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Sex differences in a rabbit eyeblink conditioning model of PTSD

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Abstract

We have developed a rabbit model of posttraumatic stress disorder (PTSD) which recapitulates several core features of PTSD, particularly hyperarousal and conditioned responding to trauma-associated cues. The work conducted with this model has all been done in male rabbits and given sex differences in PTSD prevalence, it is important to expand our animal model of PTSD to include female rabbits to determine if they develop core features of PTSD, and if those core features can be treated. This is particularly important because, contrary to human studies, nearly all animal studies have found that males are consistently more vulnerable to various forms of acute and chronic stress than females. Using eyeblink conditioning in which we paired tone with a brief periorbital shock, we found that although both male and female rabbits acquired identical levels of conditioning, females showed more hyperarousal after conditioning but seemed to respond somewhat better to treatment.

Keywords

cognitive behavioral therapy; classical conditioning; eyeblink; extinction; gender differences; post-traumatic stress disorder

Introduction

Given the increasing likelihood of women being in harm's way as first responders and in the armed forces, particularly in combat roles, the incidence of PTSD is likely to increase among women. As a result, there is a growing need for more information about the susceptibility of women to PTSD and methods for treating women with PTSD – especially among active military personnel (Crum-Cianflone and Jacobson, 2014; Hines et al., 2014) and veterans (Vogt et al., 2011; Crum-Cianflone and Jacobson, 2014; Magruder et al., 2015). There is strong epidemiological evidence for differences between men and women in the likelihood of PTSD (Olf et al., 2007; Bangasser et al., 2017), and this has been attributed to the types of trauma, younger age, stronger perceptions of threat and loss of control, and

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Conflicts of interest: none

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insufficient social support (Olff et al., 2007; Silove et al., 2017). For example, long-term exposure to trauma in cases of sexual and domestic abuse tends to occur more predominantly in women than in men (Iverson et al., 2015; Smith et al., 2015; Silove et al., 2017). When differences in some of these attributes are reduced as they might be in the military where exposure to combat, perceived threat, age, and unit cohesion may be similar between men and women, differences between men and women still exist but those differences become less clear (Vogt et al., 2011; Crum-Cianflone and Jacobson, 2014; Hines et al., 2014; MacGregor et al., 2017). There is some evidence that women may be more susceptible to combat-related PTSD and less resilient than men (Crum-Cianflone and Jacobson, 2014) although other reports suggest women combat veterans may be as resilient to combat-related stress as men (Vogt et al., 2011). In fact, meta-analyses show that there is no consensus about differences in PTSD between men and women veterans with some studies showing significant differences and others showing no differences at all (Hines et al., 2014; Hourani et al., 2015; MacGregor et al., 2017). Nevertheless, women veterans are seeking treatment more often than men (Elbogen et al., 2013) and may be exposed to more long-term sources of trauma, particularly sexual assault and intimate partner violence (Iverson et al., 2015; Ryan et al., 2015; Brignone et al., 2017). Importantly, civilian women responded better to PTSD treatment than men (Békés et al., 2016) although the evidence is less clear in the military (Tiet et al., 2015; Voelkel et al., 2015).

