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Contextually Modulated Avoidance Behavior in Rats Post-Pavlovian Extinction

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by

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Abstract

The following study sought to examine the psychological substrates of renewal (e.g.., context dependent extinction processes) for conditioned avoidance behaviors in rats. Using signaled active avoidance conditioning, rats acquired two-way shuttle responding, to two different auditory stimuli. These behaviors were then extinguished through exposure to the auditory stimuli where shuttling behavior was now without consequence. Subjects were then tested for renewal of avoidance in three distinct renewal sequences (e.g., ABA vs ABB, AAB vs AAA, and ABC vs ABB) in three separate groups of rats. It was found that subjects showed more responding to a stimulus presented outside of its extinction context compared to control tests where the cues were presented in the extinction context. This study furthers our understanding of the psychological representation of extinction as it relates to the effects of contextual modulation upon renewal of avoidance behavior.

Keywords: signaled active avoidance, renewal, extinction, context

Contextually Modulated Avoidance Behavior in Rats Post-Pavlovian Extinction

Behavioral studies have well-established the renewal effect, a robust phenomenon in which a context change following extinction can cause return of the conditioned response (Bouton, 2004). This renewal effect was borne from the study of standard Pavlovian conditioning, a well-studied form of learning, with behavioral processes that have been traced to mammalian survival circuits (LeDoux, Moscarello, Sears, & Campese et al., 2017). During Pavlovian fear conditioning, a formerly neutral stimulus (e.g., auditory tone) becomes threatening when it is repeatedly paired with an inherently aversive, unconditioned stimulus (e.g. footshock), thus establishing a CS-US association. (Bouton, 2004). During Pavlovian extinction, the CS is presented in absence of the US, thereby extinguishing the conditioned stimulus' ability to elicit a defensive (or fear) response (LeDoux et al, 2017). The renewal effect comes into play post-extinction, and is achieved when the former CS is reintroduced with a change in context relative to that of the extinction phase, and a marked return to conditioned responding is observed (Bouton, 2004). The effect of context in recovering extinguished responding has contributed to a substantial, yet mixed literature regarding the psychological processes which attenuate conditioned behavior (Delamater, 2004). As it relates to the extinction context, there are several versions of the renewal effect (e.g., ABA renewal, or ABC renewal: where letters denote physical training contexts across phases of a study) from which various, but not necessarily competing, explanations for the return of the conditioned response have been purported (Bouton, 2004).

Arguably, the most contentious aspect of extinction regards what kind of learning occurs during this phase of conditioning. Specifically, researchers have expressed competing accounts

and conceptualizations of whether the CS-US association is unlearned during extinction, or if a new, inhibitory CS-no US association is made during extinction, instead (Bouton, 2004; Delamater, 2008; Campese & Delamater, 2013). As it stands, the renewal effect serves as evidence for the latter explanation, which purports that extinction does not result in unlearning or the loss of the CS-US association, but that a new, highly context-dependent inhibitory CS-no US association is made, instead (Bouton, 2004). The context-dependent nature of renewal is evident in both the ABA and ABC renewal paradigms (Bouton, 2004; Campese & Delamater, 2013; Thrailkill & Bouton, 2015). In ABA renewal, a behavior is acquired in one context (A), extinguished in a second context (B), and then tested for renewal in context A (Bouton, 2004). Researchers have found that ABA renewal evidences a strong "occasion-setting" mechanism, such that animals demonstrate robust renewal of conditioned responding when tested outside the extinction context (Bouton, 2004; Bouton & Ricker, 1994; Campese & Delamater, 2013; Thrailkill & Bouton, 2015). The return of conditioned responding implies that the original CS-US association is maintained, and also gives reason to attribute this renewal effect to a release from the extinction context (Bouton, 2004). According to Campese and Delamater (2013), the renewal effect not only indicates that the excitatory properties of the CS have been preserved during extinction, but also suggests that testing within the extinction context (e.g. ABB renewal) mitigates the renewal effect because the inhibitory CS-US association made during extinction is retrieved, rather than the excitatory CS-US formerly acquired. For these reasons, testing within the extinction context generally results in low levels of responding. Whereas testing outside the extinction context generates a renewal effect by removing the inhibitory control over responding

acquired by the extinction context during extinction training, thus endorsing the occasion-setting mechanism underlying extinction (Campese & Delamater, 2013)

In addition to the renewal effect, there are several post-extinction testing phenomena which further support the explanation that extinction does not result in unlearning, but actually generates new, context-dependent learning (Bouton, 2004). Among these phenomena are spontaneous recovery, generalization decrement, reinstatement, and resurgence (Trask, Schepers, & Bouton, 2015).

