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Propranolol produces short-term facilitation of extinction in a rabbit model of post-traumatic stress disorder

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Abstract

Post-traumatic stress disorder (PTSD) is a learning-based anxiety disorder with significant public health challenges due to difficulties in treating the complex, multiple symptomology. We have developed an animal model of PTSD, based on Pavlovian eyeblink conditioning in rabbits, that addresses two key features: conditioned responses (CRs) to cues associated with an aversive event and a form of conditioned hyperarousal referred to as conditioning-specific reflex modification (CRM). We have found previously that unpaired extinction is ideal for reducing both CRs and CRM simultaneously and shows sensitivity to systemic serotonergic and glutamatergic manipulations. The following study aimed to extend our work to examine the role of the noradrenergic system, dysregulation of which is strongly implicated as part of the neurobiology of PTSD and which may also play a role in the balance shift from fear reconsolidation to extinction during treatment. The goal of the following two studies was to examine whether the β-adrenergic receptor antagonist propranolol combined with either a full or brief course of unpaired extinction treatment could enhance extinction of CRs and/or CRM. Results showed a within-session facilitation of propranolol on extinction of CRs, particularly during the first extinction session, and a short-term enhancement of extinction of CRM when extinction treatment was brief. However, neither benefit translated to long-term extinction retention for the majority of subjects. Findings suggest that propranolol may provide the most therapeutic benefit in situations of high arousal early in treatment, which may be more important for future patient compliance rather than long-term treatment outcomes.

Keywords

eyeblink conditioning; fear conditioning; extinction; hyperarousal; post-traumatic stress disorder; β-adrenergic

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

1. Introduction

Post-traumatic stress disorder (PTSD) is a multifaceted anxiety disorder that develops in response to trauma exposure in approximately 7% of individuals in the United States (Kessler et al., 2005), with estimates often higher in those exposed to military or combat trauma (Donoho et al., 2017; Ramchand et al., 2010; but see also Wisco et al., 2016. Treatment of PTSD remains a significant public health challenge due to difficulty in finding treatments that can address the complex nature of PTSD symptomology, which includes persistent re-experiencing of the event, avoidance of stimuli associated with trauma, and generalized hyperarousal such as increased startle reflexes and hypervigilance (American Psychiatric Association, 2013). Not surprisingly, research into the neurobiology of PTSD has suggested the involvement of multiple neural/neurochemical systems that may each play a role in one or more aspects of PTSD symptomology (Kelmendi et al., 2016; Pitman et al., 2012). In addition, there is a learning, or more specifically, a dysfunctional fear conditioning component underlying PTSD (Lissek and van Meurs, 2014; VanElzakker et al., 2014) that cannot easily be resolved by pharmacological treatment alone. The use of cognitive behavioral therapy aimed at extinguishing abnormally conditioned fear is another factor to consider in the development of PTSD treatments, further increasing the pool of pharmacological targets to include those that may be used in conjunction with therapy to improve efficacy, by enhancing fear extinction for example (Fitzgerald et al., 2014; Singewald et al., 2015). Because of the complexity of PTSD and the fact that it is often co-morbid with other disorders such as depression, animal models are crucial to further our understanding of why certain treatments work and how they may be combined to provide better treatment outcomes in the clinical population.

We have developed an animal model of PTSD that addresses two key features: conditioned responses (CRs) to trauma-associated cues and hyperarousal (Burhans et al., 2008; Schreurs and Burhans, 2015). This model is based on conditioning of the rabbit's nictitating membrane response (NMR), also known as eyeblink conditioning. One of the unique aspects of the eyeblink conditioning paradigm is that it has historically been used in *both* humans and animals as a means of assessing associative learning (Solomon, 2002), allowing strong translatability from the bench to the clinic and vice versa. While human studies have used it to document learning phenotypes for disorders like schizophrenia (Kent et al., 2015; Marenco et al., 2003) and anxiety disorders including PTSD (Burriss et al., 2007; Handy et al., 2018), parallel work in animals has delineated the behavioral laws of acquisition and extinction and the critical neural circuitry (Christian and Thompson, 2003; Freeman and Steinmetz, 2011), giving a neurobiological perspective to those phenotypes. In a typical experiment in our laboratory, rabbits are evaluated for reflexive eyeblink responding to varying intensities (from 0.1 to 2.0 mA) of a periorbital shock unconditioned stimulus (US) prior to and following eyeblink conditioning during which they learn to associate an auditory tone conditioned stimulus (CS) with a 2.0-mA US. We have established that this paradigm results in a form of hyperarousal called conditioning-specific reflex modification (CRM), manifesting as increased and exaggerated responding to shock intensities, particularly lower intensity shocks that elicited little or no responding prior to conditioning. CRM is "conditioning-specific" in that it does not develop in the same way in rabbits that receive

explicitly unpaired presentations of the CS and US. We have validated this model by demonstrating it shares many commonalities with PTSD development; for example, CRM is stronger when more aversive (i.e. more traumatic) stimuli are used as the US during conditioning (Buck et al., 2001; Seager et al., 2003) and, like PTSD in the clinic, may worsen after an incubation period (Schreurs et al., 2011a). Importantly, strong CRM only develops in a limited number of subjects (Smith-Bell et al., 2012), just as not all those exposed to trauma develop PTSD. We have also found that the amygdala, dysfunction of which is strongly implicated in PTSD (Hughes and Shin, 2011), can modulate acquisition of CRs to the tone CS and expression of CRM (Burhans and Schreurs, 2008). CRM-like changes have also been documented by others in rabbits (Gruart and Yeo, 1995; Wikgren et al., 2002) and rats (Servatius et al., 2001).

In searching for behavioral treatments that can reduce PTSD-like symptoms in our model, we have established an extinction treatment that can reduce both CRs and CRM simultaneously (Burhans et al., 2015; Schreurs et al., 2011b). While CRs to the tone CS can be extinguished by CS-alone presentations and CRM by US-alone presentations (but not vice versa), extinction sessions including unpaired presentations of the CS and US can extinguish both. Importantly, this can be achieved even when the US is reduced six-fold from the training intensity (Schreurs et al., 2011b). In translating this to the clinic, our findings suggest that adding random presentations of innately but mildly stressful stimuli like skin stimulation to traditional therapies such as exposure therapy may help address the hyperarousal symptoms of PTSD (Haesen and Vervliet, 2015). We have also been able to use the CRM model to investigate pharmacological treatments, both alone and in conjugation with behavioral extinction treatment. Previous work has delineated that different aspects of our model are sensitive to serotonergic and glutamatergic manipulations (Burhans et al., 2013, 2017), reinforcing the notion that a multi-factor approach to treatment is needed to address multiple PTSD symptoms.

The following study aimed to further extend our work in the CRM model to examine the role of the noradrenergic system. Dysregulation of norepinephrine (NE), normally released during stressful or fearful situations as part of the fight or flight response, has been strongly implicated as part of the neurobiology of PTSD (Hendrickson and Raskind, 2016; Southwick et al., 1999; Strawn and Geracioti, 2008). In support of this, for example, it has been found that NE is elevated in PTSD patients both at baseline levels and in response to trauma-associated stimuli (Blanchard et al., 1991; Geracioti et al., 2001; Liberzon et al., 1999). The crucial role NE plays in emotional arousal and modulation of emotional memory formation is believed to occur specifically through actions at β-adrenergic receptors (McIntyre et al., 2012), making these receptors a prime target for pharmaceutical intervention. Propranolol is a β-adrenergic receptor antagonist historically prescribed as a treatment for hypertension that has gained renewed interest as a treatment for PTSD, although with some mixed results (Giustino et al., 2016; Southwick et al., 1999). Systemic propranolol has also been previously examined in eyeblink conditioning paradigms and was found to impair acquisition in both rabbits (Gould, 1998) and rats (Cartford et al., 2002), although effects on extinction are less clear with some evidence for enhancement (Gould, 1998). However, there is a vast literature supporting a role for propranolol in enhancing fear extinction and blocking fear memory reconsolidation in animal models as well as healthy

humans (Giustino et al., 2016). In the following series of two experiments, we tested the hypothesis that propranolol could enhance extinction of CRs and CRM in our rabbit model of PTSD. In the first experiment, we assessed the effects of propranolol combined with a full, six-session course of unpaired extinction treatment with weak shock. In Experiment 2, we assessed the effects of propranolol combined with a brief course of unpaired extinction, which we have previously shown to be less successful at extinguishing CRs and CRM, with evidence it may even worsen CRM (Schreurs et al., 2011b).

2. Materials and Methods

2.1 Subjects

The subjects were 43 male, New Zealand White rabbits (*Oryctolagus cuniculus*), 2–3 months of age weighing approximately 1.8–2.3 kg upon delivery from the supplier (Harlan, Indianapolis, IN). Prior to behavioral training, one rabbit was removed due to a failure to adapt to restraint. 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.

