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# Transfer of Extinction Retrieval Cues Attenuates the Renewal Effect in Rats

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**Abstract:** An experiment using rats investigated whether retrieval cues for an extinction memory could also be brought under the control of cues not physically present during extinction learning. Following the extinction of a fear motivated task in a context different from original learning, rats were exposed to the training context either immediately, 30 min, or 60 min after extinction. When tested back in the original context, rats that were exposed to the training context immediately following extinction treated the context as if it had received extinction in that setting, i.e., no renewal. This attenuation of renewal was reduced or eliminated with longer post-extinction delays, which suggests the importance of an active extinction memory during exposure. These findings are consistent with other research examining the transfer of retrieval cues using original memories and old reactivated memories, and in this case demonstrate a similarity between extinction learning with original acquisition.

**Keywords:** Retrieval cues, transfer, memory, renewal effect, context, passive avoidance, rat.

## INTRODUCTION

There is increasing evidence suggesting that extinction is a process that involves new learning and memory, rather than “unlearning” or forgetting of the original learning episode. In extinction, the cues previously paired with a biologically relevant reinforcer (i.e., food or shock) are presented without that outcome, leading to a reduction in responding. Based on his observations of “spontaneous recovery” from extinction, Pavlov [1] was the first to propose that an inhibitory process was involved. That is, the decrement in responding produced by non-reinforcement (the conditioned stimulus being presented without the unconditioned event) reflected the effect of an alternative type of learning, rather than erasure or destruction of an associative bond. More recently, Bouton [2-4] has expanded the view that extinction is not a breakdown of the original association by describing three other phenomena that reflect the persistence of the conditioned response – the reinstatement effect, rapid reacquisition, and the renewal effect. All four outcomes show the persistence of the “extinguished” response.

One phenomenon in particular, the renewal effect (see [5, 6]), clearly demonstrates that extinction involves new learning. The renewal effect can be observed when extinction occurs in a context different from that of training or testing, and subjects are later tested in either the original training context (ABA) or in a third context (ABC paradigm). In these conditions, the original response returns or is “renewed,” presumably because of absence of retrieval cues

associated with extinction. This interpretation is supported by the findings that the renewal effect is attenuated if the appropriate cues are available for extinction memory retrieval (e.g., tested in the extinction context). Brooks and Bouton [7] demonstrated this by providing a cue that was present during extinction learning at the time of testing for renewal. When subjects were returned to the original training context, the presentation of the cue attenuated the renewal effect. Thus, presenting the cue associated with extinction provided the appropriate retrieval cues for the memory of extinction. Using a design modeled after the Brooks and Bouton study, Collins and Brandon [8] extended this finding to social drinking in humans. In a particular context, moderate-to-heavy social drinkers received an extinction-based cue exposure treatment consisting of repeated exposures to cues associated with drinking that typically elicit cravings (e.g., sight of beer and frequently smelling beer). The extinction exposures were administered either in the test context or a distinctly different context. The critical manipulation was the presence of a specific novel “extinction cue” consisting of a weighted textured colorful pencil and neon green clipboard used to complete an urge rating scale. Later, when asked to report on the urge to drink outside of the extinction context those who were provided the extinction cue expressed less of an urge than those who did not have the extinction cue. These results extend the evidence that an appropriate retrieval cue associated with extinction can attenuate the renewal effect. Moreover, as Collins and Brandon point out, this finding emphasizes the role that context has on relapse and cue exposure therapy and suggests possible implications that the renewal effect poses for such treatment. Additionally, other manipulations including massive extinction [9, 10], extinction in multiple contexts [11, 12], and using an explicitly unpaired procedure [13, 14] have been shown to attenuate the renewal effect.

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That there may be other ways to attenuate or eliminate the renewal effect is suggested by several recent studies on the transfer of contextual retrieval cues. Briggs, Fitz, and Riccio [15] have shown that neutral contextual (i.e., environmental) stimuli that were not present at the time of training can gain control over responding if presented shortly after a learning episode. Taking advantage of the context shift effect, in which performance is impaired when subjects are tested in a context that differs from training, Briggs *et al.* exposed rats to a new context immediately after fear conditioning. These rats showed markedly less impairment of performance than non-exposed controls when later tested in the new context. This attenuation of the context shift effect indicated that the active memory representation became associated with the novel context. Furthermore, this transfer of retrieval cues was shown to be time dependent, in that the manipulation was less effective as the interval increased between training and exposure. This time dependent function, consistent with evidence from the retrograde amnesia literature [16, 17], demonstrates that active memory representations are relatively transient. In a subsequent study, Briggs and Riccio [18] found a similar transfer effect when an older memory, whose representation was “reactivated” by presentation of the training cues, was then exposed to the new target context.

