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Effects of Extinction Treatments on the Reduction of Conditioned Responding and Conditioned Hyperarousal in a Rabbit Model of Posttraumatic Stress Disorder (PTSD)

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Abstract

We have previously characterized a model of posttraumatic stress disorder (PTSD), based on classical conditioning of the rabbit nictitating membrane response (NMR), that focuses on 2 key PTSD-like features: conditioned responses to trauma-associated cues and hyperarousal. In addition to the development of conditioned NMRs (CRs) to a tone conditioned stimulus (CS) associated with a periorbital shock unconditioned stimulus (US), we have observed that rabbits also exhibit a conditioning-specific reflex modification (CRM) of the NMR that manifests as an exaggerated and more complex reflexive NMR to presentations of the US by itself, particularly to intensities that elicited little response prior to conditioning. Previous work has demonstrated that unpaired presentations of the CS and US are successful at extinguishing CRs and CRM simultaneously, even when a significantly weakened version of the US is utilized. In the current study, additional extinction treatments were tested, including continued pairings of the CS with a weakened US and exposure to the training context alone, and these treatments were contrasted with the effects of unpaired extinction with a weakened US and remaining in home cages with no further treatment. Results showed that continued pairings only slightly decreased CRs and CRM, while context exposure had no effect on CRs and marginal effects on reducing CRM. Unpaired extinction was still the most effective treatment for reducing both. Findings are discussed in terms of applications to cognitive-behavioral therapies for treatment of PTSD, such as incorporating mild, innately stressful stimuli into virtual reality therapy.

Keywords

eyeblick conditioning; classical conditioning; nictitating membrane response; posttraumatic stress disorder; rabbit

Posttraumatic stress disorder (PTSD) is a complex stress disorder in which previous exposure to a traumatic event results in chronic symptoms including intense distress toward cues that symbolize or resemble the event and alterations in arousal such as hypervigilance and exaggerated startle response (American Psychiatric Association, 2013). There is general consensus that there is a significant learning and memory component to PTSD (Friedman et

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al., 2011; Layton & Krikorian, 2002; Orr et al., 2000; Zoellner, Rothbaum, & Feeny, 2011); however, there is no single prevailing learning theory that can explain PTSD symptoms (Lissek & van Meurs, 2014). Animal models of PTSD have traditionally focused on studying the development and consequent extinction of conditioned fear to stimuli associated with an aversive, often very acute event (Chang et al., 2009; Johnson, McGuire, Lazarus, & Palmer, 2012; Milad, Rauch, Pitman, & Quirk, 2006; Stam, 2007; VanElzakker, Dahlgren, Davis, Dubois, & Shin, 2014). The limitations of many of these past models is that they failed to address other key PTSD features such as general hyperarousal and additionally did not examine the consequences of prolonged exposure to stress as can occur in cases of combat-related PTSD following long, often repeated tours of duty. The trend of recent research, including work in our laboratory, has been to develop more clinically relevant and translatable animal models of PTSD, offering new insights into cognitive-behavioral and pharmacological strategies for PTSD treatment (Cohen, Kozlovsky, Alona, Matar, & Joseph, 2012; Corley, Caruso, & Takahashi, 2012; Schreurs, Smith-Bell, & Burhans, 2011a, 2011b; Siegmund & Wotjak, 2006; Yamamoto et al., 2009; Yehuda & LeDoux, 2007).