2.2 Apparatus

The apparatus and recording procedures for NMR conditioning have been detailed elsewhere by Schreurs and Alkon (1990) who modeled their apparatus based on those described by Gormezano (Coleman and Gormezano, 1971; Gormezano, 1966). Briefly, 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 75 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 hook that attached to a 6-0 nylon loop that was 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.

2.3 Procedure

One week after arrival, rabbits were first acclimated to restraint by being placed in restrainers for 30 minutes while under close supervision. Rabbits then received one training

session per day in the following order: adaption, US pretest, six sessions classical delay conditioning, US posttest (Post1), six sessions unpaired extinction with weak shock combined with either propranolol or saline, a second US posttest (Post2), and a CS-alone retention test (CS Test). Comparisons of Post1 with Pretest served as the initial evaluation of CRM while Post2 measured the amount of remaining CRM following extinction treatment with or without propranolol. The NMR responses to the tone CS during extinction served as a measurement of the immediate, within-session effects of propranolol on the extinction of CRs while the responses measured during the CS Test served as a post-treatment assessment of the remaining level of CRs.

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 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 three 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, 2.0 mA US (300 ms interstimulus interval). The CS-US presentations were presented with an average ITI of 60 s. Unpaired extinction with weak shock consisted of 80 presentations of the tone CS and 80 presentations of a 0.3 mA US (100 ms) that were explicitly unpaired and presented in a pseudorandom order. To maintain the session length at approximately 80 minutes, the average ITI for unpaired sessions was reduced to 30 s. The CS Test consisted of 80 presentations of the tone CS with an average ITI of 60 s.

2.4 Propranolol Injections

For each day of propranolol injections, a fresh stock solution of 24 mg/ml of propranolol hydrochloride (Spectrum Chemicals; VWR, Bridgeport, NJ) was prepared by dissolving in 0.9% sterile saline. Rabbits were divided into four groups receiving intramuscular injections of 0.9% saline or 3 mg/kg, 6 mg/kg, or 12 mg/kg propranolol 30 minutes prior to the start of each of six sessions of unpaired extinction. To equate injection volume, serial dilutions of 12 mg/ml and 6 mg/ml were prepared from the stock propranolol solution for the 6 mg/kg and 3 mg/kg groups, respectively.

2.5 Statistical Analysis

Conditioned responses (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 pre and posttests, an unconditioned response (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 millimeters. 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 = 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 (mAmp) and magnitude of the response area (mArea), 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 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, data were collapsed at the five US intensities across duration and CRM analyses were focused 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 three separate replications. Drug groups were assigned following NMR conditioning and CRM testing in order to equate groups for learning and CRM levels. Unless otherwise indicated, data were analyzed by repeated measures analysis of variance (ANOVA, SPSS 21). For violations of sphericity, p-values reflect corrections using procedures of Huynh-Feldt. Planned and follow-up comparisons were Bonferroni corrected for the number of comparisons.

Additionally, rabbits were evaluated for individual variation in the strength of CRM. Following the convention of previous publications (Smith-Bell et al., 2012; Smith-Bell and Schreurs, 2017), mean percent change in UR frequency, mArea, and mAmp from Pretest to Post1 at each US intensity was calculated for saline and propranolol groups combined. Rabbits exhibiting a percent change greater than or equal to two standard deviations above the mean percent change were categorized as having strong CRM.

3. Results

3.1 Delay Conditioning

Two rabbits were removed from all analyses due to a failure to reach a learning criterion of 80% CRs by the last day of conditioning, resulting in final n's of 10 subjects per group. The average percentage of CRs to the tone CS across six sessions of classical delay conditioning is shown on the left side of Figure 1. Rabbits rapidly acquired a high level of conditioning with averaged final percent CRs in excess of 98% (± 0.44 SEM), as confirmed by a significant effect of Session [$F(5,180)= 142.2$, $p < 0.001$] with corrected planned comparisons indicating CRs increased from the first to second session ($p < 0.001$) and remained at a similar high level for the remaining days of training. There were no significant differences between groups, demonstrating that all groups were equivalent in learning rate and level prior to drug manipulations during extinction.

3.2 Effects of Propranolol on Extinction of Conditioned Responses

The average percentage of CRs to the tone CS across six sessions of unpaired extinction with weak shock is shown in the middle of Figure 1. Treatment with propranolol thirty minutes prior to the start of the extinction session appeared to have a dose-dependent effect on CRs, with the highest dose group (12 mg/kg) showing the lowest level of CRs, followed by the middle (6 mg/kg) and then low (3 mg/kg) dose groups, with the low dose being most similar to saline injected controls. Analysis of all six sessions of extinction indicated a trend for a main effect of Drug Group [$F(3,36) = 2.43$, $p = 0.071$] and a trend for an interaction of Session and Drug Group [$F(15, 180) = 1.77$, $p = 0.086$]. When analysis was restricted to comparisons of the 12 mg/kg dose with saline controls, there was a significant effect of Drug Group [$F(1,18) = 7.57$, $p < 0.05$] but no interaction with Session, demonstrating that CRs were lower for the 12 mg/kg dose compared to saline throughout extinction.

Analyses of other CR parameters (latency, amplitude, area) at each extinction day did not reveal any effects of Drug Group with the exception of a main effect on the fifth session of extinction for CR amplitude [$F(3,24) = 4.55$, $p < 0.05$] and CR area [$F(3, 24) = 3.36$, $p < 0.05$], with corrected post hocs indicating that the 3 mg/kg group had larger CRs for both measures compared to saline and the 12 mg/kg groups (all p's < 0.05). There were no significant drug effects on the UR for US-alone trials during extinction for UR frequency (see boxed inset in Figure 1), nor for latency, area, or amplitude measures.

In order to look in more detail at whether propranolol was affecting within-session extinction, the extinction sessions were graphed by 10-trial blocks of CS-alone trials, shown in Figure 2. Importantly, at the beginning of the first extinction session, all groups appeared to be performing at a similar level. As the first extinction session proceeded, the 12 mg/kg group exhibited the greatest decrease in responding, suggesting that propranolol enhanced within-session extinction rather than producing a performance or memory deficit. Analysis of 10-trial blocks for the first versus last 10 trials of the extinction session across all six sessions yielded no significant group effects. However, a focused analysis of the 12 mg/kg group compared with the saline controls indicated a significant interaction of Session, Block, and Drug Group [$F(5,90) = 3.785$, $p < 0.05$]. After Bonferroni corrections of planned comparisons, there was only a trend for lower CRs in the 12 mg/kg dose during the last block of the first extinction session ($p = .06$), suggesting that lower overall CR levels in the propranolol group across extinction sessions was more likely the result of facilitation of extinction, rather than a performance or memory deficit. By the end of extinction, however, the saline and lower dose drug groups were catching up to the terminal levels of extinction exhibited by the 12 mg/kg group. To measure extinction retention, comparisons of the last block with the first block of the subsequent extinction session revealed the 12 mg/kg group, but not saline controls, showed an increase in percent CRs from the first to the second session, demonstrating poor extinction retention ($p < 0.01$).

During the CS Test that took place two days following the last session of extinction, the 12 mg/kg propranolol group was observed to be at a similar, but slightly higher, level of responding than saline controls (right side of Figure 1). In confirmation, analysis of the CS Test revealed that there were no group differences when analysis included either all groups or just comparisons of saline controls with the 12 mg/kg group. In addition, no group effects

emerged from examining the CS Test data divided into blocks. These results confirm that the highest dose propranolol group lost its early advantage during extinction, with all groups subsequently demonstrating similar levels of CRs at the CS Test.

3.3 Conditioning-Specific Reflex Modification

Prior to propranolol injections during extinction, all groups demonstrated significant CRM at Post1, as evidenced by Pretest to Post1 increases in several UR measures, particularly at the intermediate intensities for which CRM tends to be strongest (Burhans et al., 2008). Changes in the amplitude, area, and latency of the UR can be discerned by the UR topographies in Figure 3. Increases in area and amplitude of the UR were confirmed by significant Test (Pretest vs Post1) by Intensity interactions for mAmp [$F(4,144) = 8.20$, $p < 0.001$] and mArea [$F(4,144) = 5.94$, $p < 0.001$] with corrected planned comparisons indicating significant increases for both measures at the 0.5 mA intensity (p's< 0.05). Amplitude and area increases were also found at 0.3 mA but were not statistically significant following Bonferroni correction. Due to the limitations of empty data cells (see Methods), analyses of latency measures were conducted at each intensity separately. For onset latency, the only Pre to Post1 change occurred at the 2.0 mA intensity [$F(1,36) = 6.60$, $p< 0.05$]: however, it was the result of a *decrease* in latency. For peak latency, there were significant Pre to Post1 increases at the 0.3 [$F(1,27) = 21.76$, $p< 0.001$], 0.5 [$F(1,33) = 19.65$, $p <0.001$], and 1.0 mA [$F(1,36) = 7.63$, $p < 0.01$] intensities and a decrease at 2.0 mA [$F(1,36) = 5.61$, $p< 0.05$]. There were no significant Pre to Post1 changes in the UR frequency. There were no group differences in CRM for any of the UR measures, indicating that the groups were equivalent prior to propranolol and extinction treatment.

