Psychosocial predator-based animal model of PTSD produces physiological and behavioral sequelae and a traumatic memory four months following stress onset

Phillip R. Zoladz,⁎ Collin R. Park, Monika Fleshner, David M. Diamond

Department of Psychology, Sociology & Criminal Justice, Ohio Northern University, Ada, OH 45810, USA
Department of Integrative Physiology & Center for Neuroscience, University of Colorado, Boulder, CO 80309, USA
Medical Research Service, VA Hospital, Tampa, FL 33612, USA
Department of Psychology, University of South Florida, Tampa, FL 33620, USA
Department of Molecular Pharmacology & Physiology, University of South Florida, Tampa, FL 33620, USA
Department of Center for Preclinical & Clinical Research on PTSD, University of South Florida, Tampa, FL 33620, USA

HIGHLIGHTS
• PTSD-like sequelae induced by psychosocial stress persisted for at least 4 months.
• Predator exposure generated a persistent fear-conditioned “traumatic” memory.
• Psychosocial stress resulted in a long-term increase in anxiety and impaired cognition.
• Predator-based psychosocial stress resulted in persistent physiological changes comparable to PTSD-like symptoms.

ABSTRACT
We have a well-established animal model of PTSD composed of predator exposure administered in conjunction with social instability that produces PTSD-like behavioral and physiological abnormalities one month after stress initiation. Here, we assessed whether the PTSD-like effects would persist for at least 4 months after the initiation of the psychosocial stress regimen. Adult male Sprague–Dawley rats were exposed to either 2 or 3 predator-based fear conditioning sessions. During each session, rats were placed in a chamber for a 3-min period that terminated with a 30-s tone, followed by 1 h of immobilization of the rats during cat exposure (Day 1). All rats in the stress groups received a second fear conditioning session 10 days later (Day 11). Half of the stress rats received a third fear conditioning session 3 weeks later (Day 32). The two cat-exposed groups were also exposed to daily unstable housing conditions for the entire duration of the psychosocial stress regimen. The control group received stable (conventional) housing conditions and an equivalent amount of chamber exposure on Days 1, 11 and 32, without cat exposure. Behavioral testing commenced for all groups on Day 116. The stress groups demonstrated increased anxiety on the elevated plus maze, impaired object recognition memory and robust contextual and cued fear conditioned memory 3 months after the last conditioning session. Combined data from the two stress groups revealed lower post-stress corticosterone levels and greater diastolic blood pressure relative to the control group. These findings indicate that predator-based psychosocial stress produces persistent PTSD-like physiological and behavioral abnormalities that may provide insight into the neurobiological and endocrine sequelae in traumatized people with PTSD.

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1. Introduction

Post-traumatic stress disorder (PTSD) is a unique psychiatric condition in that its diagnosis requires a distinct etiological event, specifically, one or more intense and horrific traumatic experiences. Individuals who develop PTSD following trauma exposure experience significant psychological distress by repeatedly reliving the trauma through intrusive,
Flashback memories [1–4]. This aspect of the disorder causes PTSD patients to avoid situations that remind them of their trauma and can also facilitate the development of an array of other debilitating symptoms, such as persistent anxiety, an exaggerated startle response, cognitive impairments and an impaired ability to extinguish conditioned fear [5–7].

Trauma exposure is a necessary, but not sufficient, component for both the development and persistence of PTSD. Only a subset (10–50%) of traumatized individuals develops PTSD, depending on a multitude of interacting risk factors, including the nature of the trauma, genetics, gender, social support and early life experiences [7,8]. Our group has published a series of studies based on a predator-exposure animal model of PTSD that we developed [9]. The primary components of this model were based on trauma-induction features that are known to be associated with a greater susceptibility of a subset of traumatized people developing PTSD. Specifically, in our model, rats are immobilized and placed in close proximity to a cat on two separate occasions. The stressed rats are also exposed to daily social instability (randomized cage mates) throughout the duration of the experiment to increase the likelihood of producing long-lasting physiological and behavioral changes in the rats. Thus, our PTSD model incorporates key elements of trauma, such as uncontrollability, unpredictability, lack of social support and a re-experiencing of trauma that, in people, significantly increases the risk for the development and persistence of PTSD.

We have reported that exposing rats to our psychosocial predator-based animal model of PTSD results in a number of physiological and behavioral abnormalities, all of which are remarkably similar to those observed in people with PTSD. For instance, three weeks after the second predator exposure, rats administered psychosocial stress exhibited reduced growth rate, reduced thymus weight, greater adrenal gland weight, increased anxiety, an exaggerated startle response, impaired memory for new information, greater cardiovascular and hormonal reactivity to an acute stressor and an exaggerated physiological and behavioral response to the α2-adrenergic receptor antagonist, yohimbine [9]. Importantly, these effects depend on the combination of both cat exposures and daily social instability.

