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Extensive extinction in multiple contexts eliminates the renewal of conditioned fear in rats

Brian L. Thomas^{*}, Drina Vurbic¹, Cheryl Novak

Baldwin-Wallace College, Department of Psychology, 275 Eastland Road, Berea, OH 44017, USA

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ABSTRACT

Two studies examined whether nonreinforcement of a stimulus in multiple contexts, instead of a single context, would decrease renewal of conditioned fear in rats (as assessed by conditioned suppression of lever pressing). In Experiment 1, renewal was measured after 36 nonreinforced CS trials delivered during six extinction sessions in a single context or two extinction sessions in each of three different contexts. The number of extinction contexts did not have an effect on renewal. In Experiment 2, groups received either 36 or 144 nonreinforced CS trials during six or twenty-four extinction sessions in a single context or three different contexts. Again, renewal was not influenced by the number of extinction contexts when only 36 trials were given. However, when 144 trials were used, renewal was completely eliminated when extinction was divided between 3 contexts, but was not weakened when the sessions took place in a single context. The results suggest that the use of multiple treatment settings in exposure-based therapies is only likely to reduce relapse if a sufficient number of sessions are provided in each of the treatment settings.

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Estes and Skinner (1941) developed the conditioned suppression paradigm to study how a neutral stimulus can come to evoke fear or anxiety. Briefly, rats are trained to press a lever for reinforcement.

^{*} Corresponding author. Fax: +1 440 826 8549.

E-mail address: bthomas@bw.edu (B.L. Thomas).

¹ Now at the Department of Psychology, University of Vermont, USA.

Once this operant response is stable and on-going, a neutral stimulus is paired with an aversive unconditioned stimulus (US). Initially, the neutral stimulus does not affect lever pressing, but after several pairings with the US, rats will suppress lever pressing in the presence of the stimulus (which is now referred to as the conditioned stimulus (CS)). Once conditioned suppression has emerged, it may be extinguished by repeatedly presenting the CS in the absence of the US. Interestingly, [Bouton and Bolles \(1979\)](#) found that nonreinforcing the CS in one environment did not prevent conditioned suppression from returning in other environments. They selected the term “renewal” to describe the phenomenon where conditioned suppression is extinguished thoroughly in one context and reappears in another, without intervening CS–US pairings. Given that exposure-based psychotherapies often involve the presentation of a feared stimulus in the absence of the expected outcome and in a particularly distinctive environment (e.g., a therapist’s office), it might be expected that treatment gains would be compromised by renewal (relapse) outside the treatment setting. For this reason, [Bouton \(1991\)](#) offered a number of suggestions about how an extinguished conditioned response (CR) might be made to generalize better to settings where the CS had not been nonreinforced (i.e., reduce renewal).

One suggestion was to extinguish the CR in many different environments. The rationale was that contextual stimuli that were present while the CS was nonreinforced could help retrieve the memory of this experience if they were also present during the test for renewal. Support for this rationale can be found in all of the renewal studies that have included a control group for spontaneous recovery (i.e., a group that is extinguished and tested for renewal in the same context). For example, [Thomas, Larsen, and Ayres \(2003\)](#) showed that renewal was not observed in groups that acquired suppression in Context A and were extinguished in Context A and tested for renewal in Context A (AAA control) or extinguished in Context B and tested for renewal in Context B (ABB control). In these procedures, contextual cues that were present during extinction were also present during renewal testing and could have facilitated retrieval of extinction. In contrast, significant renewal was observed in groups that acquired suppression in Context A that was then extinguished in Context A and tested for renewal in Context B (AAB renewal), extinguished in Context B and tested for renewal in Context A (ABA renewal) or extinguished in Context B and tested for renewal in Context C (ABC renewal). In each of these procedures, a change in context between the Extinction phase and Renewal phase should have reduced the number of contextual cues that could have facilitated retrieval of the extinction experience.

[Gunther, Denniston, and Miller \(1998\)](#) were the first to directly test the suggestion that extinction in multiple contexts could increase the generalization of extinction. In Experiment 1, they used a conditioned suppression procedure where the operant response was licking from a water tube. During the Acquisition phase, rats received pairings of a white noise CS with a foot shock US in Context A. In the Extinction phase, Group E1 received 162 nonreinforced exposures (54 per session) to the CS in Context B, Group E3 received 54 nonreinforced exposures to the CS in each of three Contexts (B, C & D) and Group NE received context exposure without exposure to the CS. All of the groups were then tested for suppression to the CS in Context E. The authors found that suppression was greater in Group NE than in Group E1 showing that even extinction in a single context results in some generalization of extinction. More importantly, they found less suppression in Group E3 than Group E1, supporting the hypothesis that the generalization of extinction should be positively correlated with the number of different extinction contexts. Similar findings were reported by [Chelonis, Calton, Hart, and Schachtman \(1999\)](#) using the conditioned taste aversion paradigm to study ABA renewal.

More recently, [Bouton, Garcia-Gutierrez, Zilski, and Moody \(2006\)](#) attempted to replicate the findings of Gunther et al. using conditioned suppression of lever pressing. One justification for conducting the replication was that Gunther et al. conducted the Acquisition and Extinction phases off the operant baseline. Removal of the water tubes during these phases precluded the collection of data that might have helped to reveal the mechanism that causes extinction to generalize better with multiple contexts. More specifically, Bouton et al. describe three possible mechanisms. The first mechanism is the one described above where the amount of renewal should be influenced by the number of stimulus elements present during the renewal test that were also present during the Extinction phase. It is unclear how the CR would be influenced by this mechanism during the Extinction phase.