Evaluation of individual variation in the strength of CRM revealed that only 22.5% of rabbits exhibited strong CRM for at least one of the intermediate intensities, 0.3, 0.5, or 1.0 mA for %UR, mAmp, or mArea. This finding is similar to percentages reported in previous publications utilizing much larger databases of rabbit data (Smith-Bell et al., 2012; Smith-Bell and Schreurs, 2017). The incidence of strong CRM was not evenly distributed among groups as saline, 3 mg/kg, 6 mg/kg, and 12 mg/kg propranolol groups had 3, 0, 4, and 2 rabbits with strong CRM, respectively.

3.4 Effects of Propranolol on Extinction of Conditioning-Specific Reflex Modification

Effects of propranolol combined with six sessions of unpaired extinction were evaluated at Post2, one day following the last extinction session (see Figure 3). All groups appeared to show significant decreases in CRM regardless of drug treatment, with area, amplitude, and peak latency changes returning to Pretest levels. Analyses of mAmp and mArea focused on the intensities for which the greatest initial CRM was found, 0.3 and 0.5 mA. There was a significant effect of Test (Pre, Post1, Post2) for both mAmp [($F(2,72) = 6.36$, $p < 0.01$] and mArea [$F(2,72) = 9.72$, $p < 0.001$] with corrected post hoc tests indicating that Post2 was decreased relative to Post1 for both measures (p's < 0.001) and not statistically different from Pretest. There were no significant interactions with Intensity (0.3, 0.5 mA) or significant effects involving Drug Group. For peak latency, analyses at each of the three intensities showing significant initial CRM (0.3, 0.5, 1.0 mA) indicated a significant effect of Test at only the 0.5 mA intensity [$F(2,64) = 16.11$, $p < 0.001$], with corrected post hoc

tests indicating that Post2 was reduced compared to Post1 ($p < 0.001$) and not significantly different than Pretest. Again, there were no effects involving Drug Group. There were not enough rabbits exhibiting strong CRM in each group to conduct additional analyses to examine whether strength of CRM would affect response to propranolol treatment.

4. Experiment 2

Results from the first experiment collaborated previous studies demonstrating that six days of unpaired extinction with weak shock is effective at reducing both CRs and CRM (Burhans et al., 2015; Schreurs et al., 2011b). This raised the possibility that floor effects may have accounted for the failure to see effects of propranolol on extinction of CRM or long-term effects on the extinction of CRs. A second experiment was conducted to examine if propranolol would be more beneficial when a brief course of treatment was utilized. A single session of unpaired extinction with weak shock has previously been shown to be less effective at reducing CRs than three or six days of treatment, with evidence that it may increase the size of CRM (Schreurs et al., 2011b). In Experiment 2, we investigated the effects of propranolol combined with a single day of unpaired extinction with weak shock using the propranolol dose that enhanced within-session extinction of CRs in the first experiment (12 mg/kg).

5. Materials and Methods

5.1 Subjects

The subjects were 35 male, New Zealand White rabbits (*Oryctolagus cuniculus*), 2-3 months of age weighing approximately 1.8–2.3 kg upon delivery from the supplier (Charles River, Saint-Constant, Canada). Of some importance is the change in vendor for the rabbits used in Experiment 2 due to closure of the original vendor, which was the source for the majority of past publications on the CRM model. The old and new vendors were located in different countries (USA vs Canada), used separate stock breeds of New Zealand rabbits, and also had different handling and enrichment protocols. We had concerns when we observed rabbits from the new vendor appeared to have increased baseline sensitivity to the periorbital shock US at Pretest and a lower incidence of CRM. For this reason, we used a larger sample of animals per group for Experiment 2 than for the first experiment.

Prior to behavioral training, two rabbits were removed due to a failure to adapt to restraint. 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.

5.2 Procedure

The apparatus and stimulus parameters of the training sessions in Experiment 2 were identical to those in Experiment 1. Following restraint acclimation, rabbits received one training session per day in the following order: adaptation, US pretest, six sessions classical delay conditioning, Post1, one session of unpaired extinction with weak shock combined with12 mg/kg propranolol or saline, and Post2. One week later, rabbits received a third US

posttest (Post3) and a CS Test. NMR responses to the CS during extinction and to the US during Post2 evaluated the more immediate effects of a single day of extinction treatment with or without propranolol on CRs and CRM, respectively. Post3 and the CS Test examined the level of remaining CRM and CRs, respectively, one week after treatment. Prior to the single unpaired extinction session, a fresh stock solution of 24 mg/ml of propranolol hydrochloride (Spectrum Chemicals; VWR, Bridgeport, NJ) was prepared by dissolving in 0.9% sterile saline. Rabbits were divided into two groups receiving either saline or 12 mg/kg propranolol 30 minutes prior to the start of the session. Procedures for statistical analyses were identical to those in the first experiment.

6. Results

6.1 Delay Conditioning

One rabbit was removed from all analyses due to excessive spontaneous blinking. Two additional rabbits were removed from all analyses because they exhibited extremely large responses during Pretest and were mathematically confirmed to be outliers based on the area and amplitude of their URs. Final n's per group were 14 and 16 for the saline and propranolol groups, respectively. The average percentage of CRs to the tone CS across six sessions of delay conditioning is shown on the left side of Figure 4. Rabbits rapidly acquired a high level of conditioning with averaged final percent CRs in excess of 96% (± 1.3 SEM), as confirmed by a significant effect of Session [$F(5,140) = 148.55$, $p < 0.001$] with corrected planned comparisons indicating CRs increased from the first to second session ($p < 0.001$), further increased from the second to third session ($p < 0.05$), and then stayed at a similar high level during subsequent sessions. There were no significant differences between groups, demonstrating that both saline and propranolol groups were equivalent in learning rate and level prior to drug manipulations during extinction.

6.2 Effects of Propranolol on Extinction of Conditioned Responses

The average percentage of CRs to the tone CS across a single day of unpaired extinction with weak shock is shown in the middle of Figure 4, averaged for both the entire session and in blocks of 10 CS-alone trials. Similar to findings in Experiment 1, treatment with 12 mg/kg propranolol thirty minutes prior to the start of the extinction session produced a within-session facilitation of extinction. These observations were confirmed by a significant main effect of Drug Group for the analysis of the entire session [$F(1,28) = 6.16$, $p < 0.05$], and a significant Drug Group and Block interaction when the first and last blocks of extinction were compared [$F(1,28) = 4.54$, $p < 0.05$]. Corrected post hoc tests indicated that saline and propranolol groups did not differ at the start of the extinction session, but by the end of the session, propranolol rabbits had significantly lower CR levels than saline controls ($p < 0.05$). Analyses of other CR parameters (latency, amplitude, area) did not reveal any effects of Drug Group with the exception of CR area, which was found to be decreased in the propranolol group [$F(1,28) = 7.15$, $p < 0.05$]. Unlike analyses of percent CRs, there was no effect of Block for CR area, indicating this was an overall decrease rather than a within-session extinction effect. Analyses of URs on US-alone trials during the extinction session did not reveal any differences between saline and propranolol groups for UR frequency (see Figure 4 boxed insert) or other response parameters.

The averaged percent CRs for the CS Test, which took place eight days following the extinction session (one day after Post3), can be seen on the right side of Figure 4. Analysis of the entire session as well as the session divided into blocks did not reveal any effects of Drug Group. An analysis was also done to compare the last 10 CS-alone trials of the extinction session with the first ten trials of the CS Test in order to specifically examine extinction retention. There was a significant interaction of Drug Group and Session [$F(1,28) = 5.94$, $p < 0.05$] with corrected planned comparisons indicating that the group difference at the end of extinction ($p < 0.05$) was no longer present during the beginning of the CS Test. Increases in responding from the level exhibited at the end of extinction occurred in both saline ($p < 0.05$) and propranolol groups ($p < 0.001$), demonstrating some recovery of the CR which was greater in the propranolol group.