In more recent studies, we demonstrated that three weeks after the second predator exposure, psychosocially stressed rats exhibited a robust fear conditioned memory for the predator stress exposures, enhanced negative feedback of the hypothalamus–pituitary–adrenal (HPA) axis and increased methylation of BDNF DNA in the hippocampus [10–12]. Others have replicated and extended the findings from our model, revealing effects of predator-based psychosocial stress on neurotransmitters and neuromodulators that resemble those observed in PTSD patients [13,14]. Thus, extensive assessments fortify the conclusion that our predator-based psychosocial stress model of PTSD results in physiological and behavioral changes that are comparable to those seen in people with PTSD.

One aspect of our animal model of PTSD that has yet to be addressed is how long the physiological and behavioral changes persist. This is clearly a relevant issue for any animal model of PTSD since traumatized individuals commonly experience debilitating symptoms that can persist for years after the original trauma occurred. Thus, the purpose of the present study was to examine if the PTSD-like symptoms would persist for 4 months following the initiation of stress and whether additional predator exposure was necessary to maintain the presence of the PTSD-like effects.

2. Material and methods

2.1. Animals

Experimentally naive adult male Sprague–Dawley rats (225–250 g upon delivery) obtained from Charles River laboratories (Wilmington, MA) were used in the present experiment. The rats were housed on a 12-h light/dark schedule (lights on at 0700) in standard Plexiglas cages (two per cage) with free access to food and water. Upon arrival, all rats were given 1 week to acclimate to the housing room environment and cage changing procedures before any experimental manipulations took place. All procedures were approved by the Institutional Animal Care and Use Committee at the University of South Florida.

2.2. Psychosocial stress procedure

The experimental groups and timeline of procedures are illustrated in Fig. 1. Following the 1-week acclimation phase, rats were brought to the laboratory and assigned to one of two psychosocial stress groups or a no stress control group (N = 10 rats/group). On Day 1, rats from these groups were exposed to a chamber for 3 min. During the last 30 s of the 3-min chamber exposure, a 74-db, 2500 Hz tone was presented to the rats. The chamber (26 × 30 × 29 cm; Coulbourn Instruments; Allentown, PA) consisted of two aluminum sides, an aluminum ceiling, and a Plexiglas front and back. The floor of the chamber consisted of 18 stainless steel rods, spaced 1 cm apart. The purpose of exposing rats to the chamber was to enable rats in the psychosocial stress groups to associate the chamber (contextual fear conditioning) and tone (auditory cue) with the acute stress experiences (i.e., immobilization plus cat exposure, as described below). We then measured their fear conditioned memory by assessing immobility of the rats in the chamber when they were returned to the chamber during behavioral testing, as previously described [10,11]. Locomotor activity in the chamber was measured during the fear conditioning sessions and fear memory testing by a 24-cell infrared activity monitor (Coulbourn Instruments; Allentown, PA) mounted on the top of the chamber, which used emitted infrared body heat image (1300 nm) from the animals to detect their movement. Immobility was defined as periods of inactivity lasting at least 7 s, based on previously employed methodology [10,12].

Following the 3-min chamber exposure, rats in the psychosocial stress groups were removed from the chamber and immediately immobilized in plastic DecapitCone (Braintree Scientific; Braintree, MA), and then they were placed in a perforated wedge-shaped Plexiglas enclosure (Braintree Scientific; Braintree, MA; 20 × 20 × 8 cm). The rats, immobilized in the plastic DecapitCone within the Plexiglas enclosure, were taken to the cat housing room where they were placed in a metal cage (61 × 53 × 51 cm) with an adult female cat for 1 h. The Plexiglas enclosure prevented any contact between the cat and rats, but the rats were still exposed to all non-tactile sensory stimuli associated with the cat. Canned cat food was smeared on top of the Plexiglas enclosure to direct the cat’s attention toward the rats. An hour later, the rats were brought to the laboratory, returned to their home cages and then were returned to the vivarium. Rats in the no stress group were not immobilized or exposed to the cat; after the chamber exposure, they were placed in their home cages in the laboratory where they remained for 1 h, and then they were returned to the vivarium.