We have previously characterized a model for PTSD that focuses on two key PTSD-like features: conditioned responses to cues associated with trauma and conditioned hyperarousal (Burhans, Smith-Bell, & Schreurs, 2008). This model is based on conditioning of the rabbit's nictitating membrane response (NMR), a behavioral system for which learning parameters and neural substrates are well established and well understood (Christian & Thompson, 2003; Freeman & Steinmetz, 2011; Gormezano, 1972; Hesslow & Yeo, 2002; Kehoe & Macrae, 2002). As rabbits develop a conditioned NMR (CR) to a tone conditioned stimulus (CS) associated with a periorbital shock unconditioned stimulus (US), responding to the shock itself changes and manifests as an exaggerated and more complex response, particularly to low intensity shocks that elicited little to no responding prior to conditioning (Burhans et al., 2008; Schreurs, 2003; Schreurs, Oh, Hirashima, & Alkon, 1995). This observed hyperarousal is measured in the absence of the CS and is referred to as conditioning-specific reflex modification (CRM) as it does not develop in rabbits that receive unpaired training during which the CS and US are explicitly unpaired. Therefore, CRM is not thought to represent a form of nonassociative sensitization like that reported in *Aplysia* (Byrne & Hawkins, 2015) and in the drug abuse literature (Leyton, 2007). The development of CRM is characterized by increases in several NMR parameters that reflect the size, shape, and timing of the response, and those changes primarily occur in response to US intensities that are four- to eightfold smaller than the intensity utilized during conditioning. CRM-like changes have also been reported by others for the rabbit NMR preparation (Gruart & Yeo, 1995; Wikgren, Ruusuvirta, & Korhonen, 2002) and for eyeblink conditioning in rodents (Servatius, Brennan, Beck, Beldowicz, & Coyle-DiNorica, 2001).

The focus of recent work on CRM has been on behavioral treatments that lead to reductions in both CRs to the tone CS and CRM that could have important applications for developing PTSD treatments capable of addressing multiple symptoms. Initial work showed that traditional extinction treatments like CS-alone presentations were optimal for extinction of CRs but not CRM, whereas US-alone presentations at the intensity used during conditioning

produced the opposite results, ideal for CRM extinction but not extinction of CRs (Schreurs, Shi, Pineda, & Buck, 2000). Combining aspects of those two treatments, unpaired extinction (explicitly unpaired presentations of the CS and US) was found to be the best treatment for simultaneously extinguishing both CRs and CRM. A later experiment demonstrated that the intensity of the US utilized during unpaired extinction could be reduced eightfold and still produce successful extinction of CRs and CRM as long as training proceeded for at least 3 days (Schreurs et al., 2011b). These findings suggested that cognitive-behavioral therapies for PTSD such as exposure therapy utilizing virtual reality technology (Meyerbröcker & Emmelkamp, 2010; Rizzo et al., 2011) might be more successful at addressing hyperarousal symptoms if a mildly stressful stimulus such as skin stimulation were part of the simulation environment.

The following study examined two additional types of extinction treatments: continued pairings of tone with a weakened US and context extinction during which subjects are exposed to the training context alone without any stimulus presentations. Kehoe and colleagues have previously shown that these treatments can cause extinction-like effects on CRs in the rabbit NMR preparation (Kehoe, Weidemann, & Dartnall, 2004; Kehoe & White, 2002; Poulos, Pakaprot, Mahdi, Kehoe, & Thompson, 2006). Because it is not known what effect these treatments may have on CRM, the findings could yield important information regarding the nature of CRM extinction. For example, whether treatments that reduce CRs will consequently reduce CRM and also what role context conditioning may play in CRM extinction, with potential application to PTSD treatments capable of addressing multiple symptoms. As previously reported, unpaired extinction is more effective at simultaneously reducing CRs and CRM than CS-alone or US-alone presentations (Schreurs et al., 2000), and unpaired extinction is still effective even when the US is significantly reduced from the conditioning intensity (Schreurs et al., 2011b). Therefore, unpaired extinction with a weak US was chosen as the gold standard condition for comparison against continued pairings with a weak US and context extinction. A group remaining in home cages served as a no extinction control that has previously been shown to exhibit high remaining levels of CRM (Schreurs et al., 2000).

Method

Subjects

The subjects were 35 male, New Zealand White rabbits (*Oryctolagus cuniculus*), 2–3 months of age weighing approximately 2.0–2.2 kg upon delivery from the supplier (Harlan, Indianapolis, IN). The rabbits were housed in individual cages on a 12-hr light–dark cycle and given ad libitum access to food and water. They were maintained in accordance with the guide for the care and use of laboratory animals issued by the National Institutes of Health, and the research was approved by the West Virginia University Animal Care and Use Committee.

