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Renewal of Fear Following Immediate Extinction in a Passive Avoidance Paradigm

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Understanding the mechanisms of extinction, which is the foundation for exposure-based therapies used to treat anxiety disorders, is of theoretical and clinical importance. Recent research suggests that extinction occurring shortly after fear conditioning attenuates the renewal of fear. This experiment investigated whether extinction shortly after fear conditioning would prevent the renewal effect using a passive avoidance paradigm. Female rats received extinction either immediately (10 minutes) or one day (24 hours) after fear conditioning in either the same context as conditioning or in a different context. When testing for fear occurred in the same context as extinction, both the immediate and the delayed extinction groups showed a significant reduction of fear. However, those tested for fear in the context that differed from extinction demonstrated a significant amount of fear, i.e., the renewal of fear, regardless of extinction occurring immediately or after a delay. These results conflict with other findings that immediate extinction attenuates renewal, as well as show that the mechanisms of immediate and delayed extinction may be similar.

Keywords: Immediate extinction; renewal effect; exposure therapy; passive avoidance; rat

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It is estimated that approximately 18% of adults suffer from an anxiety related disorder in a given year, making it one of the most prevalent forms of psychopathology (Kessler, Chiu, Delmer, & Walters, 2005). Beyond the human suffering, it is also estimated that there is an economic impact of approximately $40 billion in the United States alone (Kessler, & Greenberg, 2002, Chapter 67). An effective and widely used treatment of anxiety disorders is exposure based therapies, which are based on the principles of experimental extinction (Mineka & Zinbarg, 2006; Telch, Cobb, & Lancaster, 2014, Chapter 35). Extinction is a procedure where the cues (conditioned stimuli - CS) that have been previously paired with a biologically relevant reinforcer (unconditioned stimuli - US) are presented without the reinforcement. This unreinforced cue exposure causes a reduction in the learned response (conditioned response - CR). Although there is a reduction in the CR, it is now widely accepted that extinction does not involve unlearning of the original association, but rather new learning.

Extinction is clearly seen as new learning in models in which extinction fails. Pavlov (1927) was the first to describe a failure in extinction by incorporating a long extinction-test interval. He demonstrated that the CR spontaneously recovers over time, thus showing longer delays are associated with the failure of extinction and the return of the CR. In addition to spontaneous recovery, extinction as new learning is seen in work demonstrating that extinction learning is susceptible to amnesia. For example, a study by Briggs and Riccio (2007) demonstrated amnesia for extinction memory using the amnestic agent hypothermia, and further showed that the memory for extinction shares similar characteristics as an original acquisition memory (i.e., temporally graded and recovery following reexposure to the amnestic agent). Other studies using different amnestic agents have also shown extinction to be a separate memory that is vulnerable to disruption (Briggs & Olson, 2013; Power, Berlau, McGaugh, & Steward, 2006; Suzuki et al., 2004). Demonstrating the return of the CR by the disruption of extinction indicates that the original association remained intact. Moreover, Bouton (2002) has done extensive work establishing that the original association exists following extinction, describing four models of relapse – spontaneous recovery, reinstatement, rapid reacquisition, and renewal (see also Bouton, 2004).

The relapse of fear following extinction poses a serious problem for exposure-based treatments of anxiety disorders. The renewal effect, for example, is particularly problematic. Renewal of fear is observed when the CR returns following extinction learning occurring in a distinctly different context or setting, thus demonstrating the context specificity of extinction (Bouton, 2002; Bouton & Bolles, 1979). Although much of the evidence for the renewal effect has been conducted using rodent subjects, the effect has been observed using human subjects (e.g., Hermann, Stark, Milad, & Merz, 2016; Vansteenwegen et al., 2005). Extinction being highly context specific has clear implications on the therapeutic effects of exposure therapies.

Although research supports extinction as new learning, recent evidence has emerged suggesting that short conditioning-extinction intervals may disrupt or erase the original association (Quirk et al., 2010). Myers, Ressler, and Davis (2006) were the first to show that extinction learning at a short acquisition-extinction interval (10 minutes) attenuated the spontaneous recovery, reinstatement, and renewal of fear in rats. Thus, as Myers et al. point out, the mechanisms of extinction may differ depending on the timing of the extinction learning. This immediate extinction effect has been replicated using various Pavlovian conditioning paradigms (for review see Johnson, Escobar, & Kimble, 2010); however, others have failed to find the effect using both rodent (Chang & Maren, 2009; Woods & Bouton, 2008) and human subjects (Huff, Hernandez, Blanding, & LaBar, 2009; Norrholm et al., 2008; Schiller et al., 2008).

