Delayed unpaired extinction as a treatment for hyperarousal of the rabbit nictitating membrane response and its implications for treating PTSD

Bernard G. Schreurs
*West Virginia University*

Carrie A. Smith-Bell
*West Virginia University*

Lauren B. Burhans
*West Virginia University*

Follow this and additional works at: https://researchrepository.wvu.edu/ctsi

Part of the Medicine and Health Sciences Commons

Digital Commons Citation

This Article is brought to you for free and open access by the Centers at The Research Repository @ WVU. It has been accepted for inclusion in Clinical and Translational Science Institute by an authorized administrator of The Research Repository @ WVU. For more information, please contact ian.harmon@mail.wvu.edu.
Delayed unpaired extinction as a treatment for hyperarousal of the rabbit nictitating membrane response and its implications for treating PTSD

Bernard G. Schreurs\textsuperscript{a,b,*}, Carrie A. Smith-Bell\textsuperscript{a,b}, and Lauren B. Burhans\textsuperscript{a,b}

\textsuperscript{a}Blanchette Rockefeller Neurosciences Institute, West Virginia University
\textsuperscript{b}Department of Physiology, Pharmacology and Neuroscience, West Virginia University

Abstract

Treatment for PTSD (Post-traumatic stress disorder) is rarely available immediately after trauma and often delayed for weeks or months after an event. In a rabbit eyeblink conditioning model of PTSD, we have previously shown that presentations of a tone conditioned stimulus (CS) and shock unconditioned stimulus (US) in an explicitly unpaired manner known as unpaired extinction is effective in reducing CS responding and US hyperarousal even if shock intensity is reduced eight-fold and elicits only minimal responding. Here we determined if delayed delivery of unpaired extinction would still be effective in extinguishing hyperarousal. Rabbits were tested for sensitivity to shock before CS-US pairings and after six days of unpaired extinction presented a day, a week or a month after CS-US pairings. Hyperarousal was extinguished a day and a week after conditioning but not after a month suggesting a significant delay in “treatment” can make hyperarousal persist. We next assessed if this persistence of hyperarousal was associative by comparing rabbits given CS-US pairings to those given explicitly unpaired CS and US presentations, measuring hyperarousal a day and a month later, followed by unpaired extinction and hyperarousal assessment. After four weeks, there was an increase in responding for all rabbits but only rabbits receiving CS-US pairings showed a significant increase in associatively-mediated hyperarousal. Importantly, both paired and unpaired groups showed increased levels of responding after unpaired extinction suggesting treatment delayed for too long may no longer be effective and could cause generalized hyperarousal.

Keywords

classical conditioning; eyeblink; post-traumatic stress disorder

*Corresponding author: Bernard G. Schreurs, 1024 BRNI Building, 8 Medical Center Drive, Morgantown, WV 26505.
Conflicts of interest: none

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Introduction

The treatment of Post-traumatic stress disorder (PTSD) although somewhat successful in civilians (Cusack et al., 2016, Difede et al., 2014, Hoskins et al., 2015) has proven to be more challenging in military personnel especially those exposed to combat (Crum-Cianflone and Jacobson, 2014, Fisher, 2014, Hines et al., 2014, Quartana et al., 2014, Steenkamp et al., 2015). One important factor in the efficacy of treatment has been the delay in symptom onset (Brewin et al., 2012, Fikretoglu and Liu, 2012, Goodwin et al., 2012, Horesh et al., 2010, Utzon-Frank et al., 2014). Another important factor has been the delay between trauma and treatment (Boulos and Zamorski, 2015, Maguen et al., 2014). In fact, the shorter the delay between trauma and treatment among military personnel, the better the prognosis (Boulos and Zamorski, 2015). The longer the delay, the less likely treatment will be effective (Bunting et al., 2011, Maguen, Madden, 2014) and, at least in veterans, the higher the likelihood of additional physical and mental problems (Packnett et al., 2012, Smith et al., 2016, Wisco et al., 2014).