Apparatus

The apparatus and recording procedures for NMR conditioning have been detailed by Schreurs and Alkon (1990), who modeled their apparatus based on those described by

Gormezano (Coleman & Gormezano, 1971; Gormezano, 1966). Rabbits were restrained in a Plexiglas box placed inside a sound-attenuating, ventilated chamber (Coulbourn Instruments, Allentown, PA; Model E10-20). Inside the chamber, a stimulus panel containing a speaker and houselight (10-W, 120 V) was mounted at a 45° angle 15 cm anterior and dorsal to the rabbit's head. An exhaust fan created a constant ambient noise level of 65 dB inside the chamber. Periorbital electrical stimulation was delivered by a programmable two-pole stimulator (Coulbourn Instruments, Model E13-35) via stainless steel Autoclip wound clips (Stoelting, Wood Dale, IL) that were positioned 10 mm ventral and 10 mm posterior to the dorsal canthus of the right eye. Stimulus delivery, data collection, and analysis were all accomplished using the LabVIEW software system (National Instruments, Austin, TX).

The NMRs were transduced by a potentiometer (Novotechnik US Inc., Southborough, MA; Model P2201) connected at one end, via a freely moving ball and socket joint, to an L-shaped lever containing a hook that attached to a 6–0 nylon loop sutured into but not through the nictitating membrane (NM). At the other end, the potentiometer was connected to a 12-bit analog-to-digital (A/D) converter (5-ms sampling rate, 0.05-mm resolution), and individual A/D outputs were stored on a trial-by-trial basis for subsequent analysis.

Procedure

One week after arrival, rabbits were first acclimated to restraint by placing in restrainers for 30 min while under close supervision. On subsequent days, rabbits received one session per day in the following order: adaptation, US pretest, 6 days of classical delay NMR conditioning, US posttest (Post1), 6 days of extinction treatment, a second US posttest (Post2), and a CS-alone retention test. For adaptation, subjects were prepared for delivery of the periorbital shock US and NMR recording and then adapted to the training chambers for an amount of time equivalent to subsequent training sessions (80 min). For pretest and posttests, subjects received 80 trials of US presentations with an average intertrial interval (ITI) of 60 s (range 50–70 s). Each US presentation was 1 of 20 combinations of periorbital shock intensity (0.1, 0.25, 0.5, 1.0, or 2.0 mA) and duration (10, 25, 50, or 100 ms), and these 20 unique USs were presented in four separately randomized blocks with the restriction that the same intensity or duration could not occur more than 3 times in succession. For delay conditioning, each session consisted of 80 trials of paired presentations of a 400 ms, 1 kHz, 82 dB CS that coterminated with a 100 ms, 2 mA US (300 ms interstimulus interval). The CS-US presentations were presented with an average ITI of 60 s. For the 6 daily sessions of extinction treatment, subjects were divided into four different treatment conditions: continued pairings with a weak shock (PD), unpaired extinction with a weak shock (UP), context extinction (CTX), or no further treatment (ØEXT). For the PD group, delay conditioning continued under the same parameters but with a reduced intensity US of 0.25 mA in place of the 2.0 mA US. For the UP group, each session consisted of 80 presentations of the tone CS and 80 presentations of a reduced intensity 0.25 mA US (100 ms) that were explicitly unpaired and presented in a pseudorandom order. The average ITI for unpaired sessions was reduced to 30 s to maintain the session length at approximately 80 min. For the CTX group, rabbits were set up for NMR recording and US stimulus delivery and placed in training chambers for 80 min without any CS or US presentations. Last, for the ØEXT group, rabbits remained in home

cages for the 6 days during which all other groups received daily sessions in the training chambers. The CS alone test experienced by all groups consisted of 80 presentations of the tone CS with an average ITI of 60 s.