We have developed a rabbit model of PTSD which recapitulates several core features of PTSD, particularly hyperarousal and conditioned responding to trauma-associated cues (Schreurs et al., 2000; Buck et al., 2001; Schreurs, 2003; Burhans et al., 2008; Schreurs and Burhans, 2015). In our animal model of PTSD, movement of the rabbit nictitating membrane – a response tightly coupled to the eyeblink (Berthier, 1992) – becomes exaggerated as a result of classical conditioning, a form of hyperarousal called conditioning-specific reflex modification – CRM. These exaggerated eyeblink responses are larger, reach their peak later and occur to weaker stimulus intensities when the shock unconditioned stimulus (US) is tested *without* the conditioned stimulus (CS) (reviewed in (Schreurs, 2003; Burhans et al., 2008; Schreurs and Burhans, 2015)). CRM is detected by comparing responses to a series of different US intensities *presented alone* before and after CS-US pairings. CRM occurs after CS-US pairings but not after explicitly unpaired CS and US presentations confirming it is an associative phenomenon (Schreurs et al., 1995; Seager et al., 2003). CRM is a function of conditioning parameters particularly the number of CS-US pairings (Schreurs et al., 1995) and US intensity (Seager et al., 2003), and can generalize from one US to another (Buck et al., 2001) and from one site of US application to another (Schreurs et al., 2011c). CRM is also sensitive to context (Schreurs et al., 2006), undergoes extinction (Schreurs et al., 2000; Schreurs et al., 2011b; Burhans et al., 2015), incubation (Schreurs et al., 2011a), and spontaneous recovery, can occur in both skeletal and autonomic response systems (Schreurs et al., 2005; Burhans et al., 2010; Schreurs et al., 2011c), and is susceptible to drugs (Wang et al., 2006; Burhans and Schreurs, 2008) and dietary manipulations (Schreurs et al., 2007). CRM has been reported in rabbits by a number of other laboratories (Gruart and Yeo, 1995; Wikgren and Korhonen, 2001) and can also be observed in rats (Servatius et al., 2001). There may even be historical antecedents to CRM in classical conditioning experiments conducted in the 1930s with dogs (Hilgard and Marquis, 1935) and humans (Hilgard and

Campbell, 1936) because a careful review of the data shows increased responding to the US when it was tested by itself on US-alone trials during the course of CS-US pairings. Importantly, the core features of PTSD can be successfully treated by explicitly unpaired presentations of the CS and US even if US intensity is reduced eight-fold from the training intensity (Schreurs et al., 2011b; Burhans et al., 2015; Schreurs and Burhans, 2015).

The work conducted with this rabbit model of PTSD, thus far, has all been done in males. Given the evidence for differences in PTSD prevalence and severity between men and women (Vogt et al., 2011; Crum-Cianflone and Jacobson, 2014; Hines et al., 2014; Bangasser et al., 2017), it is important to expand our animal model of PTSD to female rabbits to determine if they develop core features that are similar to PTSD, and if those core features can be treated. This is certainly in line with calls for animal models of PTSD that study sex differences (Cohen and Yehuda, 2011; Kokras and Dalla, 2014; Shansky, 2015) particularly in light of the fact that, contrary to human studies, nearly all animal studies have found that males are consistently *more vulnerable* to various forms of acute and chronic stress than females (Cohen and Yehuda, 2011; Bangasser and Valentino, 2014; Kokras and Dalla, 2014; Keller et al., 2015; Shansky, 2015).

Experiment 1

Materials and Methods

Subjects—The subjects were 14 male and 13 female, New Zealand White rabbits (*Oryctolagus cuniculus*), 2–3 months of age, and weighing approximately 2.0–2.2 kg upon delivery from the supplier (Charles River, Quebec, Canada). 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 eyeblink conditioning have been detailed by Schreurs (Schreurs and Alkon, 1990) who modeled the apparatus on those described by Gormezano (Gormezano, 1966; Coleman and Gormezano, 1971). Rabbits were restrained in a Plexiglas box placed inside a sound-attenuating, ventilated chamber (Coulbourn Instruments, Allentown, PA; Model E10–20). Inside the chamber, a stimulus panel containing a speaker and house light (10-W, 120 V) was mounted at a 45° angle 15 cm anterior and dorsal to the rabbit's head. An exhaust fan created a constant ambient noise level of 65 dB inside the chamber. Periorbital electrical stimulation was delivered by a programmable two-pole stimulator (Coulbourn Instruments, Model E13–35) via stainless steel Autoclip wound clips (Stoelting, Wood Dale, IL) that were positioned 10 mm ventral and 10 mm posterior to the dorsal canthus of the right eye. Application of the two wound clips occurred very quickly using an Autoclip wound clip applicator (Stoelting, Wood Dale, IL) without any sign of more than momentary stress. Stimulus delivery, data collection, and analysis were all accomplished using the LabVIEW software system (National Instruments, Austin, TX).