Spontaneous recovery shows that the passage of time can facilitate reemergence of the extinguished behavior similarly to changes in context (Rescorla, 2004; Bouton, 2004). Moreover, there is evidence that manipulation of both physical and temporal context factors can aggregately produce a more substantial recovery effect than manipulation of either context or time alone (Bouton, 2004). Spontaneous recovery also provides evidence that original conditioned learning associations "survive" extinction, and one can conclude that the CS takes on a newly learned inhibitory association in the extinction context (including the temporal or internal elements of what can be loosely defined as context: see Bouton, 2004), an association that is in direct conflict with the excitatory CS association formed during acquisition (Rescorla, 2001).

Second, generalization decrement, a pattern during extinction in which the animal ceases generalization between stimuli presented during conditioning and those presented during extinction, also provides support for new learning and context-dependence (Bouton, 2004). Considering generalization decrement assumes that the animal has learned that the CS means there is no US in the extinction context, it serves as support to the theory that extinction does not result in unlearning, but is actually results in new, inhibitory learning (Bouton, 2004)

A third recovery phenomenon, known as reinstatement, perhaps most simply supports the 'new learning' during extinction account. Reinstatement is said to have occurred if the formerly extinguished response is recovered when the animal is presented following presentations of the US alone. Interestingly, reinstatement is context specific, in that the effect is only achieved when the US-alone presentation is done in the acquisition context (Bouton, 2004). This has led to the suggestion that time, internal state and physical surroundings can all be understood as a constellation of contextual elements that make up the extinction experience (Bouton, 2004). In addition to Pavlovian renewal of behavior, some researchers have found that avoidance behavior -- understood as a coping mechanism -- is also subject to this renewal effect, although renewal of avoidance is largely unexplored (LeDoux et al, 2017; Nakajima, 2014) While signaled active avoidance (SigAA) and Pavlovian fear conditioning are both fear-related behavioral learning models and share commonalities -- implication of the amygdala, the "fear center" of the brain, as well as the hippocampus, associated with emotional memory -- these behavioral paradigms are distinct, and research on renewal of avoidance is both less abundant than that of standard Pavlovian conditioning (LeDoux et al, 2017; Nakajima, 2014). Considering different iterations of context renewal sequences (e.g., ABA, AAB, ABC) have demonstrated that release from the extinction context is critical in producing renewal of a Pavlovian and instrumental/operant conditioned response at test, Nakajima (2014) decided to explore this extinction context-modulated effect upon renewal of avoidance behavior.

Using signaled active avoidance (SigAA) -- an animal model of coping in which the animal learns that the presence of one stimulus cues the incoming of another stimulus -- Nakajima (2014) found that renewal of avoidance behavior was exhibited only when animals

were tested outside, or “released from” the extinction context (e.g., ABA, ABC, AAB; LeDoux et al, 2017). Thus, signaled active avoidance (SigAA) is also subject to extinction context-dependent renewal effects (Nakajima, 2014).

In general, the renewal effect of behavior has led scientists to pursue a greater understanding of the underlying psychological and neurobiological processes of extinction (Delamater, 2004). Nakajima’s (2014) identification of circumstances under which renewal of avoidance is contingent further implicates the importance of understanding why and how the extinction context -- or, more specifically, release from the extinction context -- can modulate the renewal of avoidance behavior. Studying renewal of avoidance using an animal model also has clinical implications and human applications (Nakajima, 2014). Furthering our understanding of extinction-context modulated renewal of avoidance could potentially identify and clarify under which environmental/contextual conditions human avoidance-related behaviors (e.g., relapse) are expected to be strongest. For example, a better understanding of renewal of avoidance as it relates to the extinction-context could help elucidate why people who undergo narcotics detoxification and rehabilitation in an inpatient clinic are so prone to relapse upon release from treatment. Moreover, a better understanding of extinction-context dependent processes and their impact on the renewal of maladaptive behaviors could help inform outstanding avoidance behavior-related therapies (e.g., cognitive behavioral therapy, or CBT) and potentially lead to the creation of new, empirically supported treatments for clinical disorders such as anxiety, OCD, drug addiction, substance abuse, and PTSD, too (LeDoux et al, 2017; Bouton & Trask, 2016). Understanding just how critical the extinction context is upon renewal of avoidance behavior could help prevent recidivism of avoidance behaviors, which were extinguished in a therapeutic

context. Thus, the examination of emotional fear learning in rats using signaled active avoidance informed by a Pavlovian signaling contributes to a better understanding of human avoidance and its contextually-modulated contingencies.