6.3 Conditioning-Specific Reflex Modification

Changes in the amplitude, area, and latency of the UR can be discerned by the UR topographies in Figure 5. Analysis of the initial level of CRM prior to propranolol treatment revealed significant Test (Pretest, Post1) by Intensity interactions for mAmp [$F(4,112) = 3.91$, $p < 0.01$] and mArea [$F(4,112) = 6.86$, $p < 0.001$]. Although there were Pretest to Post1 increases at the intermediate intensities (0.3 and 0.5 mA) for both measures, the comparisons were not significant. For latencies measures, there were significant Pretest to Post1 increases in onset latency at the 0.3 [$F(1,21) = 9.9$, $p < 0.01$], 0.5 [($F(1,28) = 6.16$, $p < 0.05$], and 1.0 mA [$F(1,27) = 20.25$, $p < 0.001$] intensities and in peak latency also at the 0.3 [$F(1,21) = 12.07$, $p < 0.01$], 0.5 [$F(1,28) = 15.36$, $p < 0.01$], and 1.0 mA [$F(1,27) = 17.64$, $p < 0.001$] intensities. Significant CRM was not found in analyses of UR frequency.

Compared to the first experiment, a smaller percentage of rabbits (16.6% versus 22.5%) exhibited strong CRM at the intermediate intensities (0.3, 0.5, or 1.0 mA) for %UR, mAmp, or mArea. Of those with strong CRM, two rabbits were in the saline control group and three in the 12 mg/kg propranolol group.

6.4 Effects of Propranolol on Extinction of Conditioning-Specific Reflex Modification

Effects of propranolol combined with a single day of unpaired extinction on CRM can be seen in the UR topographies in Figure 5 (Post2) and in bar graphs for the intermediate intensities in Figure 6. Following extinction, the size of CRM present at the intermediate intensities at Post1 appeared to remain relatively intact in saline controls at Post2 but diminished in the propranolol group, possibly to below Pretest levels. Analyses of mAmp and mArea focused on the two intensities where CRM occurred prior to extinction treatment, 0.3 and 0.5 mA. For mAmp, there was a trend for an interaction of Test (Pretest, Post1, Post2) and Drug Group [$F(2,56) = 2.97$, $p = 0.060$] and a trend for an interaction of Test, Drug Group, and Intensity [$F(2,56) = 0.16$, $p = 0.059$]. Corrected planned comparisons for the Test by Drug Group effect revealed that the propranolol group at Post2 had a reduced amplitude relative to Pretest ($p < 0.05$) and Post1 (trend, $p = 0.067$) whereas no differences were found at Post2 in the saline group. Corrected planned comparisons of the interaction involving Intensity indicated that for the propranolol group, responding at Post2 was decreased relative to Pretest at the 0.3 mA intensity, but was only a trend following Bonferroni correction ($p = 0.096$). For mArea, there was a significant Test, Drug Group, and

Intensity interaction [$F(2,56) = 0.96$, $p < 0.05$] with planned comparisons indicating that only the propranolol group demonstrated decreases in area at Post2 relative to both Pretest and Post1 at the 0.3 mA intensity and relative to Post1 at the 0.5 mA intensity, but only the latter comparison survived Bonferroni correction (strong trend, $p = 0.054$).

In contrast to the effect of propranolol on the size of CRM following extinction, the peak latency shift aspect of CRM appeared to decrease in both groups at Post2. Confirming this observation, there was a main effect of Test (Pretest, Post1, Post2) and no interaction with Drug Group at the intensities with significant CRM prior to treatment: 0.3 mA [$F(2,32) = 8.54$, $p < 0.01$], 0.5 mA [$F(2,52) = 5.25$, $p < 0.01$], and 1.0 mA [$F(2,54) = 11.67$, $p< 0.001$]. Corrected planned comparisons indicated that there were no differences in peak latency between Post2 and Pretest for all three intensities, and there was a decrease in latency from Post1to Post2 at 0.3 mA and 1.0 mA (p's < 0.01). For onset latency, there were also no significant effects of Drug Group. There was a main effect of Test at 1.0 mA [$F(2,54) = 11.05$, $p < 0.001$] with corrected planned comparisons indicating that onset latency at Post2 remained greater than Pretest ($p < 0.05$). Therefore, although the peak latency shift aspect of CRM was diminished after extinction, some onset latency changes remained intact.

At the assessment of CRM one week after Post2 (Post3), any benefit of combined propranolol and extinction treatment on reducing the size of CRM appeared to be transient. In confirmation, analysis of Post3 compared to Pretest and Post1 at intensities for which CRM was significant prior to treatment did not indicate any significant effects involving Drug Group for amplitude or area measures. There were also no drug effects for latency measures. Instead, at Post3, amplitude and area measures were not significantly different from Pretest or Post1, suggesting an intermediate level of CRM remained in both groups. For latency measures, the increased latency indicative of CRM at Post1 was diminished at Post3. For onset latency, there was a significant effect of Test at 0.3 [$F(2,38) = 7.32$, $p < 0.01$], 0.5 [$F(2,54) = 5.88$, $p < 0.01$] and 1.0 mA[$F(2,52) = 13.30$, $p< 0.001$], with corrected follow up comparisons indicating that CRM was decreased from Post1 levels (all p's < 0.01) and not significantly different from Pretest. The same pattern of decreased latency at Post3 compared to Post1 (all p's < 0.01) and no difference from Pretest occurred for peak latency also at 0.3 [$F(2,38) = 9.876$, $p < 0.01$] and 1.0 mA [$F(2.52) = 12.66$, $p < 0.001$]. For peak latency at 0.5 mA, corrected follow up comparisons on a main effect of Test [$F(2,54) = 3.77$, $p < 0.05$] indicated that Post3 was not statistically different from Pretest or Post1. Therefore, the initial CRM observed for latency measures showed no signs of recovery for either group one week following extinction treatment.

Although there were not enough rabbits exhibiting strong CRM in each group to statistically examine whether the strength of CRM would affect response to propranolol treatment, individual topographies were examined to see if any patterns emerged between CRM levels and treatment response. Figure 7 shows several examples of individual topographies of propranolol rabbits identified as having strong (top row) or moderate (bottom row) levels of CRM at intermediate US intensities. The top row shows two rabbits with strong CRM, with the left subject demonstrating strong CRM at the 0.5 mA and 1.0 mA intensities for both the area and amplitude of the UR and the right subject showing strong CRM for the UR amplitude at 0.3 mA. For these large changes in responding, propranolol combined with a

single day of unpaired extinction treatment appeared to reduce CRM (Post2) with effects lasting one week (Post3). But of importance to note is that within those same subjects, responding at intensities where there was more moderate CRM did not show long-lasting reductions in CRM (see Post3 responses to 0.3 mA in the top left and 0.5 mA in the top right topographies). The bottom row shows two propranolol rabbits with more typical, moderate levels of CRM, with the subject on the left showing long-lasting reductions in CRM following combined treatment, contrasted with the subject on the right showing treatment resistance. Clearly, these qualitative observations demonstrate a great deal of inter- and intra-subject variability in response to treatment, with some suggestion that propranolol may produce longer lasting benefits for the most extreme responses. However, further evaluation requires a much larger sample of subjects with strong CRM, which is beyond the scope of the current experiment.

7. Discussion

Results from Experiment 1 demonstrated that propranolol administered prior to sessions of unpaired extinction with weak shock initially facilitated extinction of CRs to the tone CS in a dose-dependent manner. However, by the end of extinction training, saline controls were able to catch up to the extinction levels exhibited by propranolol subjects. During later testing without the drug, there was no evidence that propranolol enhanced extinction retention. There was also no evidence that propranolol had any effect on extinction of CRM, as both control and propranolol groups showed similar reductions in CRM. Because of the possibility of floor effects due to the success of six sessions of unpaired extinction treatment in reducing both CRs and CRM in control subjects, Experiment 2 examined the effects of propranolol using only a single day of unpaired extinction, a treatment previously shown to be inferior to the full course of treatment that may even worsen CRM (Schreurs et al., 2011b). Similar to results from Experiment 1, propranolol facilitated within-session extinction of CRs to the tone CS but did not enhance extinction memory as propranolol subjects were back up to control levels during later retention testing. Propranolol did enhance extinction of the size of CRM with the short course of extinction, but again, this effect was transient and did not enhance retention of CRM extinction when tested a week later. However, examination of individual variations in the strength of CRM did suggest that propranolol may have longer lasting effects on responses characterized as strong CRM. Overall, these findings suggested a within-session benefit of propranolol on extinction of CRs and a short-term benefit on extinction of CRM when extinction treatment is brief/insufficient, but neither benefit translated to improved long-term treatment outcomes for the majority of subjects.

