were equivalent, $t < 1$, we combined these groups into one and compared it with the experimental group (XYX-8). A t-test revealed a significant difference between them, $t(34) = 2.53$ (all tests were two-tailed). A one-way analysis for the four groups being tested after 18 sessions of extinction yielded a significant group effect, $F(3, 44) = 3.13$. The control rats (Groups XXX-18 and XYY18, combined) statistically differed from Group XYX-18, $t(34) = 3.02$, and from Group XXY-18, $t(34) = 2.64$. The latter two experimental groups were equivalent in renewal effect, $t < 1$. A one-way analysis for three groups being tested after 28 sessions of extinction yielded a significant group effect, $F(2, 33) = 12.12$, and subsequent contrasts using Tukey's HSD tests revealed that Group XYX-28 differed from Groups XXX-28 and XXY-28, which were equivalent in their suppression ratio.

Second, we directly assessed the effect of the amount of the extinction training for each context-transition condition. A one-way analysis for the XXX condition supported the impression that there were no group differences in this condition, $F < 1$, reflecting the fact that all XXX groups equally showed no conditioned fear. A one-way analysis for the XYX failed to detect any group difference, $F < 1$, indicating that all XYX groups showed equally strong conditioned fear in testing, although Figure 2 gives a weak impression that the longer the extinction training, the stronger the fear renewal. A comparison between Groups XYY-8 and XYY-18, $t < 1$, suggests equally fearless performance of this context-transition condition, while a significant difference between Groups XXY-18 and XXY-28, $t(22) = 2.14$, implies that the additional 10 extinction training thwarted the XXY renewal effect.

Discussion

The present experiment explored impacts of the amount of extinction training on XYX and XXY renewal effects. Following 24 conditioning trials (i.e., 6 sessions), the XYX renewal was assessed after 32, 72, or 112 extinction trials (i.e., 8, 18, or 28 sessions, respectively), while the XXY renewal was assessed after 72 or 112 extinction trials. As the fear measured behaviorally was fully erased by the 32nd extinction trial, one may consider the additional 40 or 80 trials as extinction overtraining. The XYX renewal effect was equally strong independently of the number of extinction trials, while the XXY renewal effect was detected only after 72 extinction trials. These results indicate that extensive extinction training has less impact on XYX renewal than on XXY renewal.

Our finding of the similarly strong XYX renewal effects after 32, 72, 112 extinction trials is congruent with the study of Rauhut et al. (2001, Experiment 1), in which XYX fear renewal was statistically equal after 20 and 100 extinction trials. Although their graph shows unreliable alleviation of the renewal in the 100-trial group compared with the 20-trial group, there was no hint of any renewal alleviation in our experiment. If anything, longer extinction training tended to facilitate the XYX renewal effect.

On the other hand, XXY fear renewal was thwarted by extensive extinction training. As noted in the introduction of this article, there is inconsistency among the previous studies in detecting the XXY renewal effect. While it was evident in Bouton and Ricker (1994) and Tamai et al. (2000), other studies failed to demonstrate it (Bouton & King, 1983; Bouton & Swartzentruber, 1989). This inconsistency might be ascribed to the amount of extinction training received by the animals.

According to Bouton and Ricker (1994), renewal of conditioned fear takes place when the feared signal is presented in any context different from the context of extinction. In their framework (see also Bouton, 1994, 1997; Bouton & Nelson, 1998), contextual cues play a small role in the acquisition phase when a signal-shock excitatory link is established. Extinction training adds an inhibitory link between the signal and the shock, and the context gates activation of this inhibitory link. Hence, release from the extinction context leaves the original excitatory link, resulting in evoking conditioned fear to the signal. This claim predicts not only XYX renewal but also XXY renewal of extinguished fear in our experiment. Clear demonstration of XYX renewal in the rats tested after 32, 72, or 112 extinction trials and that of XXY renewal in the rats tested after 72 extinction trials supported the claim, while failure in showing XXY renewal of the rats tested after 112 extinction trials did not. The finding that extensive extinction training thwarted XXY

renewal effect poses a challenge to Bouton and Ricker's (1994) theory. This theory would predict that extensive extinction training would strengthen not only the inhibitory tone-shock link, but also the contextual gating. In order to explain the alleviation of XXY renewal by extensive extinction, the theory requires an additional assumption. For example, it could be assumed that extinction overtraining would strengthen the former effect (i.e., inhibitory learning) more than the latter (i.e., contextual gating) when the acquisition and extinction contexts are identical.

Another feature of the present experiment also requires revision of Bouton and Ricker's (1994) theory. Extinction of conditioned fear was slightly slower when the target signal was presented in the original context (Context X) rather than in the other context (Context Y). This finding suggests that the rats had learned something about the context in the preceding acquisition phase. As we equated exposure to Contexts X and Y throughout the experiment, it is hard to ascribe the effect to any difference in novelty of the contexts (cf. Lovibond, Preston, & Mackintosh, 1984). It might be worth to note that we have reported the same effect elsewhere (e.g., Tamai et al., 2000). Thus, it is unlikely that the difference in extinction speed between Groups XXX and XYX was due to chance.

There are two possible sources of renewal. One of them is that proposed by Bouton and Ricker (1994): release from the extinction context, which is gating activation of an inhibitory signal-shock link. The other is the retrieval of an excitatory signal-shock link by the acquisition context: the context acquires an occasion-setting function during the acquisition of Pavlovian conditioning. The "release" mechanism works in both XYX and XXY renewal effects, while the "retrieval" cue is available for only XYX renewal. This dual-source approach, which is similar to what Bouton (1991) himself had once claimed, fits our data, if we assume that only the former mechanism is weakened by extensive extinction training.

Whatever the ultimate explanation for the results reported here, they clearly show that XYX renewal is difficult to eliminate even after extensive extinction training, while XXY renewal is not. It is noteworthy here that these results have been successfully replicated in another experiment conduced in our laboratory. Further research is needed to elucidate the underlying mechanisms causing this difference in immunity to extinction overtraining. The on-baseline extinction procedure employed in the present experiment seems useful to monitor differences in extinction speed between Groups XXX and XYX. Additionally, if the identical procedure is employed for both extinction and testing, one has many opportunities to assess renewal effects. For example, the 6-group design of the present experiment allowed examination of the 10 different conditions shown in Figure 1.

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