The second mechanism involves protection of the CS–US association by inhibitory context conditioning ([Rescorla & Wagner, 1972](#)). Essentially, if rats learn early in the Extinction phase that contextual cues predict the absence of the US, then this might protect the CS–US association from extinction

(Lovibond, Davis, & O'Flaherty, 2000; Rescorla, 2003). Thomas and Ayres (2004) reported that extinction of an excitatory CS in compound with a discrete inhibitory CS resulted in complete protection of the CS–US association because suppression to the CS during the renewal test was not less than suppression in a group that had never received the CS nonreinforced. In contrast, extinction of an excitatory CS in compound with other discrete, excitatory CSs reduced renewal. Thus, renewal seemed to be determined by the value of stimuli that were present during nonreinforcement of the target CS. If contextual stimuli can influence renewal in the same manner as discrete CSs, greater protection from extinction would be expected when the Extinction phase occurs in a single context because those context cues should more easily become inhibitory. In contrast, changing the extinction context between sessions should increase the chances that the CS is presented against a background of more neutral contextual stimuli. Fortunately, this mechanism could be expressed through an increase in suppression between extinction sessions that should not be seen when a single context is used.

Finally, the third mechanism involves inhibition of the CR. Solomon and Wynne (1954) first proposed that the effect of nonreinforcement of the CS on the underlying CS–US association depends on how fully the CR is evoked. If the CS is presented and the CR is fully evoked, then nonreinforcement results in extinction of the fear generated by the CS. In contrast, if the CS is presented and the CR does not occur, then fear is conserved. Rescorla (2001) has recently expanded this notion to include the idea that nonreinforcement following a fully expressed CR may help to strengthen inhibition of the CR. If changing the context between successive extinction sessions increases suppression, then this might cause a weakening of the CS–US association or a strengthening of an inhibitory association with the CR. Both outcomes should result in greater generalization of extinction.

Interestingly, Bouton et al. (2006), in search of the mechanism, failed to replicate the earlier findings that extinction in multiple contexts reduced renewal. In Acquisition, rats received pairings of a tone CS with a shock US in Context A. In the Extinction phase, half of the rats received the CS nonreinforced 12 times (4 times per session) in a single context and the other half received the CS nonreinforced 4 times in each of three different contexts. Controls for spontaneous recovery (AAA) and for extinction (NE) were not included. Both groups were tested for renewal in either Context A (Experiment 2) or a novel Context E (Experiment 1). The increase in suppression from the last extinction trial to the first renewal trial was not different in rats that had received extinction in either one or three contexts. Since the Extinction phase was conducted on the baseline, it was possible to compare the amounts of suppression between extinction sessions. The increase in suppression between successive extinction sessions was significantly greater when the context was changed than when extinction was provided in a single context. The authors concluded that this result challenges the hypotheses of Solomon and Wynne (1954) and Rescorla (2001) since a more fully expressed CR during the Extinction phase did not reduce renewal. Neumann, Lipp, and Cory (2007) found results similar to Bouton et al. (2006) using a human fear conditioning paradigm. Participants that were given 3 extinction trials in each of 3 contexts or 2 extinction trials in each of 5 different contexts expected the US to occur during the renewal test as much as participants that had received the same number of extinction trials in a single context. Interestingly, Neumann (2006) reported that extinction in multiple settings abolished ABA and ABC “renewal” of conditioned suppression in human participants. However, the authors admitted that it was unclear whether the return of the CR, after a single extinction context was used, really reflected renewal because the same increase in the CR occurred to a stimulus that had never been paired with the US.

Studies that have found weaker fear renewal after extinction in multiple contexts than extinction in a single context (Chelonis et al., 1999; Gunther et al., 1998) have included more extensive extinction training than studies that failed to find this difference (Bouton et al., 2006; Neumann et al., 2007). There are several reasons why differences in renewal might only appear after more extensive extinction training. First, it is possible that the amount of time spent in a particular extinction context determines how well the contextual stimuli are encoded and whether they can serve as retrieval cues of the extinction memory during the renewal test. Second, repeatedly changing the extinction context may prevent inhibitory contextual cues from protecting the CS–US association (Rescorla & Wagner, 1972). Provided that the CS is nonreinforced sufficiently, this should allow for a more complete extinction of the CS–US association leaving little fear to be renewed. Finally, repeatedly changing the extinction context may result in renewal of the CR that, when nonreinforced, becomes more strongly inhibited (Rescorla, 2001).

The objectives of the present set of experiments were threefold (a) to add to the limited, and now discrepant, literature on renewal following extinction in one or more contexts, (b) to determine whether the discrepancy might result from differences in the amount of extinction provided and (c) to assess the various learning mechanisms that may cause extinction to become more general.

Experiment 1

The purpose of Experiment 1 was to determine if nonreinforcement of the CS in 3 different contexts reduced renewal when compared with the same amount of extinction provided in a single context.

Methods

Subjects

The subjects were 24 male, experimentally naive, 90-day-old Sprague–Dawley rats that were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN). After 1 week in the colony, their free feeding weights varied from 318 to 371 g. The rats were reduced to and maintained at 80% of those levels throughout the study. Subjects were housed individually in stainless steel cages with water available continuously. The colony room was lighted daily between 0600 and 2200 h. All of the training sessions were conducted between 0900 and 1500 h.

Apparatus

Sixteen operant chambers were used (inside dimensions = 23.2 × 20.3 × 19.5 cm). Half of the chambers were placed in each of two rooms. The chambers were placed inside sound attenuating cubes (internal dimensions = 55.9 × 55.9 × 48.9 cm) that were made of Ultra Plus foam core with a 1 mm PVC facer (United Industries, Bentonville, AR). In each room, four of the cubes were stacked 2 × 2 on a bench top affixed to the north wall, and four were similarly stacked on the adjacent west wall. A 2.5 cm sheet of foam insulation separated top and bottom boxes to further reduce any noise between them. Adjacent cubes were spaced a minimum of 20 cm apart. On the back wall of the sound attenuating cube were three white frosted light bulbs (each 7.5 W, 110 V), that provided the house lighting. The work panel and the opposite wall of each operant chamber were aluminum and the side walls were clear Plexiglas. Centered in the work panel was a response lever (5 × 1.5 cm). A weight of 20–32 g was required to close a microswitch located behind it that activated the dipper located to the left and below the response lever. The floor consisted of 18 stainless steel rods (2 mm in diameter, centers spaced 1.3 cm apart). Corrugated cardboard lined the waste tray below the rods.