Determining the mechanisms of immediate and delayed extinction is not only of theoretical importance, but also has clinical significance (Bisson, Brayne, Ochberg, & Everly, 2007; McNally, Bryant, & Ehlers, 2003). While some studies find immediate extinction to be more effective than delayed extinction and others failing to support the results, our lab recently reported that immediate extinction attenuated both spontaneous recovery and reinstatement of fear in rats using a passive avoidance task (Briggs & Fava, 2016), thus supporting the Myers et al. (2006) findings. Therefore, the current experiment sought to extend the Briggs and Fava findings by examining whether immediate extinction would also attenuate the renewal of fear using the same passive avoidance paradigm.

Method

Subjects

Forty-eight experimentally naive, adult female Long-Evans hooded rats, obtained from the Susquehanna University's animal breeding colony, served as subjects. Female rats were chosen because this study was an extension of prior work on immediate extinction from our lab that used female subjects (see Briggs & Fava, 2016). Prior research has shown no sex differences in passive avoidance learning in rats (Briggs & Olson, 2017; Lynch, Cullen, Jasnow, & Riccio, 2013). The rats were approximately 110 days old with an average weight of 248 grams at the start of the experiment. The animals were individually housed and were maintained on a 12:12-hour light:dark cycle. All experimental sessions took place during the light portion of the cycle and at the same time each day. Food and water were available *ad lib* throughout the course of the experiment. Approval of the experimental protocol was obtained by the Susquehanna University Institutional Animal Care and Use Committee prior to the start of the study. All behavioral procedures were conducted in accordance with guidelines of the National Institutes of Health guide for the care and use of laboratory animals.

Apparatus and Contexts

Training, extinction, and testing were conducted in two identical 48 X 21 X 21 cm white-black passive avoidance chambers (Ugo Basile Model 7551) with a tilting grid floor (3 mm diameter stainless steel bars spaced 1.2 cm apart, center to center). The chamber was divided into two equal compartments, a white compartment and a black compartment. An automatic sliding door separated the two sides. The white compartment had opaque white walls with a transparent lid. The black compartment had opaque black walls and a black lid. Footshocks were delivered automatically through the grid floor of the black compartment via a control unit housing a programmable scrambled shock generator. The control unit also recorded the cross latencies into the black compartment (tilting the floor) with a 0.1-second resolution.

The two avoidance chambers were located in separate rooms that served as contexts. Context A was a bright 3.05 × 2.24 m room with white walls, as well as a counter, sink, and cabinets on one wall. The avoidance chamber was located on a table along one of the long side walls. This room was illuminated by fluorescent house lights. Context A had no artificial scent, and no white noise was present. Context B was a darker 3.25 × 2.03 m room with white walls, scented with Air Wick® Wizard Virgin Islands scented oil air freshener.

White noise (70 dB) was presented at all times in this context. The room was illuminated by a single 25-W red light bulb in a corner of the room. Two walls in this context had standard lab counters along the entire walls. The avoidance chamber was located on the counter along the far back wall.

Procedure

Prior to the beginning of the experiment, all subjects were handled for 5 minutes on two consecutive days. Groups of 8 rats were randomly assigned to one of six conditions. Assignment to the contexts was counterbalanced in such that within each group 4 rats started in Context A and 4 rats started in Context B.

Training, extinction, and testing was conducted similarly to procedures used in other experiments comparing the effects of delayed extinction and immediate extinction on spontaneous recovery and reinstatement using a passive avoidance paradigm (Briggs & Fava, 2016). Twenty-four hours after their second handling, each rat received a single fear conditioning trial. During fear conditioning, each rat was brought into the context in its home cage that was placed on the counter for 15 seconds. The animal was then removed from its home cage and placed on the experimenter's arm for 15 seconds. This was done to allow time for each animal to experience the contextual cues. After being on the experimenter's arm for 15 seconds, the rat was then placed in the white compartment of the white-black chamber facing away from the closed door. After 15 seconds, the door automatically opened, and the control unit began timing the latency to cross into the black compartment (in seconds). Upon entering the black compartment which tilted the floor, the timer stopped and the door automatically shut. Two seconds after the door shut, the rat received one inescapable footshock (1 second, 0.8mA). Fifteen seconds after the door shut, the rat was removed from the apparatus and was returned to its home cage. This fear conditioning procedure produces fear of the black compartment.