The following study sought to expand upon the current literature on renewal of avoidance achieved through signaled active avoidance learning (SigAA). Expanding upon Nakajima's (2014) "Renewal of signaled shuttle box avoidance in rats," we employed a highly controlled and counterbalanced design and utilized two distinct auditory stimuli during training to control for possible generalization effects. This was done to address the possibility that the renewal effects observed in Nakajima's study were due to simple context-US learning, rather than conditional control of learning by the extinction context. For example, Nakajima trained subjects in one context, and extinguished them in a different location. Therefore, US presentations could have easily produced excitatory context-US associations for the acquisition, but not the extinction context. Subsequent tests that show more responding where there had been a history of US presentations are difficult to interpret and classify as renewal. In contrast, the procedure described below used a design that balanced contexts so that each test location had been equally as often served as an acquisition and extinction location. Renewal under these circumstances can be more safely interpreted as selective CS-US meanings based on location. Given these points, we expected the following outcomes: first (H1), we expected that testing outside the extinction context would yield the strongest renewal of avoidance behavior. Second (H2), we expected that conditioning, extinction, and renewal of avoidance behavior would be similar for both audio stimuli (Tone or Noise). Third (H3), we expected that all renewal preparations (ABA, AAB, ABC) would evidence renewal of avoidance behavior such that ABA renewal would be the most

robust, followed by AAB, and, finally, ABC. Fourth (H4), during acquisition training, we expected that avoidance behavior would significantly increase from day 1 to day 5. And lastly (H5), during extinction training, we expected that avoidance behavior would significantly decrease from day one to day 5.

Method

Subjects

Forty-eight male Sprague-Dawley rats were obtained for the purposes of this experiment. Rats were bred at Hill Top Lab Animals (Scottsdale, PA, USA) and weighed between 250 and 300 grams upon arrival in the lab. Rats were housed individually in ventilated, free-hanging plastic tubs and provided with free water and standard lab chow. The rat colony was maintained at 70 degrees Fahrenheit and kept on a 12-hour light/dark cycle. This study was conducted in compliance and according to the guidelines of Guide to the Care of the Use of Laboratory Animals of the National Institutes of Mental Health. Animal use protocol was also approved by the New York University Animal Welfare Committee.

Materials

Shuttle box. All phases of the study were conducted using two-way shuttling chambers (Coulbourn model: H10-11R-SC; Allentown, PA) manufactured by Coulbourn Instruments (5583 Roosevelt St, Whitehall, PA 18052). Over the course of the experiment, these chambers were manipulated to form distinct contexts in order to study how context contributes to avoidance behavior. Each rectangular shuttle box was constructed of Plexiglass in the front and back and metal on the sides (50.8 x 25.4 x. 30.5 cm; length x width x height) and were divided in half along the length of the chamber. The front and back walls were made of clear plexiglass and

the side walls were made of a metal alloy. A metal divider with a “rat-sized” opening (8 x 9 cm, width x height) cut in the center was constructed along the midline of the box, allowing the rat to move freely from side to side. The original shuttle box floor consisted of a series of conducive stainless steel bars.

Each shuttle box was contained within a sound-insulating chamber (Coulbourn Instruments,Whitehall, PA, USA). Two speakers were mounted on opposite sides of the metal walls and delivered a 5 kHz tone stimulus, or a white noise. A Coulbourn Precision Animal Shocker (model H13-15-220; Allentown, PA) delivered a 0.7 mA shock to the steel grid floors. Each chamber compartment was lit by two .5W light bulbs on the top of the chambers.

Shuttle behavior (movement through the midline to the other side of the shuttle box) was registered by two infrared arrays. Each array was comprised of 5 emitter-detector pairs and located on either side of the midline divider.. A PC running GraphicState (Coulbourn Instruments, Whitehall, PA, USA) software was used to deliver stimuli and collect data.

Acquisition, Extinction & Test Contexts. Two rooms, each containing 4 shuttle boxes (8 shuttle boxes total) arranged in similar fashions, were used to train, extinguish, and measure signaled-active avoidance behavior during the study. Distinct contexts were made for this experiment by manipulating these chambers’ tactile, visual, and olfactory attributes. This was done in a way that produced a total of three different context arrangements that were used to study different forms of context dependent extinction. When modified to produce a distinct context, printed patterned paper (e.g., checkers, circles) was placed outside the Plexiglass walls. Additionally, potent hand soap (Dr. Bronner’s: approximately 5 mL, either peppermint or lavender) was added to the waste trays to further distinguish the chambers from one another.