All 16 chambers were used to create a distinctive context that was used during acquisition and renewal testing (Context A). A glass furniture coaster containing 10 ml of 2% anise extract (McCormick & Co Inc., Hunt Valley, MD) was placed just outside the dipper recess and the house lighting was turned off. Following the completion of the acquisition phase, each chamber was modified to create one of the three different extinction contexts (B, C and D). Rats were moved from one box to another when a context change was required. The first configuration did not include an odor cue and the house lighting was turned on. The second configuration resembled the first except that vertical black stripes (1 cm wide spaced 1.5–2.0 cm apart) were placed on each of the Plexiglas walls of the operant chamber. Finally, the third configuration did not have an odor cue, the house lighting was left off and a metal plate was placed in each box to form a slanted back wall. It reduced the distance from the work panel to the back wall at floor level from 23.2 to 12.5 cm. In each group, rats were divided among each of the different configurations to control for box effects.

On the lid of each operant chamber were two speakers (10-cm diam.). One provided a continuous white noise background of 80 dB when superimposed on the noise from the ventilating fans. Termination of the background white noise resulted in a decrease from 80 to 68 dB which served as the CS in this study. The other speaker was not used. Sound intensity was measured using a Radio Shack sound level meter (catalog number 33-2050; C scale, slow response) with its microphone about 7 cm from the dipper tray. Throughout this research, CSs were given independently of lever pressing. It has been reported that for fear conditioning, stimulus termination of 2 min is not processed differently from stimulus presentation (Bevins & Ayres, 1991; Rauhut, McPhee, DiPietro, & Ayres, 2000). The

US was a 1-s, 0.6 mA scrambled grid shock generated by Grason-Stadler shockers (Models E1064 or 700; Grason-Stadler Co., West Concord, MA). The peak shock intensity, as measured at the grids using a Fluke 83 III multimeter (John Fluke Mfg Co. Inc., Everett, WA), was equivalent to the nominal shock setting. A computer in an adjacent room controlled all stimulus events and recorded lever presses.

Procedure

Rats were randomly assigned to three different groups ($n = 8$). Groups E1 and E3 received the CS nonreinforced in a single context or three contexts respectively and Group NE did not receive the CS nonreinforced.

Preliminary training. Preliminary training involved 2 days of training the rats to drink 32% sucrose from the dippers (magazine training), 4 days of shaping the lever response (each day with 100 reinforced responses), 4 days of training on a variable interval (VI) 60 s schedule and 2 days of preexposure (2 CS presentations per day) to the noise-off CS. During magazine training and shaping, the doors of the sound attenuating chambers had to remain open for us to see the rats. To minimize the spread of odors in the training room, anise was not used until Day 1 of VI training, when the sound attenuating doors were closed for the first time. The configuration of the boxes (Context A, B, C and D) was changed daily in order to expose all of the rats to each of the training contexts that would be used in the study. Specifically, rats were trained in Context A on Days 3, 7, 11 and 12, Context B on Days 1, 4 and 8, Context C on Days 2, 5 and 9 and Context D on Days 6 and 10.

Acquisition in Context A. All rats received five acquisition sessions in Context A. Each daily session lasted for 1 h and included two pairings of the 2 min noise-off CS and the 1 s shock US. CSs did not occur during the first 12 or final 16 min of the session. The average intertrial interval (ITI), CS offset-to-CS onset, was 23.2 min. The timing of the trials was randomized from day-to-day.

Extinction in Context B, C or D. Table 1 describes the extinction phase. Group E1 received 6 extinction sessions in a single context. Group E3 received 2 extinction sessions in each of the 3 different configurations. Group NE was placed in the chamber, was allowed to lever press for sucrose, but was not given any non-reinforced CSs. All rats were trained once daily using a 3-day cycle. For Group E1, a cycle consisted of an extinction session in Context B (Day 1), then exposure to Context C and Context D (Days 2 and 3). This cycle was repeated six times. For Group E3, the cycle varied. The first cycle was an extinction session

Table 1
Design of the Extinction phase of Experiments 1 and 2.

Experiment	Group	Extinction training ^a	Context exposure ^b	Context exposure	# of cycles ^c	# of days ^d
1	E1	B	C	D	6	18
	E3	B	C	D	2	6
		C	D	B	2	6
		D	B	C	2	6
		B	C	D	6	18
NE	B	C	D	6	18	
2	E1	B	C	D	6	18
	E3	B	C	D	2	6
		C	D	B	2	6
		D	B	C	2	6
		B	C	D	24	72
	E3-L	B	C	D	8	24
		C	D	B	8	24
		D	B	C	8	24
		B	C	D	6 or 24	18 or 72
	NE	B	C	D	6 or 24	18 or 72

Note. Letters indicate training context. Each group received a session of Extinction Training followed by two sessions of context exposure over a 3-day period.

^a CS was nonreinforced 6 times per session.

^b Exposure to the training context without CS exposure.

^c Number of times the 3-day sequence was repeated.

^d Total number of days to complete Extinction phase.

