Psychosocial animal model of PTSD produces a long-lasting traumatic memory, an increase in general anxiety and PTSD-like glucocorticoid abnormalities

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Summary Post-traumatic stress disorder (PTSD) is characterized by a pathologically intense memory for a traumatic experience, persistent anxiety and physiological abnormalities, such as low baseline glucocorticoid levels and increased sensitivity to dexamethasone. We have addressed the hypothesis that rats subjected to chronic psychosocial stress would exhibit PTSD-like sequelae, including traumatic memory expression, increased anxiety and abnormal glucocorticoid responses. Adult male Sprague-Dawley rats were exposed to a cat on two occasions separated by 10 days, in conjunction with chronic social instability. Three weeks after the second cat exposure, the rats were tested for glucocorticoid abnormalities, general anxiety and their fear-conditioned memory of the two cat exposures. Stressed rats exhibited reduced basal glucocorticoid levels, increased glucocorticoid suppression following dexamethasone administration, heightened anxiety and a robust fear memory in response to cues that were paired with the two cat exposures. The commonalities in endocrine and behavioral measures between psychosocially stressed rats and traumatized people with PTSD provide the opportunity to explore mechanisms underlying psychological trauma-induced changes in neuroendocrine systems and cognition.

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1. Introduction

Individuals exposed to intense, life-threatening trauma are at significant risk for developing post-traumatic stress disorder (PTSD). People who develop PTSD respond to a traumatic experience with intense fear, helplessness and horror...
(American Psychiatric Association, 1994) and subsequently endure chronic psychological distress by repeatedly reliving their trauma through intrusive, flashback memories (Ehlers et al., 2004; Hackmann et al., 2004; McFarlane, 1992; Reynolds and Brewin, 1998, 1999; Speckens et al., 2006, 2007). These intrusive memories are triggered by cues which were associated with the trauma and can, in extreme cases, lead to panic attacks. Therefore, PTSD patients make great efforts to avoid stimuli that remind them of their trauma. The re-experiencing and avoidance symptoms of the disorder can hinder everyday functioning in PTSD patients and foster the development of additional debilitating symptoms, including persistent anxiety, exaggerated startle, cognitive impairments and diminished extinction of conditioned fear (Brewin et al., 2000; Elzinga and Bremner, 2002; Geuze et al., 2009; Graham and Milad, 2011; Milad et al., 2009; Nemeroff et al., 2006; Newport and Nemeroff, 2000; Stam, 2007).

PTSD is also characterized by an aberrant biological profile in different endocrine and physiological systems (Krystal and Neumeister, 2009; Pervanidou and Chrousos, 2010; Vidovic et al., 2011). One of the most extensively researched endocrine systems in people with PTSD is the hypothalamic–pituitary–adrenal (HPA) axis. Empirical investigations of the adrenal hormone, cortisol, have often reported abnormally low baseline cortisol levels in people with PTSD (for reviews, see Yehuda, 2005, 2009). One explanation for the presence of low baseline cortisol levels in people with PTSD is that trauma induces an enhancement of negative feedback inhibition of the HPA axis. For example, studies have reported that people with PTSD display an increased number and sensitivity of glucocorticoid receptors (Rohleder et al., 2004; Stein et al., 1997; Yehuda et al., 1991, 1993a, 1995) and an increased suppression of cortisol and adrenocorticotropic hormone (ACTH) following the administration of dexamethasone, a synthetic glucocorticoid (Duval et al., 2004; Goenjian et al., 1996; Grossman et al., 2003; McFarlane et al., 2011; Newport et al., 2004; Stein et al., 1997; Yehuda et al., 1993b, 1995, 2002, 2004). Some studies have also observed greater increases in ACTH levels of PTSD patients, relative to controls, following the administration of metyrapone. This finding may be the result of metyrapone reducing the enhanced negative feedback inhibition present in PTSD patients (Otte et al., 2006; Yehuda and Kjos, 1996).

Studies have also employed the dexamethasone-corticotropin releasing hormone (CRH) challenge paradigm to study abnormal HPA axis functioning in people with PTSD (de Kloet et al., 2006). An advantage of this paradigm is that the subjects are treated with dexamethasone prior to CRH administration, thereby activating negative feedback mechanisms before acute HPA axis stimulation. Studies have generally reported reduced ACTH levels in dexamethasone-treated PTSD patients who were subsequently treated with CRH. These findings support the notion that PTSD patients exhibit reduced sensitivity to CRH stimulation (Rinne et al., 2002; Strohle et al., 2008).

Overall, extensive research indicates that PTSD is characterized by reduced basal levels of cortisol, reduced CRH receptor sensitivity and/or enhanced glucocorticoid negative feedback at the level of the pituitary. However, the literature in this area is not entirely consistent, which likely reflects the heterogeneity in the manifestation of trauma and the measurement of PTSD in different patient populations (Begic and Jokic-Begic, 2007; Bonne et al., 2003; Hamner et al., 2004; Klaassens et al., 2012; Marshall and Garakani, 2002; Metzger et al., 2008; Pitman and Orr, 1990; Radant et al., 2001; Shalev et al., 2008).

