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Mechanisms of Secondary Extinction

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MECHANISMS OF SECONDARY EXTINCTION

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Drina Vurbic

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Abstract

Pavlov (1927) first reported that following appetitive conditioning of multiple stimuli, extinction of one CS attenuated responding to others which had not undergone direct extinction. Four experiments with rat subjects investigated potential mechanisms of this secondary extinction effect. Experiment 1 assessed whether secondary extinction would be more likely to occur with target CSs that have themselves undergone some prior extinction. Two CSs were initially paired with shock. One CS was subsequently extinguished before the second CS was tested. The target CS was partially extinguished for half the rats and not extinguished CS for the other half. A robust secondary extinction effect was obtained with the non-extinguished target CS. Experiment 2 investigated whether secondary extinction occurs if the target CS is tested outside the context where the first CS is extinguished. Despite the context switch secondary extinction was observed. Extinction of one CS was also found to thwart renewal of suppression to a second CS when it was tested in a neutral context. Experiment 3 examined whether secondary extinction can be attributed to mediated generalization caused by association of the CSs with a common US during conditioning. Rats received conditioning with three CSs and then extinction with one of them. Secondary extinction was observed with a shock-associated CS when the extinguished CS had been associated with either food pellets or shock, suggesting that secondary extinction is not US-specific and is thus not explained by this mediated generalization mechanism. Experiment 4 examined whether intermixing trials with the two stimuli during conditioning is necessary for secondary extinction to occur. Rats were either conditioned with intermixed trials as in Experiments 1-3, or with blocked trials of each CS presented in conditioning sessions separated by a day. Secondary extinction was observed only in the former condition. The results are consistent with the hypothesis that CSs must be associated with a common temporal context for secondary extinction to occur.

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And to my fox.

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Introduction

In Pavlovian conditioning, a neutral cue acquires the ability to elicit a response as a result of pairings with a biologically-relevant unconditional stimulus (US). The response to this cue, or conditional stimulus (CS), can later be extinguished by repeatedly presenting the CS alone. Pavlov (1927) first reported that following appetitive conditioning of multiple CSs, extinction of one CS also attenuated responding to others that had not undergone any extinction. He and his associates demonstrated this effect in dogs by first pairing three neutral stimuli, a buzzer, metronome pulse, and tactile stimulation, with a US that elicited salivation. Immediately following conditioning, the buzzer was presented for several trials without the US until the salivary response was extinguished. Remarkably, when the other two CSs were tested, the elicited responses were greatly attenuated even though neither CS had undergone any extinction. In fact, responding to the metronome pulse was reportedly abolished. The finding that extinction of one CS can decrease responding to a second CS is known as "secondary extinction" (Pavlov, 1927).

Although Pavlov's findings seemed to be robust, similar effects have seldom been reported in the contemporary literature. In three conditioned suppression experiments reported by Richards and Sargent (1983), a modest secondary extinction effect was found in the first experiment, but was not replicated in the subsequent two. The first experiment compared the rates of extinction of a CS when it was the first or second of two CSs to undergo extinction. The authors reported that extinction of the tone CS was slightly more rapid in the group that first received extinction of the houselight CS than the group for whom the order of extinction was reversed. The same pattern was not observed with the houselight, however. Suppression was equivalent in groups that received extinction of the houselight first or after extinction of the tone. Two other studies using the conditioned suppression preparation also failed to demonstrate any secondary extinction effect. Bouton and King (1983) reported that extinction of a tone CS had no effect on a similarly conditioned light CS that was tested subsequently. Kasprow, Schachtman, Cacheiro and Miller (1984) similarly reported null results using both lick suppression and lever press suppression paradigms. Interestingly, these authors also reported a marginal effect of stimulus modality in one experiment that was in direct contrast to the pattern of results reported by Richards and Sargent (1983).

More recently, Ledgerwood, Richardson, and Cranney (2005) reported a secondary extinction effect in rats administered d-cycloserine (DCS) immediately following a session of fear extinction. DCS, a partial agonist of the NMDA receptor, has been shown to facilitate extinction of conditioned fear in rats and in humans (see Vervliet, 2008 for a review). Using the conditioned freezing method, Richardson and colleagues found that rats treated with DCS following extinction of a light CS froze significantly less to a tone than a group given saline after extinction. Seemingly consistent with Pavlov's (1927) findings, the reduction in freezing, although incomplete, appeared to be fairly large and robust. However, unlike the results reported by Pavlov, there was no effect of extinction in the absence of DCS. Specifically, comparison of the saline-treated extinction group to non-extinguished controls showed no differences in freezing on the test. Thus, this failure to find a secondary extinction effect with extinction alone further adds to the list of ambiguous and inconsistent findings.

Arguably, the most convincing modern examples of secondary extinction may be the “erasure” effects reported by Rescorla and Cunningham (1977, 1978). In their experiments, extinction trials with a light CS prevented recovery of responding to an extinguished tone CS produced by isolated presentations of the US (i.e., reinstatement; 1977) or a delay period between extinction of the tone and testing (i.e., spontaneous recovery; 1978). The authors suggested that extinction trials with the light, which likely had no direct effect on the tone-US association, weakened the memorial representation of the US. As a result, the weak US representation elicited less conditioned responding when it was later retrieved by the tone. Although this account explains the effects they reported, similar results have generally not been obtained in other conditioned suppression experiments, as described above. In fact, the noted failures to find secondary extinction typically employed a larger number of extinction trials conducted over multiple days. More extensive extinction treatments in these studies would be expected to more thoroughly depress the US representation, resulting in a greater secondary extinction effect. However, it should be noted that the target CS in Rescorla and Cunningham’s erasure studies were extinguished prior to the tests for reinstatement and spontaneous recovery. It is possible that this extinction training with the target CS could have rendered the US representation more sensitive to the subsequent erasure manipulation (see introduction to Experiment 1). Interestingly, given Pavlov’s use of his dog subjects in multiple experiments, it is possible that Pavlov’s successful secondary extinction experiments also involved testing with a target CS that had undergone some extinction in its previous history. Thus, if secondary extinction is dependent on existing inhibitory links between the target CS and US, then contemporary failures to replicate Pavlov's effect may be a result of testing a non-extinguished target CS. Accordingly, we began our investigation of secondary extinction with consideration of this possibility.

The foregoing analysis may help to reconcile some of the inconsistencies in the relatively small literature regarding secondary extinction. However, there are a number of potential variables that could play a role in this phenomenon and account for the failures to obtain a robust effect. We therefore conducted four experiments in an attempt to generate the secondary extinction effect reliably and isolate the contributing factors that permit its occurrence. In each experiment we investigated the effect of extinction of one CS on responding to a target CS presented later. In Experiment 1 we explored the notion that secondary extinction is primarily observed with a target CS that had undergone some extinction at an earlier point. Unexpectedly, there was evidence of secondary extinction with a target CS that had received no previous extinction. Experiment 2 then assessed the secondary extinction effect depends on the target CS being presented in context in which the other CS was extinguished. As noted by Bouton and King (1983), if the context is able to acquire inhibitory strength during extinction of a CS, the conditioned response to other excitors tested within it should be weaker (Rescorla & Wagner, 1972). Experiment 3 then explored a mediated generalization account of secondary extinction. It has previously been shown that conditioned suppression generalizes more readily from one CS to another if both stimuli had been previously paired with food (Honey & Hall, 1989), suggesting that associating two CSs with a common US might render them functionally equivalent. We thus investigated whether extinction of one CS generalizes more readily to another if both are initially paired with the same US (rather than being paired with different USs). Finally, Experiment 4 tested whether the intermixed presentation of target and non-target CSs in our conditioning procedure is necessary to obtain secondary extinction.

The specific aim of the present series of experiments was to test the predictions of a number of potential accounts for this effect and determine the conditions required for it to occur. It should also be noted that although this investigation is important from a theoretical perspective, achieving a greater understanding of the secondary extinction effect may have clinical implications that extend beyond the animal laboratory. Broadly speaking, the study of Pavlovian phenomena has contributed to a greater understanding of human psychopathology. In particular, Pavlovian conditioning and extinction have been believed to play an important role in the development and treatment of anxiety disorders since Watson and Rayner's (1920) famous early demonstration of fear conditioning in a human infant. Fear conditioning in the laboratory, typically performed on rats, has consequently become an influential model for the study of human anxiety disorders (Bouton, 1988; Mineka & Oehlberg, 2008). The practical significance of such research is evidenced by the use of extinction-based exposure therapies in the treatment of specific phobias, post-traumatic stress disorder, and other psychological disorders in humans (Foa, 2000). In the context of modern clinical practice, it is possible that the secondary extinction effect may have some therapeutic utility. For example, if extinction of a fear response to one CS can be shown to generalize to another CS, such a result might suggest that fearful stimuli may be extinguished in human patients by proxy. Exposure therapy based on a secondary extinction approach may thus be especially useful in cases where it may be difficult to extinguish each feared CS directly (e.g., after a traumatic event during which a large number of stimuli are conditioned). Given that so little is known about the mechanism that underlies this effect and the conditions under which it occurs, it is essential that secondary extinction be thoroughly investigated.