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in Context B, then exposure to Context C and D, the second cycle was an extinction session in Context C, then exposure to Contexts D and B and the third cycle was an extinction session in Context D followed by exposure to Contexts B and C. This three-cycle sequence was repeated 2 times. The purpose of using these cycles was to provide all of the groups with similar amounts of exposure to each of the 3 configurations during the extinction phase. The CS was nonreinforced 6 times during each extinction session and the timing of the trials was the same in Groups E1 and E3. CSs were not delivered during the first 10 or last 11 min of the session. The average ITI was 6.1 min. In Group E1, the extinction configuration was selected so that a similar number of rats (i.e., 3, 3 and 2) received the CS nonreinforced in each place. This was to control for the possibility that any extinction configuration more closely resembled Context A. Group NE was trained exactly like Group E1 during this phase except that it did not receive any CSs and was only allowed to lever press for sucrose.

Renewal testing in Context A. All groups were given 1 day of training in Context A where they lever-pressed for sucrose. This was to ensure that lever pressing was stable before the final renewal test was conducted. Renewal testing occurred on the following 2 days. Each day, the CS was nonreinforced twice. CSs occurred at minutes 25 and 41 during the first renewal session and at minutes 19 and 47 on the second session.

Measure of conditioned fear. A suppression ratio (Annau & Kamin, 1961) was used to quantify suppression to the CS. The ratio was defined as $D/(D + B)$, where D denotes the number of responses during the CS and B denotes the number during an equivalent period immediately prior to CS onset.

Results

The main results of Experiment 1 were that suppression was extinguished more rapidly when the CS was nonreinforced in a single context than three contexts, but this difference in extinction rate did not yield different amounts of renewal.

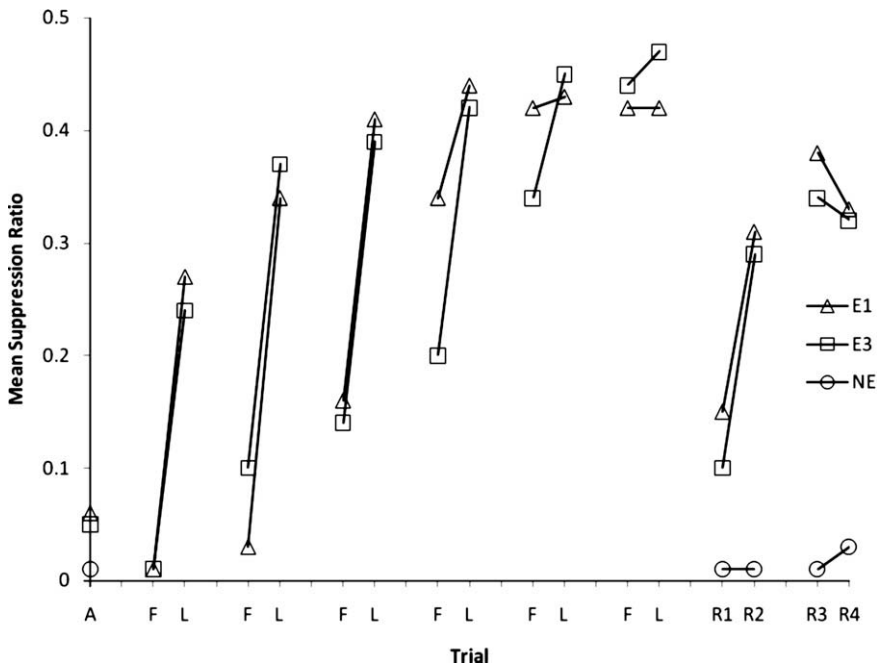


Fig. 1. Mean suppression on the last trial of the Acquisition phase (A), the first (F) and last (L) trials of each of the six sessions of the Extinction phase, and the four trials of the Renewal phase (R1–R4).

Fig. 1 shows mean suppression ratios for the last trial of the Acquisition phase (labeled “A” at the far left), the first and last trials of each of the six sessions of the Extinction phase (labeled “F” and “L” with gaps separating sessions) and the four trials of the Renewal phase (Labeled “R1” through “R4” at the far right). To be certain that groups acquired similar levels of suppression during the Acquisition phase, a one-way between groups analysis of variance (ANOVA) was conducted on the data from the last trial. It did not reveal any group differences, $F(2,21) = 2.11, p > .14$. Next, a 2×36 (Group \times Trial) ANOVA was conducted using all of the data from the Extinction phase. The main effect of Trial was significant, $F(35,490) = 38.9, p < .001$, indicating that nonreinforcement of the CS decreased suppression. More importantly, the Group \times Trial interaction was also significant, $F(35,490) = 1.55, p < .001$. This indicates that suppression decreased more rapidly in Group E1 than Group E3. To study this effect further, separate 2×2 (Group \times Trial) ANOVAs were conducted using the final trial of an Extinction session and the first trial of the very next extinction session. Using Trials 6 and 7 or Trials 12 and 13, only the main effect of Trial was significant. In contrast, using Trials 18 and 19 or 24 and 25 resulted in significant Group \times Trial interactions, $F(1,14) = 6.73$ and $F(1,14) = 5.43, p < .05$. This suggests that the difference in extinction rate was due to the daily changes in the extinction context for Group E3, changes that caused renewed suppression for that group. This effect was detectable, however, only in the second half of the Extinction phase. Given the differences in extinction rate, a one-way between groups ANOVA was performed on the final trial of the Extinction phase to ensure similar suppression levels prior to the Renewal phase. No group difference were found, $F(1,14) = 1.81, p > .20$. Finally, a 2×2 (Group \times Trial) ANOVA conducted with the data from last extinction trial and the first trial of the Renewal phase failed to reveal a main effect of Group, $F < 1$ or a Group \times Trial interaction, $F(1,14) = 2.17, p > .27$. Given that ceiling effects on suppression may mask group differences on the first renewal trial, a 3×4 (Group \times Trial) ANOVA was performed using all 4 renewal trials. This analysis yielded significant main effects of Trial and Group, $F(3,63) = 20.0$ and $F(2,21) = 43.0, p < .001$, respectively. More importantly, the Group \times Trial interaction was significant, $F(6,63) = 4.85, p < .001$. Visual inspection in Fig. 1 suggests that the interaction shows that nonreinforcement of the CS (in either 1 or 3 contexts) resulted in a more rapid elimination of suppression than in Group NE which was receiving the CS nonreinforced for the first time.