The first and second stress sessions were separated by a period of 10 days (i.e., Day 1 and Day 11), as per our previously employed methodology [9–12]. The second and third stress sessions were separated by a period of 21 days (i.e., Day 11 and Day 32). Thus, the third stress session occurred when, in previous work, we had initiated behavioral testing. Rats that had been assigned to the first psychosocial stress group (Stress × 2) were immobilized and exposed to the cat during the first two, but not the third, stress sessions. During the third stress session, rats in the “Stress × 2” group were given chamber exposure only. Rats in the second psychosocial stress group (Stress × 3) were immobilized and exposed to the cat during all three stress sessions. Rats in the control group (No Stress) were given chamber exposure, alone, during all three sessions.

The first and third acute stress sessions took place during the light cycle, between 0800 and 1300 h, and the second acute stress session took place during the dark cycle, between 1900 and 2100 h. The acute stress sessions took place during different times of the day to add an...
element of unpredictability as to when the rats could re-experience the traumatic event.

2.3. Daily social stress

Beginning on the day of the first acute stress session (Day 1), rats in the two psychosocial stress groups were exposed to unstable housing conditions, as described previously, until behavioral testing (Day 116). Rats in the psychosocial stress groups were housed two per cage, but every day, their cohort pair combinations were changed. Therefore, no rats in the psychosocial stress groups had the same cage mate on two consecutive days during the 115-day stress period. Rats in the no stress group were housed with the same cohort for the entire duration of the experiment.

2.4. Behavioral testing

Twelve weeks following the third acute stress session (on Day 116), all rats were given tests to measure their fear memory, anxiety, startle, learning and memory, cardiovascular activity and corticosterone activity. On each day of behavioral testing (Days 116–120), rats were taken to the laboratory and left undisturbed for 30 min before testing began. All behavioral testing took place during the light cycle, between 0800 and 1500 h.

2.4.1. Contextual and cued fear memory

On Day 116, immobilization of the rats in the fear conditioning chamber (context test) and in response to a tone (cue test) was assessed. First, rats were placed for 5 min in the same chamber that they were exposed to during each of the three acute stress sessions. An hour after the 5-min context test, the rats were placed in a novel chamber that had different lighting, walls and flooring texture. The rats were placed in the novel chamber for a total of 6 min for a cue-specific test. After a 3-min baseline period, a 74-dB, 2500 Hz tone was presented for the next 3 min. Immobility during the baseline period provided a measure of general fear of a novel place, and immobility during the 3-min tone provided a measure of the fear response to the cue that was, in the psychosocial stress groups, temporally associated with the cat exposures.

2.4.2. Elevated plus maze

The elevated plus maze (EPM), a routine test of anxiety in rodents, consisted of two open arms (11 × 50 cm) and two closed arms (11 × 50 cm) that intersect each other to form the shape of a plus sign. On Day 117, the rats were placed on the EPM for 5 min, and their behavior was monitored by 48 infrared photobeams located along the perimeter of the open and closed arms input to computer software (Motor Monitor; Hamilton-Kinder; San Diego, CA). The dependent measures of interest were the percent of time rats spent in the open arms and the number of ambulations (i.e., an indication of overall movement) made by each rat.

2.4.3. Startle response

One hour after the EPM assessment, acoustic startle testing was administered. The rats were placed inside a small Plexiglas box (19 × 10 × 10 cm), which was inside a larger startle monitor cabinet (Hamilton-Kinder; San Diego, CA; 36 × 28 × 50 cm). The small Plexiglas box within this cabinet contained a sensory transducer on which the rats were placed at the beginning of the trial. The sensory transducer was connected to a computer (Startle Monitor; Hamilton-Kinder; San Diego, CA).
Diego, CA), which recorded the startle responses by measuring the maximum amount of force (Newtons) that rats exerted on the sensory transducer for a period of 250 ms after the presentation of each auditory stimulus. To control for any differences in body weight, the sensitivity of the sensory transducer was adjusted prior to each trial via a Vernier adjustment with a sensitivity range of 0–7 arbitrary units. The startle trial began with a 5-min acclimation period, followed by the presentation of 24 bursts of white noise (50 ms each), eight from each of three auditory stimulus intensities (90, 100, and 110 dB). The noise bursts were presented in sequential order, and the time between each noise burst varied pseudorandomly between 25 and 55 s. Upon the commencement of the first noise burst, the startle apparatus provided uninterrupted background white noise (57 dB).