Experiment 1

The first experiment aimed to produce a secondary extinction effect in the conditioned suppression paradigm and to investigate a potential role for the target CS's reinforcement history. Specifically, we tested whether secondary extinction occurs only to target CSs that have previously undergone some extinction training, as in the method used by Rescorla and Cunningham (1977, 1978). Such a requirement might be expected if the context is assumed to acquire occasion setting properties during extinction of the non-target stimulus (Bouton, 2004; Bouton & Swartzentruber, 1986; Holland, 1992). Occasion setters are stimuli that modulate performance to CSs but do not elicit behavior directly. Though they do not function as CSs, they may have the same physical properties. For example, occasion setting stimuli can be discrete cues like tones or lights, or more diffuse stimuli like contexts. According to one view (Bouton, 2004; Bouton & Swartzentruber, 1986), the extinction context may become a negative occasion setter that inhibits performance to a CS trained within it. In this role, it reduces the conditioned response by activating the extinguished CS's existing inhibitory association with the US.

Though a number of studies with discrete occasion setters suggest that they typically act only on those targets with which they had originally been trained (Holland, 1986, 1989), others have shown that modulatory control by an occasion setter established with one CS may be able to transfer to other CSs under certain conditions (Lamarre & Holland, 1987; Rescorla, 1985; Swartzentruber & Rescorla, 1994). For example, Swartzentruber and Rescorla (1994) found that successful transfer of negative occasion setting occurred to a CS that had undergone both conditioning and extinction, but not to one that had merely been conditioned. In the former case, the CS had presumably developed two distinct associations with the US during training that were both available for modulatory control, whereas the latter acquired only an excitatory one. Thus, the transfer of occasion setting power from one CS to another may require that the new targets possess both excitatory and inhibitory associations. If secondary extinction is indeed a case of negative occasion setting by the extinction context, transfer of inhibitory control should mainly occur to target stimuli that have undergone both excitatory and inhibitory training. To test this prediction, rats were conditioned to fear two stimuli, one of which served as the target CS (X) that was tested for secondary extinction, and the second which served as the non-target CS (Y) that was extinguished prior to this test. Half of the rats were also given some initial extinction trials of X after conditioning, and thus were tested with a partially extinguished target CS. We expected to find evidence of secondary extinction in these rats, but not in those that were tested with a non-extinguished target CS. The experimental design is summarized in Table 1.

Method

Subjects

Thirty-two female Wistar rats (Charles River Laboratories, Quebec, Canada) ranging in age from 75-90 days at the start of the experiment were individually housed in suspended stainless steel cages in a room maintained on a 16-8 hr light-dark cycle. Experimental sessions were run during the light portion of the cycle at the same time each day. Prior to the start of lever press training the rats were food-deprived to 80% of their baseline body weights and maintained at that level throughout the experiment. Water was freely available in the home cages.

Apparatus

Two sets of four operant chambers (Med Associates, St. Albans, VT) located in separate rooms were used. Chambers from both sets measured 31.8 × 24.1 × 29.2 cm ($l \times w \times h$) and were individually housed in windowed sound attenuation chambers. Ventilation fans provided background noise of 65 dB, and the boxes were lit with two 7.5-W incandescent bulbs mounted to the ceiling of the sound-attenuation chamber. The front and back walls were brushed aluminum; the side walls and ceiling were clear acrylic plastic. Recessed 5.1 × 5.1-cm food cups were centered in the front wall and positioned near floor-level. In one set of boxes, a 4.8-cm long stainless steel operant lever protruded 1.9 cm from the front wall and was positioned 6.3 cm above the grid floor to the right of the food cup. The floor was composed of stainless steel rods (0.48 cm in diameter) spaced 1.6 cm apart from center to center and mounted parallel to the front wall. The ceiling and left side wall had black horizontal stripes, 3.8 cm wide and 3.8 cm apart. In the second set of boxes, the 4.8-cm long stainless steel operant lever protruded 1.9 cm from the front wall and was positioned 6.3 cm above the grid floor to the left of the food cup. The floor consisted of alternating stainless steel rods with different diameters (0.48 and 1.27 cm), spaced 1.6 cm apart from center to center. The ceiling and left sidewall were covered with rows of dark dots (1.9 cm in diameter) that were separated by approximately 1.2 cm. The 60-s light-off CS was created by terminating the houselights to produce darkness. The tone CS was a 60-s presentation of a 3000-Hz tone (80 dB) delivered through a 7.6 cm speaker mounted to the ceiling of the sound attenuation chamber. The US was a 0.5-s, 1-mA shock provided by Med Associates shock sources. Lever pressing was reinforced with 45-mg MLab Rodent Tablets (TestDiet, Richmond, IN).

Procedure

The design of Experiment 1 is summarized in Table 1. The study involved daily 84-minute sessions in which the rats lever pressed for food reinforcement. This followed one week of daily handling and food restriction to attain the target body weights.

Lever press training. The rats were first trained to lever press on a continuous reinforcement schedule, after which a variable interval (VI) 90-s reinforcement schedule was introduced and maintained throughout the remainder of the experiment. After 7 sessions of baseline training, the rats were assigned to one of four groups (*n* = 8) matched on their baseline lever pressing rates and counterbalanced across chambers.

Conditioning and baseline recovery. Two sessions of fear conditioning were conducted over two days. Each session consisted of 6 light-off and 6 tone presentations that coterminated with shock. The CSs were presented in a single alternated fashion with an average intertrial interval (ITI) of 6 min. The sequence (tone or light-off first) was counterbalanced across groups and sessions. Thus, the rats received a total of 12 conditioning trials with each CS, which were counterbalanced within each group to serve as X and Y. A baseline recovery day was conducted on the third day in order to recover lever pressing baselines that were depressed by fear conditioning. No CSs were presented during this session.

Extinction and Test. Extinction was conducted over three days following baseline recovery. On the first day, two groups of rats were given 8 extinction trials of the target CS (X) with an average ITI of 9 min. The two remaining groups were given equivalent exposure to the context but did not undergo extinction. Over the next two days, one group of rats from each of these conditions (extinguished X and non-extinguished X) were given 16 total trials (8 trials per day) of the non-target CS (Y), while the remaining control groups were given equivalent context exposure. On the final day, all rats were given 8 test trials of X. This factorial design yielded four groups, which were labeled according to the stimuli they received over the course of extinction and testing: a simple secondary extinction group (–/Y/X) and a complementary control that did not undergo extinction of Y (–/–/X), as well as a secondary extinction group in which X was partially extinguished on the first day of extinction (X/Y/X) and a control that did not undergo any extinction of Y (X/–/X).

Data Analysis. The computer recorded the number of lever presses for each rat during each 60-s CS as well as the 60-s period immediately preceding the CS. These data were used to calculate a suppression ratio, C/(C+P), where C is the number of responses made during the CS and P is the number of responses made during the pre-CS period (Annau & Kamin, 1961). A score of 0.5 denotes no lever-press suppression during the CS, whereas a score of 0 denotes complete suppression of responding during the CS. Suppression ratios were averaged across each block of two trials prior to statistical analysis. For this and the following experiments, analyses of variance (ANOVA) with a rejection criteria of $p < .05$ were used.

Results and Discussion

The results from extinction of X and Y and the test of X are presented in Figure 1, which shows suppression to the CSs averaged in two-trial blocks. As the figure suggests, suppression to X was equivalent between the two groups during the first phase of extinction (left). During the extinction of Y, however, significant group differences were present. The middle portion of Figure 1 suggests that secondary extinction of Y occurred in the X/Y/X group, which had received 8 extinction trials of X in the preceding phase. Confirming this impression, a Group × CS Modality × Block ANOVA found there was less suppression to Y in the X/Y/X group than the –/Y/X group, which had not undergone any prior extinction of X, F (1,12) = 7.25. This reduction persisted over both days of presentations of Y. Thus, inconsistent with the notion that secondary extinction occurs only with target CSs that have themselves undergone some prior extinction, the effect was observed with a target CS (in this case, Y) that had not been previously extinguished. Interestingly, there was evidence that the observed patterns were somewhat dependent on CS modality. A significant main effect of CS Modality, $F(1,12) = 16.72$, as well as significant interactions of CS Modality with Block and Group × Block (*Fs* ≥ 2.67), indicated that there was more suppression overall to the light-off CS, and that extinction of the light-off occurred more slowly than the tone. Baseline responding during the 1-min pre-CS periods were analyzed with a parallel ANOVA. No group difference in pre-CS scores were observed during the extinction of Y, *F* < 1. Average pre-CS responses were 30.6 and 28.3 in the X/Y/ X and –/ Y/X groups during the extinction of Y.