Our findings on propranolol's facilitation of extinction add to the inconsistencies in the animal literature. To our knowledge, there is only one other study that examined the effects of propranolol on extinction of eyeblink conditioning, also in rabbits and of note, also using unpaired extinction (Gould, 1998). Although results on extinction were less clear due to smaller subject size and confounding effects of subjects receiving propranolol throughout both acquisition and extinction, there was some suggestion that propranolol decreased the number of days to reach an extinction learning criterion. For rodent fear conditioning models, there have been conflicting reports on the role of noradrenergic blockade on

extinction (as reviewed in Giustino et al., 2016; Mueller and Cahill, 2010). In some cases, propranolol has been shown to impair extinction learning and retention (Fitzgerald et al., 2015; Mueller et al., 2008). In other cases, propranolol has been shown to reduce expression of fear without necessarily enhancing extinction learning or extinction retention per se (Rodriguez-Romaguera et al., 2009). Because our paradigm does not include a direct measure of fear such as freezing, our results do not rule out of the possibility that propranolol is reducing overall fear during extinction but do suggest that extinction learning itself seems to be impacted by propranolol, at least in the short-term. The lack of long-term effects of propranolol on retention of extinction of CRs and CRM may have several explanations. One interpretation to consider is that undergoing extinction in a drug state may constitute a change in context (i.e. internal state) that allows fear renewal to occur when later testing for extinction retention occurs in a drug-free state (Radulovic et al., 2017), and the other possibility is simply spontaneous recovery of responding, occurring perhaps to a greater degree in the propranolol group (Bouton, 2002).

One of the ideas about the role NE may play in extinction learning is based on the delta rule (Rescorla and Wagner, 1972), whereby the violation of expectancy that occurs during extinction (i.e. the CS is no longer paired with the US) triggers retrieval of the original CS-US association during and for some time after extinction training (Ouyang and Thomas, 2005). It is believed that retrieval of that memory makes it labile (Alberini and Ledoux, 2013; Nader and Hardt, 2009), which can lead to either fear reconsolidation or consolidation of a new extinction memory depending on the circumstances, and the retrieval process itself seems to be sensitive to β-adrenergic manipulations (Ouyang and Thomas, 2005). There is a substantial body of evidence that β-receptor blockers such as propranolol can impair fear memory reconsolidation in animals, particularly in situations where there is a high arousal level, thereby shifting the balance more towards fear extinction (as reviewed in Giustino et al., 2016). For example, recent work in rats has shown that systemic propranolol prior to extinction learning reduces within session freezing and importantly *enhances* extinction retention if extinction takes place shortly after conditioning rather than 24 hours later (Fitzgerald et al., 2015). Normally extinction deficits occur with immediate extinction, presumably because emotional arousal levels remain high from the fear conditioning session, but this can be overcome by NE blockade with propranolol. Although our paradigm involves delayed rather than immediate extinction, the fact that we used unpaired extinction where shock is still presented along with CS-alone presentations is likely to create a higher state of arousal and resulting increases in circulating NE. Indeed, unsignaled shock has been shown to increase NE levels in brain areas associated with emotional arousal such as the amygdala (Galvez et al., 1996; Ronzoni et al., 2016), and intra-amygdalar injections of propranolol prior to shock exposure can ameliorate fear behavior (Ronzoni et al., 2016). Therefore, a higher arousal level induced by unpaired extinction may explain why we found some facilitation of extinction with propranolol, possibly as a result of modulation of amygdalar NE. This is feasible with systemic propranolol as it does cross the blood-brain barrier (Neil-Dwyer et al., 1981; Pardridge et al., 1984). We have previously shown that inactivation of the amygdala abolishes CRM expression (Burhans and Schreurs, 2008), suggesting that actions of propranolol on amygdalar NE levels may also contribute to the decreased CRM observed in Experiment 2 following brief extinction.

Another possible site of action of NE in our paradigm is the cerebellum, with the interpositus nucleus (IP) in the deep cerebellar nuclei (DCN) being a critical neural substrate for eyeblink conditioning (Christian and Thompson, 2003; Freeman and Steinmetz, 2011). There is both indirect and direct evidence for an important role of cerebellar NE in delay eyeblink conditioning. The previously discussed work by Gould (1998) demonstrated that systemic propranolol given daily prior to delay eyeblink conditioning sessions slowed the rate of acquisition and increased the number of days required to reach a learning criterion in rabbits. In rats, effects of systemic propranolol were more severe, with subjects performing no better than unpaired controls after six days of conditioning under higher propranolol doses similar to those producing effects in the Gould and current rabbit study (Cartford et al., 2002). Intra-brain injections of propranolol in rats targeting cerebellar lobule HVI and the IP (Cartford et al., 2004) or cerebellar cortex (Paredes et al., 2009) have also led to impaired acquisition. In vivo microdialysis in rats has demonstrated that cerebellar NE increases during delay eyeblink conditioning, peaking early in training, with no increases observed in subjects given unpaired CS/US presentations (Paredes et al., 2009). In addition, functional β-adrenergic receptors have been documented within the rat DCN, and β-adrenergic agonists have been shown to modulate inhibition of DCN cell firing (Gould et al., 1997). Whether cerebellar NE may modulate extinction, however, is less clear. Depletion of NE via locus coeruleus lesions in rabbits trained with delay eyeblink conditioning followed by unpaired extinction showed extinction impairments that correlated with depletion of NE in the hippocampus and frontal cortex but not in the cerebellum (McCormick and Thompson, 1982). Future studies examining direct manipulations of amygdala and/or cerebellar NE with intra-brain infusions of propranolol, for example, would help further our understanding of the neural mechanisms behind the behavioral effects of systemic propranolol in our model.

As dysregulation of NE signaling has been proposed to underlie PTSD symptomology (Hendrickson and Raskind, 2016; Southwick et al., 1999; Strawn and Geracioti, 2008), the use of propranolol as a treatment to block fear memory reconsolidation or enhance extinction has been investigated in both the clinical population as well as in healthy humans. With PTSD patients, results have been mixed with some studies showing promise of persistent improvement in symptoms or lessened physiological responding to trauma recall when propranolol is used in combination with reactivation of the trauma memory (Brunet et al., 2008; Brunet et al., 2011; Brunet et al., 2014), but results are not consistently replicable (Wood et al., 2015). In addition, meta-analysis of studies examining effects of propranolol given shortly after trauma when arousal levels are high, a situation similar to immediate extinction in animals, showed that propranolol did not reduce PTSD incidence (Argolo et al., 2015). Extensive experiments conducted by Kindt and colleagues on healthy humans using fear conditioning protocols have also shown inconsistent effects of propranolol when given prior to reactivation of fear memory, with some demonstrating fear attenuation in later retention tests (Kindt et al., 2009) that can be dissociated from an intact declarative memory for the CS-US association (Soeter and Kindt, 2010), but also null results during replications (Bos et al., 2014; Schroyens et al., 2017). In a human fear conditioning study where propranolol was given prior to extinction (repeated CS presentations), propranolol did not affect the physiological fear response but did impair cognitive extinction memory.

Replication challenges and complications from conscious versus unconscious fear memories in human studies further highlight the need to also examine animal models for further clarification on how and why propranolol may benefit the clinical population.

Overall, findings from our study suggest that propranolol may provide the most therapeutic benefit in situations where arousal level is elevated, such as at the beginning of treatment or when the cognitive behavioral therapy itself evokes a higher arousal level. One such treatment is the use of virtual reality therapy (VRT) where subjects are immersed in multi-sensory virtual environments representative of the trauma experience. VRT has shown great promise to be an effective PTSD treatment, particularly in military combat PTSD (Maples-Keller et al., 2017; Rizzo et al., 2011). We are unaware of any clinical or healthy human studies combining propranolol with VRT, so it would be interesting to see if propranolol may be uniquely suited to enhance efficacy of this type of therapy. The lack of long-term beneficial effects of propranolol, however, suggests that propranolol may have the most impact early in therapy. For example, if propranolol facilitates extinction during the first cognitive behavioral therapy session, patients may be less inclined to drop-out if they feel like therapy is working early on, especially if a more intense/distressing treatment like VRT is used. Importantly, propranolol also has the potential to address more than one PTSD symptom, such as conditioned fear to trauma-associated cues as well as hyperarousal. Because traditional exposure therapies tend to focus solely on extinguishing fear to cues associated with trauma, we had previously examined whether extinction treatment involving CS-alone presentations had any effect on CRM (Schreurs et al., 2000). This earlier work and a later extinction study that also examined context extinction and continued CS-US pairings with a weak US (Burhans et al., 2015), demonstrated that only treatments that incorporated US-alone presentations had any significant impact on CRM. In summary, these findings suggested that traditional exposure therapy may not adequately address hyperarousal symptoms. Adding presentations of unsignaled, innately stressful (but mild) stimuli such as skin stimulation may help alleviate hypervigilance symptoms, which would be easy to incorporate into the immersive, multisensory environment of VRT therapy.