Sweeps of the nictitating membrane (NM) 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 NM under local anesthesia (0.5% Tetracaine HCl Ophthalmic Solution, Henry Schein Animal Health, Dublin, OH). 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), a second US Post-Test four weeks later (Post2), and a CS-alone retention test (CS Test). For adaptation, subjects were prepared for delivery of the periorbital shock US and NM recording and then adapted to the training chambers for an amount of time equivalent to subsequent training sessions (80 min). To assess URs during pretesting 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 80% CRs during CS-US pairings to be included in the analyses. The CS 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 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; Kehoe et al., 2008; Kehoe et al., 2009). 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 NM movement 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 univariate analysis of variance (Systat 8.0, SPSS Inc).

Results

Responding to the CS during CS-US pairings—One male rabbit did not reach 80% CRs and was excluded from further analysis. Figure 1 depicts mean percent responding to the tone CS (%CRs) during CS-US pairings and CS Test. The figure shows the remaining 13 male rabbits and the 13 female rabbits acquired CRs quickly and to uniform levels of more than 95% CRs by the end of CS-US pairings. Analysis of variance (ANOVA) of %CRs yielded a significant effect of Days ($F(5, 120) = 93.92, p < 0.001$) but no main effect or interaction of the Sex factor ($F's < 1.44, p > 0.217$). There was no difference in %CRs during the CS Test ($F < 1$).

Responding to the US during US testing

Pretest: Analysis of responding on Pretest showed a significant main effect of US Intensity for percent URs ($F(4,96) = 136.34, p < 0.001$), magnitude of UR amplitude ($F(4,96) = 105.011, p < 0.001$) and magnitude of UR area ($F(4,96) = 26.87, p < 0.001$) but no main effects or interactions of Sex ($F's < 1.10, p's > 0.370$). There were also no significant differences in response threshold between males and females (Chi square = 1.38, $p = 0.848$).

Post1: The average response topographies of Figure 2 and the bar graphs (Mean $\pm$ SEM) in Figure 3 show several important outcomes across US testing on Pretest, Post1, and Post2. First, there is very clear evidence of increases in responding from Pretest to Post1 especially at intermediate US test intensities – indicative of CRM for both male and female rabbits. These observations were supported by analyses which yielded a main effect of Pretest versus Post1 for percent URs ($F(1,24) = 7.73, p = 0.010$) and interactions of Pretest versus Post1 and US Intensity for magnitude of UR amplitude ($F(4, 96) = 3.47, p = 0.011$) and magnitude of UR area ($F(4,96) = 2.77, p = 0.032$). Importantly, there were no main effects or interactions of Sex ($F's < 2.6, p's > 0.121$). Figure 2 also shows that the increases in response amplitude and area from Pretest to Post1 were accompanied by a shift to the right in the peak latency of the UR indicating response timing had changed and rabbit responses were reaching their 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 a peak latency (0.3, 0.5, 1.0 and 2.0mA) yielded a significant effect of Pretest versus Post1 ($F(1,24) = 9.28, p = 0.006$). There were no significant effects of Sex ($F's < 1.32, p's > 0.278$).

Post2: The second important outcome that can be seen in Figures 2 and 3 was that four weeks after CS-US pairings, responding on Post2 remained well above Pretest levels for both male and female rabbits but peak latencies tended to return to Pretest values. These observations were corroborated by significant differences between Pretest and Post2 for

percent URs ($F(1,24) = 65.28, p < 0.001$), magnitude of UR amplitude ($F(1,24) = 14.19, p = 0.001$), magnitude of UR area ($F(1,24) = 9.04, p = 0.006$) and a lack of a Pretest Post2 difference for peak latency ($F(1,24) = 0.16, p = 0.697$). Interestingly, the only significant effect of Sex in comparing Pretest to Post2 was for percent URs ($F(1,24) = 4.48, p = 0.045$) that varied as a function of US Intensity ($F(2,48) = 3.86, p = 0.028$) which the top of figure 3 suggests may have been due to larger differences in the frequency of responding between Pretest and Post2 as a result of lower levels of responding on Pretest at intermediate intensities for female rabbits. However, as noted above, there were no significant differences between male and female rabbits on Pretest including percent URs even when analysis was limited the intermediate intensities at which we see CRM ($F(1,24) = 2.75, p = 0.110$). However, the response topographies in Figure 2 and bar graphs of Figure 3 do suggest a significant difference in UR amplitude between Post2 and Post1 that is more pronounced for females at higher US intensities which was supported by a Test x Intensity x Sex interaction ($F(2,48) = 4.06, p = 0.024$).