The final test of X is displayed on the right portion of Figure 1. As expected, there was less overall suppression to X in the two groups for whom X had been partially extinguished. This was confirmed by a significant main effect of extinction of X in an Extinction-X Group × Extinction-Y Group × CS Modality × Block ANOVA, *F*(1,24) = 25.18. However, there was no evidence that extinction of Y affected suppression to X, whether X was partially extinguished or not, *Fs* < 1. As during extinction of Y, a significant main effect of CS Modality indicated greater overall suppression to the light, *F*(1,24) = 4.86, though an interaction with Extinction-X suggested that this disparity was greater in the groups for whom X was not previously extinguished, *F*(1,24) = 4.53, reflecting a possible ceiling effect in the extinguished-X groups. Analysis of pre-CS responding revealed a significant Extinction-Y Group × CS Modality interaction, *F*(1,24) = 4.02. There was greater responding before light-off trials in the extinguished-Y groups, which averaged 32.0 pre-CS responses, than the control groups, which averaged 22.4 responses. Average scores per group were 29.2, 25.0, 27.7, and 25.7 for the X/Y/X, X/–/X, –/Y/X, and –/–/X groups, respectively. There were no other group differences on the test, *Fs* < 1.

The results suggest that secondary extinction can be observed in the conditioned suppression preparation. However, contrary to expectations, the effect found in Experiment 1 did not depend on prior extinction of the target CS. The group that received 8 extinction trials of X in the first phase of extinction (X/Y/X) clearly showed reduced suppression to Y in the second phase. This reduction was evident even on the first two-trial block of extinction, $F(1,16) = 4.70$, at which point Y would not yet have acquired an inhibitory association with the US, and thus would not have been a suitable target for transfer of negative occasion setting by the context. It is therefore unlikely that secondary extinction observed with Y was a consequence of the mechanism described above. It is unclear why the same secondary extinction effect was not observed during the final test of X, especially in the group that had no prior extinction of X (–/Y/X). In this group the treatment of stimuli at test was analogous to that given to the X/Y/X group in the second phase of extinction where the secondary extinction effect was observed with Y. Both groups received extinction of one CS before presentations of the other CS. The two conditions differed most notably by the number of extinction trials of the non-target CS (8 vs. 16) and the interval between the end of conditioning and the start of extinction (72 hrs vs. 120 hrs).

Experiment 2

The fact that a secondary extinction effect in Experiment 1 was obtained with a non-extinguished target CS suggests that the target CS was not under the control of a negative occasion setter. These results do not, however, rule out a contextual role in the secondary extinction effect observed with Y. Indeed, a possible role for the context has been predicted by a number of influential models of Pavlovian conditioning. The Rescorla-Wagner model (Rescorla & Wagner, 1972), for instance, provides a very simple account for this phenomenon. Essentially, the model assumes that the context acts as a CS that acquires its own excitatory or inhibitory strength during training. When a discrete excitor is extinguished in its presence, the context is thought to develop inhibition. Consequently, as a conditioned inhibitor, it would reduce responding to a second excitor by canceling its excitatory strength. According to this view, extinction of X in Experiment 1 could have allowed the context to acquire inhibitory strength in the X/Y/X group, which subsequently reduced the response to Y. Though previous studies have failed to find evidence for such a summation effect (e.g., Bouton & King, 1983), the secondary extinction effect found in Experiment 1 suggests that it might occur under some conditions. However, if that effect was solely a result of the extinction context becoming a conditioned inhibitor, it would not have affected responding to Y if the subsequent test of Y occurred in a different context. The primary goal of Experiment 2 was thus to examine whether the secondary extinction effect evident in Experiment 1 would occur when Y is presented outside of the context where X is extinguished. To test this idea, we modified the design of Experiment 1 so that Y was tested in a context different from the one in which the rats received extinction of X. The experimental design is summarized in Table 1.

A secondary goal of Experiment 2 was to investigate the generality of the erasure effect. As described above, Rescorla and Cunningham reported that nonreinforced presentations of one CS just before tests of reinstatement (1977) and spontaneous recovery (1978) of a second, extinguished, CS successfully abolished those recovery effects. A related recovery phenomenon that has yet to be investigated for erasure is renewal. Numerous studies using a wide range of experimental preparations have demonstrated that responding to an extinguished CS is renewed when it is tested outside of the extinction context (e.g., Bouton & Bolles, 1979; Bouton & King, 1983; Bouton & Peck, 1992; Rosas & Bouton, 1997). Robust renewal effects have been observed when testing occurs back in the original conditioning context after extinction in a novel one (i.e., ABA renewal), as well as when testing occurs in a neutral context (i.e., ABC renewal). The design of Experiment 2 permitted us to determine whether extinction of Y is able to prevent (or "erase") ABC renewal of X, which was conditioned in Context A, extinguished in Context B, and tested for renewal in Context C.

Method

Subjects

Thirty-two female Wistar rats of the same age and purchased from the same supplier were used. The rats were housed and maintained under the same conditions as in Experiment 1.

Apparatus

The two sets of four operant chambers used in Experiment 1 were modified slightly for use as the extinction contexts (B and C, counterbalanced). To create distinct odors in the respective contexts, a polystyrene weighing dish containing 5ml of a 2% anise solution or 4% coconut solution (McCormick) was placed outside of each chamber near the front wall. Two additional counterbalanced sets of four chambers were used as the conditioning context (A). Chambers in the first set measured 26 × 25 × 19 cm. The front, back, and one side wall were made of aluminum; the remaining side wall and ceiling were clear acrylic plastic. The floor consisted of tubular steel rods, 1.6 cm in diameter and spaced 3.2 cm from center to center. A recessed 5.5 × 5.5-cm food cup was centered on the front wall near the grid floor, and was located to the left of the operant lever. Odor was provided by 5 ml of white vinegar in a dish outside the chamber. Boxes in the second set measured 32 × 25 × 21 cm; the front and rear walls, as well as the ceiling, were made of clear acrylic plastic, while the sidewalls were made of aluminum. The floor was made of stainless steel rods, 0.5 cm in diameter and spaced 1.5 cm apart. A recessed 5 × 5-cm food cup was centered along the front wall at floor level, and was located to the right of the operant lever. This set of chambers was scented with 1.0 ml of Vick's Vaporub^{TM} in a dish outside the chamber. Both sets of chambers were housed in windowed sound attenuating chambers illuminated by two 7.5-watt incandescent bulbs mounted to the ceiling. A fan in each chamber provided 65 dB of background noise. The CSs and food pellets were the same as those used in Experiment 1. The US was a 1-s 1-mA shock produced from the same shock sources.

Procedure

The design of Experiment 2 is a modified version of the previous experiment that was based partly on Rescorla and Cunningham's (1977, 1978) erasure method. The procedure was the same as that in Experiment 1 except as noted. Lever press training occurred over 6 daily sessions, with half of the sessions conducted in each of the extinction and test contexts; three sessions occurred in Context B (Days 1, 2, and 5) and three sessions occurred in Context C (Days 3, 4 and 6). The rats were subsequently matched on their baseline lever pressing rates and assigned to groups. Conditioning was conducted in Context A using the same sequence and arrangement of stimuli as in Experiment 1. A longer 1-s shock was employed to produce strong conditioning and, consequently, a robust renewal effect. Baseline recovery was conducted in Context B in preparation for the extinction of X in that context.

Extinction and Test. Eight extinction trials with X were then presented in an 84-min session in Context B. On the following day, the rats were placed in Context C for extinction of Y and test of X, which were both conducted in a single 112-min session with an average ITI of 9 min. One group of rats from each of these conditions (extinguished X and non-extinguished X) were given 8 nonreinforced trials of the non-target CS, Y, while the remaining control groups were given equivalent context exposure. If the extinction of X on the previous day causes secondary extinction because it allows Context B to acquire inhibition, this treatment should have no effect on Y after the switch to Context C. Four test trials of X were subsequently presented to all groups immediately following the last extinction trial of Y. Comparison of the X/Y/X and X/–/X groups on this test should show whether the extinction trials of Y preceding the test of X have the ability to “erase” renewed fear to X, for which conditioning, extinction, and test occurred in Contexts A, B and C.

Results and Discussion

Figure 2 summarizes the data from the extinction of X in Context B, and the extinction of Y and test of X in Context C. As expected, the initial extinction of X proceeded without issue, though group differences were again present during subsequent extinction of Y. There, a Group × CS Modality × Block ANOVA found that the X/Y/X group showed less suppression to Y than the –/Y/X control group, which did not undergo any prior extinction, *F*(1, 12) = 4.84. The result appears comparable to the result reported in Experiment 1. However, this secondary extinction effect was observed outside the context in which X had been extinguished. Thus, it appears that secondary extinction does not require testing of the second CS in the context in which the first CS was extinguished.