2.4.4. Novel object recognition

The novel object recognition (NOR) task was a modified version of that which was employed by Baker and Kim [15]. On Day 118, the rats were placed in an open field (Hamilton-Kinder; San Diego, CA; 40 × 47 × 70 cm) for 5 min to acclimate to the environment. A Logitech camera that was mounted on the ceiling overlooking the open field monitored rat behavior. This camera was connected to computer software (ANY-Maze; Stoelting; Wood Dale, IL) which scored rat behavior. Twenty-four hours later (Day 119), the rats were placed in the same open field with two identical (plastic/metal) objects for 5 min. The objects were in opposite corners of the open field and secured to the flooring to prevent the rats from displacing them. The objects were counterbalanced across rats, as were the corners in which the objects were placed. Three hours later, the rats were returned to the open field for a final 5-min test trial, but this time, the open field contained a replica of the object that had been there before and a novel object. During this testing session, greater time spent by the rats in proximity to the novel versus familiar object was an indication of intact memory for the familiar object. The time that each rat spent with the objects during training and testing was quantified by specifying a zone around the objects for the ANY-Maze software to score the duration of investigatory behavior. These data were then used to calculate a discrimination index [novel object investigatory time / novel object investigator time + familiar object investigatory time]. This index was then converted into a percentage score by multiplying the result by 100. Chance performance (i.e., spending an equivalent amount of time with the novel and familiar objects) was thus 50%.

2.4.5. Blood sampling, cardiovascular activity and post-mortem dissections

On the final day of behavioral testing (Day 120), rats were brought, one cage at a time, to a nearby procedure room for blood sampling. The saphenous vein of each rat was punctured with a sterile, 27-gauge syringe needle. A 0.2 cm³ sample of blood was then collected from each rat. This first blood sample was collected within 2 min after the rats were removed from the housing room. After obtaining this sample, the rats were immobilized in plastic DecapCones for 20 min, and then another 0.2 cm³ sample of blood was collected via saphenous vein puncture. Immediately after collecting the second blood sample, the rats were placed in Plexiglas tubes within a warming test chamber (-32 °C) for 5 min to increase their body temperature, which enhanced blood flow to their tails. Heart rate (HR) and blood pressure (BP) were assessed using a tail cuff fitted with photoelectric sensors (ITC Life Science; Woodland Hills, CA). Three HR and BP recordings were obtained from each rat (these three recordings were averaged to create single HR and BP data points for each rat). Following the HR and BP measurement, the rats were returned to their home cages. An hour later, the third and final blood sample (trunk blood) was collected following rapid decapitation. Then, the adrenal glands and thyromaxes were removed and weighed. Once all of the blood had clotted at room temperature, it was centrifuged (3000 rpm for 8 min), and the serum was extracted and stored at -80 °C until assayed by M.F. at the University of Colorado at Boulder.

2.4.6. Body weights

All rats were weighed before each acute stress session, as well as on the first day of behavioral testing.

2.5. Statistical analyses

In most cases, one-way ANOVAs were used to analyze the data, with group serving as the between-subjects factor. When repeated measures variables were involved, mixed-model ANOVAs were utilized. Planned comparisons (t-tests) were conducted between groups that were predicted to differ a priori. For all analyses, alpha was set at 0.05, and Holm–Sidak post hoc tests were employed when necessary.

3. Results

3.1. Fear conditioning and memory testing (see Fig. 2)

3.1.1. Fear conditioning sessions

Analysis of immobility during the first [F(2,27) = 1.00, p > 0.05] and second [F(2,27) = 2.87, p > 0.05] 3-min chamber exposures revealed no significant effect of group. Analysis of immobility during the third fear conditioning trial revealed a significant effect of group [F(2,25) = 3.64, p < 0.05]. Both psychosocial stress groups exhibited greater immobility than the no stress group.

3.1.2. Fear conditioned contextual memory test

Analysis of immobility during the 5-min context test on Day 115 revealed a significant effect of group, F(2,26) = 16.65, p < 0.001. Both psychosocial stress groups exhibited greater immobility than the no stress group, and the “Stress × 3” group exhibited greater immobility than the “Stress × 2” group. Analysis of overall movement on the EPM (i.e., ambulations) during the 5-min trial revealed no significant group effect [F(2,25) = 2.05, p > 0.05]. Both psychosocial stress groups exhibited greater movement than the no stress group.

3.1.3. Fear conditioned cue memory test

Analysis of immobility during the 6-min cue test revealed significant effects of group, F(2,25) = 3.73, and tone, F(1,25) = 47.87, and a significant Group × Tone interaction, F(2,25) = 4.13 (p’s < 0.05). There were no group differences in immobility during the first 3 min of the cue test, which was prior to tone delivery. During the 3-min tone delivery period, both psychosocial stress groups exhibited greater immobility than the no stress group.