Highlighting the translatability of our CRM model from rabbits to humans, there is a growing body of literature utilizing the eyeblink conditioning paradigm to study patients with PTSD (Ayers et al., 2003; Burriss et al., 2007; Handy et al., 2018). Newer reports using eyeblink conditioning with a partial reinforcement schedule (CS- or US-alone trials interspersed with paired CS-US trials) have demonstrated that individuals with PTSD or personality traits that increase vulnerability to anxiety-disorders like PTSD have enhanced acquisition of delay eyeblink conditioning (Allen et al., 2018; Allen et al., 2016) and can also show resistance to extinction (Allen et al., 2014; Handy et al., 2018). Interestingly, Wistar-Kyoto mice, used to model anxiety-vulnerability, also show enhanced delay eyeblink conditioning (Ricart et al., 2011). By analyzing large pools of rabbit data collected with our CRM model, we have previously sought to determine whether factors, such as conditioning rates or levels, can predict individual susceptibility or resilience to CRM (Smith-Bell et al., 2012; Smith-Bell and Schreurs, 2017). We have found that subjects that went on to develop strong CRM took fewer trials to reach a learning criterion (Smith-Bell and Schreurs, 2017) and also that CR amplitude and area was positively correlated with the size of CRM (Smith-Bell et al., 2012). Interestingly, larger amplitude CRs have also been reported in veterans

with PTSD in addition to increased amplitude URs during trace eyeblink conditioning (Burriss et al., 2007), suggesting that CRM-like changes in PTSD patients may be detectable as well. Also of note, US-alone pre-exposure in anxiety- prone individuals disrupts enhanced conditioning (Allen and Miller, 2016), suggesting that our model, which includes US-alone presentations during Pretest prior to conditioning, may preclude us from detecting a stronger relationship between conditioning levels and CRM susceptibility. In addition, our rabbits are conditioned daily for a total of six sessions yielding high, sustained performance levels prior to CRM assessment, unlike human studies that typically only use a single session. To further bridge the gap to the clinical data, we might consider future studies using a more challenging partial reinforcement schedule, which may improve the ability of our model to detect and predict a PTSD-like phenotype and enhance sensitivity to drug effects. Likewise, clinical studies may benefit from looking for CRM-like changes in the UR, as it may be another defining feature of PTSD patients or anxiety-prone individuals. Overall, these findings from both animals and humans support the utility of eyeblink conditioning as a tool to identify a PTSD phenotype, predict susceptibility, and test behavioral and pharmacological treatments that can be easily translated from the bench to bedside and vice versa.

8. Conclusions

In conclusion, findings presented here further validate our CRM eyeblink conditioning model of PTSD by showing that extinction of trauma-associated cues and conditioned hyperarousal are both sensitive to noradrenergic manipulation, dysfunction of which is strongly implicated in PTSD. Our results suggest that propranolol may provide the most benefit early during extinction therapy when arousal levels are high, which may offer incentive to patients to continue with treatment, thereby improving patient outcomes. The rabbit CRM model of PTSD and complimentary, ongoing work using the eyeblink paradigm to examine PTSD and anxiety-vulnerability in humans demonstrate the important utility of eyeblink conditioning to further our understanding of the neurobiological mechanisms of PTSD and the neuropharmacology of treatments.

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Highlights

- Propranolol facilitates within-session extinction of conditioned eyeblink responses
- Propranolol enhances extinction of conditioned hyperarousal short-term
- Propranolol provides the most benefit early in extinction when arousal level high
- Propranolol may improve compliance rather than long-term treatment outcomes

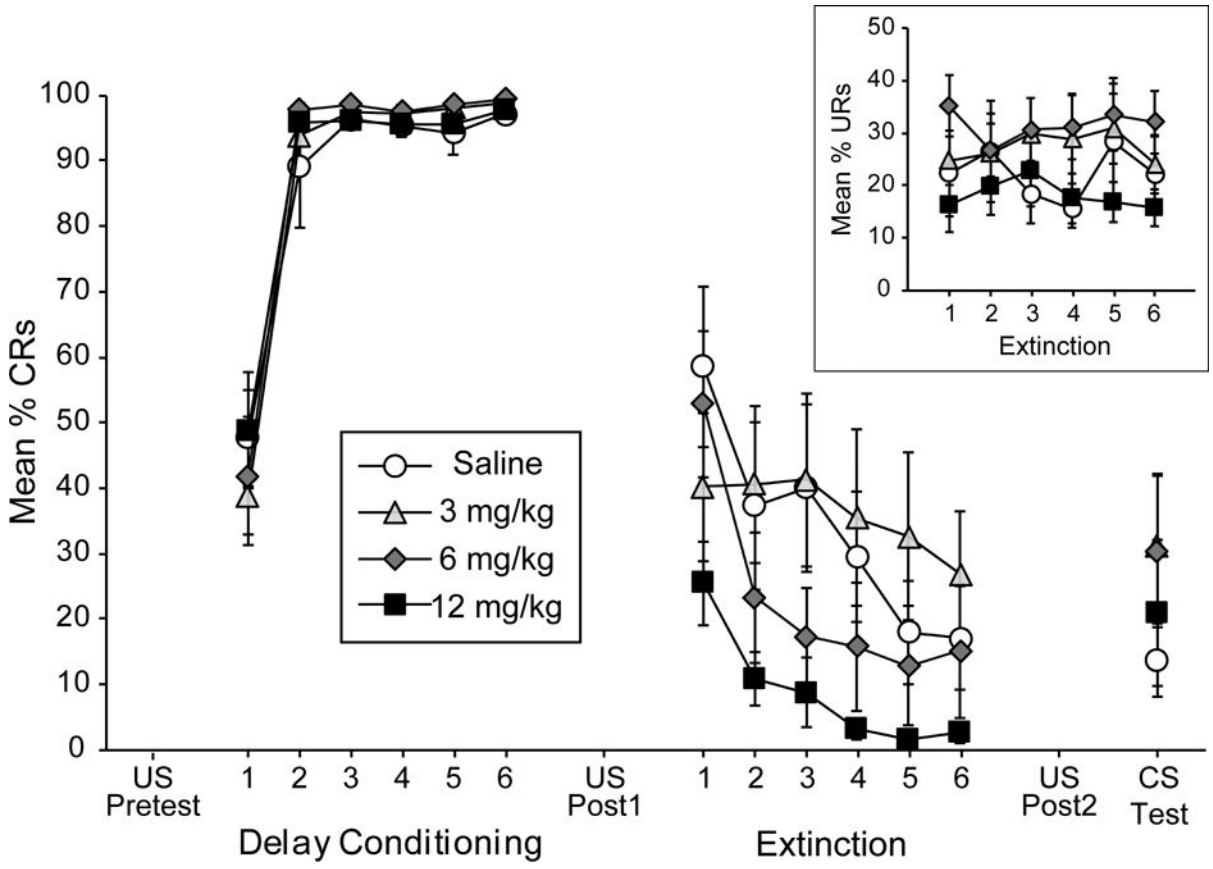

Figure 1.

The mean percentage (± SEM) of conditioned responses (% CRs) to the tone conditioned stimulus (CS) during six daily sessions of delay conditioning, six daily sessions of unpaired extinction with weak shock combined with saline or propranolol injection, and a CR retention test consisting of CS-alone presentations (CS Test). The boxed inset panel shows the mean percentage (± SEM) of unconditioned responses (% URs) to the 0.3 mA shock presented during extinction. Prior to the unpaired extinction sessions, rabbits received saline (open circle) or 3 mg/kg (light grey triangle), 6 mg/kg (dark grey diamond), or 12 mg/kg propranolol (black square).

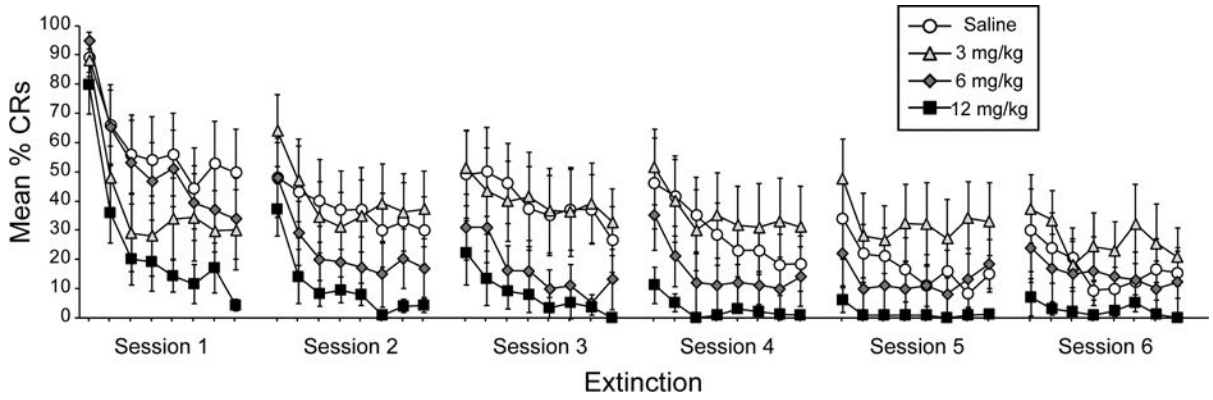

Figure 2.