On the final test of X, an Extinction-X Group × Extinction-Y Group × CS Modality × Block ANOVA failed to find a main effect of extinction of Y, *F*(1,24) = 2.15. However, a separate Extinction-X Group × Extinction-Y Group × CS Modality ANOVA conducted on the first two-trial block of the test revealed a main effect of extinction of Y, *F*(1,24) = 4.13. Thus, secondary extinction was observed on the test. There was also less overall suppression in the groups for which X had been previously extinguished, *F*(1,24) = 12.57, and less overall suppression to the tone, *F*(1,24) = 7.21. Note that unlike Experiment 1, though, conditioning and extinction of X were performed in different contexts (A and B, respectively) in order to produce renewal during testing in Context C. To isolate that renewal effect, we compared performance at the end of X's extinction with suppression to X at the start of testing. A Group × CS Modality × Block ANOVA failed to find a statistically significant block effect, *F*(1,12) = 4.23, or Group × Block interaction, *F*(1,12) = 4.07. However, different patterns emerged when the two groups were analyzed separately in parallel CS Modality × Block ANOVAs. Here was a significant increase in suppression from extinction to test in the X/–/X control group, confirming an ABC renewal effect, *F*(1,6) = 10.01. However, the corresponding difference did not approach significance in the X/Y/X group, *F*(1,6) < 1, suggesting that extinction of Y immediately prior to the test of X prevented (or “erased”) ABC renewal in this group. A main effect of CS Modality was significant only for the groups that had not undergone extinction of X, *F*(1,6) = 9.20, consistent with the likelihood of a ceiling effect in the extinguished-X groups.

As usual, pre-CS response rates were analyzed with ANOVAs that paralleled the ones conducted on the suppression ratios. No group differences in pre-CS responding were found in any phase of the experiment, largest *F* = 3.94. The average pre-CS responses during extinction of Y were 20.0 and 21.2 in the X/Y/X and X/–/X groups. On the final test of X, the scores were 21.6, 25.4, 19.3, and 21.7 in the X/Y/X, X/–/X, –/Y/X and –/–/X groups, respectively.

The results replicate a key finding from Experiment 1: extinction of X resulted in less responding to Y on the following day. However, as noted above, in the present experiment these two phases were conducted in different physical contexts. Although this finding does not conclusively eliminate a possible contextual role, because there could have been some generalization between the present contexts, it suggests that context inhibition alone might not explain the secondary extinction effect. Additionally, Experiment 2 further asked whether an erasure effect that Rescorla and Cunningham reported for reinstatement (1977) and spontaneous recovery (1978), occurred with ABC renewal. In keeping with their reports, we found that nonreinforced presentations of Y in

Context C shortly before a renewal test of X prevented renewal of conditioned suppression. Although the present design employed a larger number of nonreinforced trials of the non-target CS than did Rescorla and Cunningham, the lack of ABC renewal in the X/Y/X group is consistent with their findings with reinstatement and spontaneous recovery, and thus extends the generality of the erasure effect to renewal of extinguished fear.

Experiment 3

The finding that secondary extinction occurs outside of the extinction context undermines the view that secondary extinction is primarily mediated by learning about the extinction context. Experiments 3 and 4 therefore aimed to isolate other features of the conditioning process that might contribute to this phenomenon. Honey and Hall (1989) reported that conditioned suppression generalized more readily from one CS to another if both stimuli had been previously associated with the same US (food pellets). Their findings indicate that generalization between two physically different stimuli can be increased if they are associated with a common event. Such a mechanism could readily contribute to secondary extinction because X and Y are associated with a common US during the initial conditioning phase.

Experiment 3 therefore attempted to determine whether secondary extinction is another example of such mediated generalization. The design is illustrated in Table 1. We first conditioned three stimuli (X, Y, and Z) with either a food or a shock US. In each group, two of the CSs were associated with one US, and the third CS was associated with the other US. In one condition, X and Y were paired with shock during conditioning, and

Z was paired with food. We then extinguished Y (an aversive CS) and tested the two targets (X, also an aversive CS) and Z (an appetitive CS) for secondary extinction. According to the mediated generalization hypothesis, there should be a reduction in the response to the target X (which was paired with the same shock US as Y), but not the target Z (which had previously been paired with food). A second condition set up a complimentary arrangement that tested appetitive and aversive CSs after extinction of an appetitive CS.

It is important to note that unlike Experiments 1 and 2, all groups were conditioned with both appetitive CSs that signaled food and aversive CSs that signaled shock. In order to measure the responses elicited by both types of cues, we used a novel arrangement in which we first trained rats to regularly check the food cup by delivering food pellets noncontingently. After establishing a stable baseline of food cup entries, we then paired X, Y and Z with their respective USs. We expected that each of the CSs would have an effect on the food cup entry baseline, but that CSs paired with shock would suppress the baseline while CSs paired with food would elevate it. Thus, the method allowed us to assess responding to both aversive and appetitive CSs by measuring changes to the same baseline behavior.

Method

Subjects

The subjects were thirty-two female Wistar rats purchased from the same supplier and maintained under the same housing conditions.

Apparatus

The apparatus was the same as that used in Experiment 1. The 45 mg food pellets used to reinforce lever pressing in Experiments 1 and 2 were also used as the appetitive US during conditioning. The aversive US was a 0.5-s, 0.6-mA shock. Use of a less intense shock was intended to minimize the loss of baseline responding, which would hinder conditioning on appetitive trials presented in the same session. The CSs were three 30-s auditory stimuli delivered through a 7.6 cm speaker mounted to the ceiling of the sound attenuation chamber. These included the 3000-Hz tone used in the previous experiments, an intermittent white noise (clicker; 70 dB at 4 pulses/sec), and continuous white noise (80 dB). Previous experiments conducted in our lab have shown that rats discriminate between these stimuli (Bouton, Vurbic & Woods, 2008; Morris & Bouton, 2007). Infrared photocells mounted inside each of the food cups allowed a computer located in an adjacent room to count the number of photobeam breaks produced by head entries.

Procedure

The design is summarized in the third portion of Table 1. Consecutive daily 84-min sessions were employed throughout the experiment following one week of daily handling and food restriction to attain the target weights.

Food cup entry baseline. The rats were given three daily sessions in which individual food pellets were delivered noncontingently on a variable time (VT) 120-s delivery schedule, which created and maintained a stable baseline of food cup entry behavior. The VT 120-s schedule was employed in all phases of the experiment. We expected that a fear-conditioned CS would suppress this baseline (just as it would suppress a lever pressing baseline) whereas a CS associated with food would elevate it.

Conditioning. After the appetitive baseline was established, the rats were assigned to four groups ($n = 8$) matched on their baseline rates of food cup entry and counterbalanced across chambers. Two sessions of conditioning were then conducted over the following two days, with each session consisting of four conditioning trials of each of the three auditory CSs, denoted as X, Y and Z. The average ITI was 6 min. For each group, the CSs were presented in a repeating sequence (XYZXYZ or ZYXZYX) that counterbalanced the serial positions of X and Z, which were the two target CSs. The order presented in the first session was reversed in the second session. Thus, the rats received a total of 8 trials with each CS during this phase. For all rats, the clicker and noise were counterbalanced to serve as X and Z. The aversive target, X, was always paired with a shock US, whereas the appetitive target, Z, was always paired with a food US consisting of 5 pellets. The tone served as Y, the CS that was later extinguished. Y was paired with food in two groups (ExtF and NoExtF), and with shock in the remaining two groups (ExtS and NoExtS).

Extinction and Test. Following a baseline recovery day in which the rats were given a session of lever pressing for VI-90 reinforcement, two groups received eight extinction trials of Y and two groups did not. For one of the extinguished groups, Y was an aversive CS that had been paired with shock (ExtS). For the other one, Y was an appetitive CS that had been paired with food (ExtF). The remaining control groups (NoExtS and NoExtF) were given equivalent context exposure but no extinction trials were presented. On the following day, X and Z were each tested four times. The trials with each CS were presented in double alternating fashion (XXZZ or ZZXX, counterbalanced within groups) with an average ITI of 9 minutes. Thus, each group was tested with two target CSs; one which shared a US with Y during conditioning, and one which did not. If secondary extinction is mediated by shared associations with a US, extinction of Y should only generalize to one of the two target CSs. Specifically, extinction of an aversive Y (ExtS) should generalize to X but not Z, and extinction of an appetitive Y (ExtF) should generalize to Z but not X.

Data Analysis. A computer recorded the number of photobeam breaks in the food cups during each 30-s CS as well as the 30-s period immediately preceding the CS. Suppression of baseline food cup entries was calculated for each trial with an aversive CS (X in all four groups) with the same formula used to calculate suppression ratios for lever pressing in the above experiments. Elevation of baseline food cup entries was calculated for each trial with an appetitive CS (Z in all four group) by subtracting the number of food cup entries made during the 30-s pre-CS period from those made during the 30-s CS (such “elevation scores” are widely used in appetitive conditioning, e.g., Bouton & García-Gutiérrez, 2006; Bouton & Sunsay, 2003). Unlike suppression ratios, greater responding is indicated by higher, rather than lower, numbers. Suppression ratios and elevation scores were averaged across each block of two trials. The test scores from two rats in the NoExtS group who failed to perform any head entries during the pre-CS periods were not included in the analyses.