Our understanding of how trauma affects the HPA axis in people may be enhanced by animal models that generate PTSD-like behavioral and physiological abnormalities. To this end, we have developed an animal model of PTSD that includes trauma induction procedures which are analogous to those that induce PTSD in people, including a threat to survival, a lack of control, an intrusive re-experiencing of a traumatic event and social instability (Roth et al., 2011; Zoladz et al., 2008; Zoladz and Diamond, 2010). Specifically, our animal model of PTSD is based on a combination of acute traumatic experiences (two 1-h periods of inescapable confinement of rats in close proximity to a cat) embedded within a 1-month-long period of social stress. We reported that rats administered this psychosocial stress regimen exhibited changes in physiology and behavior in common with people diagnosed with PTSD, including heightened anxiety, exaggerated startle, impaired cognition, increased cardiovascular reactivity and an exaggerated response to yohimbine administration (Brewin et al., 2000; Elzinga and Bremner, 2002; Nemeroff et al., 2006; Newport and Nemeroff, 2000; Stam, 2007). Moreover, we recently demonstrated that our psychosocial predator stress model of PTSD produced hippocampus-specific increases in DNA methylation (Roth et al., 2011), a finding which may be relevant toward understanding how traumatic memories can persist for a lifetime (Yehuda and Bierer, 2009).

The purpose of the present experiments was to extend our animal model of PTSD to determine if rats administered psychosocial stress would exhibit two hallmark features of PTSD: (1) a long-lasting memory of the traumatic event (inescapable live cat exposure); and (2) abnormalities in glucocorticoid levels under baseline conditions and in response to stress and dexamethasone administration.

2. Methods

2.1. Subjects

Experimentally-naive adult male Sprague-Dawley rats (225–250 g upon delivery) obtained from Charles River laboratories (Wilmington, MA) were used in all experiments. The rats were pair-housed on a 12-h light/dark schedule (lights on at 0700 h) in standard Plexiglas cages with free access to food and water. The colony room temperature and humidity were maintained at 20 ± 1°C and 60 ± 3%, respectively. After the rats were given a 1-week vivarium acclimation period, their weights increased to 304 g (±2.3 g), which was when all experimental manipulations began. All procedures were approved by the Institutional Animal Care and Use Committee at the University of South Florida.

2.2. Psychosocial stress paradigm

Following the 1-week acclimation phase, rats were brought to the laboratory and randomly assigned to “psychosocial stress” or “no psychosocial stress” groups. Rats in the psychosocial stress groups were given two 1-h cat exposures,
separated by 10 days, in conjunction with daily social stress in the form of randomized housing, as described previously (Zoladz et al., 2008). During each of the two cat exposures, rats in the psychosocial stress groups were immobilized in plastic DecapiCones (Brantree Scientific; Braintree, MA) and placed in a perforated wedge-shaped Plexiglas enclosure (Brantree Scientific; Braintree, MA; 20 cm × 20 cm × 8 cm). The rats, still immobilized in the plastic DecapiCones within the Plexiglas enclosure, were taken to the cat housing room where they were placed in a metal cage (61 cm × 53 cm × 51 cm) with an adult female cat for 1 h. The Plexiglas enclosure prevented any physical contact between the cat and rats, but enabled the rats to be exposed to all non-tactile sensory stimuli associated with the cat. Canned cat food was smeared on top of the Plexiglas enclosure to increase cat motor activity because a moving cat provokes a greater fear response in rats than a non-moving cat (Blanchard et al., 1975).

The two cat exposures were separated by 10 days, with the first exposure taking place during the light cycle (between 0800 h and 1300 h), and the second exposure taking place during the dark cycle (between 1900 h and 2200 h). Beginning on the day of the first cat exposure, rats in the psychosocial stress group were exposed to unstable housing conditions for the next 31 days. Rats in the psychosocial stress group were housed two rats per cage, with their cohort pair combination changed on a daily basis during the entire 31-day stress period. That is, rats in the stress groups were never housed with the same rat on consecutive days. Rats in the no psychosocial stress group remained in their home cages in the laboratory instead of receiving the 1-h acute stress sessions, and these rats were housed with the same cage cohort for the duration of the experiment.

2.3. Experiment 1: Traumatic memory expression and general anxiety

Fig. 1 provides a summary of the timeline and procedures for the two experiments in this study. In Experiment 1, 20 rats were assigned to “psychosocial stress” (n = 10) or “no psychosocial stress” (n = 10) groups and exposed to the stress manipulations described above. Since a major component of PTSD is the persistent memory of a traumatic experience, we have developed a method with which to measure a rat’s memory for the cat exposure experiences. To accomplish this goal, rats in the psychosocial stress group were given a predator-based form of fear conditioning. The rats were placed in a chamber for 3 min immediately prior to each of the two cat exposures. The chamber (26 cm × 30 cm × 29 cm; Coulbourn Instruments; Allentown, PA) consisted of two aluminum sides, an aluminum ceiling and clear Plexiglas on the front and back walls and speaker on one wall. The floor of the chamber consisted of 18 stainless steel rods, spaced 1 cm apart. A 74 dB, 2500 Hz tone was presented during the last 30 s of each chamber exposure. Rats in the no psychosocial stress group were also given the 3-min chamber and tone exposures, but without subsequent immobilization and cat exposure.