CRs of the NMR were defined as any extension of the NM exceeding 0.5 mm that was initiated following CS onset but prior to US onset. For US testing, a UR was defined as any extension of the NM exceeding 0.5 mm that was initiated within 300 ms following US onset. The definition of the UR was based on prior observations that responses to the US following CS-US pairings had onset latencies within the same range as CRs (Schreurs et al., 2000). Amplitude of the response was calculated as the maximum extension of the NM in mm. Onset latency of the response was the latency in ms from stimulus onset to when the NM rose 0.1 mm above baseline, while peak latency was the latency in ms from stimulus onset until maximum NM extension occurred. Area of the response was calculated as the total area of the response curve (arbitrary units, au) from stimulus onset until the end of trial (trial length = 2,000 ms). For URs during US testing, two additional measures were calculated in order to overcome the statistical limitations of empty data cells produced by subthreshold responses to periorbital shock, particularly at the lower intensities and durations. These measures, magnitude of the response amplitude and magnitude of the response area, included the amplitudes and areas of all NMRs above baseline regardless of whether the 0.5 mm criterion was met (Garcia, Mauk, Weidemann, & Kehoe, 2003). A significant pre- to posttest increase in any of the UR response measures as a function of classical conditioning is a defining feature of CRM. To increase the sensitivity for detection of CRM and to follow the convention of previous CRM studies, we collapsed data at the five US intensities across duration and focused CRM analyses on the first 20 trial US sequence where the strongest CRM is observed (Schreurs et al., 2000). To examine the shape and timing of NMRs during US tests, response topographies were generated at each US intensity by averaging across rabbits and across US durations within each experimental group.

The experiment was conducted in two separate replications. Unless otherwise indicated, data were analyzed by repeated measures analysis of variance (ANOVA; SPSS 21). Planned and follow-up comparisons were Bonferroni corrected for the number of comparisons.

Results

CR Acquisition, Extinction, and Retention

All rabbits acquired the delay conditioning task with the exception of one rabbit whose data were eliminated for failure to reach a learning criterion of 80% CRs by the last day of conditioning. Final *n* values per group were 9, 8, 9, and 8 for PD, UP, CTX, and ØEXT groups, respectively. Figure 1 illustrates the percent CRs to the tone CS across delay conditioning, extinction, and CS-alone presentations. By the 6th day of delay conditioning, rabbits had mean CRs in excess of 96% ($= 97.52 \pm 0.55$ SEM). Acquisition of delay conditioning was confirmed with repeated measures ANOVA, which indicated a significant main effect of days, $F(5, 150) = 150.63$, $p < .001$, with no effects or interactions involving group. Bonferroni corrected planned comparisons showed that CRs increased from the first to second day of conditioning, $p < .004$, and remained at a similar, high level during the subsequent sessions.

The middle of Figure 1 shows the percent CRs during extinction for the two groups receiving CS presentations. For the group receiving continued CS-US pairings with a weak shock US (PD), CRs moderately decreased on the first day of extinction relative to the last day of delay conditioning but did not decrease further over the course of extinction. For the group receiving explicitly unpaired CS/US presentations with a weak shock US (UP), CRs decreased steadily over the 6 extinction sessions, reaching a terminal value of less than 15% ($= 12.24 \pm 5.78$ SEM). Analysis comparing the last day of delay conditioning with the first day of extinction indicated a significant effect of session, $F(1, 15) = 23.16, p < .001$, but no effects involving group, demonstrating that CRs dropped to a similar level in both groups initially. The continued CR decrease in the UP group but not the PD group was confirmed by a significant interaction of days and group, $F(5, 75) = 3.48, p < .01$, in the analysis of all 6 extinction sessions. Bonferroni corrected planned comparisons indicated that CRs were significantly lower in the UP group compared with the PD group during extinction days four, $p < .05$, five, $p = .05$, and six, $p < .01$.