To determine whether the elevated measures of responding one month after CS-US pairings persisted beyond the beginning of Post2, we examined response topographies during the second block of US testing (Trials 21–40) shown in Figure 4. ANOVAs revealed a continued difference between Pretest and Post1 for percent URs ($F(1,24) = 9.56, p = 0.005$) but not magnitude of UR amplitude or area (F 's < 1). There was also a significant difference between Pretest and Post2 for percent URs ($F(1,24) = 32.75, p < 0.001$) and the magnitude of UR amplitude ($F(1,24) = 4.30, p = 0.049$) but not magnitude of UR area ($F(1,24) = 2.15, p = 0.156$). As in Figure 2, the differences in magnitude of UR amplitude between Post2 and Post1 for female but not male rabbits seen in Figure 4 were supported by a significant Test x Sex interaction ($F(1,24) = 9.80, p = 0.005$). These observations were confirmed by analyses of Post2 data on the first and second 20-trial block of US testing which yielded significant differences between males and females for both magnitude of UR amplitude ($F(1,24) = 5.07, p = 0.034$) and magnitude of UR area ($F(1,24) = 4.31, p = 0.049$) between the two blocks of Post2 testing.

Taken together, the results of Experiment 1 show that, given virtually identical rates and levels of eyeblink conditioning during CS-US pairings, females showed comparable levels of hyperarousal to males and may have shown higher levels of responding that persist for longer through testing one month after CS-US pairings than those shown by male rabbits. This is contrary to animal studies showing that males are consistently more vulnerable to stress and show more PTSD-like behavior than females (Cohen and Yehuda, 2011; Bangasser and Valentino, 2014; Kokras and Dalla, 2014; Keller et al., 2015; Shansky, 2015).

Experiment 2

The results of Experiment 1 suggested that, unlike other animal models of PTSD where males are more susceptible to stress than females, both male and female rabbits showed significant hyperarousal following CS-US pairings that persisted as an increase in response amplitude for at least 4 weeks after training and testing. We have shown previously that as few as three days of explicitly unpaired presentations of the CS and a weak US can extinguish hyperarousal successfully in male rabbits (Schreurs et al., 2011b; Burhans et al.,

2015). The purpose of Experiment 2 was to determine whether hyperarousal could be extinguished in female rabbits using just one session of explicitly unpaired presentations of the CS and a weak US (Schreurs et al., 2011b; Burhans et al., 2015).

Material and Methods

Subjects—The subjects were 7 male and 6 female New Zealand White rabbits (*Oryctolagus cuniculus*), 2–3 months of age weighing approximately 2.0–2.2 kg upon delivery from the supplier (Charles River, Quebec, Canada). The rabbits were housed and treated in the same manner as those in Experiment 1 and, unless otherwise noted, the apparatus and procedures were the same as those used in Experiment 1.

Procedure—Following restraint habituation, rabbits received one session per day in the following order: adaptation, US Pretest, six days of CS-US pairings, Post1, one day of unpaired extinction with a weak shock followed by Post2 and a CS-alone retention test (CS Test). For unpaired extinction, the session consisted of 80 presentations of the tone CS and 80 presentations of the 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.

Results

Responding to the CS during CS-US pairings—Figure 5 depicts mean percent responding to the tone CS (%CRs) during CS-US pairings, unpaired extinction and CS Test. The figure shows that both male and female rabbits acquired CRs quickly and to uniform levels of more than 95% CRs by the end of CS-US pairings. ANOVA of %CRs yielded a significant effect of Days ($F(5, 55) = 50.07$, $p < 0.001$) but no main effect or interaction of Sex ($F's < 1$). There was no overall difference in %CRs during unpaired extinction or CS Test ($F's < 1$). Response levels to the US during unpaired extinction averaged 18.5 ± 8.64 Percent URs for males and 19.9 ± 4.70 Percent URs for females and were not significantly different ($F < 1$).