Solid plastic floor inserts were also used as needed. More specifically, ABA, AAB, and ABC renewal were measured in 3 separate groups of rats using a mixture of the alterations described above over the acquisition, extinction, and test phases of the study.

General Procedure

The study consisted of 3 phases: 1) SigAA acquisition, 2) Pavlovian extinction, and 3) test for SigAA behavior. Each subject was given training experience with two stimuli, Noise and Tone, and the capacity for each cue to elicit avoidance responding was evaluated and compared in two different test locations. The particular arrangement used here produced 3 groups referred to as ABA, AAB, and ABC. These letters denote the context locations where the different phases of the experiment took place for each group as a function of the stimulus history across the study. For example, the ABA group experienced conditioning of avoidance for each stimulus in distinct contexts (e.g., CS1 in context 1 and CS2 in context 2). In contrast, extinction occurred in in the opposite context (e.g., CS1 in context 2 and CS2 in context 1). Subjects were tested with each cue in both locations, so that each cue served the ABA as well as the control ABB role (e.g., CS1 is ABA context 1 and ABB in context 2, while CS2 is ABA in context 2 and ABB in context 1). In other words, because the studies were run using a within-subjects approach, these arrangements actually applied to two different stimuli for each group in a highly controlled way (See Table 1); this will be described in more detail below.

INSERT TABLE 1 HERE

Acquisition. During SigAA training, an auditory stimulus (Noise or Tone in different sessions) was presented after a 5 min baseline to signal an ensuing footshock. Following an inescapable first trial, rats learned that a shuttle response through the midline of the box in

response to the auditory stimulus (CS) would result in termination of the auditory stimulus and prevention of the scheduled footshock (US). If the rat failed to shuttle during the 15 s auditory stimulus (tone or noise), then the the scheduled footshock was delivered, which lasted a maximum of 15 s. In total, each SigAA training session consisted of 1 Pavlovian acclimation trial, 30 CS trials, with an inter-trial-interval (ITI) that averaged 120 s; a single session lasted no more than one hour and 20 minutes.

Rats were trained to avoid shock over a period of 5 days and were subject to 2 acquisition training sessions each day; for the ABA and AAB groups, these sessions were in different contexts. For the ABC group they were in the same space. In all cases, at least 2 hours of rest time in the rodent colony was interpolated between these sessions. The order of these sessions was alternated so that each day began with a different cue-context association.

Extinction. Extinction training was executed in a fashion similar to acquisition training, but with a few modifications. Rats underwent extinction training over a period of 5 days and were subject to 2 extinction training sessions each day, one in each training context. There was at least 2 hours of rest time between sessions, as in acquisition training. Extinction training schedule followed the same schedule as acquisition training. As in acquisition training, audio stimulus exposure and context was counterbalanced by training day. Extinction protocols were similar to acquisition protocols, but with two critical differences: first, when the 15 s audio stimulus (Tone or Noise) was presented, it was not followed with a scheduled footshock (US) and, secondly, shuttle behavior did not terminate the audio stimulus In total 30 CS-no US trials, with an inter-trial-interval (ITI) that averaged 120 s; a single extinction session lasted no more

than one hour and 20 minutes. During extinction, each group had each cue extinguished in a different location.

Test. Rats were tested in two separate sessions following extinction training. One was conducted 24-hours after extinction concluded, and the other a week later, to encourage response recovery. Each cue was tested in each test session with a block of 15 trials before moving to the other cue. The stimulus testing order was counterbalanced across test context. For the subsequent test session, the subject was placed in the alternative context and presented with the stimuli a second time, in the opposite order, in a counterbalanced fashion across a given group. The testing protocol was similar to the extinction protocol in that there were 30 CS-no US trials, with an inter-trial-interval (ITI) that averaged 120 s. However, one-half of the trials delivered a tone stimulus, and the second-half delivered a noise stimulus. Additionally, a single session lasted no more than one hour and 20 minutes.

Results

Acquisition

A 3 x 2 x 5 mixed factorial ANOVA was conducted to compare renewal type, audio stimulus-type, and training day and the interaction of the aforementioned variables upon fear behavior -- measured as number of shuttle responses -- during the acquisition phase of training. The ANOVA consisted of the following variables: 1) between-subjects variable of renewal type (3 levels: ABA, AAB, ABC), 2) within-subjects variable of audio stimulus-type (2 levels: Tone, Noise), and 3) within-subjects variable of acquisition training day (5 levels: Day 1, Day 2, Day 3, Day 4, Day 5). Unless otherwise noted, all effects are reported at $p < 0.05$.