Following fear conditioning, two groups received standard extinction training 24 hours after conditioning (Delayed Extinction groups) and two groups received immediate extinction 10 minutes after conditioning (Immediate Extinction groups). Of the four groups, one group from the Delayed Extinction condition and one group from the Immediate Extinction condition received the extinction session in the same context as conditioning (Extinction). The other two groups received extinction in the context different from the conditioning context (Renewal). During the single extinction trial, the rat was brought into the context in its home cage which was placed on the counter for 15 seconds, followed by 15 seconds on the experimenter's arm. Following the exposure time to the context, the animal was first placed in the white side of the chamber for 15 seconds and then manually moved to the black compartment for 10 minutes. The door remained closed at all times and no shocks were delivered during extinction. After 10 minutes of being confined to the black compartment, the rat was removed and returned to its home cage. As mentioned above, the parameters and procedure chosen for extinction were similar to those in previous studies (see Briggs & Fava, 2016). Two fear only groups received only the fear conditioning and no extinction to serve as a control showing a baseline level fear of the black compartment. The design of the experiment is summarized in Table 1.

Twenty-four hours after extinction (48 hours after conditioning for the Delayed Fear group; 24 hours after conditioning for the Immediate Fear group), all animals underwent a passive avoidance test. This delay was used to maintain a consistent extinction-to-

Table 1. *Experimental design*

Group	Training	*Delay*	Extinction	*Delay*	Test
Delayed Fear	Context A	24 hr	No	24 hr	Context A
Immediate Fear	Context A	No	No	24 hr	Context A
Delayed Extinction	Context A	24 hr	Context A	24 hr	Context A
Immediate Extinction	Context A	10 min	Context A	24 hr	Context A
Delayed Renewal	Context A	24 hr	Context B	24 hr	Context A
Immediate Renewal	Context A	10 min	Context B	24 hr	Context A

Note: $N = 48$, $n = 8$. *Contexts A and B were counterbalanced within each group.*

testing interval for the experimental conditions of interest. The fear groups from both the Delayed Extinction and Immediate Extinction conditions were tested in the same context as conditioning. The extinction groups from both the Delayed Extinction and Immediate Extinction conditions were also tested in the same context in which the conditioning and extinction trials took place to assess the effectiveness of the extinction session in reducing fear. The renewal groups (both Delayed Extinction and Immediate Extinction) were tested in the original conditioning context, which differed from the extinction session context. This was to assess the recovery/renewal of fear when tested in the original training context. Test trials were conducted identically to conditioning trials, except no shocks were delivered upon the rat entering the black compartment. After the rat crossed to the black side, the door automatically closed, and it was removed from the chamber and returned to its home cage. Testing lasted for 5 minutes, and the rats that did not cross to the black compartment were removed from the white side. The latency to cross to the black compartment (in seconds) was automatically recorded by the control unit and was used as the dependent measure.

Results

Counterbalancing

Independent samples *t*-tests were calculated for each of the six groups comparing cross latencies recorded in Context A to cross latencies recorded in Context B for both training and testing. The results revealed that there were no differences (all p's > .14) within any of the six groups at training or testing (i.e., the animals in each group treated the contexts similarly). Independent samples *t*-tests were also calculated to test for differences in cross latency scores during training and testing in Context A and in Context B overall by collapsing groups. Again, results showed no significant difference between latency scores recorded in Context A and in Context B during training, $t(46) = .305$, $p = .762$, $d = .087$, or at testing, $t(46) = .081$, $p = .936$, $d = .023$. Accordingly, since the contexts were treated similarly, and no significant differences were found, the two contexts were collapsed within each group for all further analyses.

