Predator-based psychosocial stress animal model of PTSD: Preclinical assessment of traumatic stress at cognitive, hormonal, pharmacological, cardiovascular and epigenetic levels of analysis

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Abstract

Research on post-traumatic stress disorder (PTSD) is faced with the challenge of understanding how a traumatic experience produces long-lasting detrimental effects on behavior and brain functioning, and more globally, how stress exacerbates somatic disorders, including cardiovascular disease. Moreover, the design of translational research needs to link animal models of PTSD to clinically relevant risk factors which address why only a subset of traumatized individuals develop persistent psychopathology. In this review, we have summarized our psychosocial stress rodent model of PTSD which is based on well-described PTSD-inducing risk factors, including a life-threatening experience, a sense of horror and uncontrollability, and insufficient social support. Specifically, our animal model of PTSD integrates acute episodes of inescapable exposure of immobilized rats to a predator with chronic daily social instability. This stress regimen produces PTSD-like effects in rats at behavioral, cognitive, physiological, pharmacological and epigenetic levels of analysis. We have discussed a recent extension of our animal model of PTSD in which stress exacerbated coronary pathology following an ischemic event, assessed in vitro. In addition, we have reviewed our research investigating pharmacological and non-pharmacological therapeutic strategies which may have value in clinical approaches toward the treatment of traumatized people. Overall, our translational approach bridges the gap between human and animal PTSD research to create a framework with which to enhance our understanding of the biological basis of trauma-induced pathology and to assess therapeutic approaches in the treatment of psychopathology.

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1. General characteristics of post-traumatic stress disorder

Individuals who are exposed to life-threatening trauma, such as wartime combat, motor vehicle accidents, terrorist attacks or rape, are at risk for developing post-traumatic stress disorder (PTSD). People with PTSD endure chronic psychological distress by repeatedly reliving their trauma through intrusive, flashback memories (Reynolds and Brewin, 1999; Speckens et al., 2007). These individuals also exhibit several other physiological and behavioral symptoms, such as persistent anxiety, hyperarousal, cognitive impairments and autonomic and immune system dysfunction (Schmidt et al., 2013; Vanelzakker et al., 2013; Zoladz and Diamond, 2013). Only a subset of traumatized individuals develops PTSD, depending on a multitude of interacting risk factors, including the nature of the trauma, sex, genetics, social support and early life experiences (Koenen et al., 2009; Voisey et al., 2014; Zoladz and Diamond, 2013). Therefore, understanding susceptibility factors that promote persistent and intrusive traumatic memory expression, as well as more global somatic PTSD symptoms, is of great scientific and practical value.

2. The utility of animal models of PTSD

Whereas clinical research is vital for improving our understanding of the basic phenomenology of PTSD and the implementation of novel therapeutics, animal models of PTSD provide a crucial complementary component to this process. In addition to their key role in establishing the safety and initial efficacy of novel therapeutic compounds, animal models are valuable in three key areas of treatment development. First, animal models facilitate the rapid cost effective development of proof of concept studies to identify the most promising pharmacological candidates which can block trauma-induced behavioral and physiological abnormalities. This approach, with direct molecular assays of neural tissue, can improve our understanding of the mechanisms of action of these compounds. Second, animal research provides for assessment of the effects of interventions initiated prior to, or soon after, trauma occurs. This approach provides for the opportunity to develop preventive strategies which would be high risk, expensive and potentially unethical to undertake in people. Finally, animal studies provide for the study of direct tests for different PTSD comorbidities and risk factors that might influence treatment responses in people, such as early life abuse, sex, social support and traumatic brain injury.