There was no significant main effect of renewal type, indicating that the number of shuttle responses was similar during acquisition training across each renewal type, $F(2, 45) = .410$, $p > .05$. However, there was a significant main effect of audio stimulus-type, $F(1, 45) = 51.754$, $p < .001$. Simple contrasts revealed that rats, in general, shuttled significantly more in response to the audio stimulus-type noise ($M = 21.033$) than they did to tone ($M = 14.7130$). Training day also yielded a significant main effect, $F(4, 180) = 11.372$, $p < .001$ (See Figure 1). Review of Bonferroni-adjusted pairwise comparisons indicate that the number of shuttle responses significantly increased from acquisition training day 1 to training day 5. Simple contrasts of acquisition training demonstrated that shuttling responses increased most between day 2 ($M = 14.01$) and day 5 ($M = 19.698$) of acquisition training, $F(1, 180) = 46.906$, $p < .001$. Of the four potential interaction effects, only one was significant: there was a significant two-way interaction effect of stimulus type x training day, $F(4, 180) = 6.556$, $p < .001$. Further inspection of Bonferroni-adjusted pairwise comparisons and estimated marginal means indicated that, on average, rats shuttled more in response to noise than they did tone on each training day. Interestingly, this shuttle response increase was not linear according to training day.

Extinction

A 3 x 2 x 5 mixed factorial ANOVA was conducted to compare renewal type, audio stimulus-type, and training day and the interaction of the aforementioned variables upon fear behavior -- measured as number of shuttle responses -- during the extinction phase of training. The ANOVA consisted of the following variables: 1) between-subjects variable of renewal type (3 levels: ABA, AAB, ABC), 2) within-subjects variable of audio stimulus-type (2 levels: Tone,

Noise), and 3) within-subjects variable of extinction training day (5 levels: Day 1, Day 2, Day 3, Day 4, Day 5). Unless otherwise noted, all effects are reported at $p < 0.05$.

There was no significant main effect of between-subjects variable of renewal type $F(2, 45) = .682$, $p > .05$. In contrast, there was a significant main effect of audio stimulus-type $F(1, 45) = 65.452$, $p < .001$. An examination of both estimated marginal means and simple contrasts further detailed that rats during extinction training shuttled more in response to noise ($M = 11.967$) than they did tone ($M = 6.571$). Finally, there was a significant main effect of training day $F(4, 180) = 38.054$, $p < .001$ (See Figure 1). There were 4 potential interaction effects, of which only the two-way interaction between audio stimulus-type and training day was significant, $F(4, 180) = 57.413$, $p < .001$. Inspection of estimated marginal means and Bonferroni-adjusted pairwise comparisons illustrated that although shuttle responding did steadily decrease from extinction training Day 1 to Day 5, rats shuttled more in response to tone during extinction training than they did noise.

INSERT FIGURE 1 HERE

Test

A preliminary 3 x 2 x 2 mixed factorial analysis of variance (ANOVA) was conducted to compare the renewal behavior outcomes of Test 1 and Test 2. The ANOVA took into account between-subjects factor, renewal type (ABA, AAB, ABC), within-subjects factor, test context (extinction context or non-extinction context), and test day (Test 1: 24-hours after final extinction training; Test 2: One week following Test 1).

There was a significant main effect of between-subjects factor, renewal type, $F(2, 45) = 5.136$, $p = .010$. Additionally, there was a significant main effect of within-subjects factor, test

day, $F(1, 45) = 23.386$, $p < .001$. Estimated marginal means indicated that renewal of avoidance was greater in Test 2 ($M = 4.719$) than renewal of avoidance was in Test 1 ($M = 2.760$). There was also a main effect of the within-subjects factor, test context, $F(1, 45) = 9.999$, $p = .003$. None of the three possible two-way interactions were significant. Finally, there was no significant three-way interaction. Because there were no interactions between test and group, or test and context, we collapsed across this factor (i.e., Test) and analyzed data on the basis of whether it was the animal was texted in the extinction or non-extinction context.

A 3 x 2 mixed factorial analysis of variance (ANOVA) was conducted to compare the influence of between-subjects factor, renewal type (ABA, AAB, ABC), and within-subjects factor, test context (extinction context, non-extinction context) upon fear behavior -- measured as number of shuttle responses -- during the test phase.

There was a significant main effect of between subjects factor, renewal type, $F(2, 45) = 5.136$, $p = .010$. Estimated marginal means indicated that rats shuttled least during testing when assigned ABC renewal ($M = 2.50$), followed by ABA renewal ($M = 3.859$), and lastly, AAB renewal ($M = 4.859$). To further clarify, AAB renewal elicited the strongest renewal of shuttling behavior, while ABC renewal elicited the weakest renewal of shuttling behavior. There was also a significant main effect of within-subjects factor, test context, $F(1, 45) = 9.999$, $p = .003$. Estimated marginal means illustrated that rats shuttled more when tested in the non-extinction context ($M = 4.323$) than they did when tested within the extinction context ($M = 3.156$) (See Figure 2). There was no significant interaction effect of renewal type x test context, $F(2, 45) = .431$, $p > .05$.