Training

The rats in all six groups exhibited short cross latencies during training with group means ranging from 13.9 seconds for the Immediate Extinction Fear group to 15.8 seconds for the Delayed Extinction group. Mean cross latencies for the Delayed Extinction Fear group, the Immediate Extinction group, the Delayed Renewal group, and the Immediate

Renewal group were 14.1, 15.6, 15.3, and 15.5 seconds, respectively. A one-way analysis of variance (ANOVA) revealed no differences among the groups, $F(5, 42) = .068$, $p = .997$, $\eta_p^2 = .008$, showing that all groups had similar latencies to cross at training.

Testing

Figure 1 shows the mean cross latencies for all six groups at testing. As can be seen, both delayed (left panel) and immediate (right panel) extinction treatments (Extinction groups) were effective in reducing fear when extinguished in the same context as training (denoted A-A-A) regardless of receiving the cue exposure 10 minutes or 24 hours after fear conditioning. Moreover, there was also significant renewal of fear (Renewal groups) for both the delayed and immediate extinction conditions when testing occurred back in the original training context, i.e., the context that differed from extinction (denoted A-B-A).

Confirming these observations, a two-way delay (Delayed, Immediate Extinction) X group (Fear, Extinction, Renewal) ANOVA performed on the testing cross latencies revealed a significant effect of group, $F(2, 42) = 30.266$, $p < .001$, $\eta_p^2 = .590$, but a nonsignificant effect of delay, $F(1, 42) = .058$, $p = .810$, $\eta_p^2 = .001$. As well, the delay by group interaction did not reach significance, $F(2, 42) = 1.800$, $p = .178$, $\eta_p^2 = .079$. To test for the effectiveness of extinction in reducing fear, planned comparisons were completed comparing the Extinction groups to the no extinction Fear control groups for both the delayed and immediate extinction conditions. Separate independent samples *t*-tests confirmed that extinction significantly reduced fear in both the delayed extinction condition, $t(14) = 5.926$, $p < .001$, $d = 2.963$, 95%*CI* = 266.6, 124.9, and the immediate extinction condition, $t(14) = 7.173$, $p < .001$, $d = 3.587$, 95%*CI* = 307.4, 165.9.

The critical comparison to evaluate renewal of fear is between the animals that received training, extinction, and testing in the same context (A-A-A) compared to those that received extinction in a different context from training and testing (A-B-A). Separate planned comparisons using independent samples *t*-tests analyzing the Extinction groups and Renewal groups in both the delayed and immediate extinction conditions were calculated to assess renewal. Renewal of fear was significant in both the delayed extinction condition, $t(14) = 3.65$, $p = .003$, $d = 1.826$, 95%*CI* = 63.5, 244.1, and the immediate extinction condition, $t(14) = 3.255$, $p = .006$, $d = 1.627$, 95%*CI* = 30.7, 149.1. Thus, significant renewal of fear was observed in both the delayed and immediate extinction conditions, although slightly not as robust in the immediate extinction condition.

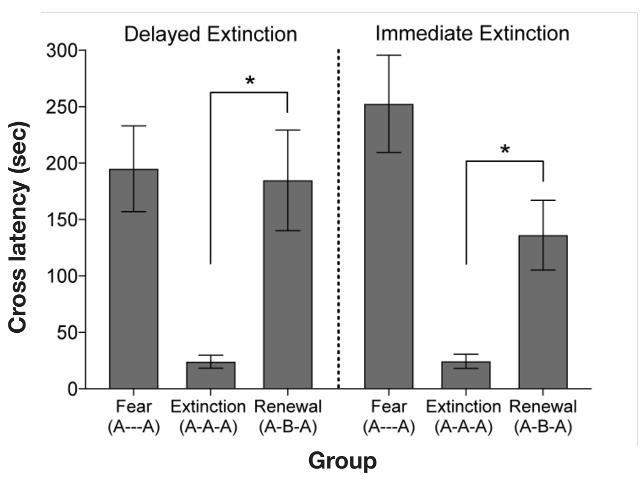

Figure 1. *Mean (± SEM) cross latencies to the black compartment in seconds for all groups. The left panel contains groups from the Delayed (24 hr) Extinction condition. The right panel contains the groups from the Immediate (10 min) Extinction condition. Asterisks represents a significant difference (p < .05) between the Extinction groups and Renewal groups.*

Discussion

The purpose of this experiment was to examine whether immediate extinction would prevent the renewal of fear in a passive avoidance task. The results showed significant renewal of fear in both immediate and delayed extinction treatment conditions. Thus, these results suggest that the mechanisms of immediate extinction may not differ from those of delayed extinction. Moreover, the results presented here indicate that both immediate and delayed extinction involve separate competing memories that are context specific (see Bouton, 2002, 2004).