We have previously characterized a model for PTSD that focuses on two key PTSD-like features: conditioned responses (CRs) to cues associated with trauma and conditioned hyperarousal (Schreurs and Burhans, 2015, Schreurs et al., 2011b). This model is based on classical conditioning of the rabbit’s nictitating membrane response (NMR) with well-understood behavioral and neural underpinnings (Gormezano, 1972, Kehoe and Macrae, 2002). As rabbits develop a CR to a tone conditioned stimulus (CS) associated with a periorbital shock unconditioned stimulus (US), unconditioned responses (URs) to shock change becoming exaggerated and more complex, particularly to low intensities that elicit little responding prior to conditioning. This hyperarousal is measured in the absence of the CS and referred to as conditioning-specific reflex modification (CRM) because it does not develop in rabbits that receive explicitly unpaired CS and US presentations – a control condition designed to assess non-associative contributors to responding that does not result in the development of CRs (Gormezano and Kehoe, 1975).

The development of CRM is characterized by increases in response parameters that reflect size, shape, and timing of the response, in response to US intensities below the intensity used during CS-US pairings. Specifically, CRM is an overall increase in the size of the response which also peaks later than responses to the US before classical conditioning. CRM-like changes have been reported by others in the rabbit (Gruart and Yeo, 1995, Wikgren and Korhonen, 2001) and rat (Servatius et al., 2001). Increases in responding to the US as a result of classical conditioning are discernable during presentations of the US by itself in early eyeblink conditioning experiments in dogs (Hilgard and Marquis, 1935) and humans (Hilgard and Campbell, 1936).

In the rabbit model of PTSD, we have also shown that explicitly unpaired presentations of the CS and US effectively reduce CS responding and US hyperarousal (Buck et al., 2001, Schreurs et al., 2000). Because using the traumatic event as a means of treating PTSD is untenable, we since showed that extinguishing US hyperarousal as well as CRs using explicitly unpaired presentations of the CS and US can also be achieved when the intensity
of the shock US is reduced eight-fold – an intensity that only elicits minimal responding (Burhans et al., 2015, Schreurs and Burhans, 2015).

In two experiments, we determined if a delay in explicitly unpaired presentations of the CS and US termed “unpaired extinction” one day, one week, or four weeks after CS-US pairings would be effective in extinguishing hyperarousal to the US as well as CRs to the CS and whether the effect was associative in nature.

Materials and Methods

Subjects—The subjects were 25 male, New Zealand White rabbits (Oryctolagus cuniculus), 2–3 months of age, and 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-hour 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 who modeled their apparatus based on those described by Gormezano (Gormezano, 1966). Rabbits were restrained in a Plexiglas box placed inside a sound-attenuating, ventilated chamber (Coulborn 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 (Colbourn 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 retractable 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 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 being placed in restrainers for 30 minutes while under close supervision. On subsequent days, rabbits received one session per day in the following order: adaptation, US Pretest, six days of CS-US pairings, US Post Test (Post1), and then six days of explicitly unpaired presentations of the CS and US – unpaired extinction – with a weak US (0.3 mA) presented one day (0 Week, n=9), one week (1 Week, n=7) or one month (4 Week, n=7) later. Rabbits remained in their home cages during the interval between CS-US pairings and unpaired extinction. All rabbits then received a second US Post Test (Post2), and a CS-alone retention test (CS 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). To assess URs on pretest and on post-tests, subjects received 80 trials of US presentations with an average inter-trial interval (ITI) of 60 s (range 50–70 s). Each US presentation was one of 20 combinations of periorbital shock intensity (0.1, 0.3, 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 the CS-US pairings used to establish 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. Rabbits were required to reach a criterion of 85% CRs during CS-US pairings to be included in the analyses. For unpaired extinction, each session consisted of 80 presentations of the tone CS and 80 presentations of a weak shock US (100 ms, 0.3 mA) 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 minutes. The CS alone test consisted of 80 presentations of the tone CS with an average ITI of 60 s.

CRs 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. Amplitude of the response was calculated as the maximum extension of the NM in millimeters. 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 = 2000 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 et al., 2003). A significant pre- to post-test increase in any of the UR response measures as a function of CS-US pairings is a defining feature of CRM. To increase the sensitivity for detecting 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. 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. Unless otherwise indicated, data were analyzed by repeated measures analysis of variance (ANOVA, Systat 8.0, SPSS Inc). Follow-up comparisons between means were conducted using Psy (School of Psychology, University of New South Wales, 2000).