INSERT FIGURE 2 HERE

General Discussion

It was found that rats of each renewal type group (ABA, AAB, ABC) had effectively acquired avoidance behavior, extinguished avoidance behavior, and then exhibited renewal of avoidance behavior at test as a function of whether a particular CS was tested in its acquisition or extinction context. Acquisition data results ensured that rats did learn avoidance behavior during signaled active avoidance (SigAA) training, seeing as the average number of shuttle responses significantly increased from day one to day five of training. The opposite trend was found in the extinction data, meaning that rats shuttled significantly less on the last day of extinction training relative to the first day of extinction training. Interestingly, there was a consistent significant main effect of audio stimulus-type in both the acquisition and extinction training in which rats, on average, exhibited more shuttling behavior in response to noise as opposed to tone. A possible account for this increased responding to noise is that the noise stimulus was comprised of several different frequencies, in contrast with tone, which was comprised of a single, steady frequency; rats may find noise a more excitatory stimulus than tone due to its multiple frequencies; this effect of greater responding to noise has been replicated in other studies of signaled active avoidance (Campese, Kim, Rojas, & LeDoux, 2017; Darvas, Fadok, & Palmiter, 2011).

Extinguished avoidance behavior in rats was significantly affected by release from the extinction context. More specifically, release from the context in which avoidance behavior was extinguished resulted in a stronger renewal of avoidance behavior. This outcome is aligned with Nakajima's (2014) finding that renewal of avoidance behavior is consistently stronger when rats are tested outside of the context in which the avoidance behavior was extinguished. However, simple contrasts of the main effect of between-subjects factor, renewal type (ABA, AAB, ABC),

although significant, did not align with Nakajima's (2014) outcome. More specifically, simple contrasts revealed that AAB renewal exhibited more overall responding during test in comparison with ABA renewal, and ABC renewal with the least amount of responding. Considering Nakajima (2014) used a strictly within-subjects design with a single audio CS, and we employed a between-subjects factor of renewal type and added a two-level within-subjects audio stimulus (Tone or Noise), it is possible that Nakajima's findings actually reflect context-US associations, whereas our results more so reflect conditional learning. Additionally, the fact that ABA renewal was found to be weaker than AAB renewal may indicate that return to the acquisition context upon text may not be as strong a predictor of renewal of avoidance behavior as previously thought (Bouton, 2004). In fact, it may be that avoidance behavior is renewed when one is confronted with similar stimuli in an equivalent but distinct context. Second, other studies of renewal of avoidance behavior have replicated this finding that an AAB renewal sequence can elicit stronger renewal of avoidance than ABA renewal (Bouton, 2004).

Although the results of this study generally support the hypotheses (H1, H3, H4, H5), there are a few limitations which much be acknowledged. Biological sex differences were not considered in the execution of this study, in that only male rats were used as subjects. There is a possibility renewal of avoidance behavior may be modulated by sex, but that is beyond the scope of the current study; this is an avenue worth pursuing. Another limitation is that the subjects used have poor eyesight, rendering visual context cues (e.g., checkered paper or circular patterned paper) perhaps less salient than olfactory or tactile cues in contributing to the creation of distinct training contexts. To accommodate for this possibility, it may be fruitful to create more distinct context cues by attending more to tactile or olfactory cues. For example, using another floor

insert (e.g., silicone) to achieve a more distinct tactile cue may produce a more distinct context, and, in turn, perhaps a stronger renewal effect, too. Surgical lesion manipulations are another line of research worth pursuing to further our understanding of the neurophysiological structures and pathways involved in extinction-context dependent renewal of avoidance. The central amygdala (CeA), and both the dorsal hippocampus (DH) and ventral hippocampus (VH) are known to affect extinction and renewal of avoidance behavior, and are also involved in contextual and emotional learning (Moscarello & LeDoux, 2013; Tovote, Fadok, & Lüthi, 2015; Xu et al., 2016). If one were to replicate or expand upon replicate this experiment and include a group of VH or DH lesioned subjects, this would likely further our understanding of how context and/or extinction is neurophysiologically circuited within the brain.