It is important to emphasize that the rats in both groups were not given any noxious stimulation, such as foot shock, while they were in the chamber. It is also important to emphasize that the tone was delivered to the rats while they were in the chamber, and then they were removed from the chamber and then immobilized and brought to a different room, where the traumatic experience (immobilization during cat exposure) occurred.

Three weeks following the second cat exposure, the rats were tested for their conditioned fear memory by assessing their freezing response (degree of immobility) when they were returned to the chamber and exposed to the tone. Rats were placed in the chamber, which had been previously paired with the 2 cat exposures, for 5 min (context test). One hour later, the rats were placed in a novel box for 6 min, with a 74 dB, 2500 Hz tone presented during the last 3 min of the exposure (cue test). The novel box exposure consisted of a chamber (25 cm × 23 cm × 33 cm; Coulbourn Instruments, Allentown, PA) with two aluminum sides, an aluminum ceiling and Plexiglas front and back. Unlike the conditioning chamber, which contained metal rods on the floor, the cue-testing chamber had a solid metal floor. Also, a house light was on while the rats were in the cue-testing chamber. During the context and cue tests, a 24-cell infrared activity monitor (Coulbourn Instruments; Allentown, PA) mounted on top of the chambers detected the infrared body heat image from the animals to detect their movement. Immobility in the chambers was operationally defined as continuous periods of inactivity lasting at least 7 s.

Twenty-four hours after fear conditioning memory testing, the rats were placed on an elevated plus maze (EPM) for 5 min. The EPM is a routine test of anxiety in rodents (Korte and De Boer, 2003) and consists of two open arms (11 cm × 50 cm) and two closed arms (11 cm × 50 cm) that intersect each other to form the shape of a plus sign. At the beginning of each trial, the rats were placed in the intersection area of the EPM, facing one of the open arms. Rat behavior on the EPM was monitored by computer from 48 infrared photobeams located along the perimeter of the open and closed arms (Motor Monitor, Hamilton Kinder; San Diego, CA). The primary dependent measures of interest were the amount of time rats spent in the open arms and their overall ambulations. An arm entry was scored by the computer program only when a rat’s entire body moved from one arm into a new arm. An ambulation was scored by the computer program each time a rat crossed a photobeam sensor.

2.4. Experiment 2: Basal glucocorticoid levels and dexamethasone suppression test

The rats used in Experiment 2 were not administered any post-psychosocial stress behavioral testing. Instead, these rats were used solely for the purpose of obtaining blood samples at the conclusion of the psychosocial stress regimen to evaluate corticosterone levels under undisturbed baseline, acute stress and dexamethasone injection conditions (see timeline; Fig. 1). Twenty days after the second cat exposure, the hind legs of all rats were shaved to allow access to their saphenous veins. The rats were then taken back to the housing room and left undisturbed for the remainder of the day. The hind legs of all rats were shaved 1 day prior to blood sampling to minimize the amount of time it took the experimenter to obtain baseline blood samples on the following day.
The next day, between 1100 h and 1400 h, a subset of the rats was administered subcutaneous injections of dexamethasone (10 μg/kg, 25 μg/kg, 50 μg/kg; 1 ml/kg) or vehicle. These doses of dexamethasone were chosen because previous work indicated that they produce a modest suppression of corticosterone levels in rats (Lurie et al., 1989). Ten rats from each of the psychosocial stress and no psychosocial stress groups were assigned to receive an injection of one of the three doses of dexamethasone or the vehicle solution. Dexamethasone (Sigma–Aldrich; St. Louis, MO) was dissolved in a vehicle solution consisting of sodium sulfite (1 mg/ml) and sodium citrate (19.4 mg/ml), which were both dissolved in distilled water. Immediately following the administration of dexamethasone or vehicle, the rats were returned to the housing room until the commencement of blood sampling. An additional ten rats from each of the psychosocial stress and no psychosocial stress groups were not injected to allow for the analysis of undisturbed baseline corticosterone levels.

Six hours following the injections (or at an equivalent time point for un.injected rats, between 1700 h and 2000 h), the rats were taken individually to a procedure room for blood sampling (Lurie et al., 1989). Petroleum jelly was applied to each rat’s hind leg to reduce clotting while the blood sample was being collected. The saphenous vein of each rat was punctured with a sterile, 27-gauge syringe needle. A 0.2 cc sample of blood was then collected from each rat in a
microcentrifuge tube. The first blood sample was considered a baseline measure of corticosterone and was collected within 2 min after the rats were removed from the housing room. After obtaining this sample, the rats were immobilized in plastic DecapiCones for 20 min. The rats were then removed from the DecapiCones, and another 0.2 cc sample of blood was collected in a microcentrifuge tube via saphenous vein puncture. This blood sample served to examine the hormonal responses of rats to acute immobilization stress. We employed this manipulation because in previous work utilizing the single prolonged stress paradigm (Kohda et al., 2007), it was necessary to stress the rodents after dexamethasone administration to observe an exaggerated suppression of corticosterone in single prolonged stress-exposed animals. In addition, examining stress-induced changes in corticosterone levels provides an assessment of HPA axis functioning comparable to the one used in dexamethasone-CRH challenge paradigms in PTSD patients.