3.2. Elevated plus maze (see Fig. 3)

3.2.1. Open arm time

Analysis of open arm time for the entire 5-min trial revealed no significant effect of group, F(2,26) = 0.81, p > 0.05. More specific analyses revealed that, during the first minute of the 5-min trial, there was a significant effect of group, F(2,26) = 9.13, p < 0.001, indicating that rats in both psychosocial stress groups spent less time in the open arms than the no stress group.

3.2.2. Ambulations

Analysis of overall movement on the EPM (i.e., ambulations) during the entire 5-min trial, F(2,27) = 0.63, and the first minute of the 5-min trial, F(2,27) = 1.90, revealed no significant effects of group (p’s > 0.05).

3.3. Startle response (see Fig. 4)

Analysis of startle responses revealed a significant effect of dB, F(2,50) = 87.15, p < 0.001, indicating that rats exhibited greater startle responses as the intensity of the white noise bursts increased. However, there was no significant effect of group, F(2,25) = 2.85, and the Group × dB interaction was not significant, F(4,50) = 0.88 (p’s > 0.05).
Fig. 2. Immobility during the three stress sessions and the context and cue tests. There were no significant group differences during the first two sessions (a and b). By the third session (c), both psychosocial stress groups exhibited greater immobility with chamber exposure than the no stress group. During the 4-month context memory test (d), both psychosocial stress groups exhibited greater immobility than the no stress group, and the Stress × 3 group exhibited greater immobility than the Stress × 2 group. In the cue test (e), the psychosocial stress groups displayed greater immobility than the no stress group during the tone (diagonal lines) compared to the pre-tone period (solid bars). Data are means ± SEM *p < 0.05 vs. the no stress group; **p < 0.05 vs. all other groups.

Fig. 3. Behavior on the elevated plus maze (EPM). Both psychosocial stress groups spent less time in the open arms than the no stress group (left). This effect was not attributable to a difference in overall activity level, as there were no significant group differences in ambulations (right). Data are means ± SEM *p < 0.05 vs. the no stress group.
3.4. Novel object recognition (see Fig. 5)

3.4.1. Novel object recognition: habituation

The analysis of locomotor activity in the open field during the 5-min habituation phase revealed no significant effect of group, $F(2,27) = 0.13$, $p > 0.05$. The analysis of time that the rats spent in each area of the open field revealed no significant effect of group, $F(2,27) = 0.22$, or quadrant, $F(3,81) = 0.60$, and the Group × Quadrant interaction was not significant, $F(6,81) = 0.28$ ($p’s > 0.05$). These findings indicate that the rats did not display a preference for one area of the open field over another.

3.4.2. Novel object recognition: training

Within-group comparisons showed that the no stress group, $t(9) = 0.12$, “Stress × 2” group, $t(9) = 0.72$, and “Stress × 3” group, $t(9) = 1.03$, spent a comparable amount of time with each of the object replicas that were placed in the open field during object recognition training ($p’s > 0.05$), indicating that no object preference effects were present. Moreover, a between-groups comparison of the total amount of time spent with both objects during training revealed no significant effect of group, $F(2,27) = 1.92$, $p > 0.05$, indicating that all groups spent a comparable amount of time with both objects during training.

3.4.3. Novel object recognition: memory test

The analysis comparing the discrimination indices of all groups during the 5-min object recognition testing session revealed no significant effect of group, $F(2,27) = 1.74$, $p > 0.05$. The analysis comparing the discrimination indices of all groups during the first minute of the testing trial revealed a significant effect of group, $F(2,27) = 5.24$, $p < 0.05$, indicating that both psychosocial stress groups exhibited lower discrimination indices than the no stress group. Further exploration of the time that rats spent with the novel and familiar objects indicated that the “Stress × 2” group spent significantly less time with the novel object than the familiar object during the first minute of testing, $t(9) = 2.55$, $p < 0.05$.

3.5. Body weights (see Fig. 6)

The analysis of weight gained over the course of the experiment revealed a significant effect of time, $F(2,52) = 614.76$, $p < 0.001$, and a significant Group × Time interaction, $F(4,52) = 2.79$, $p < 0.05$. Both psychosocial stress groups gained less weight than the no psychosocial stress group between the first stress session and the second and third stress sessions. However, the psychosocial stress groups both recovered and gained the same amount of weight as the no stress group by the first day of behavioral testing. Overall, there was no significant effect of group, $F(2,26) = 1.54$, $p > 0.05$.