Preclinical research on traumatic stress has spawned a vast amount of research on the effects of exposing animals, primarily rodents, to strong stressful experiences followed by physiological and behavioral testing, which, in theory, provides insight into PTSD in traumatized people (Stam, 2007). Such studies have employed different types of stressors, such as electric shock (Garrick et al., 2001; Li et al., 2006; Milde et al., 2003; Pynoos et al., 1996; Rau et al., 2005; Sawamura et al., 2004; Servatius et al., 1995; Shimizu et al., 2004, 2006; Siegmund and Wotjak, 2007a, b; Wakizono et al., 2007), underwater trauma (Cohen et al., 2005; Richter-Levin, 1998), stress–restress and single prolonged stress paradigms (Harvey et al., 2003; Khan and Liberzon, 2004; Kohda et al., 2007; Liberzon et al., 1997; Takahashi et al., 2006) and exposure to predators (Adamec, 1997; Adamec et al., 1997, 1999a, b, 2006a, c, 2007; Adamec and Shallow, 1993; Blundell and Adamec, 2007) or predator-related cues (Cohen et al., 2000b, 2005, 2006a, b; Daskalakis et al., 2013b; Goswami et al., 2013; Matar et al., 2006). Studies utilizing these stressors have shown that they result in physiological and behavioral changes in rodents that are comparable to those observed in people with PTSD, such as heightened anxiety, an exaggerated startle response, cognitive impairments, enhanced fear conditioning, resistance to fear extinction and reduced social interaction.

All of these paradigms have contributed toward our understanding of how traumatic stress changes aspects of physiology and behavior. However, there remain conceptual limitations to linking the study of stress in animals to generating a syndrome which resembles the clinical features of PTSD. For example, a routine observation of people who experience a horrific event or rats that are exposed to a strong aversive stimulus, such as a shock, is a powerful and persistent memory of the event. However, having a memory of the traumatic event, alone, is not the determinant of whether PTSD develops since only a subset of the individuals with disturbing memories of a horrific experience actually develop PTSD. Moreover, although traumatic memories are a hallmark feature of PTSD, the intrusive memory of the experience is only one component of the entire PTSD syndrome. Our view is that the cluster of symptoms in PTSD represents the inability to cope with the memory of the trauma. The challenge for an animal model of PTSD, therefore, is not only to generate a conditioned fear memory for a traumatic experience, but also to produce physiological and behavioral abnormalities which resemble the susceptibility and great complexity of the entire PTSD syndrome.

3. The usefulness of predator-based animal models of PTSD

Pioneering research by Caroline and Robert Blanchard described how rats exhibit a strong, innate fear of a predator, such as a cat (Blanchard et al., 1975, 1990). Their work also contributed to the development of studies examining predator scent, provided, for example, by cat or fox urine, as a stress-provoking stimulus which can substitute for the live animal (Apfelbach et al., 2005; Rosen, 2004). Further evidence of the effectiveness of predator exposure as a means with which to generate a fear response are findings in which predator exposure activates the hypothalamic–pituitary–adrenal (HPA) axis (Masini et al., 2006; Park et al., 2006, 2008; Vanelzakker et al., 2013, 2015; Mac Callum et al., 2014). Their work also contributed to the development of the profound capacity for predator exposure to impair spatial memory and synaptic plasticity in the hippocampus, and to enhance synaptic plasticity in the amygdala (Diamond et al., 1999, 2006; Mesches et al., 1999; Park et al., 2006, 2008; Vanelzakker et al., 2013; Vouimba et al., 2006; Woodson et al., 2003, 2012b). Therefore, the ethical relevance and potency of predator exposure provide a highly relevant approach toward producing an intense, purely psychological, fear response in rodent models of PTSD.

The laboratories of Robert Adamec, Jacqueline Blundell and Hagit Cohen have provided important basic research findings by employing live predator exposure or predator scent to induce PTSD-like symptoms in rodents. The Adamec and Blundell laboratories have shown that exposing rodents to physical contact with a live cat, typically for a period of 5 min, produces PTSD-like changes in physiology and behavior assessed 3 weeks later. Behaviorally, the 5-min cat exposure resulted in heightened anxiety on the elevated plus maze and in the light/dark box, reduced locomotor activity and exploratory behavior in the hole board test and an exaggerated startle response (Adamec et al., 1997, 2006b, 2008a, b, 2009; Adamec and Shallow, 1993; Mitra et al., 2009). These investigators have also examined the neural mechanisms underlie the behavioral effects of unprotected predator stress. They have shown that cat exposure induced molecular and electrophysiological evidence of synaptic plasticity in brain regions important for fear and defensive behavior, such as the amygdala and periaqueductal gray, and that the effects of predator stress can be prevented by NMDA receptor antagonists and the beta-adrenergic receptor antagonist, propranolol (Adamec, 1997, 1998, 2001; Adamec et al., 2001, 2005a, b, 2006c, 2007, 2011, 2012a, b; Blundell et al., 2005; Blundell and Adamec, 2006, 2007; Mitra et al., 2009). More recent work by Blundell and her co-workers has focused on mechanistic and behavioral approaches toward improving our understanding of the formation and persistence of a “traumatic” memory, enhancing its extinction in cat-exposed rodents and preventing the development of PTSD-like behavioral changes (Clay et al., 2011; Fifield et al., 2013, 2015; Mac Callum et al., 2014).