Responding to the US during US testing

Pretest: Analysis of responding on Pretest showed a significant main effect of US Intensity for percent URs ($F(4,44) = 72.70$, $p < 0.001$), magnitude of UR amplitude ($F(4,96) = 89.63$, $p < 0.001$) and magnitude of UR area ($F(4,96) = 7.65$, $p < 0.001$), no main effects of Sex ($F's < 2.98$, $p's > 0.111$), but an interaction of Sex with US Intensity for magnitude of UR amplitude ($F(4,44) = 2.94$, $p = 0.031$) reflecting larger responses at higher US intensities in male rabbits (Figure 7) but not at intermediate intensities ($F(2,44) = 2.20$, $p = 0.135$). There were no significant differences in response threshold between males and females (Chi square = 4.30, $p = 0.367$).

Post1: The average topographies of Figure 6 and the bar graphs in Figure 7 show several important outcomes across US testing on Pretest, Post1, and Post2. First, there was once again very clear evidence of increases in responding from Pretest to Post1 – indicative of CRM in female as well male rabbits replicating the results of Experiment 1. These observations were supported by analyses which yielded a main effect of Pretest versus Post1

for percent URs ($F(1,11) = 16.48$, $p=0.002$), magnitude of UR amplitude ($F(1,11) = 17.53$, $p=0.002$), and magnitude of UR area ($F(1,11) = 27.01$, $p<0.001$). Interestingly, there was an interaction of Pretest versus Post1 testing with Sex for percent URs ($F(1,11) = 12.12$, $p=0.005$) that can be seen in Figure 5 as larger differences between Pretest and Post1 for female rabbits than for males.

Figures 6 and 7 also show that the increases in response amplitude and area from Pretest to Post1 were accompanied by a shift to the right in the peak latency of the UR (see Table 1) indicating response timing had changed and rabbit responses 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 a peak latency (0.3, 0.5, 1.0 and 2.0mA) yielded a significant effect of Pretest versus Post1 ($F(1,24) = 27.95$, $P<0.001$). Analysis of peak latencies also yielded significant effects of Sex suggesting male and female rabbits differed in the shifts in timing of the response peak from Pretest to Post1 with females have a very large shift at 0.3 mA. The observed shifts in peak latency were corroborated by a significant interaction of Sex and US Intensity ($F(3,33) = 8.97$, $p<0.001$) and an interaction of Pretest versus Post1 with Sex and US Intensity ($F(3,33) = 6.44$, $p<0.001$).

Post2: Another important outcome that can be seen in Figures 6 and 7 is that after a session of unpaired extinction, response size on Post2 decreased back to Pretest levels indicative of successful extinction of CRM. These observations were corroborated by several significant differences between Pretest and Post2 including percent URs ($F(1,11) = 6.81$, $p=0.024$) that interacted with Sex ($F(4,44) = 3.30$, $p=0.019$) and magnitude of UR amplitude ($F(1,11) = 7.31$, $p=0.023$) that also interacted with Sex ($F(1,11) = 6.94$, $p=0.023$). These results suggest that responses stayed somewhat larger in females than in males following unpaired extinction. A Pretest versus Post2 x US Intensity x Sex interaction ($F(3,33) = 8.69$, $p<0.001$) for peak latencies provided support for the observation that response peaks shifted back to or even below pretest levels in female rabbits compared to males. Indeed, a comparison of peak latencies on Post1 versus Post2 yielded a significant interaction of Post1 versus Post2 x US Intensity x Sex ($F(3,33) = 8.34$, $p<0.001$) suggesting that female rabbits extinguished peak latency shifts – a key feature of CRM – somewhat better than males.