After the second blood sample was collected, the rats were returned to their home cages. One hour later, a final blood sample was collected from all rats to examine corticosterone levels following the termination of the acute immobilization stress. Once the blood samples had clotted at room temperature, they were centrifuged (3000 rpm for 8 min), and the serum was extracted and stored at −80 °C until assayed for corticosterone with an Enzyme ImmunoAssay kit from Assay Design, Inc. (cat#901-097, Ann Arbor, MI). All samples were diluted 1:50 and assayed per kit instructions.

2.5. Body and organ weights

In each experiment, body weights were recorded on the day of the first cat exposure and on the first day of behavioral/physiological testing. Average growth rates (g/day) were calculated for statistical analysis. At the end of each experiment, the rats were euthanized, and the adrenals (left and right adrenals were pooled) and thymus were removed and weighed. Organ weights were expressed as mg/100 g body weight.

2.6. Statistical analyses

Alpha was set at 0.05 for all analyses, and Holm-Sidak post hoc comparisons were employed when an omnibus F test indicated a significant ANOVA. Outlier data points greater than 3 standard deviations from the exclusive group means were eliminated from the analyses (less than 1% of the data were outliers).

In Experiment 1, independent samples t-tests were used to compare growth rates, adrenal glands weights, thymus weights, degree of immobility during the 5-min context test and the amount of time spent in the open arms of the EPM between the psychosocial stress and no psychosocial stress groups. A two-way mixed-model ANOVA was used to analyze the degree of immobility by rats during the cue test, with psychosocial stress (psychosocial stress, no psychosocial stress) serving as the between-subjects factor and tone (no tone, tone) serving as the within-subjects factor.

In Experiment 2, two-way between-subjects ANOVAs were used to analyze the growth rates, adrenal gland weights and thymus weights, with psychosocial stress (psychosocial stress, no psychosocial stress) and injection condition (no injection, vehicle, 10 μg/kg, 25 μg/kg, 50 μg/kg) serving as the between-subjects factors. A three-way mixed-model ANOVA was used to analyze corticosterone levels, with the psychosocial stress and injection condition serving as the between-subjects factors and time point (0 min, 20 min, 80 min) serving as the within-subjects factor.

3. Results

3.1. Experiment 1: Fear conditioning and anxiety testing

3.1.1. Growth rates and organ weights

The psychosocial stress group exhibited a significantly lower growth rate, t(17) = 3.43, significantly larger adrenal glands, t(14) = 2.24, and a significantly smaller thymus, t(16) = 2.78, than the no psychosocial stress group (p’s < 0.05; Table 1).

3.1.2. Traumatic memory expression

The psychosocial stress group exhibited significantly greater immobility than the no psychosocial stress group during the context test, t(14) = 4.55, p < 0.001. The analysis of the cue test revealed significant main effects of psychosocial stress, F(1,16) = 6.26, and tone, F(1,16) = 12.59, as well as a significant Psychosocial Stress × Tone interaction, F(1,16) = 7.62 (p’s < 0.05). The psychosocial stress group demonstrated significantly greater immobility than the no psychosocial stress group in the novel environment only when the tone was presented (Fig. 2).

3.1.3. General anxiety

The analysis of EPM behavior revealed that the psychosocial stress group spent significantly less time in the open arms than the no psychosocial stress group, t(15) = 2.44, p < 0.05, thus corroborating our previously-reported anxiogenic effects of the psychosocial stress paradigm (Zoladz et al., 2008). There was no significant difference between the two groups in their overall movement (i.e., number of ambulations) on the EPM, t(16) = 0.16, p > 0.05 (Fig. 3).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Average growth rates and organ weights (±SEM) for Experiment 1.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Growth rate (g/day)</td>
</tr>
<tr>
<td>No psychosocial stress</td>
<td>5.68 (0.53)</td>
</tr>
<tr>
<td>Psychosocial stress</td>
<td>3.88* (0.13)</td>
</tr>
</tbody>
</table>

* p < 0.05 relative to no psychosocial stress.
3.2. Experiment 2: Influence of psychosocial stress on basal glucocorticoid levels and dexamethasone suppression test

3.2.1. Growth rates and organ weights
Each of the growth rate and organ weight analyses revealed significant main effects of psychosocial stress, indicating that the psychosocial stress groups exhibited significantly lower growth rates, $F(1,90) = 13.65$, significantly larger adrenal glands, $F(1,88) = 16.53$, and a significantly smaller thymus, $F(1,86) = 36.77$, than the no psychosocial stress groups ($p's < 0.001$; Table 2).