Results

Responding to the CS and US during delay conditioning and unpaired extinction—The top of Figure 1 depicts mean percent responding to the tone CS (%CRs)
during delay conditioning, unpaired extinction with a weak US, and CS-alone test. Two rabbits did not reach a criterion of 85% CRs and were not included in the analyses. The left side of the top of the figure shows the remaining 23 rabbits acquired CRs quickly and to uniform levels higher than 95% CRs by the end of delay conditioning. ANOVA of %CRs yielded a significant effects of Days (F(5, 105) = 16.65, p<0.001) but no main effect or interaction for the dummy variable Delay (F’s < 1.85, p>0.164). The center-top of Figure 1 shows that responding to the CS during unpaired extinction with a weak US declined as a function of days but there were no clear differences in %CRs as a function of the delay to extinction (Delay) although the 0-week delay group seems to have responded at higher levels than the 1- or 4-week delay groups. ANOVA yielded a significant effect of Days (F(5,100) = 12.95, p<0.001) but no main effect of or interaction with Delay (F’s < 1.90, p’s >0.175). Comparisons of %CRs on individual days across the different delays to extinction yielded significant differences between the 0-week group and the 1- and 4-week groups on Days 4 and 5 of unpaired extinction p=0.034 and 0.029, respectively. There were no significant differences in %CRs during the CS Test (F(2,20) = 1.55, p=0.237).

The bottom of Figure 1 shows mean percent responding to the US (%URs) as a function of the 6 days of unpaired extinction with a weak US and there appears to be a higher level of responding, at least initially, to the US 4 weeks following delay conditioning than either 0 or 1 week after delay conditioning. However, ANOVA only yielded a main effect of Days (F(5,100) = 3.88, p=0.003) and no main effect or interaction of Delay (F’s < 2.13, p’s >0.123).

Responding to the US during US testing—The average topographies of Figure 2 and the bar graphs in Figure 3 show several important outcomes across US testing on Pretest, Post Test 1 (Post1), and Post Test 2 (Post2). First, there is very clear evidence of increases in responding from Pretest to Post1 especially at intermediate US test intensities (0.3, 0.5, 1.0 mA) – indicative of CRM. These observations were supported by analyses of the intermediate intensities which yielded a main effect of Pretest versus Post1 for UR frequency (F(1,20) = 11.77, p =0.003) and interactions of Pretest versus Post1 and US intensities for the magnitude of UR amplitude (F(4, 80) = 7.22, p<0.001) and magnitude of UR area (F(4,80) = 7.93, p<0.001). Figure 2 also shows that the increase in response area from Pretest to Post1 was accompanied by a shift to the right in the peak latency of the UR (Table 1) indicating response timing had changed and were reaching a peak later than they had on Pretest. ANOVA of peak latencies for US intensities at which there were a sufficient number of responses to measure peak latency (0.5 and 1.0mA) without the ceiling effect at 2.0 mA yielded a significant effect of Pretest versus Post1 for peak latency (F(1,20) = 5.64, P=0.028). There were no significant effects of the dummy variable Delay (F’s < 2.13, p’s>0.144)

The second important outcome was that following a delay of one day or one week but not a delay of four weeks, six days of unpaired extinction with a weak US resulted in clear reductions in responding to intermediate US intensities on Post2 that appeared to return to levels of responding on Pretest. In contrast, following a 4-week delay between CS-US pairings and unpaired extinction, response amplitudes were higher on Post2 (following unpaired extinction) than on Pretest. There were no effects of Delay nor differences between...
Pretest and Post2 for UR frequency \((F(1,20) = 3.22, p=0.088)\) or magnitude of UR area \((F(1,20) = 2.13, p=0.145)\) but there was a main effect of Delay \((F(2,20) = 3.81, p=0.04)\) and an interaction of Delay and US intensity \((F(8,80) = 2.80, p=0.03)\) for the magnitude of US amplitude. Finally, peak latencies on Post2 appeared to return to Pretest levels because there was no difference in peak latency between Pretest and Post 2 \((F(1,20) = F = 0.24, p=0.628)\).