Figure Captions

TABLE 1. Table 1 illustrates the experimental training design for each renewal group (ABA, AAB, ABC). A total of three contexts were employed (e.g., Cx 1, Cx 2, Cx 3), and two auditory stimuli (e.g., Tone, Noise) were used. Table 1 shows that each group underwent 5 days of acquisition training, followed by 5 days of extinction training, test 1 the day following the last day of extinction, and test 2 a week following test 1. Table 1 denotes, according to group assignment, which auditory stimulus was presented in which context according during each phase of renewal training.

FIGURE 1. Figure 1 depicts the mean avoidance responses (mean ARs) of each training day during both acquisition and extinction, according to renewal group (ABA, AAB, ABC). Furthermore, the mean ARs to each auditory stimulus (e.g., Tone, Noise) is represented by a distinct, colored trend line with unique markers; the mean ARs to Tone is represented by the blue line with circular markers, and the mean ARs to Noise is represented by the red line with square markers.

FIGURE 2. Figure 2 illustrates the mean avoidance responses (mean ARs) in each test context (e.g. extinction context, or the non-extinction context) according to renewal group (ABA, AAB, ABC). It must be noted that the data were collapsed across auditory stimuli, meaning that each distinct bar represents the mean ARs to both Tone and Noise according to test context. For example, the light green bar in AAB renewal represents the mean ARs to both Tone and Noise when subjects were tested in the non-extinction context.

Table 1.

2 day break 7 day break

	Acquisition	Extinction	Test 1	Test 2
Group ABA	Cx 1: Tone Cx 2: Noise	Cx 2: Noise Cx 1: Tone	Cx 1: Tone, Noise Cx 2: Tone, Noise	Cx 2: Tone, Noise Cx 1: Tone, Noise
Group AAB	Cx 1: Tone Cx 2: Noise	Cx 1: Noise Cx 2: Tone	Cx 1: Tone, Noise Cx 2: Tone, Noise	Cx 2: Tone, Noise Cx 1: Tone, Noise
Group ABC	Cx 1: Tone, Noise Cx 1: Noise, Tone	Cx 2: Tone Cx 3: Noise	Cx 2: Tone, Noise Cx 3: Tone, Noise	Cx 3: Tone, Noise Cx 2: Tone, Noise

Figure 1.