Results and Discussion

In the present experiment each group of rats was tested with two target CSs, one that had been paired with shock during conditioning (X) and the other paired with food (Z). In two groups extinction was carried out with a third CS (Y), which had been conditioned with either US. Separate ANOVAs isolating each of these two groups were conducted for the extinction phase. A significant Block effect indicated that extinction proceeded normally for the group that underwent extinction of the shock-associated CS $F(3,15) = 6.92$. However, the group extinguished with the food-associated CS showed a less consistent pattern of responding; the Block effect was not significant in the corresponding analysis, $F(3,21) = 1.64$.

The data from the test of X, the aversive CS, is depicted in Figure 3A. The figure suggests that extinction of Y decreased suppression to X in both extinction groups, irrespective of whether Y had been associated with shock or food. This impression was supported by an Extinction Group (yes or no) × US Type (shock or food US paired with Y) × CS Type (clicker or noise) × Block ANOVA, which found a significant main effect of extinction group, $F(1,22) = 6.94$. This effect did not interact with the US Type factor, $F(1,22)<1$, indicating that extinction of both the aversive (ExtS) and appetitive (ExtF) Y reduced suppression to X on the test. Although this effect was expected to occur in the former group since X and Y shared the same US during conditioning, its occurrence in the latter group suggests that secondary extinction is not specific to the US used in conditioning, and is thus not an instance of mediated generalization. There was a significant main effect of CS Type, $F(1,22) = 7.55$, as well as a CS Type × US Type interaction, *F*(1,22) = 7.23, indicating that suppression to the tone was greater than suppression to the click, particularly in the groups for which Y had previously been paired with shock. Analysis of the pre-CS food cup entries revealed no differences on the test, largest *F* = 1.44. Average responses were 8.2, 10.3, 12.0 and 11.6 for the ExtS, NoExtS, ExtF and NoExtF groups.

The results from the tests of Z, the appetitive target CS, are presented in Figure 3B. Though the pattern suggests that extinction of Y reduced responding to Z if and only if Y had been paired with food, none of the corresponding comparisons approached significance, and there were no other significant main effects or interactions, largest *F* = 1.06. The appetitive conditioning results were thus not conclusive. This experiment introduced a new method in which appetitive conditioning was measured by the CS's ability to increase food cup entry behavior above a baseline that was maintained by presenting food pellets on a VT 120-s schedule. The method was less successful than expected. However, the failure to obtain clear results with the appetitive target Z does not necessarily compromise interpretation of the results with the aversive target X. In the latter case, secondary extinction was obtained when the extinguished and target CSs were associated with different USs. That result was clearly not consistent with the mediated generalization hypothesis.

Experiment 4

In each of the above experiments, conditioning occurred over two sessions in which the target and non-target stimuli were intermixed in an alternating fashion within each session. Such intermixing was explicitly not used by Bouton and King (1983), who failed to find the present secondary extinction effect. Although the arrangement of CS presentations used by Richards and Sargent (1983) and Ledgerwood, Richardson, and Cranney (2005) is unclear, it is possible that those authors, who also did not obtain evidence of secondary extinction, did not intermix the CSs in the same fashion as in the present experiments. To investigate the role of this variable, we returned to the basic conditioned suppression method used in Experiments 1 and 2. X and Y were paired with shock over two days, either intermixed as before, or in separate sessions conducted 24 h apart.

Method

Subjects

Thirty-two female Wistar rats of the same age and purchased from the same supplier were used. The rats were housed and maintained under the same conditions as in the previous experiments.

Apparatus

The operant chambers and all stimuli (tone and light-off CSs, and 0.5-s, 1.0 mA shock US) were the same as those used in Experiment 1.

Procedure

The design is summarized at the bottom of Table 1. All phases of the experiment were conducted in the same physical context. After one week of daily handling and food restriction, the rats were trained to lever press on a VI 90-s reinforcement schedule over 8 daily 84-min sessions, and then matched on response rates and assigned to groups as before.

Conditioning. The rats were given two 84-min sessions of fear conditioning with the tone and light-off CSs, counterbalanced to serve as X and Y. Four trials were presented in each session. Two groups (Y/X Mix and –/X Mix) were given two tone and two light-off trials in each session, presented in an alternating fashion with an average ITI of 18 min. For the remaining groups (Y/X Separate and –/X Separate), all four conditioning trials of one CS occurred in the first session, and all four trials of the other CS in the second. The order of CS presentation was counterbalanced for both CS modality (tone or light-off first) and CS type (X or Y first).

Extinction and Test. The extinction of Y and test of X were conducted in a single 112-min session following a baseline recovery day. Two groups of rats were given 6 extinction trials of Y while the remaining groups were given equivalent context exposure. Four test trials of X were presented after the last extinction trial. An average ITI of 9 min was maintained throughout the session.

Data Analysis. The baseline lever pressing rates from three subjects greatly declined after the first test trial of X (fewer than three pre-CS responses) and did not recover. The data from those rats, two from the –/X Mix group and one from the Y/X Separate group, were consequently not included in the analysis.

Results and Discussion

Figure 4 summarizes the data from the extinction of Y and test of X. As the graph makes evident, suppression to Y during extinction was equivalent between groups and comparable to the levels of suppression observed in the previous experiments. The fact that both groups performed similarly during this phase, particularly on the first block of extinction, suggests that the conditioning treatments (intermixed and separate CS presentations) conditioned comparable fear to the CS. More important, however, the test data revealed a secondary extinction effect only in the group that received the intermixing treatment. A Conditioning Group × Extinction Group × CS Modality × Block ANOVA conducted on the test data found a main effect of extinction, $F(1,21) = 6.30$, a factor which importantly interacted with the conditioning treatment, $F(1,21) = 5.86$. Planned comparisons confirmed that extinction of Y reduced suppression to X in the Y/X Mix group only, $F(1,10) = 11.07$. The same effect was not observed in the Y/X Separate group, $F(1,11) < 1$. Thus, it appears that mixing the conditioning trials of the target and non-target stimuli is necessary for secondary extinction to occur in this method. Significant main effects of CS Modality indicating greater suppression to the light-off CS were also observed in the main ANOVA, $F(1,21) = 19.72$, as well as both of the subsequent comparisons, $F(1,10) = 7.58$, and $F(1,11) = 12.65$, respectively. No differences in pre-CS scores were observed between groups in either the extinction or test phases, largest $F = 2.96$. Pre-CS responding during the extinction phase averaged 20.2 and 24.7 for the Y/X Mix and Y/X Separate groups, and 22.6, 16.7, 24.2, and 24.5 on the test for the Y/X Mix, –/X Mix, Y/X Separate and –/X Separate groups.

General Discussion

Since Pavlov's (1927) initial discovery of secondary extinction, few studies have reported a similar pattern of results. In the present series of experiments, we (1.) demonstrated that a robust and replicable secondary extinction effect can be obtained in Pavlovian fear conditioning and (2.) uncovered a number of distinctive features that begin to characterize the phenomenon. In Experiment 1 we demonstrated that secondary extinction is not dependent on the extinction history of the target CS. Although Experiment 2 demonstrated that our manipulation was sufficient to "erase" ABC renewal, the results of Experiment 1 led us to conclude that secondary extinction is not merely an instance of the erasure of extinguished and recovered fear reported by Rescorla and Cunningham (1977, 1978). In Experiment 2 we also found that secondary extinction might not be fully explained by context inhibition or negative occasion setting acquired by the extinction context. Experiment 3 investigated the possibility that secondary extinction is mediated by the shared US. This notion was first suggested by Rescorla and Cunningham (1977, 1978), who hypothesized that a weakened US representation was responsible for the erasure effect. It also follows from the findings reported by Honey and Hall (1989), who showed that generalization of conditioned suppression was greater between CSs that had shared a common food US in an earlier phase of training. We therefore asked if secondary extinction depends on the extinguished and target CSs sharing an association with a common US. Surprisingly, our results suggest that it does not. Finally, in Experiment 4 we showed that intermixing conditioning trials with both CSs is critical for secondary extinction to occur. Although we consistently observed secondary extinction in groups that received an intermixed conditioning procedure (Experiments 1-4), the effect was abolished when we conditioned X and Y in separate sessions.

Fundamentally, a test for secondary extinction is a test of generalization of extinction between X and Y. There is little unconditional generalization between the CSs used in these experiments, as demonstrated by the failures to observe secondary extinction under some conditions (Experiment 4). However, various psychological (learned) mechanisms are known to encourage generalization between physically different stimuli. Mediated generalization created by the shared US (Honey & Hall, 1989) is one such possibility. However, as noted above, it cannot account for the pattern of results observed in Experiment 3. Alternatively, a study by Bennett and Mackintosh (1999) suggests another possibility. Using conditioned taste aversion, the authors showed that the amount of generalization between two taste stimuli is influenced by how closely in time they are spaced when they are presented during an initial exposure phase. When the interval between preexposure trials was 2 minutes or greater, generalization between the stimuli was not high. But a more rapid alternation of less than 1 minute between the stimuli increased generalization between them. It is thus possible that the close spacing of conditioning trials in the present intermixed procedure increased generalization between X and Y in a similar fashion. However, the effective interval in the experiments reported by Bennett and Mackintosh (1999) was much shorter than the ones used in the present experiments. Furthermore, it is notable that alternated exposures to stimuli can sometimes enhance their discriminability (e.g., Prados, Hall & Leonard, 2004; Symonds and Hall, 1995), as opposed to increase the generalization between them, although such a result theoretically depends on the two stimuli being physically similar, unlike the present auditory and visual CSs that played the role of X and Y.