To better understand the differences in responding during US testing as a function of the delay between CS-US pairings and unpaired extinction, we analyzed the relationship between Pretest, Post1, and Post2 by using within-group orthogonal contrasts for linear and quadratic trend across the three tests as well as between-group orthogonal contrasts to compare among the three delay groups. Specifically, if responding increased as a function of CS-US pairings indicative of CRM (Pretest < Post Test 1) and then remained high or even increased on Post Test 2 following unpaired extinction (Post Test 1 ≤ Post Test 2), there would be a linear trend across the three tests (Pretest < Post Test 1 ≤ Post Test 2). If, on the other hand, responding increased as a function of CS-US pairings indicative of CRM (Pretest < Post Test 1) and then decreased again following unpaired extinction (Pretest < Post Test 1 > Post Test 2) there would a quadratic trend across the three tests. As was the case for the ANOVAs conducted above, these outcomes would be expected to be most clear among the intermediate US intensities (0.3, 0.5, 1.0 mA) where there was no floor (0.1 mA) or ceiling (2.0 mA) in the level of responding (%UR). Indeed, there was a significant quadratic trend at intermediate US intensities across the three US tests for UR frequency \((F(1,20) = 5.36, p= 0.031)\), magnitude of UR amplitude \((F(1,20) = 7.38, p=0.013)\) and magnitude of UR area \((F(1,20) = 9.67, p=0.006)\). Finally, there was a significant increase in magnitude of UR amplitude for the 4-week group compared to the 0- and 1-week groups across testing \((F(1,20) = 4.94, p=0.038)\) corroborating the observed increase in amplitude for the 4-week group on Post2 compared to Pretest and confirming the ANOVA results for magnitude of UR amplitude presented above.

Experiment 2

The significant increase in amplitude of responses one month following unpaired extinction but not in the peak latency of those responses was reminiscent of increases in amplitude we have seen previously following unpaired presentations of the CS and US (Burhans, Smith-Bell, 2015, Schreurs, Shi, 2000, Seager et al., 2003). Consequently, we designed an experiment in which we could assess the non-associative contributors to responding by comparing responding to the US in a group of rabbits given explicitly unpaired presentations of the CS and US one month before delayed unpaired extinction with a weak US to another group given paired presentations of the CS and US one month before the delayed unpaired extinction with a weak US.

Materials and Methods

Subjects—The subjects were 18 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 and treated in the same manner
as those in Experiment 1 and, unless otherwise note, the apparatus and procedures were the same as those used in Experiment 1.

Rabbits received one session per day in the following order: adaptation, US Pretest, six days of CS-US pairings (Paired, n=9) or explicitly unpaired CS and US presentations (Unpaired, n=8), US Post Test (Post1), a second US Post Test (Post2) one month later, and then six days of unpaired extinction with a weak US (0.3 mA) followed by a third US Post Test (Post3). For explicitly unpaired stimulus presentations, each session consisted of 80 presentations of the tone CS and 80 presentations of the shock US (100 ms, 2 mA) 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 minutes.

Results

Responding to the CS and US during delay conditioning and unpaired CS and US presentations—The top of Figure 4 depicts mean percent responding to the tone CS (%CRs) for rabbits given six days of delay conditioning followed one month later by six days of unpaired extinction with a weak US (Paired, n=9) and those given six days of explicitly unpaired stimulus presentations followed one month later by six days of unpaired extinction with a weak US (Unpaired, n=8). One rabbit in the Paired group had not reached a criterion of 85% CRs and was not included in the analyses. The top of the figure shows all rabbits in the Paired group acquired CRs quickly and to uniformly high levels exceeding 95% CRs by the end of delay conditioning. Rabbits in the Unpaired group showed levels of responding that did not exceed 3% across the six days of unpaired stimulus presentations. ANOVA of %CRs during the delay conditioning phase yielded significant effects of Pairings (F(1,15) = 3270.94, p<0.001), Days (F(5,75) = 67.20, p<0.001) and an interaction of Pairings and Days (F(5,75) = 62.57, p<0.001). The right-top of Figure 4 shows rabbits in the Paired group showed a gradual decrease in %CRs from 50% during presentations of the tone CS during unpaired extinction with a weak US whereas rabbits in the Unpaired group responded at levels that did not exceed 5%. ANOVA of %CRs during unpaired extinction yielded significant effects of Pairings (F(1,15) = 19.61, p<0.001), Days (F(5,75) = 4.30, p=0.002), and an interaction of Pairings and Days (F(5,75) = 4.58, p=0.001).