3.2.2. Serum corticosterone levels
The analysis of serum corticosterone levels revealed a significant main effect of time point, $F(2,158) = 122.58$, $p < 0.001$. Rats demonstrated a significant increase in corticosterone levels following 20 min of immobilization, and these levels significantly declined, yet still remained elevated relative to baseline, 1 h later ($p's < 0.001$). There was a significant main effect of injection condition, $F(4,79) = 54.99$, $p < 0.001$, indicating that, relative to vehicle- and non-injected groups, dexamethasone led to significantly lower corticosterone levels. The Time Point $\times$ Injection Condition, $F(8,158) = 6.35$, and Time Point $\times$ Psychosocial Stress $\times$ Injection Condition, $F(8,158) = 3.10$, interactions were also significant ($p's < 0.01$). The non-injected psychosocial stress group displayed significantly lower corticosterone levels than the non-injected no psychosocial stress group at the 0 min time point ($p < 0.01$).

Following administration of 10 $\mu$g/kg dexamethasone, the psychosocial stress group exhibited a greater suppression of post-immobilization corticosterone levels than the no psychosocial stress group. Additionally, 25 $\mu$g/kg dexamethasone prevented the immobilization-induced increase in
cortisol levels in the psychosocial stress group only. Administration of 50 µg/kg dexamethasone suppressed baseline and post-immobilization cortisol levels equivalently in the psychosocial stress and no psychosocial stress groups (p > 0.05; Fig. 4).

4. Discussion

The overall goal of our research program has been to develop an animal model of PTSD based on clinically-relevant features of the disorder. To this end, we previously demonstrated that two episodes of inescapable cat exposure, in conjunction with daily social instability, caused rats to exhibit heightened anxiety, exaggerated startle, impaired cognition, increased cardiovascular reactivity and an exaggerated response to yohimbine administration (Zoladz et al., 2008; Zoladz and Diamond, 2010), all of which are commonly observed symptoms in people with PTSD (Brewin et al., 2000; Elzinga and Bremner, 2002; Nemeroff et al., 2006; Newport and Nemeroff, 2000; Stam, 2007). In the present work, we have extended our animal model of PTSD by demonstrating that rats exposed to two acute predator stress experiences, embedded within a 31-day period of social instability, resulted in a long-lasting, classically-conditioned fear memory for the predator exposure experiences. We have also shown that rats exposed to the psychosocial stress regimen exhibited increased general anxiety and a significant reduction of glucocorticoid levels at baseline and following dexamethasone administration.

4.1. Predator-based fear conditioning

In Experiment 1, we exposed rats to a unique chamber (context) and tone (cue), which served as the two conditioned stimuli (CSs). The CSs were presented immediately prior to immobilization in conjunction with cat exposure, which served as the unconditioned stimulus (US) (Pavlov, 1928). The use of a live moving cat, alone, is well-established as a powerful and unlearned fear-provoking stimulus to rats (Blanchard et al., 1975, 1990; Blanchard et al., 1991; Pentkowski et al., 2006). Psychosocially stressed rats expressed a fear conditioned memory, as indicated by significant immobility, upon their re-exposure, three weeks later, to the context and cue that were temporally associated with the two cat exposures (Fig. 2). Importantly, during the cue test, the psychosocially stressed rats did not exhibit significant immobility in the novel environment until the tone was presented. This finding indicated that their fear response during the context test was not an indication of a nonspecific increase in anxiety, but was, instead, a selective anxiogenic response to the contextual stimuli that were associated with cat exposure.

The expression of learned fear that the psychosocially stressed rats exhibited to the conditioned stimuli in this study can be considered analogous to the fear and panic that people with PTSD display when they are exposed to a cue that reminds them of their trauma (Garakani et al., 2006; Izquierdo et al., 2004; Redgrave, 2003). These findings confirm that our animal model of PTSD involves a fear conditioning (traumatic memory) component, which is central to the expression of PTSD in people (Bryant et al., 2007; Debiec and LeDoux, 2006; Elzinga and Bremner, 2002; Johnson et al., 2012; Jovanovic and Ressler, 2010; Kolb, 1984; Liberton and Sripada, 2008). The present paradigm, therefore, may reveal mechanisms involved in the formation and persistence of intrusive memories in traumatized people. For example, in recent work, we reported that the same psychosocial stress regimen used here produced selective epigenetic alterations (methylations) of the hippocampal brain-derived neurotrophic factor (BDNF) gene (Roth et al., 2011). We have also demonstrated that cat exposure impaired memory-related functions of the hippocampus (Diamond et al., 1999, 2007; Mesches et al., 1999; Vanelzakker et al., 2011; Youimba et al., 2006), and, conversely, enhanced synaptic plasticity (Youimba et al., 2006) and phosphorylation of calcium/calmodulin-dependent protein kinase II (CaMKII), a critical molecular component of memory formation and synaptic plasticity (Cammarota et al., 2002; Listman et al., 2002), in the amygdala (Zoladz et al., 2012). These and related findings on an animal model of intrusive memories (Zoladz et al., 2010) are consistent with the view that traumatic memory reactivation involves impaired functioning of the hippocampus, in conjunction with enhanced functioning of the amygdala (Brewin, 2001; Debiec and LeDoux, 2006; Metcalfe and Jacobs, 1998; Milad et al., 2009; Nadel and Jacobs, 1998).
In related work, Cohen and colleagues (Cohen et al., 2006, 2008; Matar et al., 2009; Zohar et al., 2008) incorporated a fear conditioning-like component into their animal model of PTSD. These investigators exposed rats to well-soiled cat litter (predator scent stress) and subsequently measured their freezing behavior in response to fresh, unused cat litter. They found that predator scent stress-exposed rats exhibited significant freezing behavior upon exposure to unused cat litter, which suggested that the rats had a fear-provoking memory of the predator scent stress experience. Their work...
is important because it potentially provides insight into how a fear-provoking experience produces a long-term change in rat behavior. However, their approach did not provide evidence that the stressed rats had formed an explicit association between a previously neutral cue (CS) and a biologically relevant, arousing stimulus (US), which is an essential feature of classical conditioning (Pavlov, 1928). The increased freezing of their stressed rats exhibited in response to clean litter may have developed as a result of increased generalized fear to all novel stimuli. Thus, the possibility that the predator scent stress experience produced a non-associative sensitization of stress responses was not addressed in their work.