3.6. Organ weights (see Fig. 6)

The analysis of adrenal gland weights revealed no significant effect of group, $F(2,27) = 1.04$, $p > 0.05$. However, the effect of group on thymus weight was significant, $F(2,26) = 5.23$, $p < 0.05$. This effect indicated that the “Stress × 3” group exhibited reduced thymus weight relative to the no stress group.

3.7. Cardiovascular activity (see Fig. 7)

The analysis of cardiovascular activity revealed no significant effects of group for HR, $F(2,16) = 0.12$, systolic BP, $F(2,16) = 1.43$, or diastolic BP, $F(2,16) = 1.79$ ($p’s > 0.05$). In order to more thoroughly examine the possibility that our animal model of PTSD resulted in long-term cardiovascular changes, we conducted additional statistical analyses by combining HR and BP data from both psychosocial stress groups and comparing these data to the no stress group. The two psychosocial stress groups were not significantly different from one another on any cardiovascular measure; thus, this analysis allowed us to increase statistical power and address whether 2 or 3 cat exposures, in general, exerted any long-term effects on cardiovascular activity. These analyses revealed no significant differences for HR and systolic BP; however, the effect for diastolic BP was borderline significant and revealed that the psychosocial stress group (“Stress × 2” + “Stress × 3”) exhibited greater diastolic BP than the no stress group, $t(17) = −1.948$, $p = 0.068$. 

Fig. 4. Startle responses to the three auditory stimulus intensities. Although startle responses increased as sound intensity increased, there were no significant group differences at any one intensity. Data are means ± SEM.

Fig. 5. Novel object preference (expressed as a discrimination index) during novel object recognition testing. Over the entire 5-min testing trial (right), there were no significant group differences. Upon examination of the first minute of the trial (left), both psychosocial stress groups exhibited significantly less preference for the novel object than the no stress group. The dashed line at 50% is indicative of chance performance. Data are means ± SEM. *$p < 0.05$ vs. the no stress group.
3.8. Corticosterone levels (see Fig. 7)

The analysis of corticosterone levels revealed a significant effect of time point, $F(2,54) = 104.22, p < 0.001$, indicating that all groups displayed elevated serum corticosterone levels, relative to baseline, following 20 min of acute immobilization stress and that these levels remained elevated an hour later. There was no significant effect of group, $F(2,27) = 0.77$, and the Group × Time interaction was not significant, $F(4,54) = 0.64 (p's > 0.05)$.

To more thoroughly examine the possibility that our animal model of PTSD resulted in long-term hormonal changes, we conducted additional statistical analyses by combining the corticosterone data from both psychosocial stress groups and comparing these data to the no stress group. These analyses revealed a significant main effect of group, indicating that the two psychosocial stress groups exhibited lower corticosterone levels than the no stress group, overall, $F(1,26) = 4.19, p = 0.05$, and a reliable Group × Time interaction, $F(2,52) = 3.08, p = 0.05$. Post hoc comparisons revealed that 1 h following the cessation of acute immobilization stress, the psychosocial groups exhibited significantly lower corticosterone levels than the no stress group ($p's < 0.05$).

4. Discussion

We have reported that this psychosocial predator-based animal model of PTSD produces PTSD-like physiological and behavioral changes that are present 4 months following the initiation of stress. In addition to testing the persistence of our original paradigm (i.e., 2 cat exposures combined with daily social instability), we also examined whether a third cat exposure was necessary to permit the effects to be observed so long following stress onset. Although we found that 2 cat exposures were sufficient to produce a long-lasting contextual and cue-based fear memory, as well as heightened anxiety on the elevated plus maze and impaired object recognition memory, 3 cat exposures significantly increased the strength of the contextual fear memory and led to a significant reduction of thymus weight, which was not observed following 2 cat exposures. The combined data from both psychosocial stress groups revealed lower corticosterone levels 1 h following acute immobilization stress, relative to the no stress group. Moreover, psychosocial stress produced a trend for an increase in diastolic BP following 20 min of acute immobilization stress. We did not observe any significant effects of psychosocial stress on startle response, and the body weight reduction that was observed in the psychosocial stress groups between the first and third stress sessions normalized by the time of behavioral testing. Combined, these findings suggest that over the course of the 4 months, some of the effects decreased in magnitude. Most importantly, our experimental paradigm produced contextual and cue-based fear memory, detectable changes in anxiety-like behavior and cognition and alterations in cardiovascular and hormonal activity that were observable more than 4 months following stress onset. This great durability of a large subset of effects indicates that our PTSD model is useful for testing the neurobiological mechanisms of, and novel treatments for, long-term traumatic memories and chronic PTSD symptomatology.
4.1. Collective effects of the psychosocial predator-based animal model of PTSD