The bottom of Figure 4 depicts mean percent responding to the shock US (%URs) for Paired and Unpaired rabbits. The bottom of the figure shows all rabbits in the Unpaired group responded to the US at uniformly high levels that exceeded 99% URs across the six days of unpaired stimulus presentations. There are no data for the Paired group because the CS and US coterminated, and their responding is shown in the top of the figure. The right side of the bottom of Figure 4 shows %URs during the six days of unpaired extinction with the weak US. Rabbits in the Paired group showed levels of responding to the weak US that were higher than those shown by rabbits in the Unpaired group across the six days of unpaired extinction suggestive of the increased response frequency seen with CRM. ANOVA of %URs to the weak US during unpaired extinction yielded a significant main effect of Pairings (F(1,15) = 14.34, p=0.002) but no main effect or interaction of Days (F’s < 0.65, p’s>0.60).

J Psychiatr Res. Author manuscript; available in PMC 2019 April 01.
Responding to the US during US testing—The average topographies of Figure 5 and the bar graphs in Figure 6 show several important outcomes across US testing on Pretest, Post1, Post2 four weeks later, and Post3 after six days of unpaired extinction with the CS and a weak US. First, there is very clear evidence of increases in responding from Pretest to Post1 especially at intermediate US test intensities (0.3, 0.5, 1.0 mA) for rabbits in the Paired group but not in the Unpaired group replicating and confirming previous observations that CRM is the result of CS-US pairings not simply a function of repeated CS and US presentations, and is therefore associative in nature (Schreurs et al., 1995, Seager, Smith-Bell, 2003). This observation was confirmed at intermediate US intensities (0.3, 0.5, 1.0 mA) by a main effect of Pairing for UR frequency (F(1,15) = 4.61, p=0.049), magnitude of UR amplitude (F(1,15) = 4.56, p=0.050) and magnitude of UR area (F(1,15) = 5.24, p=0.037). Figure 5 also shows the increase in response area from Pretest to Post1 was accompanied by shift to the right in the latency to the peak of the response for rabbits in the Paired group. These changes were not observed in the Unpaired group. ANOVA of peak latencies for intermediated US intensities at which there were a sufficient number of responses to measure peak latency without a floor or ceiling effect (0.5 and 1.0mA) yielded a significant main effect of Pairings (F(1,15) = 12.56, P=0.003).

The second result was that following a delay of four weeks, response frequency and size on Post2 was elevated for rabbits in both the Paired and Unpaired groups which was supported by an increase from Pretest to Post2 in %URs (F(1,15) = 9.30, p=0.008), magnitude of the amplitude (F(1,15) = 8.39, p=0.011), and magnitude of response area (F(1,15) = 8.45, p=0.011) but no significant effects of Pairings (F’s < 3.98, p’s>0.064). There was, however, as suggested in Figure 5 for the Paired rabbits especially at 0.5 mA, a significant difference between Paired and Unpaired rabbits in the peak latency of their responses suggesting that at least one index of CRM had survived the four-week delay between CS-US pairings and unpaired extinction. This was supported by a significant main effect of Pairing (F(1,15) = 5.28, p=0.036).

Finally, following six days of unpaired extinction with a weak US, rabbits from both Paired and Unpaired groups showed continued elevated responding in the frequency and size of responding reflected in significant differences between Pretest and Post3 in %URs (F(1,15) = 21.08, p<0.001), magnitude of the amplitude (F(1,15) = 10.32, p=0.006), and magnitude of response area (F(1,15) = 10.59, p=0.005) without any differences between rabbits in the Paired and Unpaired groups (F’s < 3.46, p’s>0.082). Interestingly, although response frequency and size remained elevated after unpaired extinction, inspection of Figure 5 suggests and an analysis confirms that latencies to the peak of responses to shock returned to Pretest levels following unpaired extinction (Pretest versus Post3, (F(1,15) = 1.41, p=0.254) confirming that at least one index of CRM was extinguished by unpaired extinction with a weak US.