Discussion

The principal findings of the current experiments were that female rabbits showed more CRM than males but a key feature of CRM – a shift to longer peak latencies – extinguished better. Experiment 1 showed that male and female rabbits had comparable levels of CRM but that hyperarousal persisted in females with continued testing whereas it was not in evidence in males. Experiment 2 showed that one session of unpaired extinction with a weak shock reduced hyperarousal in males and to a lesser extent in females but a key feature of CRM – shifts in peak latency – extinguished somewhat better in females than in males. In other words, conditioning-specific features of PTSD may be more treatable in females but generalized fear/hyperarousal may be harder to treat. Taken together, these results suggest that female rabbits show more CRM – an index of hyperarousal – than male rabbits but at least one aspect can be treated more effectively. As such the rabbit model recapitulates key features of PTSD including sex differences that are found in clinical populations (Olff et al.,

2007; MacGregor et al., 2017) and contrasts with other animal models where males are consistently *more vulnerable* to various forms of acute and chronic stress than females (Cohen and Yehuda, 2011; Bangasser and Valentino, 2014; Kokras and Dalla, 2014; Keller et al., 2015; Shansky, 2015).

Sex differences in PTSD

Epidemiological data have long reported a higher incidence of PTSD in women than in men (Olff et al., 2007; MacGregor et al., 2017), and this has been attributed to the types of trauma, younger age, stronger perceptions of threat and loss of control, and insufficient social support (Olff et al., 2007; Silove et al., 2017). For example, long-term exposure to trauma in cases of sexual and domestic abuse tends to occur more predominantly in women than in men (Iverson et al., 2015; Smith et al., 2015; Silove et al., 2017). In the present experiments, female rabbits showed more CRM than males but conditioning-specific aspects of CRM particularly peak latency shifts extinguished better in females than in males. Importantly, conditioning itself was acquired and extinguished equally well in both sexes. When differences in some of the attributes of risk of trauma are reduced as they might be in the military where exposure to combat, perceived threat, age, and unit cohesion may be similar between men and women, differences between men and women still exist but those differences become less clear (Vogt et al., 2011; Crum-Cianflone and Jacobson, 2014; Hines et al., 2014; MacGregor et al., 2017). Indeed, when handling, pretesting and the levels of eyeblink conditioning were equivalent between male and female rabbits in the current experiments, female rabbits generally showed higher levels of CRM than males but the effects were not large. Evidence from limited studies of sex differences in response to treatment show that in civilians women respond better to treatment than men (Békés et al., 2016) and in the military there are no sex differences in treatment response (Voelkel et al., 2015) although evidence has been mixed (Blain et al., 2010). In Experiment 2, unpaired extinction was more effective in extinguishing at least one aspect of CRM for female rabbits than males – a finding that is consistent with the PTSD treatment literature.

PTSD and eyeblink conditioning

There are several studies of eyeblink conditioning comparing veterans who suffer from PTSD to those who do not, and these studies show rather mixed results. The first documented study of eyeblink conditioning in combat veterans found impaired eyeblink conditioning in combat veterans compared to non-combat veterans regardless of whether or not they suffered from PTSD (Ayers et al., 2003). Subsequent studies showed similar rates and levels of eyeblink conditioning during acquisition and extinction between veterans with PTSD and those without (McGlinchey et al., 2014); similar conditioning but greater sensitivity to glucocorticoid (Vythulingam et al., 2006); faster acquisition but delayed extinction (Myers et al., 2012); and similar acquisition but heightened overall responsivity to the stimuli – hyperarousal (Burriss et al., 2007). The equivalence in acquisition and extinction and heightened responsivity seen in veterans seems to be have some correspondence to the present eyeblink and CRM data in rabbits.

Sex differences in eyeblink conditioning

Although there is good evidence for the effects of stress on differences between the sexes in rat eyeblink conditioning because males condition more strongly than females (Wood and Shors, 1998), there is very little evidence of differences between the sexes in rabbit eyeblink conditioning. In fact, there is only one study showing ovariectomized female rabbits learn and extinguish eyeblink conditioning marginally faster than males (Orlowska-Majdak et al., 2001). The data from the current experiments show no sex differences in response rate or terminal levels of delay conditioning. In contrast, a recent human eyeblink conditioning study showed that females, both children and adults, showed more eyeblink conditioning than males although, in contrast to the present experiments, the overall levels of conditioning were rather weak (Löwgren et al., 2017). We may not have detected sex differences in rabbit eyeblink conditioning because of ceiling effects resulting from a salient US and six days of conditioning.