In previous work, we have found that our psychosocial predator-based animal model of PTSD resulted in a number of physiological and behavioral abnormalities, all of which are similar to those observed in people with PTSD. Three weeks following the second predator exposure, psychosocially stressed rats exhibit reduced growth rate, reduced thymus weight, greater adrenal gland weight, increased anxiety, an exaggerated startle response, impaired memory for new information, greater cardiovascular and hormonal reactivity to an acute stressor and an exaggerated physiological and behavioral response to the \( \alpha_2 \)-adrenergic receptor antagonist, yohimbine [9]. We have also shown that, three weeks after the second predator exposure, psychosocially stressed rats exhibit a robust fear memory for the predator stress exposures, increased negative feedback of the HPA axis and increased methylated BDNF DNA in the hippocampus [10–12]. These findings highlight a unique aspect of our animal model of PTSD — it manifests evidence of associative and non-associative fear in rats. That is, psychosocially stressed rats exhibit a memory for the initial “traumatic” experience, as evidenced by classically-conditioned fear expression, and they demonstrate hyperarousal, cognitive symptoms and physiological symptoms that appear to be independent of this memory.

Other investigators have replicated some of these findings and have extended our animal model to characterize its effects on neurotransmitter levels and markers of inflammation. Wilson and colleagues reported that our psychosocial predator-based model of PTSD resulted in decreased serotonin and increased norepinephrine and dopaminergic activity in the prefrontal cortex and hippocampus [13]. These investigators also demonstrated that psychosocially stressed rats displayed increased measures of inflammation and oxidative stress in the hippocampus, prefrontal cortex, adrenal glands and systemic circulation [14], effects that have been observed 70 days following the initiation of stress in a paradigm of 3 cat exposures similar to the one used in the present study [16]. Importantly, most of the effects of this animal model on neurotransmitter levels and markers of inflammation were reversed by administration of valproic acid or sertraline [16,17].

One empirical question that had yet to be addressed in our animal model was how long the effects induced by the psychosocial stress paradigm would last. In the present study, we have shown that although some of the effects appeared to have diminished over time, a novel and important finding is that psychosocially stressed rats displayed intact conditioned fear memory for the cat exposures, heightened anxiety and impaired cognition 4 months following the initiation of the stress experience. To our knowledge, the present study is the first to report PTSD-like physiological and behavioral changes in rats this long after the initiation of stress, potentially making it uniquely relevant to understanding the mechanisms underlying long-term, or chronic, forms of PTSD.

Another question that was addressed in this study was whether a third cat exposure was necessary to produce physiological and behavioral alterations so long after the initiation of stress. The only differences that were produced by the additional cat exposure were a greater contextual fear memory and a greater reduction in thymus weight, relative to rats given only 2 cat exposures. A reduction in thymus weight is significant because it is suggestive of compromised immune system function. That we observed a significant decrease in thymus weight in the Stress × 3 group only suggests that a third cat exposure may be necessary for this model to produce longer-lasting immune system alterations. We did observe additional effects upon combining the cardiovascular and endocrinological data from both psychosocial stress groups and comparing this combined group to the no stress group. These analyses revealed a statistical trend indicative of elevated diastolic BP in the combined psychosocial stress group, which is consistent with our previous findings [9,12] and cardiovascular abnormalities observed in PTSD patients [7]. It is important to note, though, that tail cuff measures of BP are notoriously less sensitive to small changes in BP, relative to in vivo biotelemetry. Thus, the use of more sensitive measures could have revealed a greater effect of psychosocial stress on cardiovascular measures. We also observed a greater reduction of corticosterone levels in the combined psychosocial stress group one hour following exposure to acute immobilization stress. This finding is consistent with our previous work showing significantly lower baseline levels of corticosterone and a greater recovery of stress-induced corticosterone levels following dexamethasone administration in psychosocially stressed rats [10]. Psychosocially stressed animals may have demonstrated lower corticosterone levels one hour after the immobilization stress because they had enhanced negative feedback of the HPA axis, a finding that would be consistent with a majority of the PTSD literature [18,19].