Discussion

The principal findings of the present experiments were that: (1) the hyperarousal observed following CS-US pairings can be extinguished with explicitly unpaired presentations of the CS and a weak US presented a day or a week after delay conditioning but is much less
effective if explicitly unpaired presentations of the CS and a weak US are presented a month after conditioning; (2) conditioned responding to the CS can be extinguished successfully with explicitly unpaired presentations of the CS and US presented a day, a week, or a month after CS-US pairings; (3) responding to the US a month after conditioning may have both associative and non-associative components because rabbits given explicitly unpaired CS and US presentations one month before testing also developed increased responding to the US; (4) an important index of CRM – an increase in the peak latency of responding to the US – shifted to the right and remained there one month later in rabbits given CS-US pairings but did not shift in rabbits given unpaired stimulus presentations; and (4) the shift in peak latency seen in rabbits given CS-US pairings returned to pretest levels following explicitly unpaired presentations of the CS and a weak US.

Delays in seeking treatment particularly among military personnel (Maguen et al., 2012, Maguen, Madden, 2014) and loss of effectiveness of therapy for PTSD the longer treatment is delayed have been well-documented (Boulos and Zamorski, 2015). There are several factors that contribute to the problem, particularly in the military populations where patient-centered issues such as delay or avoidance of treatment for fear of being stigmatized for seeking mental health treatment are prevalent (Iversen et al., 2011, Mittal et al., 2013, Quartana, Wilk, 2014, Rosen et al., 2011) and where delays in treatment occur due to system-generated bureaucratic inefficiencies such as scheduling (Mattocks et al., 2017, Vanneman et al., 2017). The current data add to the evidence that such delays are detrimental to treatment effectiveness and the ability to recover from PTSD. Despite the fact that the actual prevalence of PTSD in the military is a controversial issue (Fisher, 2014, Magruder et al., 2015), it is clear that treatment of PTSD in active military and veterans is less successful than among civilians (Cusack, Jonas, 2016, Difede, Olden, 2014, Hermes et al., 2012) and delays in treatment may be a significant contributor (Boulos and Zamorski, 2015). Indeed, the current data show that responding to shocks of varying intensities comprise a hyperarousal that, although “treated” to some extent because shifted peak latencies return to pretest levels after unpaired extinction with a weak US, still shows increased magnitudes despite such “treatment”.

The elevated responding in both the paired and unpaired groups one month after stimulus presentations provides an interesting perspective on the events surrounding trauma. In combat, as in civilian life, there are both signaled and unsignaled dangers. For example, if troops are attacked by mortars or gunfire but are not at the front of the line, there are usually sights and sounds that provide a warning of imminent danger. However, if they are the first to get hit by gunfire or a roadside bomb, there may be no warning. Likewise, civilians are often warned well in advance of life-threatening weather events but may be blindsided by a drunk driver and have no warning in a vehicle accident. In the laboratory, humans exposed to unsignaled aversive events can develop learned helpless whereas those expose to signaled aversive events do not (Grillon, 2008, Maier and Seligman, 2016). Interestingly, both civilians and combat veterans with PTSD show more reactivity to unsignaled aversive events than those without PTSD (Grillon et al., 2009, Simmons et al., 2013). Although possible, it is not clear whether unsignaled aversive events are more likely to cause PTSD. They do, however, play a significant role in anxiety disorders (Grillon et al., 2008, Lissek et al., 2008).
In the current experiment, both signaled and unsignaled events were modeled by the paired (warning of the US by the CS) and explicitly unpaired (no warning of the US) procedures in the second experiment, and both resulted in forms of hyperarousal. An important factor to keep in mind with this analogy is that these dangers exist in a context where danger might be expected (e.g., being on combat patrol or traveling in a speeding vehicle) including a specific context in which unpaired presentations of the CS and US took place. It is possible that there is some level of conditioning to the context in the rabbits given explicitly unpaired stimulus presentations where the shock only occurs in the training chambers after the rabbits are restrained and set up to measure responding. Although rodents are exquisitely sensitive to novel contexts particularly in contextual fear conditioning (Izquierdo et al., 2016, Maren, 2011), rabbits do not seem nearly as sensitive to changes in context because we have demonstrated previously that CRM is only affected by a change in context if the second context is familiar and not if it is novel (Schreurs et al., 2006). Nevertheless, sensitivity to the context may be more important after the period of incubation afforded by the month between CS and US presentations and US testing and unpaired extinction. The only evidence we have that incubation may not have played a role is that there was no CRM in rabbits given six days of incubation between CS-US pairings and US testing (Schreurs et al., 2011a). However, rabbits in that former experiment were only given a single day of CS-US pairings, leaving open the possibility that incubation effects may still be at play after a longer delay if rabbits are given a full course of CS-US pairings (six days). Another form of sensitivity to the context may occur as a result of the reinstatement (Bouton et al., 2006, Myers and Davis, 2007) inherent in repeated US presentations during US testing, particularly a month after unpaired stimulus presentations. However, a comparison of responding on US testing after unpaired extinction for the 4-week group in Experiment 1 (Figure 2) and the Paired group in Experiment 2 (Figure 5) shows virtually identical levels of responding, despite the Paired group receiving a US test session one day prior to the start of unpaired extinction. Another consideration is that six days of unpaired extinction may have been enough to overcome any reinstatement effect resulting from US testing.