The mean percentage (± SEM) of conditioned responses (% CRs) to the tone conditioned stimulus (CS) during six daily sessions of unpaired extinction with weak shock combined with saline or propranolol injection, averaged in 10 trial blocks of CS presentations for each session. Prior to the unpaired extinction sessions, rabbits received saline (open circle) or 3 mg/kg (light grey triangle), 6 mg/kg (dark grey diamond), or 12 mg/kg propranolol (black square).

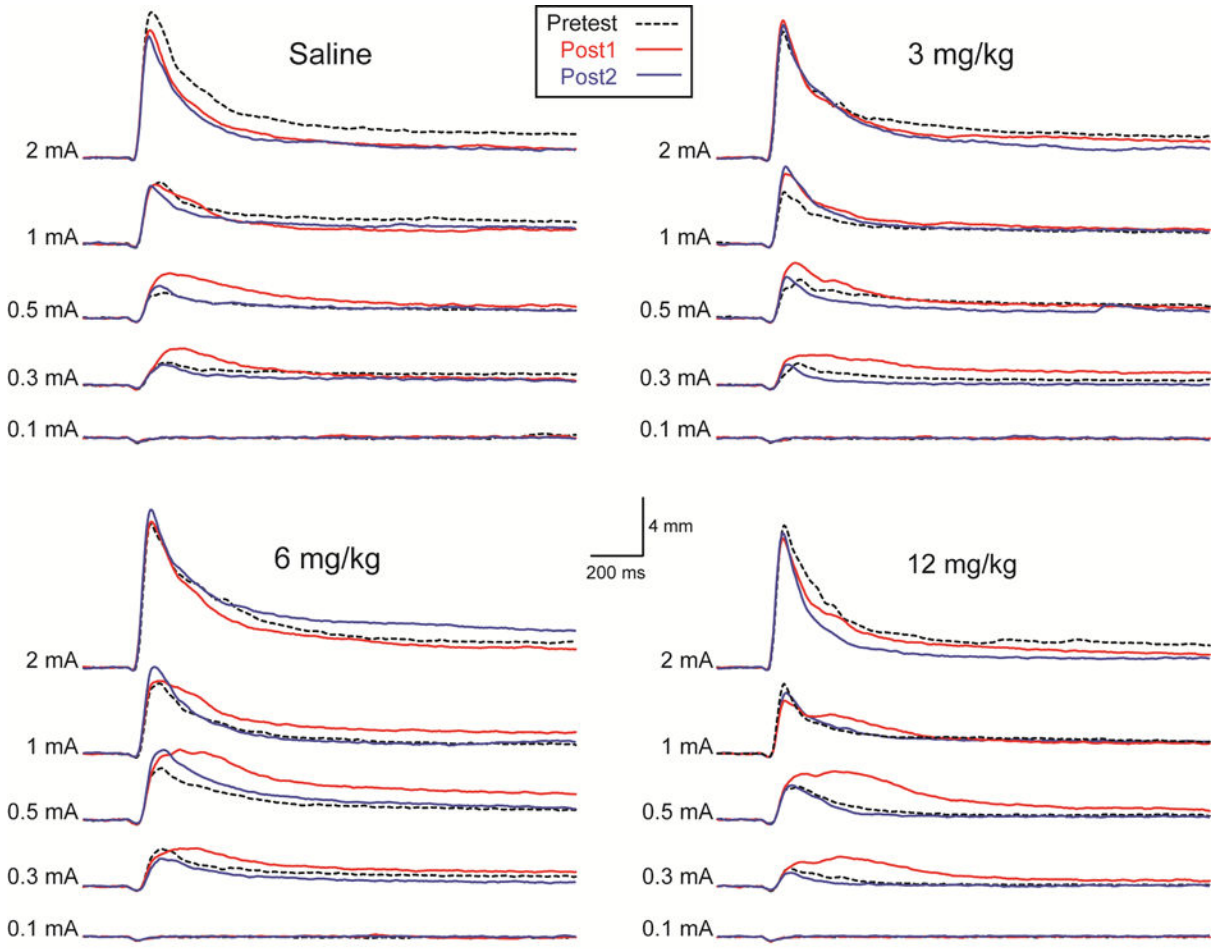

Figure 3.

Topographies for the averaged unconditioned response to the periorbital shock unconditioned stimulus (US) during the first 20 trials of the US pretest (Pretest, black dotted line), the posttest following delay conditioning (Post1, red line), and the posttest following extinction combined with saline or propranolol injection (Post2, blue line). Topographies are shown at the five unconditioned stimulus intensities (2.0, 1.0, 0.5, 0.3, 0.1 mA) presented during US testing, collapsed across duration.

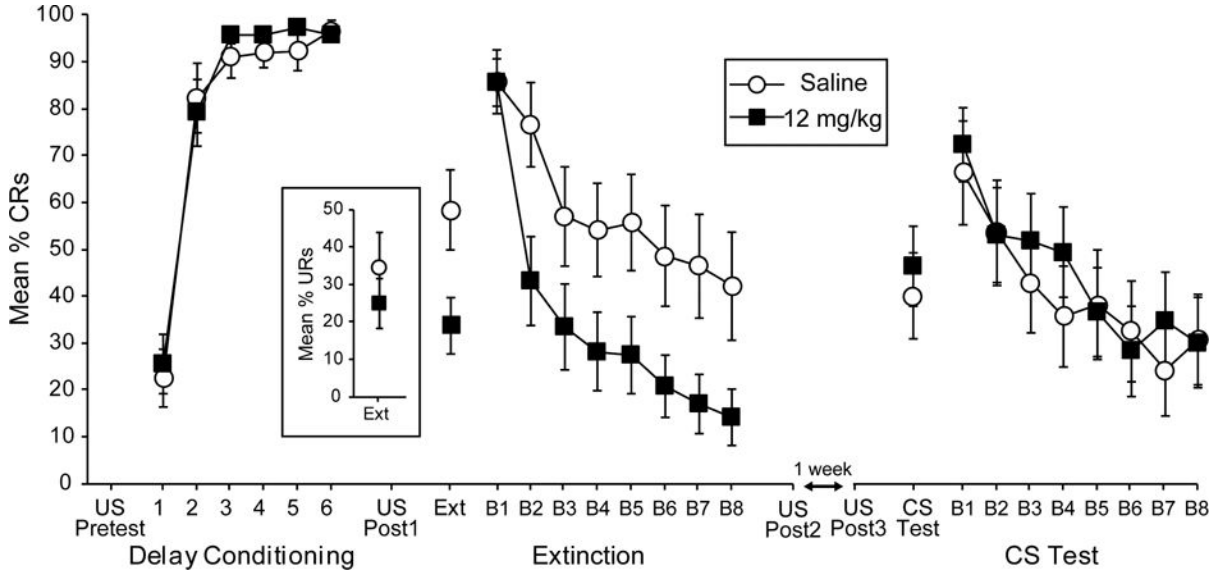

Figure 4.

The mean percentage (± SEM) of conditioned responses (% CRs) to the tone conditioned stimulus (CS) during six daily sessions of delay conditioning, one session of unpaired extinction with weak shock following saline (open circle) or 12 mg/kg propranolol injection (black square), and a CR retention test consisting of CS-alone presentations eight days later (CS Test). CRs during extinction and the CS Test session are shown averaged across the entire session and in 10 trial blocks of CS presentations. The boxed inset panel shows the mean percentage (± SEM) of unconditioned responses (% URs) to the 0.3 mA shock presented during extinction, averaged across the entire session.