The remaining level of CRs in all groups was assessed during the CS-alone retention test (CS test) and is illustrated in the right side of Figure 1. The highest level of CRs was found in the CTX group whereas the lowest level was found in the UP group. One-way ANOVA for the entire 80-trial session of the CS test indicated a significant effect of group, $F(3, 30) = 3.95, p < .05$, with post hoc tests indicating a significant difference between the CTX and UP groups, $p < .05$, and a trend for CRs in the PD group to also be elevated compared with the UP group, $p = .091$. Additional analyses were then focused on only the first 10 trials of the CS test in order to measure CR retention without any confounding extinction-producing effects of repeated CS-alone presentations (Pavlov, 1927). The right inset of Figure 1 illustrates the first 10 trials of the CS test along with the last 10 trials for comparison. In contrast to results from the entire session, CR retention was revealed to be highest in the CTX and ØEXT groups. This finding was confirmed by a significant effect of group, $F(3, 30) = 6.29, p < .01$, with Bonferroni corrected post hoc tests indicating that the UP group had significantly lower CRs than the CTX, $p < .01$, and ØEXT, $p < .05$, groups. As a further test to evaluate the effectiveness of the various extinction treatments on extinction of CRs to the tone CS, the last session of delay conditioning was compared with the first 10 trials of the CS test. Repeated measures ANOVA revealed a significant interaction of session and group, $F(3, 30) = 6.45, p < .01$, with Bonferroni corrected, within group planned comparisons indicating that only the UP group had a significant reduction in CRs, $p < .05$. In summary, unpaired extinction with a weak shock was the most effective treatment at reducing CRs to the tone CS.

CRM Acquisition and Extinction

The averaged NMR topographies from US testing are shown in Figure 2 and illustrate the timing, size, and shape of the response to the periorbital shock US prior to and following delay conditioning (Pretest, Post1) and following extinction treatment (Post2). Changes in the frequency of the UR, which cannot be discerned from topographies, are illustrated in Figure 3 (left column). Initial comparisons of the UR changes from Pretest to Post1 were conducted to determine the level of CRM prior to extinction treatment. All groups demonstrated CRM, as shown by enhanced responding at Post1 compared with Pretest at the

lower US intensities. The development of CRM was confirmed by a significant interaction of US test and US intensity for UR frequency (% URs), $F(4, 120) = 13.75, p < .001$, magnitude of the response amplitude (mAmp), $F(4, 120) = 27.05, p < .001$, and magnitude of the area (mArea), $F(4, 120) = 18.39, p < .001$. Bonferroni corrected planned comparisons showed that Pretest to Post1 increases were found at the 0.25 mA and 0.5 mA intensities for %URs, mAmp, and mArea, all p values < 0.01 . Analyses for latency measures were more restricted due to the limitation of empty data cells (see Methods). For this reason, Pretest versus Post1 differences were analyzed while US intensity was held constant. Analyses indicated a significant effect of test at the 0.25 mA, $F(1, 6) = 8.84, p < .05$, and 0.5 mA, $F(1, 28) = 7.032, p < .05$, intensities for UR peak latency resulting from a Pretest to Post1 increase that was reflected as a latency shift of the peak of the UR to the right (see Figure 2).

To determine the effect of extinction treatments on CRM, analyses were focused on the intensities at which CRM was significant prior to extinction, 0.25 mA and 0.50 mA. Comparison of the UR topography at Post2 with Post1 (see Figure 2) suggested that CRM was reduced in the UP and CTX groups, moderately reduced in the PD group, but intact in the ØEXT group. However, repeated measures ANOVA with factors of group, test (Pretest, Post1, Post2), and intensity (0.25, 0.5 mA) revealed no significant group effects. The change in CRM was then further evaluated in each group separately. Differing patterns of change within individual groups are illustrated more clearly in the bar graphs for %URs, mAmp, and mArea shown in Figure 3. Two distinct, consistent patterns occurred in the ØEXT and UP group across all three UR parameters at both the 0.25 and 0.5 mA intensities. While the ØEXT group demonstrated a lack of CRM extinction manifested as a continued increase from Pretest through Post2, the UP group exhibited extinction of CRM manifested as a decrease at Post2 following the initial increase at Post1. The PD and CTX also demonstrated the increase-decrease pattern seen in the UP group but not at all parameters and intensities examined for CRM change.