As mentioned in the introduction, it has been suggested by researchers that immediate extinction disrupts the original association, serving as "unlearning," interfering with the fear memory or disrupting consolidation of original learning (Myers, Ressler, & Davis, 2006). The present results are in contrast with Myers et al. The fact that renewal occurred with a short interval is consistent with the results of Maren and Chang (2006) and Woods and Bouton (2008). For example, Maren and Chang found that rats receiving extinction 15 minutes after conditioning showed more fear (i.e., greater freezing) than those that received extinction 24 hours following conditioning. In addition, Woods and Bouton showed that immediate extinction (10 minutes after training) resulted in greater spontaneous recovery and renewal compared to delayed extinction in both appetitive and aversive tasks. Although our results failed to show elevated renewal of fear following immediate extinction, the current findings did show significant renewal of fear following immediate extinction.

A possible explanation of why immediate extinction failed to attenuate the renewal of fear in the current report, but that a similar short-delayed extinction procedure was found to disrupt both spontaneous recovery and reinstatement using the same paradigm (Briggs & Fava, 2016) may be due to the context specificity of extinction. Evaluating the immediate extinction effects on the phenomena of spontaneous recovery and reinstatement involved the acquisition, extinction, and test phases all occurring in the same context. Thus, when extinction occurred shortly after fear learning while the information was still actively being encoded, the competing inhibitory learning could have interfered with original learning for behavioral control when tested the following day. In the current study evaluating the renewal effect, the extinction phase was conducted in a distinctly different context from the acquisition and test phases. Therefore, although the original learning was still actively being encoded during the extinction phase, the change in context compartmentalized the competing inhibitory event making it less likely to influence the original association at test. This explanation is similar to the dominant trace notion proposed by Dudai (2004; see also Eisenberg, Kobilo, Berman, & Dudai, 2003). However, this explanation of the current results is not sufficient in accounting for other findings where immediate extinction attenuated renewal of fear using various conditioning paradigms (e.g., Myers, Ressler, & Davis, 2006).

It is important to note that the current results were obtained using a consistent extinction-test interval of 24 hours, while manipulating only the acquisition-extinction interval. It should be noted that some have found differential effects of immediate extinction by manipulating post extinction test intervals. Using a conditioned emotional response (CER) paradigm with rats, Johnson, Escobar, and Kimble (2010) evaluated the effects of immediate extinction (using a 10-minute acquisition-extinction interval) compared to delayed extinction (24-hour acquisition-extinction interval) on the spontaneous recovery of fear using various extinction-test intervals. The authors were able to replicate the immediate extinction effect (i.e., less spontaneous recovery of fear) when testing occurred 72 hours after extinction (Experiment 1) and 7 days after extinction (Experiment 2). That is, at both extinction-test intervals the immediate extinction groups showed less spontaneous recovery of fear compared to the delayed extinction groups. However, in the second experiment when the extinction-test interval was shortened to 48 hours the opposite results were obtained. Using the shorter post-extinction test interval resulted in less spontaneous recovery for the delayed extinction condition compared to the immediate extinction condition. Therefore, as the authors point out, the results show that delayed extinction is more beneficial in preventing the spontaneous recovery of fear in the short term and immediate extinction is more beneficial in the long term. Conversely, Maren (2014) describes evidence suggesting that extinction occurring soon after fear conditioning fails to suppress fear long term. Because of these findings, Maren suggests that extinction is most effective when extinction occurs at longer conditioning-extinction delays, possibly due to the arousal levels from fear and the brain mechanisms involved interfering with extinction learning.

Along with determining the mechanisms of shorter acquisition-extinction effects on eliminating fear, evaluating the manipulation of the extinction-test interval has theoretical and clinical importance. Moreover, that immediate extinction has been found to reduce spontaneous recovery, reinstatement, and renewal of fear in some instances while others fail to observe the immediate extinction effects makes investigating the mechanisms of short delayed extinction worthy of further study. Understanding the mechanisms of extinction in attempts to enhance its effects is of clear clinical importance to the treatment of anxiety related disorders.

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