Other investigators have also studied different forms of fear memory testing in rodents based on their forming an association between neutral stimuli and exposure to a predator (Adamec, 1997; Adamec et al., 2004, 2011) or predator odor (Blanchard et al., 2003a,b; Hubbard et al., 2004; Munoz-Abellan et al., 2009; Rosen et al., 2008; Takahashi et al., 2008). Unlike the other approaches, we embedded predator-based fear conditioning within a prolonged (31-day) period of chronic social stress. As such, our work mimics a clinically relevant situation in which a person is exposed to acute trauma as a component of prolonged periods of daily anxiety. The combination of acute traumatic experiences delivered in conjunction with chronic daily stress may explain why our model, unlike some predator odor-based work (Munoz-Abellan et al., 2009; Zangrossi and File, 1992), generates a persistent fear-based memory, as well as a long-lasting increase in behavioral anxiety.

It is important to note that unlike conventional classical conditioning training, in the current work, the CS (context/cue) and US (immobilization during cat exposure) occurred in completely different locations. That is, in traditional classical conditioning, the CS and US are always presented together in the same context. For example, in typical fear conditioning training, rats are administered foot shock and a tone in the same context (Fanselow and Gale, 2003; Rudy et al., 2004). A rat’s memory for the shock is then tested by observing the rat’s behavior when it is returned to the same environment where the shock occurred. We have shown here that rats can associate two neutral stimuli (CSs; chamber and tone) with an aversive experience (US; immobilization during predator exposure) that occurred in two different places. That is, the rats were removed from the chamber and brought to another room, where cat exposure occurred, and yet, the rats exhibited fear when they were returned to the chamber or exposed to the tone. Thus, our demonstration that rats can associate cues that occurred across time and space is unique, and potentially relevant toward understanding how traumatic stress can produce powerful, long-lasting and context-independent intrusive memories and phobias in people (Bryant and Harvey, 1996; Cuthbert et al., 2003; Jacobs and Nadel, 1985; Wild et al., 2007).

4.2. PTSD-like HPA axis alterations

In Experiment 2, psychosocially stressed rats exhibited abnormal, PTSD-like endocrine profiles, including significantly lower corticosterone levels at baseline and following dexamethasone administration (Fig. 4). Although investigations of HPA axis function in PTSD patients have produced mixed results (Bonne et al., 2003; Hawk et al., 2000; Klaassens et al., 2012; McFarlane et al., 1997, 2011; Metzger et al., 2008; Pitman and Orr, 1990; Shaley et al., 2008), numerous studies have reported that people with PTSD exhibit abnormally low baseline cortisol levels (for reviews, see Yehuda, 2005, 2009), an increased number and sensitivity of glucocorticoid receptors (Rohleder et al., 2004; Stein et al., 1997; Yehuda et al., 1991, 1993a, 1995) and increased suppression of cortisol and ACTH following the administration of dexamethasone (Duval et al., 2004; Goenjian et al., 1996; Grossman et al., 2003; Newport et al., 2004; Stein et al., 1997; Yehuda et al., 1993b, 1995, 2002, 2004). However, given the heterogeneity of findings in this area of research, some glucocorticoid abnormalities may be present only in a subtype of the disorder under restricted conditions (e.g., combat trauma in males versus sexual assault in females). It is also possible that such glucocorticoid abnormalities may occur as a result of trauma, per se, rather than being tied specifically to the diagnosis of PTSD (de Kloet et al., 2007), which may explain the difficulty in considering glucocorticoid abnormalities to be ubiquitous biomarkers of all forms of PTSD (Klaassens et al., 2012). Thus, inclusion of other biomarkers, perhaps at the molecular level (Kolassa et al., 2010; Su et al., 2009; Xie et al., 2009; Zhang et al., 2008), may identify measures unique to trauma-induced psychopathology.