The shift in the peak latency of responses to the US and the change in shape of the response so that the response is timed differently and less stereotypic is only seen in rabbits given paired presentations of the CS and US and is the hallmark of an associative effect we see consistently in experiments examining CRM (Burhans et al., 2008, Schreurs and Burhans, 2015) and which has also been observed by others (Gruart and Yeo, 1995). This exaggerated response is returned to more stereotypic responding after explicitly unpaired presentations of the CS and US and the response begins to return to pretest levels. If viewed as an index of hyperarousal, it is tempting to speculate that the return of the response to baseline as successful “treatment” of that hyperarousal (Burhans, Smith-Bell, 2015, Schreurs and Burhans, 2015).

Taken together, the current data provide evidence that unpaired extinction with a weak US soon after CS-US pairings is effective in reducing both responding to the cues associated with the US and hyperarousal to the US. In contrast, a significant delay in unpaired extinction results in persistent hyperarousal that may have both associative and non-associative components. As such, rabbit CRM recapitulates another aspect of the realities of
seeking and obtaining treatment for PTSD particularly among those currently serving in the military and our veterans.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**References**

Boulos D, Zamorski MA. Do shorter delays to care and mental health system renewal translate into better occupational outcome after mental disorder diagnosis in a cohort of Canadian military personnel who returned from an Afghanistan deployment? BMJ Open. 2015; 5:1–12.


*J Psychiatr Res*. Author manuscript; available in PMC 2019 April 01.