Conclusions

We have developed a rabbit eyeblink conditioning model of PTSD which recapitulates several core features of PTSD, particularly hyperarousal and conditioned responding to trauma-associated cues but until now, the work has all been done in male rabbits. We found that although both male and female rabbits acquired identical levels of conditioning, females showed more hyperarousal after conditioning than males but had conditioning-specific aspects that responded somewhat better to treatment. The increased hyperarousal in females is particularly important because previous animal studies modeling PTSD show that males are consistently more vulnerable to stress and show more PTSD-like behavior than females (Cohen and Yehuda, 2011; Bangasser and Valentino, 2014; Kokras and Dalla, 2014; Keller et al., 2015; Shansky, 2015).

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Highlights

- An animal model that shows core features of PTSD including hyperarousal
- Sex differences in this PTSD model recapitulate sex differences that are found in clinical populations
- These findings contrast with other animal models where males are consistently more vulnerable to various forms of acute and chronic stress than females

Figure 1. Responding to the conditioned stimulus (CS) during conditioned stimulus-unconditioned stimulus (US) pairings and CS-alone testing (CS). The figure depicts mean ($\pm$ SEM) percent responding to the tone CS (%CRs).

Figure 2.

Responding to the unconditioned stimulus (US) during the first 20-trial block of US testing. 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 a second US post-test (Post2, blue line) presented four weeks later. 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 ($\pm$ SEM) percentage of unconditioned responses (Percent URs, first row), magnitude of the UR amplitude (second row), magnitude of the UR area (third row) and peak latency of URs for the first 20 trials of presentations of the periorbital shock unconditioned stimulus (US) during pretest (Pretset, white bar), the post-test following CS-US pairings (Post1, black bar), and the second post-test four weeks later (Post2, 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. Peak latencies are not shown for the lowest US intensity (0.1 mA) because there were very few responses.

Figure 4.

Responding to the unconditioned stimulus (US) during the second 20-trial block of US testing. 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 a second US post-test (Post2, blue line) presented four weeks later. 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 5. Responding to the conditioned stimulus (CS) during conditioned stimulus-unconditioned stimulus (US) pairings, unpaired extinction with a weak 0.3-mA US (EXT), and CS-alone testing (CS Test). The figure depicts mean ($\pm$ SEM) percent responding to the tone CS (%CRs).

Figure 6.

Responding to the unconditioned stimulus (US) during the first 20 trials of US testing. 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 a second US post-test (Post2, blue line) presented following 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 7.

Mean (± SEM) percentage of unconditioned responses (Percent URs, first row), magnitude of the UR amplitude (second row), magnitude of the UR area (third row) and peak latency of URs 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 black bar), and the post-test following unpaired extinction with a weak US (0.3 mA) (Post2, 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. Peak latencies are not shown for the lowest US intensity (0.1 mA) because there were very few responses.

Mean ($\pm$ SEM) peak latencies for the unconditioned responses to the periorbital shock unconditioned stimulus (US) for male and female rabbits during the first 20 trials of the US pretest (Pretest), the US post-test following CS-US pairings (Post1), and the US post-test following one day of 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 1

Group	Test	US Intensity			
		0.3 mA	0.5 mA	1.0 mA	2.0 mA
Males	Pretest	94.67 $\pm$ 1.90	88.06 $\pm$ 6.42	74.44 $\pm$ 5.67	81.61 $\pm$ 9.41
	Post1	135.00 $\pm$ 3.82	215.28 $\pm$ 61.89	96.07 $\pm$ 9.18	69.52 $\pm$ 3.94
	Post2	128.13 $\pm$ 10.14	96.96 $\pm$ 9.28	69.64 $\pm$ 4.16	70.18 $\pm$ 2.03
Females	Pretest	132.50 $\pm$ 5.81	110.00 $\pm$ 1.29	72.64 $\pm$ 10.35	71.18 $\pm$ 3.71
	Post1	314.69 $\pm$ 28.42	136.81 $\pm$ 17.07	91.88 $\pm$ 15.77	65.07 $\pm$ 4.54
	Post2	120.33 $\pm$ 9.04	82.29 $\pm$ 7.54	84.17 $\pm$ 7.54	71.04 $\pm$ 6.29