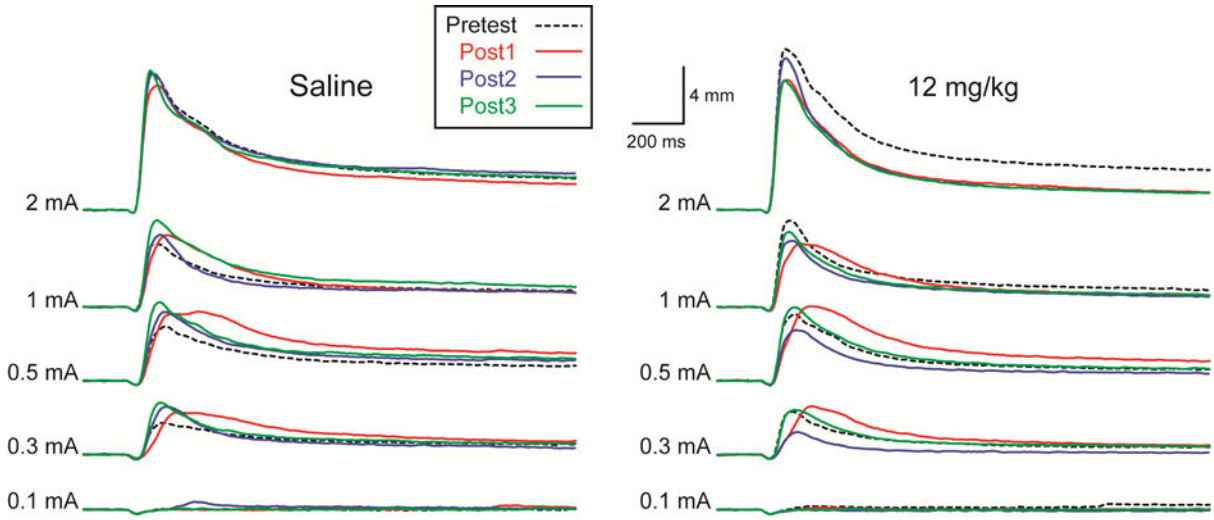

Figure 5.

Topographies for the averaged unconditioned response to the periorbital shock unconditioned stimulus (US) during the first 20 trials of the US pretest (Pretest, black dotted line), the posttest following delay conditioning (Post1, red line), the posttest following a single extinction session combined with saline or 12 mg/kg propranolol injection (Post2, blue line), and a third posttest one week later (Post3, green). Topographies are shown at the five unconditioned stimulus intensities (2.0, 1.0, 0.5, 0.3, 0.1 mA) presented during US testing, collapsed across duration.

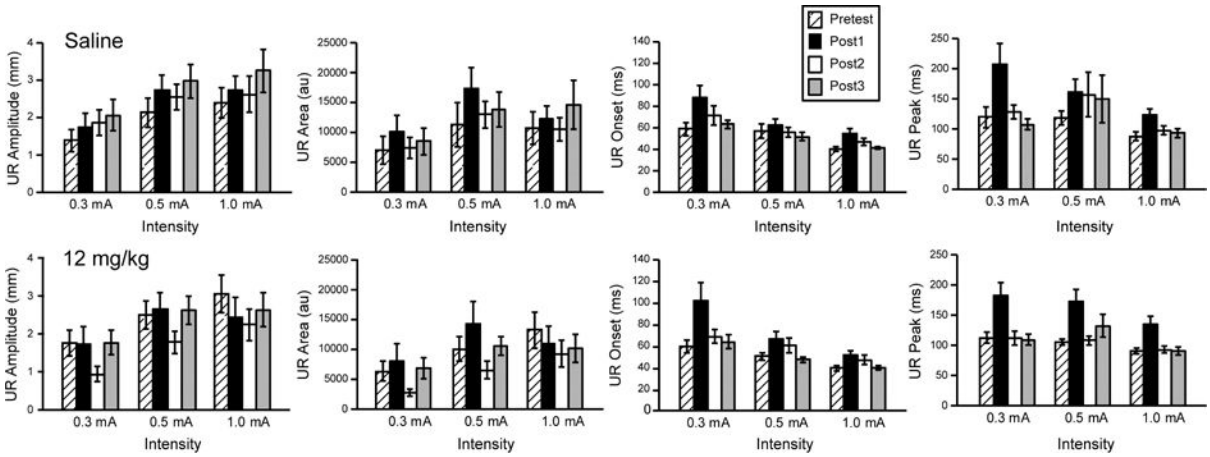

Figure 6.

Mean (± SEM) magnitude of the unconditioned response (UR) amplitude (left column) and area (left middle column), onset latency (right middle column), and peak latency (right column) to the intermediate unconditioned stimulus intensities (1.0, 0.5, 0.3 mA) for the first 20 trials of each of four US tests, collapsed across duration. Pretest (striped bar) and Post1 (black bar) took place prior to and following delay conditioning, respectively, whereas Post2 (white bar) and Post3 (gray bar) occurred one day and one week following a single session of unpaired extinction with weak shock combined with saline (top row) or 12 mg/kg propranolol (bottom row) injection.

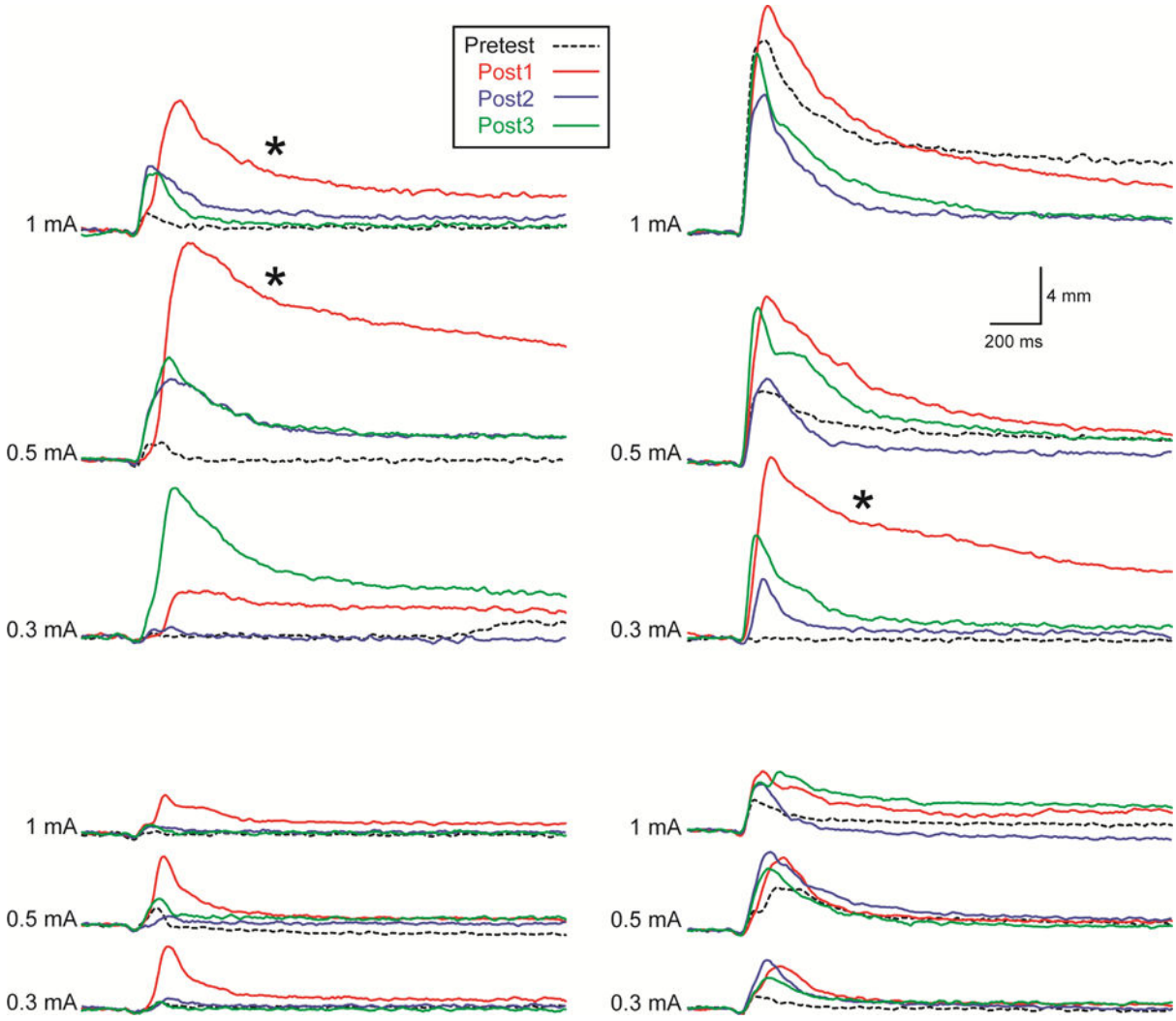

Figure 7.

Unconditioned response (UR) topographies for four individual propranolol subjects, two exhibiting strong CRM (top row, strong CRM responses indicated by asterisks) and two exhibiting more moderate CRM (bottom row). Data shown are the UR to the periorbital shock unconditioned stimulus (US) during the first 20 trials of the US pretest (Pretest, black dotted line), the posttest following delay conditioning (Post1, red line), the posttest following a single session of unpaired extinction combined with a 12 mg/kg propranolol injection (Post2, blue line), and a third posttest one week later (Post3, green). Topographies are shown at three intermediate US intensities (1.0, 0.5, 0.3 mA) presented during US testing, collapsed across duration.