Given the findings of transfer of retrieval cues and those suggesting that the renewal effect is caused by a lack of appropriate retrieval cues for extinction, the present experiment was designed to investigate whether contextual retrieval cues for an extinction memory could also be brought under the control of contextual cues not physically present during extinction learning. To evaluate any transfer of extinction cues, the renewal paradigm was employed. Thus, if transfer of extinction cues does take place, then the renewal effect should be diminished or eliminated. Additionally, if transfer of extinction information is a time dependent phenomenon, as is the case with transfer of contextual control over excitatory conditioning, then a delay between extinction and the contextual exposure should reduce or eliminate the transfer effect. Accordingly, a second aim was to determine if the transfer of extinction cues shows a temporal gradient.

## METHOD

### Subjects

The subjects were 60 experimentally naïve, adult male Long-Evans hooded rats, approximately 85 days of age, purchased from Harlan (Indianapolis, IN). Rats were individually housed with free access to food and water, and were maintained on a 15/9 hr light/dark cycle. All experimental sessions took place during the light portion of the photocycle and at the same time each day. The animals were maintained in our colony for a minimum of two weeks before being used.

### Apparatus and Contexts

Training, extinction, and testing were conducted in two identical 43 X 18 X 18 cm black–white shuttle boxes with grid floors (2 mm grids spaced 1 cm apart center to center). Each shuttle box was divided into two compartments of

equal size by a guillotine door. The exposure chamber was a 22 X 22 X 23.5 cm box made of clear Plexiglas walls and lid. The chamber was placed near the shuttle box in each context during exposure.

The two shuttle boxes were located in separate rooms that served as contexts. Context A was a 1.62 X 2.33 m room with white walls and scented with Airwick Wizard® air freshener with Country Berries® scented oil. White noise (76 dB) was presented at all times in this context. The room was illuminated by a 25 W red light bulb above the shuttle box. Context B was a brightly lit 1.83 X 2.74 m room with white walls. Posters were placed on each wall to provide visual cues. This room was illuminated by fluorescent houselights. The context was not artificially scented, and no white noise was present.

### Procedure and Design

Prior to the beginning of the experiment, all subjects were handled for 2 min on three consecutive days and were then randomly assigned to one of six conditions ( $n = 10$  per group). Each rat received a single punishment training trial in either Context A or Context B. Assignment to the contexts was counterbalanced such that within each group five rats were trained in Context A and five in Context B. For simplicity of exposition, we refer to context shifts (A to B) generically, regardless of the actual context used.

At training, the rat was brought into the context (A) on the experimenter’s arm and remained there for 15 sec to provide brief exposure to the context. The rat was then placed in the white compartment of the shuttle box facing away from the closed guillotine door. After 15 sec, the guillotine door was raised and the latency to cross into the black compartment (all four paws) was recorded. The door was then lowered and two inescapable footshocks (1 sec, 0.5 mA) were delivered 5 sec and 10 sec after the door was lowered. Five seconds after the last footshock, the animal was removed and returned to its home cage.

Twenty-four hours after training, five groups received a single extinction session in the context that differed from training (B). Prior to the extinction session, rats received a 1 min probe trial to assess fear to the novel context (B). The probe trial was similar to the training described above, except that the probe trial lasted 1 min and no shocks were administered. On each probe trial, the rat was placed in the white compartment of the white–black shuttle box facing away from the closed guillotine door. After 15 sec, the guillotine door was raised. The probe trial lasted for 1 min once the guillotine door was opened allowing the rat to enter the black compartment. Latency to cross to the black side of the shuttle box and the total time spent on the white (safe) side (TTS) were recorded.

After 1 min, the extinction session was begun. Regardless of which compartment the animal was in, it was removed and was immediately placed into the black compartment for 9 min. The rats were not able to cross between compartments, as the guillotine door was closed. No shocks were delivered during this period of time.

Following extinction, three transfer groups (Tran-0, Tran-30, and Tran-60) received exposure to the original

training context (A). Exposure consisted of bringing the rat into the training context (A) and placing it into the white compartment of the shuttle box with the guillotine door closed for 15 sec in an attempt to maintain the activity of the extinction learning. After 15 sec of exposure to the white side, the rat was removed and placed in the clear Plexiglas chamber for 4 min 45 sec in order to expose the rats to the contextual cues. Following the total of 5 min exposure treatment, the rat was returned to its home cage. Group Tran-0 received exposure immediately following extinction. Groups Tran-30 and Tran-60 received exposure treatment 30 min and 60 min following extinction, respectively, to assess whether transfer of extinction cues was less effective following a delay in exposure to the target context.