Similar analyses were performed on the Pre-CS lever pressing rates during the Extinction and Renewal phases to be certain that between group differences in baseline lever pressing did not complicate the interpretation of the suppression ratios. A 2×36 (Group \times Period) ANOVA including all 36 Pre-CS periods of the Extinction phase failed to reveal a main effect of Group, $F(1,14) = 1.03$, or a Group \times Trial interaction, $F < 1.0$. Mean lever pressing rates were 42 and 48 responses per minute for Groups E1 and E3 respectively. A similar 3×4 ANOVA using all of the pre-CS periods in the Renewal phase showed that the main effect of Group, $F(2,21) = 2.68, p > .09$, and the Group \times trial interaction, $F < 1.0$, were not significant. Mean lever pressing rates were 51, 63 and 41 responses per minute for Groups E1, E3 and NE, respectively.

Discussion

The results of Experiment 1 extend those reported by Bouton et al. (2006) that increasing the number of extinction contexts from 1 to 3 increased suppression between extinction sessions, but did not decrease ABC renewal. One procedural difference between the studies of Bouton et al. and Gunther et al. (1998) was that Gunther et al. equated exposure to the different extinction contexts and Bouton et al. did not. In the present study, Groups E1 and E3 spent the same amount of time in each of the different configurations, as in Gunther et al., but the extinction and renewal results resembled those of Bouton et al. This suggests that equating exposure to the different extinction contexts is not sufficient to ensure that extinction in multiple contexts will reduce renewal. Experiment 1 also differed from Bouton et al. in the number of extinction sessions (and thus the number of nonreinforced CSs) provided. Interestingly, Bouton et al. observed a greater increase in suppression between Days 1 and 2 and 2 and 3 of the Extinction phase with multiple contexts than one. In the present study, suppression was strong in both groups between Days 1 and 2 and 2 and 3. The greatest differences in suppression appeared between Days 3 and 4 and Days 4 and 5 once suppression became weaker in Group E1. Perhaps the failure to find differences in renewal in the present study and in Bouton et al. resulted

from either an insufficient number of context changes or an insufficient number of extinction trials. Gunther et al. increased the generalization of extinction by providing 162 extinction trials and 1 session in each of three contexts. That is, they used the same number of context changes as Bouton et al., but many more extinction trials (162 vs. 12). Compared with Experiment 1, Gunther et al. provided many more extinction trials (162 vs. 36) but half as many context changes (3 vs. 6). Given that they did not collect data during the Extinction phase, it would be speculation to state which of these two variables might be operating to cause the discrepancies between studies. Finally, Experiment 1 included Group NE that did not receive the CS nonreinforced during the Extinction phase. Data from the Renewal phase showed that initial extinction of the CR in Group NE was retarded when compared with re-extinction of the CR in Groups E1 and E3. This suggests that there was some transfer of extinction from the extinction context to the renewal context in Groups E1 and E3. Given that Bouton et al. also found an incomplete renewal effect when a single extinction context was used, it is possible that a weak renewal effect made it impossible to detect any additional reductions in renewal when three extinction contexts were used. Experiment 2 was designed to address some of these issues.

Experiment 2

The primary purpose of Experiment 2 was to determine if renewal is affected by (a) the number of times the CS is nonreinforced, (b) the number of different contexts the CS is nonreinforced in or (c) an interactive effect of these variables. Groups E1 and E3 received 6 extinction sessions with the CS nonreinforced 6 times per session in either 1 or 3 different contexts (just as in Experiment 1). Groups E1-L and E3-L (L for “Larger” number of extinction trials) received 24 extinction sessions with the CS nonreinforced 6 times per session in either 1 or 3 different contexts.

Methods

Subjects

The subjects were 40 male, experimentally naive, 90-day-old Sprague–Dawley rats from Harlan Sprague Dawley Inc. (Indianapolis, IN). After 1 week in the colony, their free feeding weights ranged from 330 to 415 g. The rats were reduced to and maintained at 80% of those levels. Subjects were housed singly in hanging stainless steel and steel mesh cages with water always available. The room was lighted daily between 0600 and 2200 h. We conducted all training sessions between 0900 and 1500 h.

Apparatus

The same operant chambers and the same configurations used in Experiment 1 were used in Experiment 2.

Procedure

Rats were assigned to one of five different groups ($n = 8$). Group E1 and E1-L received the CS nonreinforced in a single configuration (with animals divided between the three configurations as in Experiment 1) and Groups E3 and E3-L received the CS nonreinforced in 3 different contexts. Groups E1 and E3 were given 6 extinction sessions (36 nonreinforced CSs) and Groups E1-L and E3-L were given 24 extinction sessions (144 nonreinforced CSs). Half of the rats in Group NE were trained like Group E1 except that they did not receive any nonreinforced CSs and the other half of the Group were trained like Group E1-L (again without nonreinforcement of the CS). Preliminary training, the Acquisition phase and the Renewal phase were conducted just as they were in Experiment 1.