Animal studies involving chronic stress have frequently reported significant elevations of baseline glucocorticoid levels (Blanchard et al., 1993; Kant et al., 1987; Lepesch et al., 2005; Marin et al., 2007; Mizoguchi et al., 2001; Patterson-Buckendahl et al., 2001; Touyarat and Sandi, 2002). Few studies, however, have produced abnormally low baseline glucocorticoid levels similar to those reported here. Those animal models that have reported significantly reduced baseline glucocorticoid levels have employed either the single prolonged stress or a stress—restress paradigm, consisting of situational reminders of the original stress experience (Diehl et al., 2007; Harvey et al., 2003; Harada et al., 2008; Iwamoto et al., 2007; Kohda et al., 2007; Liberonz et al., 1997; Takahashi et al., 2006; Wang et al., 2008). The present experiment, therefore, extends these findings by demonstrating that similar HPA axis abnormalities can be produced in rats by exposure to two acute predator stress episodes, in conjunction with daily social instability, three weeks after the last predator exposure occurred.

It is important to note that we observed significantly lower baseline corticosterone levels only in the uninjected psychosocially stressed rats (Fig. 4). This baseline effect is contrasted with the lack of a psychosocial stress effect on “baseline” corticosterone in the rats that were injected with vehicle 6 h before the baseline blood sample was obtained (Fig. 4). These findings illustrate the importance of obtaining blood samples from completely undisturbed rats to obtain a true measure of baseline HPA axis activity. This finding also illustrates the sensitivity of corticosterone levels to relatively mild environmental manipulations, such as an intraperitoneal injection. This finding may also be relevant to inconsistent findings in the PTSD literature as single time point basal HPA and cardiovascular measures may be affected, for example, by how long subjects spend in an experimental (e.g., hospital) environment before a baseline measure is obtained (McFall et al., 1992).
We did not find that corticosterone levels declined to baseline 60 min after the termination of 20 min of acute immobilization stress. The persistent elevation of corticosterone levels 1 h post-stress could have resulted, in part, because of the time of day that we initiated blood sampling. We began blood sampling in the early evening hours, at the time when lights are off and corticosterone levels rise in rats. This timing of blood sampling was designed to increase the likelihood that we could observe group differences in baseline corticosterone levels. The increase in corticosterone levels in the rat's dark cycle could have interacted with the acute immobilization stress response to prevent a full recovery of corticosterone levels to baseline at the 80 min time point.

As expected, dexamethasone-treated animals displayed significantly lower baseline corticosterone levels than vehicle-treated animals. However, there was no effect of psychosocial stress on baseline corticosterone levels following dexamethasone administration. The reason why we did not observe group differences in baseline corticosterone levels is because each of the three doses of dexamethasone produced an almost complete suppression of baseline corticosterone levels in the stress and control groups. Additional study with lower doses of dexamethasone may reveal a subtle influence of psychosocial stress on the dexamethasone-induced suppression of baseline corticosterone levels.

In contrast to equivalent baseline corticosterone levels in dexamethasone-treated groups, we found that the 25 μg/kg dose of dexamethasone blocked the immobilization-induced increase in corticosterone levels in the psychosocial stress group. This finding replicates observations by Kohda et al. (2007), who found significantly lower corticosterone levels in rats exposed to the single prolonged stress paradigm following the administration of dexamethasone. Akin to the present study, Kohda et al. (2007) exposed rats to an acute stressor following dexamethasone administration to observe group differences in corticosterone levels. We also found that psychosocially stressed rats exhibited significantly lower post-immobilization (80 min time point) corticosterone levels than the no psychosocial stress group following administration of 10 μg/kg of dexamethasone. This dose of dexamethasone did not selectively blunt the immobilization-induced increase of corticosterone levels in psychosocially stressed rats, as there were no significant group differences in corticosterone levels at the 20 min time point. However, following termination of the immobilization stress, psychosocially stressed rats displayed a significantly greater decline of corticosterone levels 60 min later.

The finding of reduced corticosterone levels in psychosocially stressed animals following dexamethasone administration supports the hypothesis that our PTSD regimen generates enhanced negative feedback of the HPA axis. We may further speculate that dexamethasone prevented the immobilization-induced increase (25 μg/kg) and exacerbated the post-immobilization recovery (10 μg/kg) of corticosterone levels in the psychosocial stress groups because the rats in these groups had a greater number and/or sensitivity of glucocorticoid receptors or reduced sensitivity of CRH receptors in the pituitary. It is also possible that rather than enhanced negative feedback of the HPA axis, reduced adrenal output could explain the reduced corticosterone levels that were observed in the dexamethasone-treated stressed groups. Arguing against this interpretation is the finding that the psychosocial stress groups exhibited normal levels of corticosterone in response to acute stress in the uninjected and saline-treated groups (Fig. 4). Moreover, we found that psychosocial stress produced adrenal hypertrophy (Tables 1 and 2), which would seem inconsistent with adrenal insufficiency as an explanation of reduced corticosterone responses in the dexamethasone-treated psychosocial stress groups. However, previous work has demonstrated that chronic psychosocial stress in rodents can produce adrenal hypertrophy in conjunction with reduced ACTH responsiveness of the adrenal glands (Reber et al., 2007). Therefore, future work will need to be performed to identify the neuroendocrine basis of the psychosocial stress-induced disturbance of HPA axis function we have observed here, including an examination of CRH and glucocorticoid receptor expression, levels of circulating ACTH and adrenal gland responsiveness to ACTH.