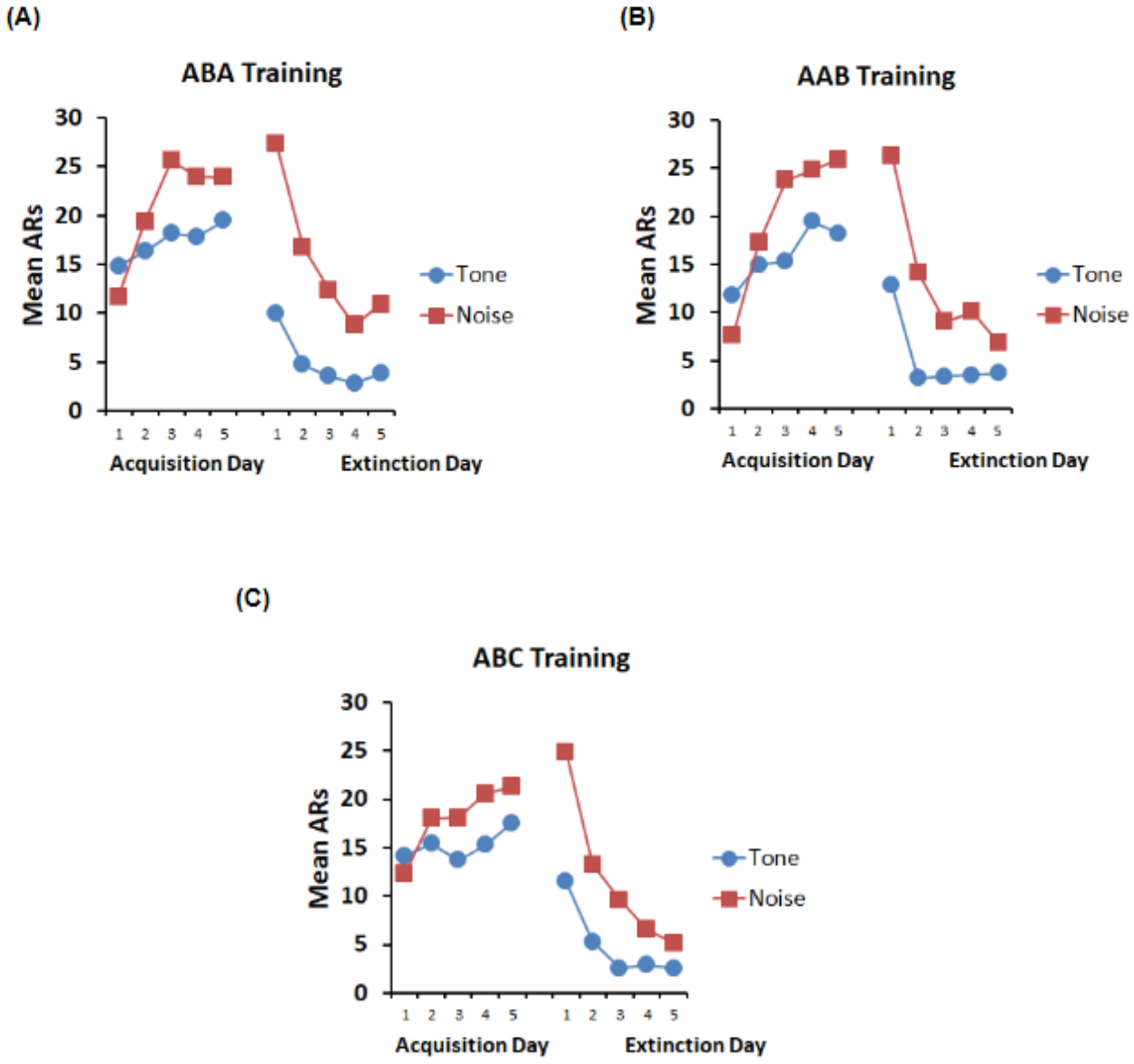
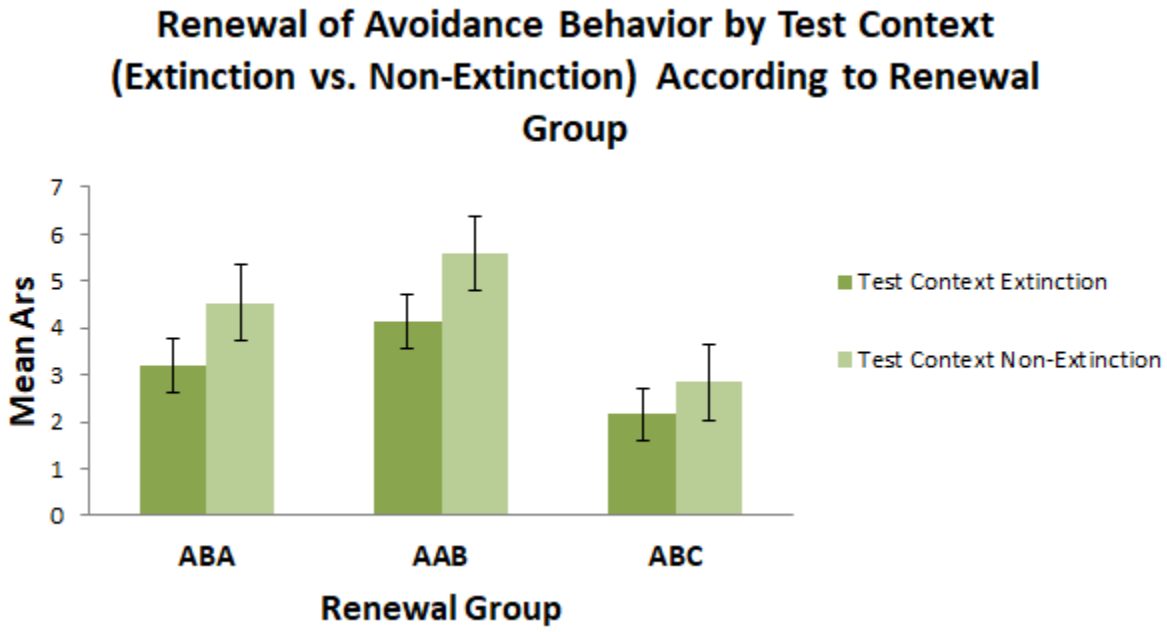

(A)
ABA Training
Mean ARs
30
25
20
15
10
5
0
1 2 3 4 5
1 2 3 4 5
Acquisition Day
Extinction Day
Tone
Noise
(B)
AAB Training
Mean ARs
Acquisition Day
Extinction Day
Tone
Noise
(C)
ABC Training
Mean ARs
Acquisition Day
Extinction Day
Tone
Noise

Figure 2.


Renewal of Avoidance Behavior by Test Context (Extinction vs. Non-Extinction) According to Renewal Group
Mean Ars
0
1
2
3
4
5
6
7
ABA
AAB
ABC
Renewal Group
Test Context Extinction
Test Context Non-Extinction

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