The lack of CRM extinction observed in the ØEXT group at the 0.25 and 0.5 mA intensities was confirmed by repeated measures ANOVA, which indicated a main effect of test, significant for mArea, $F(2, 1) = 7.14, p < .01$, and marginally significant for mAmp, $F(2, 14) = 3.57, p = .056$, in addition to significant linear trends for both measures, mArea: $F(1, 7) = 12.08, p < .02$; mAmp: $F(1, 7) = 6.11, p < .05$. These results indicated that for the ØEXT group, the enhanced amplitude and area of the NMR at Post1 compared with Pretest (indicative of CRM) continued to increase at Post2, demonstrating that CRM remained intact if not enhanced. The increase-decrease pattern of the UP group was confirmed by a significant effect of test and significant quadratic trends for % URs, $F(2, 14) = 9.62, p < .01$; $F(1, 7) = 20.76, p < .01$, mAmp, $F(2, 14) = 7.86, p < .01$; $F(1, 7) = 10.91, p < .05$, and mArea, $F(2, 14) = 5.01, p < .05$; $F(1, 7) = 6.33, p < .05$. These analyses therefore demonstrated that the enhanced frequency, amplitude, and area of the response at Post1 (i.e., CRM) decreased at Post2. In support of partial extinction for the PD group, there was a significant effect of test for all three UR parameters, %URs: $F(2, 16) = 5.13, p < .05$; mAmp: $F(2, 16) = 7.94, p < .01$; mArea: $F(2, 16) = 4.15, p < .05$, with significant quadratic trends for mAmp, $F(1, 8) = 23.79, p < .01$, and mArea, $F(1, 8) = 7.78, p < .05$, but a linear trend for %URs, $F(1, 8) = 5.73, p < .05$. These findings demonstrated that CRM was

reduced by continued pairings in terms of the area and amplitude of the response but not response frequency. For the CTX group, there were no significant effects of test, only trends for mAmp, $F(2, 16) = 3.31, p = .063$, and mArea, $F(2, 16) = 3.10, p = .073$, with marginally significant quadratic trends for mAmp, $F(1, 8) = 5.11, p = .054$, confirming that the increase-decrease pattern was exhibited in the CTX group but was less pronounced. These results verified that context exposure lead to only a marginal CRM reduction and for only the amplitude of the response.

Discussion

The major finding of the current experiment was that compared with continued pairings with a weak US, exposure to the context, or remaining in home cages, unpaired presentations of the CS with a weak US was by far the most effective treatment for extinguishing both CRs and CRM. This extends our earlier work showing that unpaired extinction is more effective at reducing CRM than CS-alone or US-alone presentations (Schreurs et al., 2000) and is consistent with previous findings on unpaired extinction with a weak US (Schreurs et al., 2011b). Additional results were that continued pairings with a weak US resulted in moderate reductions in CRM and CRs, while context alone exposure led to a slight reduction in CRM but had no effect on reducing CRs. Last, rabbits that remained in home cages maintained a high level of CRs and CRM, consistent with findings that long-term memory of NMR delay conditioning remains robust for up to a month (Schreurs, 1993) and unpublished observations that CRM endures for at least a month.

Based on the work of Kehoe and White (2002), it was expected that continued pairings with a weak shock would result in more significant reductions in CRs and consequently, CRM. To make sure we were not masking extinction effects by analyzing CRs across the entire extinction session, we examined CR data in blocks of 10 trials as done in the aforementioned Kehoe and White (2002) study. Looking at the last 10 trials of CS-weak US presentations across extinction sessions, average CR levels never dropped below 50%, compared with levels below 20% achieved by our colleagues. This may be accounted for by variations in NMR conditioning parameters that in our experiment led to faster acquisition and higher terminal CR levels. The most significant differences included our use of an easier delay conditioning paradigm with a longer duration tone CS (400 ms) and US (100 ms) versus a zero trace paradigm used by Kehoe and White with shorter duration CS (250 ms) and US (50 ms). Our initial higher acquisition level may therefore have contributed to an increased resistance to extinction. Despite the more moderate level of CR reductions observed in this study, an important finding is that the CR reduction was paralleled by a slight reduction in CRM. We have previously suggested that CRM may represent a generalization of the CR due to the close resemblance between the NMR topography for CRs to the tone CS and URs the US during expression of CRM (Burhans et al., 2008; Schreurs et al., 2000). The current findings add further support to the idea that CRM is at least in part a generalization of the CR because here we show that a treatment that can reduce CRs can also lead to a reduction in CRM. However, our work has also demonstrated the dichotomy that can exist between CRs and CRM (Burhans & Schreurs, 2008; Schreurs et al., 2000; Smith-Bell, Burhans, & Schreurs, 2012), highlighting that some of the

mechanisms responsible for the acquisition and consequent extinction of CRM may be independent of those involved in CRs.