Despite the positive outcomes observed in the present study, we did not observe an exaggerated startle response in psychosocially stressed animals. This would appear to contradict our previous work, as well as the PTSD literature. However, as we have reviewed previously [7], the literature regarding startle response in PTSD patients is inconsistent and has failed to conclusively provide evidence that an exaggerated startle response is a cardinal symptom of the disorder. One alternative explanation for the exaggerated startle response observed in some studies involving PTSD patients is that an exaggerated startle response is a pre-existing risk factor for a subset of traumatized individuals to develop the disorder and is therefore not present in every person with PTSD [20]. Another explanation is that when tested in a laboratory setting, PTSD patients exhibit a context-dependent enhancement of startle due to heightened reaction to novelty stress or anticipatory anxiety, rather than exhibiting startle enhancement under true baseline conditions, such as in the safety of their home [21]. Thus, a failure to detect an exaggerated startle response in our psychosocially stressed rats is not inconsistent with the broader PTSD literature and may reflect the inherent variability in startle response data that is observed in such studies. It might also suggest that, over time, greater variability in the startle response develops, which means an exaggerated startle response would be more difficult to detect in individuals having PTSD for a longer period of time [22,23].

We also did not observe an impairment of object recognition memory across the entire 5-min testing trial. Indeed, data suggestive of impaired object recognition memory in psychosocially stressed animals was observed only during the first minute of testing. Perhaps more important is the finding that the “Stress × 2” group spent significantly less time with the novel object than the familiar object during the first minute of testing. This finding raises the issue of whether the “Stress × 2” group had an intact memory for the familiar object and chose to avoid the novel object as a result of neophobia. Although the “Stress × 3” group did not spend significantly less time with the novel object than with the familiar object during the first minute of testing, the data from this group were in the same direction as that of the “Stress × 2” group. These findings could indicate that over time, rats exposed to our predator-based psychosocial stress regimen develop PTSD-related fear of novelty, especially because we have not observed neophobic behaviors in psychosocially stressed animals in our previous work. However, because we did not observe increased immobility of psychosocially stressed animals during the first 3 min of the cue test, which took place in a novel environment, additional work is necessary to explore further this possibility.

4.2. Essential components of animal models of PTSD

Animal models of PTSD are essential to understand the disorder because they provide control over environmental factors and stress-inducing stimuli, thus affording investigators a greater opportunity to make cause–effect inferences than can be accomplished in clinical research. They also enable investigators to examine neurochemical mechanisms that potentially underlie PTSD and test the efficacy of novel therapeutic agents in reversing any stress-induced alterations of
physiology and behavior, which could lead to more treatment options for PTSD patients in the future. However, PTSD is a complex physiological disorder that consists of several symptom clusters. The ability to reproduce all symptoms of the disorder in a non-human animal model is likely unrealistic, but a valid animal model of PTSD should model as many aspects of the disorder as is possible to achieve in the species being studied. Furthermore, these models should evidence phenomena that are known to exist in the human condition, such as a dose-dependent relationship between the stressor and PTSD-like symptoms, a long-term manifestation of the PTSD-like sequelae and associative and non-associative fear-related behaviors [24].

Numerous stress paradigms have been employed to model PTSD in rodents, such as repeated exposure to conditioned fear [25,26], electric shock [27–38], underwater trauma [39–41], stress–restress and single prolonged stress paradigms [42–46], and exposure to predators or predator-related cues [47–57]. These paradigms have resulted in heightened anxiety, exaggerated startle, cognitive impairments, enhanced fear conditioning, reduced social interaction and hormonal changes that correlate with PTSD. Still, many animal models of PTSD have modeled limited aspects of the human condition, and the effects that have been produced last a short period of time. In addition, some models provide evidence for a "traumatic" memory without demonstrating changes in anxiety levels, symptoms of hyperarousal and physiological alterations that are comparable to those observed in PTSD. Other models have provided the opposite — that is, numerous behavioral and physiological changes without evidence for a memory component of PTSD. What is unique about our psychosocial predator-based animal model of PTSD is that it produces a wide array of PTSD-like physiological and behavioral alterations, in addition to a memory for the "traumatic" cat exposures. Furthermore, based on the present findings, at least some of the effects produced by our model persist for 4 months following stress onset. Thus, our animal model of PTSD can be used to study the mechanisms underlying multiple facets of PTSD, as well as its chronic nature.

5. Conclusions

We have found that our well-validated animal model of PTSD produces physiological and behavioral disturbances that can be observed 4 months following stress onset. Two cat exposures, as employed in our original animal model of PTSD [9], resulted in an intact fear memory for the predator stress experiences, heightened anxiety and impaired cognition. A third cat exposure enhanced the contextual fear memory for the predator stress and resulted in a significant reduction of thymus weight. Data from the two psychosocial stress groups revealed greater diastolic BP and lower corticosterone levels in these groups. Collectively, our findings provide further validation of our predator-based psychosocial model of PTSD and suggest that it is useful for testing the mechanisms and novel therapeutic strategies for persistent PTSD symptoms.

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