To control the possibility of the exposure alone producing new learning or acting as an extinction session, an exposure-only control group (Exp Only) did not receive the extinction session in the shifted context (B), but received the exposure to the training context (A) 24 hours after training.

To assess the renewal effect, two groups (Renewal and Ext) did not receive the exposure treatment to the training context after extinction. Following training (A) and extinction (B), these groups were merely tested either in the same context (A) as training (Renewal) or in the same context (B) as extinction (Ext). Although the absolute level of extinction is not known in the absence of a group trained, extinguished, and tested all in the same context, the central question addressed by the present design concerns the presence or absence of the renewal effect.

Twenty-four hours after extinction/exposure, all groups underwent a 10 min passive-avoidance test in which the rat was placed on the white side and allowed to cross into the black compartment. The cross-through latency and total time spent on the white (safe) side (TTS) were recorded as the dependent measures. The exposed groups (Tran-0, Tran-30, and Tran-60) and expose only control group (Exp Only) were tested in the original training context (A). Thus, with the exception of the extinction control group (Ext), all groups were tested in the original training context (A). Testing was identical to training trials, except that no shocks were delivered and the guillotine door remained open. The design of the experiment is summarized in Table 1.

**Table 1. Experimental Design**

Group	Training	-24 hr-	Extinction	Delay	Exposure	-24 hr-	Test
Renewal	Context A		Context B	-	No		Context A
Ext	Context A		Context B	-	No		Context B
Tran-0	Context A		Context B	0 min	Context A		Context A
Tran-30	Context A		Context B	30 min	Context A		Context A
Tran-60	Context A		Context B	60 min	Context A		Context A
Exp Only	Context A		No	0 min	Context A		Context A

Note: Contexts A and B were counterbalanced within each group.

## RESULTS

### Training

Rats in all six groups exhibited short cross latencies at training with group means ranging from 10.2 sec to 11.7 sec. An analysis of variance (ANOVA) performed on training cross latencies revealed no differences between the six groups ( $F(5, 54) = .08, p > .50$ ).

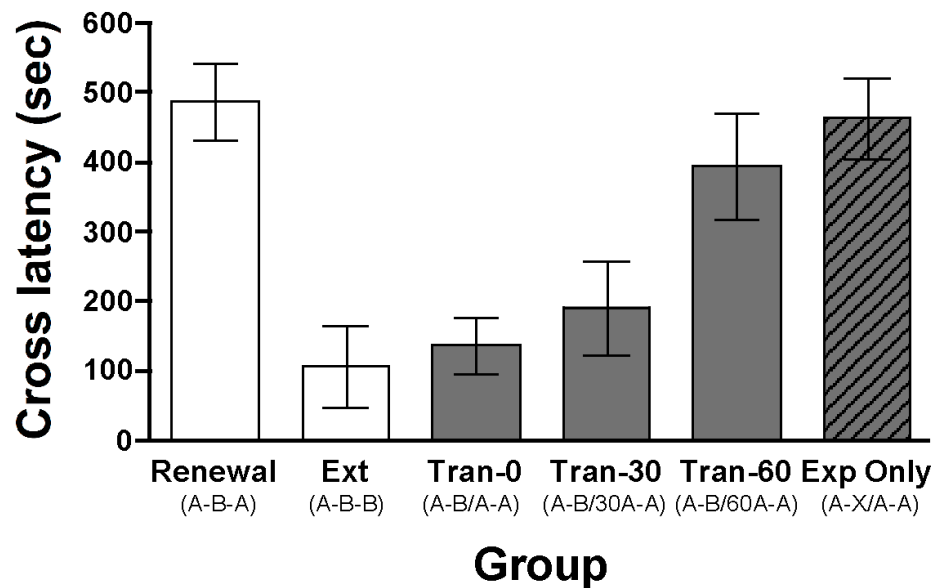
### Counterbalancing

There were no differences between any groups in training cross latencies, cross latencies during the extinction probe and at test, as well as no differences between TTS scores during extinction and test in either context. Accordingly, the contexts were collapsed within each group for all further analyses.

### Extinction: Cross Latency and TTS

All five groups that received the extinction probe trial appeared to have an insignificant amount of fear (short latencies) with little variation between groups. These results were confirmed by an ANOVA that revealed no differences between groups (cross latency –  $F(4, 45) = .79, p > .50$ ; TTS –  $F(4, 45) = .76, p > .50$ ).