Although the precise temporal arrangement of conditioning trials has not yet been investigated, the fact that X and Y must be conditioned in the same session does suggest that time between trials may play an important role in the secondary extinction effect. One possibility is that the animals simply associated X and Y over the intertrial interval in the intermixed condition. However, there is little independent evidence that rats associate visual and auditory cues over the 18-min intervals used here. Alternatively, Bouton (1993; Bouton, Westbrook, Corcoran & Maren, 2006) has argued that time can function as a context, and that the passage of time can produce effects that parallel those created by a change in physical context. For example, adding a retention interval after extinction produces spontaneous recovery of the conditioned response akin to a renewal effect (e.g., Brooks & Bouton, 1993). Following this hypothesis it may be reasoned that the 24-hr interval between the two conditioning sessions functioned as a change in context. It can thus be stated that X and Y are conditioned in the same temporal context when they are intermixed within the same session, but in different temporal contexts when trained separately. In that case, the increased generalization between X and Y may be a function of their shared associations with the common temporal context during conditioning.

The temporal context, and perhaps even the physical context, may function in the following way. During the course of intermixed conditioning, both X and Y might develop associations with the context such that the context retrieves a representation of X during Y+ conditioning trials and vice versa. This arrangement might allow the formation of excitatory associations between X and Y. In that case, subsequent extinction trials with Y might also excite a representation of X, allowing extinction of X to occur indirectly. Such an effect would be analogous to a mediated extinction effect reported by Shevill and Hall (2004). In three experiments, rats were initially given conditioning with two compound stimuli, AX and BY. In the following phase, A was extinguished alone. The authors reported that on a subsequent test of mediated extinction, suppression to X was attenuated compared to Y, whose associate (B) had not been extinguished. Similarly, Ward-Robinson and Hall (1996) reported a mediated extinction effect with a target CS that had been paired with its associate during a sensory preconditioning phase.

Further consideration of this mechanism may help to explain the absence of secondary extinction in the groups that received separate sessions of conditioning of X and Y. In the case of separate conditioning all of the trials with the first CS preceded all of the trials with the second CS. On Day 1 of conditioning the first CS would have developed an association with the physical and/or temporal context just as in intermixed training. However, a representation of the absent CS would not have been associatively activated by the context at this time since it had not yet been presented at any point. Therefore, it would not have been possible for an association to form between X and Y on the first day of conditioning. Of course, on Day 2 the physical context would subsequently have been able to retrieve a representation of the first CS during conditioning of the second CS, mediating the formation of an X-Y association. It is also possible, however, that the CS1-context association was extinguished during the second session since only CS2 was presented on this day. In that case, any association between X and Y would likely be very weak. Thus, if secondary extinction depends on the formation of an excitatory association between X and Y during conditioning, then the effect would be stronger after intermixed conditioning.

As discussed earlier, several published failures to obtain secondary extinction did not use the particular conditioning procedure that we have shown to be essential for the effect to occur. For example, Bouton and King (1983) presented all of the trials of the light CS on the first day of conditioning and all of the tone trials on the second day. Additionally, it does not appear that the conditioning methods used by Richards and Sargent (1983) or Ledgerwood, Richardson and Cranney (2005) were entirely consistent with those used in the present experiments, although the precise order of conditioning trials was not explicitly outlined by the authors. More puzzling, however, are the null results reported by Kasprow et al. (1984), who failed to find a secondary extinction effect with both lick suppression and lever press suppression paradigms with an intermixed conditioning procedure in which trials with X and Y were single alternated. It should be noted, however, that there are a number of potentially important methodological differences between those experiments and the ones reported here. Of course, further research would be needed to analyze the differences empirically. In spite of this inconsistency, however, the present findings are most consistent with the notion that the conditions that enable secondary extinction arise from intermixed training during the initial conditioning phase.

Although the actual mechanism that mediates the secondary extinction effect is still not well understood from a theoretical view, the experiments presented here provide a number of insights into this phenomenon and have uncovered a critical factor in its occurrence. The findings are also intriguing from a clinical perspective. In particular they suggest that in a therapeutic setting, the secondary extinction effect may allow exposure therapy to generalize across stimuli. This approach may be useful when feared stimuli can not be targeted through direct exposure. However, the results of Experiment 4 suggest that the effect may be restricted to stimuli conditioned during the same episode. More research is still needed to investigate exactly how the intermixing of conditioning trials functions to facilitate the generalization of extinction from one CS to another, and to explore additional boundary conditions that may reconcile the remaining inconsistencies in the literature.

References

Annau, Z. & Kamin, L.J. (1961). The conditioned emotional response as a function of intensity of the US. *Journal of Comparative and Physiological Psychology, 54,* 253-272.

Bennett, C.H. & Mackintosh, N.J. (1999). Comparison and contrast as a mechanism of perceptual learning? *Quarterly Journal of Experimental Psychology, 52,* 253-272.

Bouton, M. E. (1988). Context and ambiguity in the extinction of emotional learning: Implications for exposure therapy. *Behaviour Research & Therapy, 26*, 137-149.

Bouton, M.E. (1993). Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. *Psychological Bulletin, 114,* 80 -99.

Bouton (2004). Context and behavioral processes in extinction. *Learning and Memory, 11,* 485-494.

Bouton, M.E. & Bolles, R.C. (1979). Contextual control of the extinction of conditioned fear. *Learning and Motivation, 10,* 445 -466.

Bouton, M. E., & García-Gutiérrez, A. (2006). Intertrial interval as a contextual stimulus. *Behavioural Processes, 71*, 307-317.

Bouton, M.E. & King, D.A. (1983). Contextual control of the extinction of conditioned fear: Tests for the associative value of the context. *Journal of Experimental Psychology: Animal Behavior Processes, 9,* 248–265.

Bouton, M.E. & Peck, C.A. (1992). Spontaneous recovery in cross-motivational transfer (counter-conditioning). *Animal Learning and Behavior, 20,* 313 -321.

Bouton, M.E. & Sunsay, C. (2003). Importance of trials versus accumulating time across trials in partially-reinforced appetitive conditioning. *Journal of Experimental Psychology: Animal Behavior Processes, 29,* 62-77.

Bouton, M.E. & Swartzentruber, D. (1986). Analysis of the associative and occasion-setting properties of contexts participating in a Pavlovian discrimination. *Journal of Experimental Psychology: Animal Behavior Processes, 12,* 333 -350.

Brooks, D.C. & Bouton, M.E. (1993). A retrieval cue for extinction attenuates spontaneous recovery. *Journal of Experimental Psychology: Animal Behavior Processes, 19,* 77 -89.

Foa, E.B. (2000). Psychosocial treatment of posttraumatic stress disorder. *Journal of Clinical Psychiatry, 61,* 43-51.

Holland, P.C. (1986). Temporal determinants of occasion setting in feature-positive discriminations. *Animal Learning and Behavior, 14,* 111-120.

Holland, P.C. (1989). Transfer of negative occasion setting and conditioned inhibition across conditioned and unconditioned stimuli. *Journal of Experimental Psychology: Animal Behavior Processes, 15,* 311-328.

Holland, P.C. (1992). Occasion setting in Pavlovian conditioning. In: D. Medin (Ed.), *The psychology of learning and motivation, Vol. 28* (pp. 69-125). Academic Press, San Diego.

Honey, R.C. & Hall, G. (1989). Acquired equivalence and distinctiveness of cues. *Journal of Experimental Psychology: Animal Behavior Processes, 15,* 338-346.

Kasprow, W.J., Schachtman, T.R., Cacheiro, H., & Miller, R.R. (1984). Extinction does not depend upon degradation of event memories. *Bulletin of the Psychonomic Society, 22,* 95-98.

Lamarre, J. & Holland, P.C. (1987). Transfer of inhibition after serial Feature Negative discrimination training. *Learning and Motivation, 18,* 319 -342.

Ledgerwood, L., Richardson, R., & Cranney, J. (2005). D-Cycloserine facilitates extinction of learned fear: Effects on reacquisition and generalized extinction. *Biological Psychiatry, 57,* 841-847.

Mineka, S. & Oehlberg, K, (2008). The relevance of recent developments in classical conditioning to understanding the etiology and maintenance of anxiety disorders. *Acta Psychologica, 127*, 567–580.