Extinction in Context B, C or D. Table 1 describes the Extinction phase. Rats in Groups E1 and E1-L received sessions of extinction in a single context, B, and Rats in Groups E3 and E3-L received extinction sessions divided equally between three contexts, B, C and D, using the 3-day cycles described in Experiment 1 (6 complete cycles for E1 and E3 and 24 complete cycles for E1-L and E3-L). All groups started the Extinction phase on the same day. Therefore, Groups E1 and E3 finished the phase 54 days before Groups E1-L and E3-L. In order to equate the time interval between the last extinction session and the Renewal phase, all Groups were tested for renewal 2 days after the conclusion of the final extinction

session. As in Experiment 1, a recovery session was provided on the day preceding the start of the Renewal Phase. Of course, in equating the interval between the end of the Extinction phase and the Renewal Phase, a confound was created between the amount of extinction training given and the time interval between the end of the Acquisition phase and the start of the Renewal phase. Rats in the Group NE were divided equally so that half were tested after the shorter acquisition-to-test interval (like Groups E1 and E3) and the other half were tested with the longer acquisition-to-test interval (like Groups E1-L and E3-L). If rats in Group NE suppress similarly during the Renewal phase, then differences between Groups E1, E3, E1-L and E3-L during the Renewal phase cannot be attributed to differences in how strongly fear is retained over time.

Results

The results of Experiment 2 show that renewal was eliminated when 24 sessions of extinction training were distributed evenly across three distinctive contexts. In contrast, 24 extinction sessions provided in a single context did not reduce renewal when compared with 6 extinction sessions in a single context. Similarly, 6 extinction sessions divided across three distinctive contexts did not reduce renewal when compared with the same number of sessions given in a single context. Finally, changing the context between consecutive extinction sessions resulted in an increase in suppression during the Extinction phase that was not seen when a single context was used.

Fig. 2 shows suppression to the CS on the last trial of the Acquisition phase (labeled A), the first and last trial of extinction Sessions 1 through 4 (labeled F and L with gaps separating sessions), the first and last trial of the final extinction sessions (Session 6 for Groups E1 and E3 and Session 24 for Groups E1-L and E3-L) and the 4 renewal test trials (labeled R1 through R4). A one-way between groups ANOVA was performed on the data from the last acquisition trial. That ANOVA was not significant, $F(4, 35) = 1.78, p > .15$, showing that suppression levels were similar prior to the start of the Extinction phase. Based on the results of Experiment 1, it was expected that nonreinforcing the CS

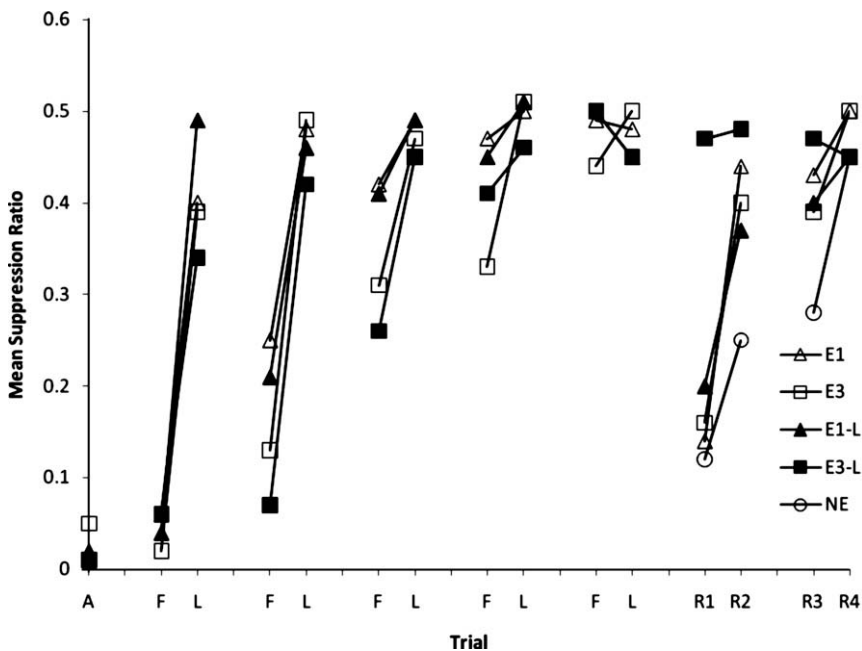


Fig. 2. Mean suppression on the last trial of the Acquisition phase (A), the first (F) and last (L) trials of the first four and the very last session of the Extinction phase, and the four trials of the Renewal phase (R1–R4).

in three different contexts would retard extinction of suppression. A 2×24 (Number of extinction contexts (1 vs. 3) \times Trial) ANOVA using data from the first 24 extinction trials confirmed this expectation with a significant interaction, $F(23,644) = 2.91, p < .01$. To determine if renewal at the start of each Extinction session was causing the retardation of extinction in rats in Group E3 and E3-L, subsequent 2×2 (Contexts (1 vs. 3) \times Trial) ANOVAs were conducted on the final extinction trial of a session and the first trial of the very next extinction session. For Trials 6 and 7, the main effects of Trial and Contexts reached significance, $F(1,29) = 61.1$ and $F(1,29) = 6.1, ps < .02$. For Trials 12 and 13, the main effects of Trial and Group were significant, $F(1,29) = 14.9$ and $F(1,29) = 6.8, ps < .02$. The Group \times Trial interaction was also significant, $F(1,29) = 4.3, p < .05$. For Trials 18 and 19, the main effects of Trial and Group were significant, $F(1,29) = 7.2$ and $F(1,29) = 5.7, ps < .03$. Taken together, these results support the finding from Experiment 1 that extinction in multiple contexts permits renewal to occur between sessions and that renewal contributes to the retardation of extinction. A final one-way between groups ANOVA was conducted on data from the last extinction trial to determine if the extra extinction sessions provided to Groups E1-L and E3-L resulted in less suppression than in Groups E1 and E3. Differences in final suppression levels would complicate the interpretation of the data from the Renewal phase. The one-way between groups ANOVA was not significant, $F < 1$. Therefore, suppression levels were similar between the groups prior to the Renewal phase.