Figure 1.
Responding to the conditioned stimulus (CS) and unconditioned stimulus (US) during delay conditioning and unpaired extinction. The top of Figure 1 depicts mean (± SEM) percent responding to the tone CS (%CRs) during CS-US pairings with a strong US (2 mA), unpaired extinction with the CS and a weak US (0.3 mA) presented after a delay of one day (0 Week), one week (1 Week) or four weeks (4 Week), followed by CS-alone testing one day later. The bottom of Figure 1 shows mean (± SEM) percent responding to the shock US (%URs) during unpaired presentations of the CS and a weak US (0.3 mA, Unpaired Extinction) presented after a delay of one day (0 Week), one week (1 Week) or one month (4 Week).
Figure 2.
Responding to the unconditioned stimulus (US) during testing of the US. 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 post-test following CS-US pairings (Post1, red line), and the US post-test presented one day (0 Week), one week (1 Week) or 4 weeks (4 Week) following unpaired extinction (Post2, blue line) during which rabbits received unpaired extinction with a weak US (0.3 mA). Topographies are shown at the five US intensities (2.0, 1.0, 0.5, 0.3, 0.1 mA) presented during US testing, collapsed across duration.
Figure 3.
Mean (± SEM) percentage of unconditioned responses (Percent URs, top row), magnitude of the UR amplitude (middle row), and magnitude of the UR area (bottom row) for the first 20 trials of presentations of the periorbital shock unconditioned stimulus (US) during pretest (Pretest, white bar), the post-test following delay conditioning (Post1, light gray bar), and the post-test following extinction (Post2, gray bar) presented one day (0 Week Delay), one week (4 Week Delay) or 4 weeks (4 Week Delay) following unpaired extinction with a weak US (0.3 mA). Bar graphs are shown for the five US intensities (0.1, 0.3, 0.5, 1.0, 2.0 mA) presented during US testing, collapsed across duration.
Figure 4.
Responding to the conditioned stimulus (CS) and unconditioned stimulus (US) during delay conditioning and unpaired extinction for rabbits in the Paired and Unpaired groups. The top of Figure 4 depicts mean (± SEM) percent responding to the tone CS (%CRs) during CS-US pairings with a strong US (2 mA) and unpaired extinction with the CS and a weak US (0.3 mA) for rabbits in the Paired group and rabbits in the Unpaired group. The bottom of Figure 4 shows mean (± SEM) percent responding to the shock US (%URs) during unpaired presentations of the CS and a weak US (0.3 mA, Unpaired Extinction) for rabbits in the Paired group and rabbits in the Unpaired group.
Figure 5.
Responding to the unconditioned stimulus (US) during testing of the US for rabbits in the Paired and Unpaired groups. 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 post-test following CS-US pairings (Post1, red line), the US post-test presented 4 weeks later (Post2, blue line), and the post-test following unpaired extinction (Post3, green line) during which rabbits received unpaired extinction with a weak US (0.3 mA). Topographies are shown at the five US intensities (2.0, 1.0, 0.5, 0.3, 0.1 mA) presented during US testing, collapsed across duration.
Figure 6.
Mean (± SEM) percentage of unconditioned responses (Percent URs, top row), magnitude of the UR amplitude (middle row), and magnitude of the UR area (bottom row) for the first 20 trials of presentations of the periorbital shock unconditioned stimulus (US) during pretest (Pretest, white bar), the post-test following CS-US pairings (Post1, light gray bar), the post-test presented 4 weeks later (Post2, gray bar), and the post-test following unpaired extinction with a weak US (0.3 mA, Post 3, dark gray bar). Bar graphs are shown for the five US intensities (0.1, 0.3, 0.5, 1.0, 2.0 mA) presented during US testing, collapsed across duration.
Table 1

Mean (± SEM) peak latencies for the unconditioned responses to the periorbital shock unconditioned stimulus (US) during the first 20 trials of the US pretest (Pretest), the US post-test following CS-US pairings (Post1), and the US post-test presented one day (0 Week), one week (1 Week) or 4 weeks (4 Week) following unpaired extinction (Post2) during which rabbits received unpaired extinction with a weak US (0.3 mA). Peak latencies are listed at the four US intensities (0.3, 0.5, 1.0, 2.0 mA) at which some level of responding occurred during US testing. The data are collapsed across US duration.

<table>
<thead>
<tr>
<th>Group</th>
<th>Test</th>
<th>US Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.3 mA</td>
</tr>
<tr>
<td>0 Week</td>
<td>Pretest</td>
<td>252.85 ± 150.20</td>
</tr>
<tr>
<td></td>
<td>Post1</td>
<td>148.47 ± 20.51</td>
</tr>
<tr>
<td></td>
<td>Post2</td>
<td>106.33 ± 18.89</td>
</tr>
<tr>
<td>1 Week</td>
<td>Pretest</td>
<td>88.75 ± 10.38</td>
</tr>
<tr>
<td></td>
<td>Post1</td>
<td>252.5 ± 36.06</td>
</tr>
<tr>
<td></td>
<td>Post2</td>
<td>124.06 ± 37.55</td>
</tr>
<tr>
<td>4 Week</td>
<td>Pretest</td>
<td>229.50 ± 135.28</td>
</tr>
<tr>
<td></td>
<td>Post1</td>
<td>234.82 ± 81.81</td>
</tr>
<tr>
<td></td>
<td>Post2</td>
<td>89.10 ± 5.48</td>
</tr>
</tbody>
</table>