Morris, R.W. & Bouton, M.E. (2007). The effect of yohimbine on the extinction of conditioned fear: A role for context. *Behavioral Neuroscience, 121,* 501-514.

Pavlov, I.P. (1927). *Conditioned Reflexes.* New York: Courier Dover.

Prados, J., Hall, G., & Leonard, S. (2004). Dissociation between the Espinet and perceptual learning effects in flavour aversion conditioning. *Behavioral Processes, 65,* 221-229.

Rescorla, R.A. (1985) Conditioned Inhibition and Facilitation, in R.R. Miller & N.S. Spear (Eds.), *Information Processing in Animals: Conditioned Inhibition,* Hillsdale, N.J.: Erlbaum.

Rescorla, R.R. & C.L. Cunningham (1977). The erasure of reinstated fear. *Animal Learning and Behavior, 5,* 386–394.

Rescorla, R.R. & C.L. Cunningham (1978). Recovery of the US representation over time during extinction. *Learning and Motivation, 9,* 373–391.

Rescorla, R. A., & Wagner, A. R. (1972). A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In A. H. Black and W. K. Prokasy (Eds.), *Classical conditioning II: Current research and theory*, New York: Appletone-Century-Crofts.

Richards, R.W., & Sargent, D.M. (1983). The order of presentation of conditioned stimuli during extinction. *Animal Learning and Behavior, 2,* 229-236.

Rosas, J.M. & Bouton, M.E. (1997). Additivity of the effects of retention interval and context change on latent inhibition: Toward resolution of the context forgetting paradox. *Journal of Experimental Psychology: Animal Behavior Processes, 23,* 283-294.

Shevill, I. & Hall, G. (2004). Retrospective revaluation effects in the conditioned suppression procedure. *Quarterly Journal of Experimental Psychology, 57,* 331-347.

Swartzentruber, D. & Rescorla, R.A. (1994). Modulation of trained and extinguished stimuli by facilitators and inhibitors. *Animal Learning and Behavior, 22,* 309-316.

Symonds, M. & Hall, G. (1995). Perceptual learning in flavour aversion conditioning: Roles of stimulus comparison and latent inhibition of common stimulus elements. *Learning and Motivation, 26,* 203-219.

Vervliet, B. (2008). Learning and memory in conditioned fear extinction: Effects of D-cycloserine. *Acta Psychologica, 127,* 601-613.

Ward-Robinson, J. & Hall, G. (1996). Backward sensory preconditioning. *Journal of*

Experimental Psychology: Animal Behavior Processes, 22, 395-404.

Watson, J.B. & Rayner, R. (1920). Conditioned emotional reactions. *Journal of Experimental Psychology, 3,* 1-14.

Table 1:

Group	Conditioning	Extinction X	Extinction Y	Test of X
Experiment 1 – All phases conducted in the same context				
—/Y/X	[12X+ 12Y+]	—	16Y-	8X-
—/—/X	[12X+ 12Y+]	—	—	8X-
X/Y/X	[12X+ 12Y+]	8X-	16Y-	8X-
X/—/X	[12X+ 12Y+]	8X-	—	8X-
Experiment 2	Context A	Context B	Context C	
—/Y/X	[12X+ 12Y+]	—	[8Y-	4X-]
—/—/X	[12X+ 12Y+]	—	[—	4X-]
X/Y/X	[12X+ 12Y+]	8X-	[8Y-	4X-]
X/—/X	[12X+ 12Y+]	8X-	[—	4X-]
Experiment 3 – All phases conducted in the same context				
ExtF	[8X(F)+ 8Y(F)+ 8Z(S)+]		8Y-	4X- 4Z-
NoExtF	[8X(F)+ 8Y(F)+ 8Z(S)+]		—	4X- 4Z-
ExtS	[8X(F)+ 8Y(S)+ 8Z(S)+]		8Y-	4X- 4Z-
NoExtS	[8X(F)+ 8Y(S)+ 8Z(S)+]		—	4X- 4Z-
Experiment 4 – All phases conducted in the same context				
Y/X Mix	[4X+ 4Y+]		[6Y-	4X-]
—/X Mix	[4X+ 4Y+]		[—	4X-]
Y/X Sep	[4X+] [4Y+]		[6Y-	4X-]
—/X Sep	[4X+] [4Y+]		[—	4X-]

Table 1. Designs of Experiments 1-4. Group labels for Experiments 1, 2 and 4 correspond to the treatments given during extinction of X, extinction of Y, and testing of X. Symbols: — indicates context exposure only; X+, Y+, and Z+ are conditioning trials with the three CSs (+ indicates a US presentation); X-, Y-, and Z- are CS trials with no US; (F) is a food pellet US; (S) is a shock US. Stimuli contained within brackets were presented in the same experimental session.

Figure 1:

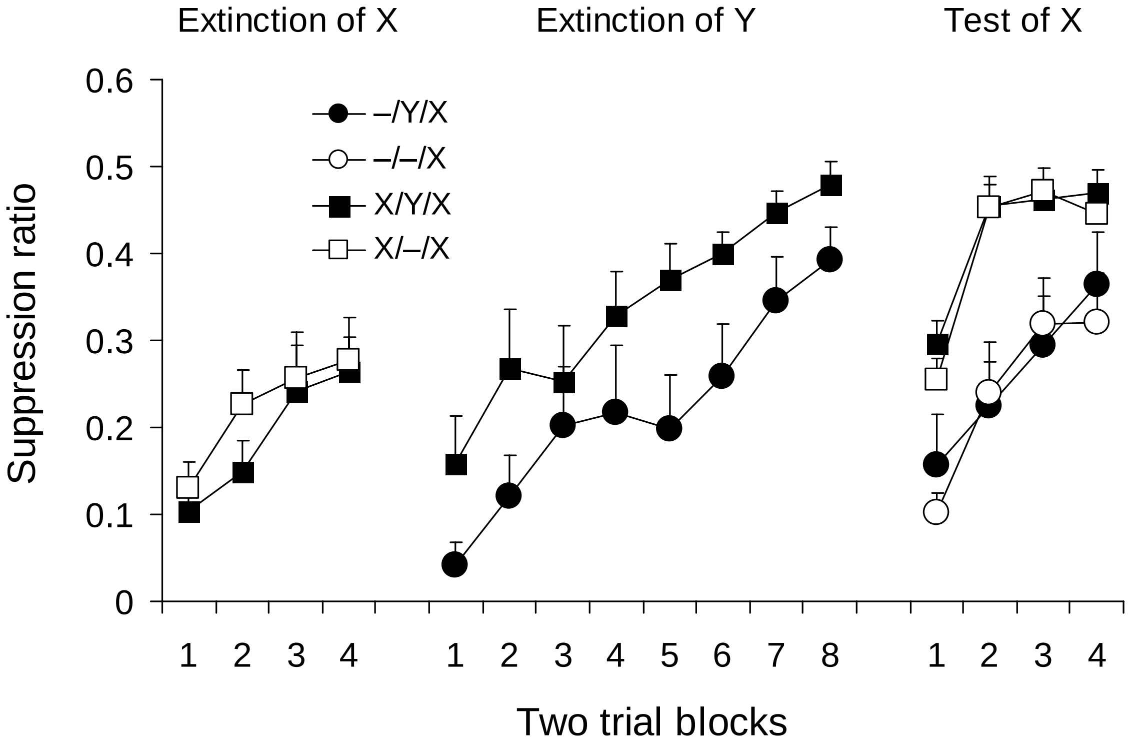

Figure 1. Conditioned suppression during extinction of X and Y and the test of X in Experiment 1. During the extinction of Y (center) the X/Y/X group, which received extinction of X on the previous day (left), showed significantly less suppression than the –/Y/X group, which had not been given previous extinction of X. Thus, secondary extinction was observed during extinction of Y, which served as a non-extinguished target CS at this point in the experiment. However, no secondary extinction effect was observed during the test of X (right). Both the X/Y/X and –/Y/X groups, which had been given extinction of Y in the preceding phase, showed similar levels of suppression to X as their respective controls groups, X/–/X and –/–/X.