To determine if fear is forgotten over time, a one-way between groups ANOVA was conducted on the first trial of the Renewal phase using only the rats in Group NE. Half of these rats were tested 8 days after the last Acquisition session and the other half were tested 26 days after the last Acquisition session. The ANOVA did not reach significance, $F(1,6) < 1.0$. Mean suppression ratios for the two halves were .10 (shorter interval) and .13 (longer interval). Maintenance of conditioned suppression over long forgetting intervals has been reported in a number of papers (Gale, Anagnostaras, Godsil, Mitchell, Nozawa, Sage, Wiltgen, & Fanselow, 2004; Hoffman, Selekman, & Fleshler, 1996; Rauhut, Thomas, & Ayres, 2001; Thomas, Longo, & Ayres, 2005).

To determine if the increase in the number of extinction trials, the number of extinction contexts or both variables influenced renewal, a $2 \times 2 \times 2$ ANOVA (Number of extinction contexts (1 vs. 3) \times Number of extinction Sessions \times Trial) was conducted using the data from the last extinction trial and first renewal trial. Most importantly, the triple interaction was significant, $F(1,28) = 5.2, p < .03$. This indicates that the number of contexts that the CS was nonreinforced in had different effects depending on the number of extinction sessions (and trials) conducted. Visual inspection of Fig. 2 suggests that nonreinforcing the CS in multiple contexts was not different from nonreinforcement in a single context when only 2 sessions were provided in each of the three contexts. In comparison, non-reinforcement of the CS during 8 sessions in each of three contexts completely eliminated renewal, but the same increase in the number of sessions in a single context was without consequence. The same three-way ANOVA was performed without Group E3-L and the only significant result was the main effect of Trial, $F(1,21) = 70.5, p < .001$. This analysis confirms that suppression was not different in Groups E1, E3, and E1-L. Next, a paired samples *t*-test was conducted using the last extinction and first renewal trial data for Group E3-L. The test was not significant, $t(7) = 2.0, p > .08$. This confirms that renewal did not occur when extensive extinction was provided in multiple contexts. Lastly, to determine if Groups E1, E3 and E1-L differed from Group NE, a 4×4 ANOVA (Group \times Trial) was conducted using all of the trials of the Renewal phase. Only the main effect of Trial reached significance, $F(3,84) = 52.3, p < .001$. Therefore, 6 sessions of extinction in 1 or 3 contexts or 24 extinction sessions in a single context did not result in any less suppression in the Renewal phase than in a group that had never received the CS nonreinforced.

Similar analyses were conducted on the extinction and renewal data using the Pre-CS lever pressing rates. A 4×24 (Group \times Trial) ANOVA using the first 24 Pre-CS periods of the Extinction phase, showed that the main effect of Group, $F(3,28) = 1.07$, and the Group \times Trial interaction, $F(69,644) = 1.11$, were nonsignificant, $ps > .27$. Mean lever pressing rates were 54, 53, 47, and 59 responses per minute for Groups E1, E3, E1-L and E3-L, respectively. A similar analysis using the 4 pre-CS periods of the renewal phase did not reveal a main effect of Group, $F(4,39) = 1.45$ or a Group \times Trial interaction, $F < 1.0, ps > .23$. Mean lever pressing rates were 56, 61, 46, 57 and 42 responses per minute for Groups E1, E3, NE, E1-L and E3-L, respectively.

Discussion

The primary purpose of Experiment 2 was to determine if renewal is affected by (a) the number of extinction sessions (and trials), (b) the number of different extinction contexts or (c) an interactive effect of these variables. The results favored possibility c. That is, extinction in three contexts did not reduce renewal more than extinction in a single context when only 6 extinction sessions were conducted, but did so when 24 extinction sessions were conducted. Taken with the results of [Gunther et al. \(1998\)](#), it appears that the failure to observe more generalized extinction with multiple contexts in [Bouton et al. \(2006\)](#) and the present studies resulted from insufficient amounts of extinction training in each of the contexts. When 162 extinction trials were given over 3 sessions ([Gunther et al.](#)) or in the present Experiment 2 when 144 extinction trials were given over 24 sessions in three different places, extinction generalized more completely than when the same events were experienced in a single setting. Failure to find an effect (on renewal) of increasing the number of nonreinforced CSs given in a single context is consistent with most of the animal literature that has investigated this variable ([Bouton & Swartzentruber, 1989](#); [Rauhut et al., 2001](#); [Tamai & Nakajima, 2000](#)) but see [Denniston, Chang and Miller \(2003\)](#) for an exception.

In Experiment 2, the increase in the number of extinction sessions was deliberately confounded with an increase in the total number of extinction trials because it was expected that therapists would use additional session time for additional nonreinforced CS exposure. To isolate the impact of the increase in extinction sessions from the increase in extinction trials, a 2×2 factorial study manipulating the number of extinction trials (36 or 144) and the number of Sessions (6 or 24) would be an appropriate strategy.