4.3. Clinical relevance

The importance of the current work is that our psychosocial stress regimen generated, in rats, three cardinal features of PTSD: evidence of a long-lasting traumatic memory, persistent anxiety and hormonal abnormalities. One feature of our work which may appear inconsistent with clinical research is that psychosocial stress produced PTSD-like sequelae in most, if not all, rats, but only a subset (10—25%) of traumatized people develops PTSD (Aupperle et al., 2012; Delahanty and Nugent, 2006; Marmar et al., 2006; Skelton et al., 2012; Yehuda and Flor, 2007). In this context it is important to point out that our animal model of PTSD was explicitly designed to expose rats to conditions which are known to maximize the likelihood of PTSD developing in people. Specifically, DSM-IV criteria for the diagnosis of PTSD includes the following three conditions: (1) PTSD can be triggered by an event that involves threatened death or a threat to one’s physical integrity; (2) a person’s response to the event involves intense fear, helplessness or horror; and (3) in the aftermath of the trauma, the person feels as if the traumatic event were recurring, including a sense of reliving the experience (American Psychiatric Association, 1994). All of these components of the DSM-IV criteria for PTSD have been incorporated into our animal model of PTSD. That is, rats exhibit an intense fear response when exposed to a cat (Adamec et al., 2005, 2011; Blanchard et al., 2005; Hubbard et al., 2004), which is a threat to their survival. The immobilization component of our animal model is a rodent analogue to a loss of control over traumatic conditions, which is known to exacerbate behavioral and physiological responses to stress (Amat et al., 2005; Bland et al., 2006, 2007; Kavushansky et al., 2006; Maier et al., 1993; Maier and Watkins, 2005; Shors et al., 1989). The re-experiencing the rats were exposed to with the second cat exposure was designed to be relevant to the increased likelihood of PTSD developing with multiple traumatic experiences (Koenen et al., 2002; Kolassa et al., 2010; Xie et al., 2009) and the unstable psychosocial component of our paradigm is based on the well-described finding of a link between a lack of social support reported by people who progress from short-term to long-lasting PTSD (Andrews et al., 2003; Boscariol, 1995; Brewin et al., 2000; Solomon et al., 1989; Ullman and Filipas, 2001; Vogt et al., 2011).
Taken together, the basis of the high rate of PTSD-like endocrine and behavioral effects we have reported here and in previous studies (Roth et al., 2011; Zoladz et al., 2008; Zoladz and Diamond, 2010) can be ascribed to the explicit design of our psychosocial stress manipulations to mimic clinical features of trauma that are known to increase the likelihood that persistent PTSD will occur in people. This approach, therefore, is a tool with which to identify mechanisms that are activated specifically in the subset of individuals who are exposed to environmental conditions that are most likely to progress from an acute stress state to chronic PTSD.

5. Concluding remarks

We have developed an animal model of PTSD that includes trauma induction procedures which are analogous to those that induce PTSD in people, including a threat to survival, a lack of control, an intrusive reminder of a traumatic experience and social instability. Rats administered this psychosocial stress regimen exhibited changes in physiology and behavior in common with people diagnosed with PTSD, including heightened anxiety, exaggerated startle, impaired cognition, increased cardiovascular reactivity and an exaggerated response to yohimbine administration (Roth et al., 2011; Zoladz et al., 2008; Zoladz and Diamond, 2010). In the current work we have extended our model to demonstrate that psychosocially stressed rats exhibited a powerful and long-lasting fear memory for the context and cue which were temporarily associated with immobilization and predator exposure. We also observed significant changes in HPA activity, specifically, reductions in basal glucocorticoid levels and enhanced dexamethasone-induced inhibition of glucocorticoid levels, which have been shown to occur in traumatized people. Overall, the numerous commonalities in stress-induced changes in physiology and behavior in traumatized people and in the psychosocially stressed rats studied here further validates the use of our animal model of PTSD to explore cognitive and neuroendocrine mechanisms underlying emotional trauma-induced changes in brain and behavior.

Conflicts of interest

All other authors declare that they have no conflicts of interest.

Contributors

David Diamond designed the study and contributed to the editing of the manuscript. Phillip Zoladz contributed to the design of the study, conducted all laboratory procedures and wrote the first draft of the manuscript. Monika Flesnher contributed to the design of the study, conducted the corticosterone assays and contributed to the editing of the manuscript. All authors contributed to and have approved the final manuscript.

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