Figure 2:

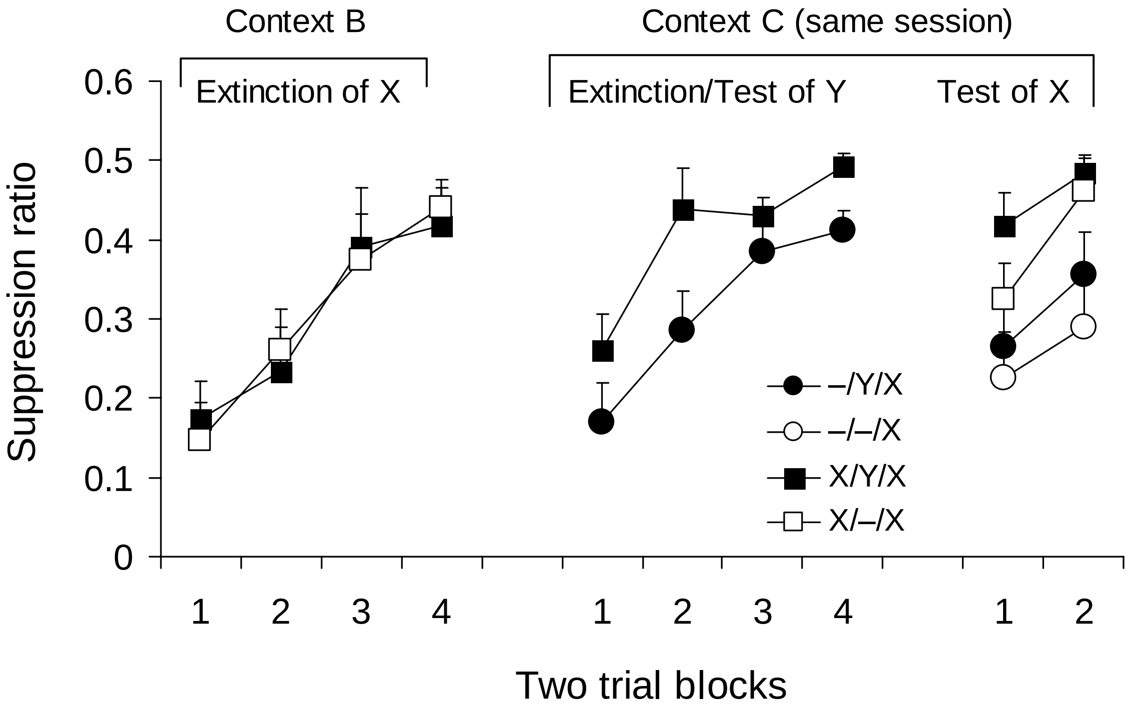

Figure 2. Conditioned suppression during extinction of X and Y and the test of X in Experiment 2. Following conditioning in Context A (not shown), extinction of X (left) was conducted in Context B. Extinction of Y (center) and the test of X (right) occurred in a single session in Context C. Despite a context switch between extinction of X and extinction of Y, the X/Y/X group, which had first received extinction of X, showed significantly less suppression to Y than the –/Y/X group, which had not been given previous extinction of X. The occurrence of a secondary extinction effect here indicates that secondary extinction is not context specific. Additionally, on the test of X in Context C (right), the X/–/X group, which had been given extinction of X in Context B, showed

renewal of responding to X. However, renewal was thwarted in the X/Y/X group, which had received extinction of Y in Context C immediately before the test of X. The finding that extinction trials of one CS prevented renewal of a second CS is analogous to the studies reported by Rescorla and Cunningham on the “erasure” of reinstatement (1977) and spontaneous recovery (1978).

Figure 3:

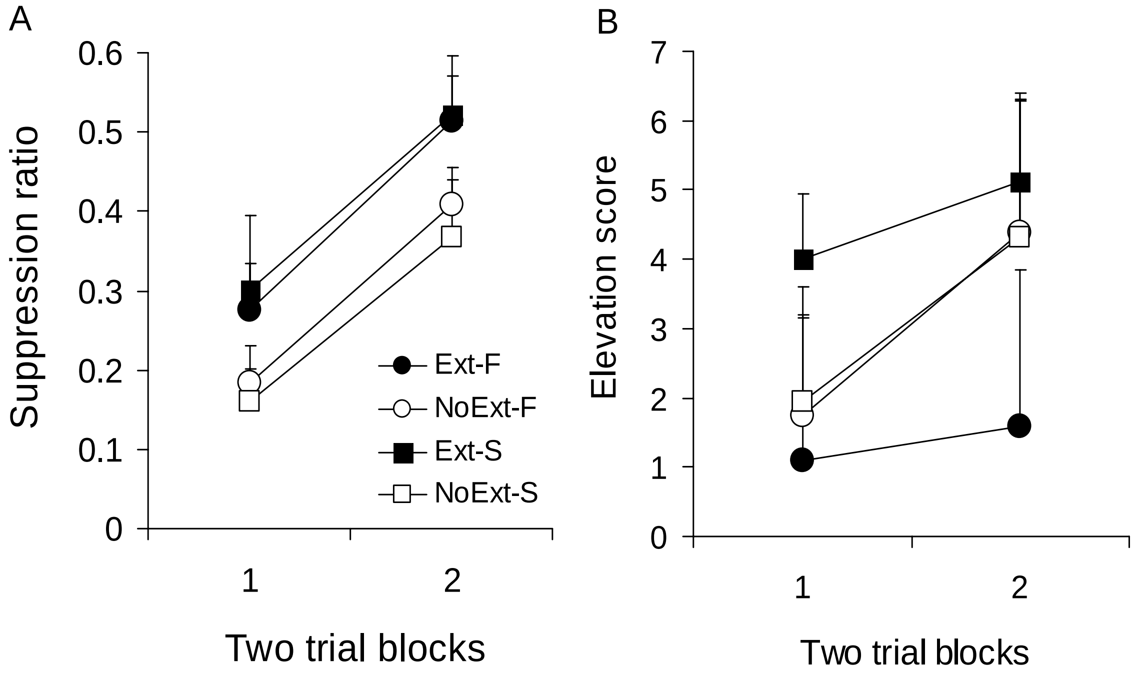

Figure 3. Test of the aversive target CS (Fig. 3A) and appetitive target CS (Fig. 3B) in Experiment 3. Figure 3A shows suppression of food cup entry behavior during the test of X, a CS previously conditioned with shock. There was less overall suppression to X after extinction of Y whether Y was an appetitive CS that was previously conditioned with food pellets (ExtF) or an aversive CS that was previously conditioned with shock (ExtS). Thus, secondary extinction is not specific to the shock US paired with the target CS. Figure 3B shows elevation of food cup entry behavior during the test of Z, a CS previously conditioned with food. Higher scores indicate greater elevation during the CS compared to the baseline. There were no significant main effects or interactions.

Figure 4:

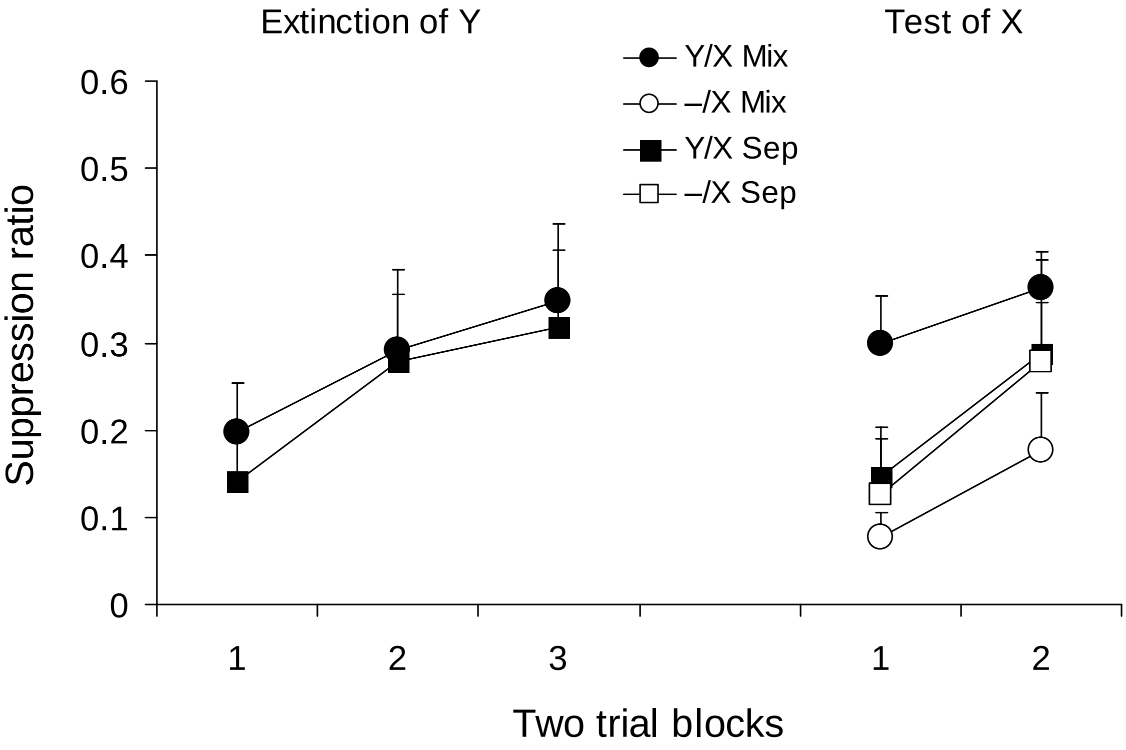

Figure 4. Extinction of Y and Test of X in Experiment 4. On the test of X (right) a secondary extinction effect occurred between groups that received intermixed X and Y trials during conditioning (Y/X Mix vs. –/X Mix). However, secondary extinction was not observed between the groups that received separate conditioning sessions of X and Y (Y/X Sep vs. –/X Sep).