Animals in all five extinction groups crossed into the black compartment under the 1 min ceiling during the extinction probe. Because extinction took place in a context different from training there appears to be a context shift effect, whereby there is a tendency for performance to be impaired when the test context differs from that of training [19-22]. Thus, all groups showed little fear of the black compartment when probed in the novel context. Despite the apparent lack of fear during the extinction session, there still appears to be an effect of the extinction exposure on the training memory when rats were tested in either the original training context or in the extinction context (see Fig. 1 for test cross latency results). That the extinction session modified a memory that appeared not to be active due to a change in context appears to contradict the usual view of memory processing, in which memories are only modifiable when they are active. However, research has shown that extinction can modify a fear memory that is amnesic or inaccessible [23]. Thus, although the fear was not expressed as a result of



**Fig. (1).** Mean latency to cross from the white compartment to the black compartment in seconds for all groups. Error bars represent standard errors of the means. Renewal and Ext groups (open bars) represent the renewal effect. The shaded bars represent the experimental groups. The Tran-0 group received exposure to the original context immediately following extinction in the shifted context and was tested back in the original training context. Group Tran-60 demonstrates that with longer extinction to exposure delays the transfer of extinction cues is less effective. Group Exp Only (shaded slashed bar) shows that the exposure alone did not act as new learning or an extinction session.

the lack of appropriate retrieval cues when probed before extinction in the shifted context, the training memory was in an active and liable state and extinction did have an effect as is evident in the test cross latency results.

#### Testing: Cross Latency

Fig. (1) shows the mean cross latencies for all six groups at test. As can be seen, the Renewal group ( $M = 488$ ,  $SEM = 55.88$ ) exhibited a considerable amount of fear (long cross latency) compared to the extinction (Ext) group ( $M = 107$ ,  $SEM = 57.08$ ), which demonstrates the renewal effect. The groups that were exposed to the shifted context either immediately following extinction (Tran-0;  $M = 138$ ,  $SEM = 40.29$ ) or 30 minutes after extinction (Tran-30;  $M = 191$ ,  $SEM = 66.33$ ) showed fear similar to the Ext group and less than the Renewal group. This transfer of extinction cues appears to be time dependent. The group that was exposed to the training context 60 min after extinction (Tran-60;  $M = 359$ ,  $SEM = 77.13$ ) showed as much fear as the Renewal group and more than both the shorter delay groups (Tran-0 and Tran-30) and the Ext group. The exposure only control group (Exp Only;  $M = 464$ ,  $SEM = 58.81$ ) demonstrated that the exposure to the original training context alone did not contribute to the reduction of fear (short cross latencies), as they showed as much fear as the Renewal group and the Tran-60 group.

These results were confirmed by an ANOVA that revealed an overall significant difference between groups ( $F(5, 54) = 8.05$ ,  $p < .001$ ). A Tukey post-hoc test confirmed that the Renewal group showed more fear compared to the extinction (Ext) group ( $p < .01$ ). Post-hoc tests also confirmed that the Tran-0 group that was immediately exposed to the training context following extinction showed the same low level of fear as the extinction (Ext) group ( $p > .50$ ), as well as significantly less fear than the Renewal group

( $p < .01$ ). Tukey's post-hoc tests also revealed that the group that received the exposure to the training context 30 min after extinction (Tran-30) showed the same low level of fear as the immediately exposed (Tran-0) group and the extinction (Ext) group ( $p$ 's  $> .50$ ). The temporal gradient was confirmed by post-hoc tests revealing that the group that received the exposure to the training context 60 min after extinction (Tran-60) had significantly more fear than both the Ext group and the Tran-0 group ( $p$ 's  $< .05$ ). Group Tran-60 showed as much fear as the Renewal group ( $p > .50$ ). Post-hoc tests also confirmed that the exposure alone did not cause a decrease in fear at test by revealing that the exposure alone (Exp Only) group showed as much fear as the Renewal group ( $p > .90$ ) and significantly more fear than the Ext group ( $p < .01$ ).

#### Testing: TTS

Although the TTS measure produced a pattern similar to the cross latency measure and an ANOVA confirmed an overall significant difference between the groups ( $F(5, 54) = 3.72$ ,  $p < .05$ ), Tukey post-hoc tests failed to reveal any significant differences between them. As the groups showed more fear overall than was seen in the latency measure, the lack of significant differences between any groups is likely due to the increase in TTS scores for the Ext and transfer groups, producing a ceiling effect. One reason for the increase in fear (long TTS scores) may have been that upon crossing into the black compartment the rats were reminded of the shock experienced during training. Thus, reexposure to the cues associated with shock reminded them of training, which increased the likelihood of the avoidance of the black (unsafe) compartment and led to an increase in the total time spent on the white (safe) side (see [24, 25], for a similar finding and interpretation of higher fear scores with the TTS measure).