There have been previous accounts using the rabbit NMR preparation of CR decrements following context alone exposure (Kehoe et al., 2004; Poulos et al., 2006). The current study found no decrement in CR performance and in fact, during the first block of 10 trials of the CS-alone retention test, rabbits in the context exposure group were performing at the same level as rabbits that had remained in home cages without any treatment. One procedural difference in our study is that there was a session of US posttesting after extinction but before the CS-alone retention test. Reinstatement of CRs previously extinguished is a learning phenomenon that occurs following presentations of the US in the training context, typically 24 hr prior to testing for CRs (Bouton, 2002). One possibility therefore is that the US alone presentations during the posttest masked any context exposure extinction effects due to reinstatement of CRs. Although reinstatement has been documented in other paradigms (Bouton, 2002; Pavlov, 1927; Rescorla & Heth, 1975; Shaham, Shalev, Lu, De Wit, & Stewart, 2003), it has not been observed for rabbit NMR conditioning (Kehoe et al., 2004; Napier, Macrae, & Kehoe, 1992). For example, Napier et al. (1992) had a group that received 5 days of context alone exposure after which reinstatement was assessed by presenting several US alone trials followed by CS alone trials. The level of percent CRs following the unsignaled shocks was only 3% in stark contrast to the nearly 100% CR level we saw in the first block of our CS-alone retention test. In Kehoe et al. (2004), 5 days of context exposure with the US present also did not appear to create any reinstatement effects on CRs. Our data suggest that reinstatement may take place if US presentations occur 24 hr prior to CR testing, and if US exposure is limited to a single session rather than several consecutive sessions across days. Alternatively, NMR conditioning to the tone CS in our paradigm may have been strong enough to withstand any effects of context exposure. There are also some reports of context exposure after conditioning actually enhancing rather than reducing CRs to a discrete CS in other learning paradigms (Chang, Stout, & Miller, 2004; Grahame, Barnett, & Miller, 1992).

The finding that context alone exposure, despite having no effect on CRs, can lead to slight reduction in CRM suggests a possible role for context conditioning in CRM expression and extinction. Previous work has shown that like classical conditioning of the NMR (Penick & Solomon, 1991), CRM expression is sensitive to shifts in context between NMR conditioning and CRM testing (Schreurs, Gonzalez-Joekes, & Smith-Bell, 2006). Interestingly, however, a context shift-induced reduction in CRM was not found if the test context was novel; instead, it only occurred if the amount of exposure to the test context was equated with that of the training context in which NMR conditioning took place. To equate contexts, rabbits were exposed to the future CRM test context for 6 days prior to 6 days of NMR conditioning in a different context. Context exposure in the absence of fearful stimuli, innate or conditioned, can be argued to produce an association of the context with safety. Therefore, another interpretation of this previous study's data is that CRM expression will generalize across contexts unless previous experience has assessed a particular context as "safe." In the case of our current study's data, context alone exposure *after* conditioning requires the rabbit to update the previous association of the context with the CS and US to

an association of the context with the *absence* of the CS and US, that is, safety. This new association would then consequently result in a reduction in CRM expression. However, this interpretation seems contrary to the result that US presentations are so effective in reducing CRM. The presentation of distributed, unsignaled shocks during unpaired extinction and during US-alone extinction (Schreurs et al., 2000) might be expected to maintain fear to the context (Fanselow, DeCola, & Young, 1993), and yet both consequently reduce CRM.