General discussion

The present pair of studies was conducted in an attempt to add to, and bring consistency to, the surprisingly limited literature on extinction and renewal using multiple extinction contexts. Several studies involving human participants have demonstrated renewal of fear. For example, [Mystkowski, Craske, and Echiverri \(2002\)](#) found ABC renewal of a spider phobia when extinction was conducted in one room and renewal testing was conducted in another. Similarly, [Mystkowski, Mineka, Vernon, and Zinbarg \(2003\)](#) found the same ABC renewal effect when caffeine was used, in lieu of external context changes, to differentiate the extinction and renewal test contexts. Assuming that renewal of fear in animals resembles relapse of fear in humans, techniques that reduce renewal might uncover processes that would help in the treatment of anxiety disorders (e.g., phobias, Post-Traumatic Stress Disorder). To date, there have been four papers published on extinction in multiple contexts. Two papers reported that a CR extinguished in multiple contexts was more resistant to renewal than a CR extinguished in a single context ([Chelonis et al., 1999](#); [Gunther et al., 1998](#)) and two papers failed to find an effect of the number of contexts ([Bouton et al., 2006](#); [Neumann et al., 2007](#)). The present findings support the original suggestion made by [Bouton \(1991\)](#), that therapists attempting to eliminate fear and prevent its relapse should conduct treatment sessions in a variety of different settings. However, when several extinction contexts are used, the CS must be nonreinforced sufficiently in each context or relapse will be as likely to occur as in cases where a single extinction context is used. Moreover, simply exposing subjects extensively to the “extinction” settings without nonreinforced exposure to the feared CS did not affect relapse.

Additionally, increasing the number of times the CS was nonreinforced did not affect relapse when only a single extinction context was used. This result is consistent with several other reports that used a single extinction context. For example, [Bouton and Swartzentruber \(1989\)](#) found significant renewal of fear after the CS had been nonreinforced 84 times. [Tamai and Nakajima \(2000\)](#) showed that 112 extinction trials attenuated AAB renewal, but did not reduce ABA renewal. Lastly, [Rauhut et al. \(2001\)](#) showed that 100 extinction trials did not reduce ABA renewal more than 20 extinction trials. These results are consistent with the present finding that simply increasing the number of extinction trials from 36 to 144, without increasing the number of extinction contexts, did not reduce the renewal of fear. Notably, [Denniston, Chang, and Miller \(2003\)](#) found that increasing the number of extinction trials from 160 to 800 reduced, but did not eliminate, the renewal of fear. Taken together, these results

suggest that increasing the number of nonreinforced CS trials is unlikely to prevent the relapse of fear when a single extinction context is used.

Three reasons why extensive extinction distributed over a number of contexts might increase the generalization of extinction are (a) it could increase the number of possible stimuli that could help to retrieve extinction memories, (b) it could reduce the likelihood that contextual stimuli become safety cues that prevent extinction of the CS-US association (the underlying source of the fear) and (c) it could strengthen inhibition of the CR.

Brooks and Bouton (1994) provided evidence that renewal represents a retrieval failure. Using an appetitive learning task, they showed that an extinguished CR was attenuated in the Renewal phase when a discrete stimulus from the Extinction phase, a “reminder”, was also provided. More recently, Vansteenwegen, Vervliet, Hermans, Beckers, Baeyens, and Eelen (2006) used a human fear conditioning paradigm and showed that participants' expectation for a loud noise US, measured through self-report and galvanic skin response, was reduced during a renewal test by a reminder stimulus from the Extinction phase. These studies suggest that the presence of a single, salient, discrete stimulus from the Extinction phase can improve the generalization of extinction. One interpretation of the present results is that conducting extinction in a variety of different contexts, and for an extended number of sessions, increased the likelihood that one or more contextual stimuli were encoded during extinction and acted as a retrieval cue during the Renewal test.

The associative model developed by Rescorla and Wagner (1972) generates the hypothesis that non-reinforcement of an excitatory CS in a neutral context should simultaneously weaken the excitatory strength of the CS and increase the inhibitory strength of contextual stimuli. Once the inhibition and excitation are equal in strength, the contextual stimuli are expected to protect the excitatory CS from further extinction on subsequent trials. Unlike the retrieval hypothesis, this hypothesis leads to the prediction that changing the context between extinction sessions should result in an increase in suppression because the CS would be presented in the absence of inhibitory context stimuli. Experiments 1 and 2 confirmed this prediction. Another way to reduce protection from extinction, without changing extinction contexts, is to provide USs during the Extinction phase at times when the CS is absent (i.e., explicitly unpaired extinction, EU). These USs should cause excitatory conditioning of contextual stimuli that would offset the normal accrual of inhibition expected with more conventional, CS only, extinction. Rauhut et al. (2001) first reported that EU extinction conducted in a single context completely eliminated ABA renewal (see also Thomas, Longo, and Ayres (2005)). Perhaps the effects of changing context during extinction and providing unpaired USs are the result of a common mechanism.

Finally, Rescorla (2001) has proposed that responses that are made more vigorously or more often during the Extinction phase become more strongly inhibited as a result of the nonreinforcement. In the present study, a CS that evoked fear that was nonreinforced should have a strengthened tendency to inhibit that fear on future trials. If one assumes that this inhibition is not context specific, then the amount of suppression in the Renewal phase should be inversely related to the amount of suppression in the Extinction phase. The results of Experiments 1 and 2 are only partially supportive of this hypothesis. When extinction was scheduled in different contexts, suppression increased between sessions. This increase in the CR during the Extinction phase should have resulted in a decrease in suppression in the Renewal phase. When 24 extinction sessions were used, suppression in the Extinction phase was inversely related to suppression in the Renewal phase. However, when only six extinction sessions were given, increased suppression in the Extinction phase did not affect suppression in the Renewal phase. Notably, context changes only resulted in increased suppression between the first few extinction sessions so the difference between Group E3 and E3-L in renewal cannot be attributed to suppression that occurred during extinction Sessions 7 through 24 in Group E3-L.

To summarize, the present results may resolve a discrepancy in the literature on extinction in multiple contexts. It appears that extinction in multiple contexts only increases the generalization of extinction when a sufficient number of extinction sessions and nonreinforced CS trials are provided. A few extinction sessions or a limited number of trials may not allow the subjects to adequately encode contextual stimuli that could assist in retrieval of extinction in non-extinction settings. Alternatively, limited training may not result in effective inhibitory context conditioning and protection of the CS from extinction, processes that would reveal the benefit of varying the extinction settings.

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