## DISCUSSION

The findings presented here provide evidence that exposing rats to the fear-conditioning context shortly after an extinction exposure in a different context serves to attenuate the renewal effect. Thus, background contextual stimuli not present at the time of an extinction exposure can later act as retrieval cues that extend the effectiveness of extinction to an otherwise ineffective context. As we did not have an online measure of extinction, one question that might arise is whether the poor performance in the Ext condition (A-B-B) merely reflects a context shift effect or generalization decrement, rather than extinction of the fear response. However, if extinction had not occurred then the fear response should have remained strong in the transfer groups tested in the training environment. But as the data indicate, the renewal effect was eliminated or attenuated in the groups exposed to the training context immediately or 30 minutes after their CS no-shock exposure in context B, reflecting the transfer of an extinction effect. (It should be noted that the renewal effect itself can be viewed as a context shift effect, but one that occurs after extinction rather than after acquisition.)

Independently, and using rather different methods, Richardson's lab (R. Richardson, personal communication, June 19, 2007) has recently replicated the pattern of outcomes described here. One interpretation of the time dependent outcome is that exposure to the original training contextual cues while the extinction memory is active allows the information to become encoded with those contextual cues. Thus, when subjects are placed in the original training for testing (A) the memory for extinction is retrieved, and less fear is expressed. However, as the delay between extinction and exposure to the target context increases, the level of activity of the memory representation presumably decreases, resulting in less re-encoding of extinction information.

The issue of specificity was not addressed directly in this particular study. That is, was the transfer effect obtained specific to returning the rats to the training context following the extinction treatment, or might the same outcome have been found if rats had been exposed to an irrelevant context (C) and then tested in the training context (A)? The experimental design did not include such a potential control because, in two other studies examining transfer of retrieval cues following fear conditioning, we found no evidence that exposure to an irrelevant context influenced performance in the test context [15, 18]. While it is possible that learning of extinction is not affected in the same way, given other similarities between extinction and acquisition it seems highly likely that the transfer is context specific. This, however, remains to be tested. Moreover, further research is needed to determine whether the transfer of extinction demonstrated here had any effect on performance in the actual extinction context (B). That is, did the transfer of memory for extinction result in a loss of extinction to the context in which non-reinforced exposures had been given, a kind of "erase and update" effect? Recent research investigating the transfer of original memory retrieval cues to a novel context demonstrated that such transfer had no effect on the original memory [26]. Thus, using the current paradigm, testing the rats back in the extinction context following the exposure

treatment would determine the fate of the extinction retrieval cues.

These results parallel findings that a memory representation for original acquisition can be transferred to a new context [15, 27, 28], see also [18]). The findings presented here are also consistent with Brooks and Bouton's [7] experiments demonstrating that the renewal effect is the result of a retrieval failure from the absence of extinction cues outside the extinction context. Although a number of different studies have obtained the renewal effect, the evidence in our controls of renewal in a passive avoidance task adds further to the generality of the phenomenon.

Just as the severity of retrograde amnesia is inversely related to the interval between training and the amnesic treatment [17, 29, 30], these results indicate that memory of extinction-related cues is more malleable shortly after the non-reinforced exposure than after a delay: the attenuation of renewal was greatest with the immediate exposure. A further similarity is provided in a recent study examining retrograde amnesia for extinction. Assuming that extinction is another type of learning, Briggs and Riccio [31] showed that, as with acquisition, retrograde amnesia could be induced in a time-dependent manner following an extinction treatment. Moreover, the amnesia could be reversed by re-application of the amnesic agent (hypothermia) shortly prior to testing, which suggests that the memory loss was based on a retrieval failure (for review, see [32]). Presumably, processing of the extinction episode continued for a brief period and became associated with, or embedded in, the amnesic state. Returning that salient internal context then permitted retrieval of the extinction information. Conceptually, the exposure to the original training context (A) in the present study can be viewed as analogous to exposure to the amnesic context, where testing in context A can then provide the cues necessary for retrieval.

The attenuation of renewal reported here bears upon a question of importance to clinical investigators: given the robust nature of renewal, how does therapy provided in one (limited) context extend to other situations in daily life? Although "stimulus generalization" is often mentioned as the basis for such transfer, an intriguing possibility is that the processing of the treatment (such as extinction) persists in time and can become linked with other, new contexts outside of the treatment situation.<sup>1</sup> Such a transfer of retrieval cues could provide a potent counterforce to reduce renewal effects.

## AUTHORS NOTE

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<sup>1</sup>We thank Dr. Rick Richardson for calling this general implication to our attention.

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