In terms of specific applications to treatments for PTSD, the results suggest that therapies that incorporate exposure to trauma-associated cues with unpaired presentations of mild, innately stressful stimuli may help reduce fear responses to trigger cues in addition to reducing symptoms of hyperarousal. This type of treatment could be incorporated into virtual reality therapy by programming a temporal separation between the presentation of trauma-associated cues and a mildly stressful stimulus such as electrical stimulation of the skin. Indeed, a human fear conditioning paradigm involving both cued and unsignaled shock presentations has already been previously demonstrated using a virtual reality environment (Baas, Nugent, Lissek, Pine, & Grillon, 2004; Grillon, Baas, Cornwell, & Johnson, 2006).

The current work extends the usefulness of the CRM paradigm as an important corollary to current animal models of PTSD. We have previously demonstrated that the strength of CRM increases with the number of days of NMR conditioning (Schreurs et al., 1995) and with the aversiveness of the US (Buck, Seager, & Schreurs, 2001; Seager, Smith-Bell, & Schreurs, 2003), demonstrating that the length and intensity of exposure to an aversive event can elevate the level of observed conditioned hyperarousal. Similar to clinical reports on PTSD, there is an incubation period following exposure during which CRM can be exacerbated (Schreurs et al., 2011a). In addition, CRM and PTSD may share similar neural substrates as the amygdala has been shown to play an important role in modulation of CRM (Burhans & Schreurs, 2008) while human imagining studies have implicated amygdalar dysfunction in PTSD (Hughes & Shin, 2011). There is also considerable individual variation in CRM levels (Burhans et al., 2008; Smith-Bell et al., 2012), just as PTSD does not develop in all those exposed to trauma (Packnett, Gubata, Cowan, & Niebuhr, 2012; Ramchand et al., 2010; Stein et al., 2014). Last, CRM has been found to be sensitive to fluoxetine (Burhans, Smith-Bell, & Schreurs, 2013), a drug that belongs to the class of selective serotonin uptake inhibitors that are often the first line of defense in pharmacological treatment of PTSD (Antai-Otong, 2007; Davidson, 2006; Hoskins et al., 2015; Ipser & Stein, 2012). The results presented here add to this work and demonstrate that the CRM paradigm may serve as a useful tool to evaluate cognitive-behavioral PTSD treatment strategies.

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Figure 1.

The mean percentage ($\pm$ SEM) of CRs to the tone CS during 6 daily sessions of delay conditioning, 6 sessions of extinction, and a CR retention test consisting of CS-alone presentations (CS test). The right inset panel shows the first and last block of 10 trials for the CS test. During extinction, rabbits received either continued pairings of the CS with a weak shock US (PD, solid circle), exposure to the training context with no stimulus presentations (CTX, square), unpaired extinction with a weak US (UP, open circle), or remained in home cages (ØEXT, triangle). Data during extinction are pictured only for groups receiving CS presentations (PD, UP).

Figure 2.

Averaged response topographies for the unconditioned response to the periorbital shock US during the first 20 trials of the US pretest (Pretest, dotted black line), the US posttest following delay conditioning (Post1, red line), and the US posttest following extinction (Post2, blue line) during which rabbits received either continued pairings of the CS with a weak shock US (PD, top left), exposure to the training context with no stimulus presentations (CTX, top right), unpaired extinction with a weak US (UP, bottom right), or remained in home cages (ØEXT, bottom left). Topographies are shown at the five US intensities (2.0, 1.0, 0.5, 0.25, 0.1 mA) presented during US testing, collapsed across duration.

Figure 3.

Mean ($\pm$ SEM) % URs (left column), magnitude of the UR amplitude (mAmp, middle column), and magnitude of the UR area (MArea, right column) for the first 20 trials of presentations of the periorbital shock US during pretest (Pretest, white bar), the posttest following delay conditioning (Post1, black bar), and the posttest following extinction (Post2, gray bar) during which rabbits received either continued pairings of the tone CS with a weak US (PD, top row), exposure to the training context with no stimulus presentations (CTX, 2nd row from top), unpaired extinction with a weak US (UP, bottom row), or remained in home cages (ØEXT, 2nd row from bottom). Bar graphs are shown for the five US intensities (0.1, 0.25, 0.5, 1.0, 2.0 mA) presented during US testing, collapsed across duration.