Hagit Cohen’s group is well-known for their use of predator scent stress to produce PTSD-like sequelae in rats. These investigators...
typically expose rats to soiled cat litter for a 10 min period and then examine rat physiology and/or behavior 7 days later. In addition to providing evidence of a number of PTSD-like changes in rat physiology and behavior, such as heightened anxiety on the elevated plus maze, an exaggerated startle response and numerous structural and molecular changes in brain areas involved in PTSD (Chertkow-Deutsher et al., 2010; Cohen et al., 2006a, 2008, 2012, 2014; Hoffman et al., 2015; Kozlovska et al., 2007; Matar et al., 2006), a seminal feature of their model is showing that only a subset of rats exposed to predator scent stress develops PTSD-like symptoms (Cohen et al., 2004; Cohen and Zohar, 2004). The Cohen lab employs “cutoff behavioral criteria” to divide stress-exposed animals into those exhibiting an “extreme behavioral response (EBR),” a “partial behavioral response” or a “minimal behavioral response (MBR).” Frequently, Cohen and colleagues report significant physiological and behavioral differences between EBR and MBR groups, potentially providing a basis for the identification of biomarkers of susceptibility versus resilience in response to traumatic stress. Thus, the animal model of PTSD employed by Cohen and her colleagues could provide insight into why only a subset of traumatized individuals develop PTSD.

4. Psychosocial predator stress (PPS) model of PTSD

In 2008 our group developed a predator exposure–based psychosocial stress animal model of PTSD (Zoladz et al., 2008a). The components of this model were based on the hypothesis that a synergistic effect develops as a result of the interaction of multiple risk factors for PTSD. It was designed, therefore, to integrate multiple risk factors in one PTSD model to maximize the likelihood of producing PTSD-like effects in all stressed animals, not just a subset. Specifically, PTSD is often triggered by an event that involves threatened death or a threat to one’s physical integrity, and a person’s response to the event involves intense fear, helplessness or horror. In the aftermath of the trauma, the person feels as if the traumatic event were recurring, including a sense of reliving the experience (Gershuny et al., 2003; Keane et al., 2006; McCloskey and Walker, 2000; Reynolds and Brewin, 1999). Therefore, to mimic these risk factor components of PTSD susceptibility, in our work, rats are immobilized (loss of control) and placed in close proximity to a cat. Rats have an instinctual and intense fear of cats (Blanchard et al., 1990), which, in theory, would be intensified by their inability to have control over escape from the cat (Maier and Watkins, 2005). Although there is no physical contact between the rats and cat, the experience produces a profound physiological stress response in the rats, including increased heart rate, blood pressure and corticosterone levels (Zoladz et al., 2008a).

A core symptom of PTSD is the repeated “re-experiencing” of the traumatic event that people with PTSD suffer from in response to the reactivation of intense and intrusive memories of their trauma. For this reason, we included a “re-experiencing” component in our animal model of PTSD by giving rats a second cat exposure 10 days after the first. Moreover, research has shown that there is hypertrophy of the amygdala, an area heavily involved in generating anxiety-like behavior, 10 days following acute stress exposure (Mitra et al., 2005). Therefore, we hypothesized that stressing the rats a second time, 10 days after the first, would reactivating a sensitized amygdala, further exacerbating a rat’s stress response and promote the development of persistent, PTSD-like physiological and behavioral sequelae. The second exposure of rats to the cat following a substantial time delay is also based on research showing that PTSD develops in some people only after they have repeated traumatic experiences, as well as work revealing that prolonged exposure to trauma increases the likelihood of the development of PTSD (Gurvits et al., 1996; Resnick et al., 1995).

The manipulations described above all relate to the trauma memory, itself, but the PTSD syndrome is more than the response to the experience, alone. How one copes with trauma is a crucial component of PTSD susceptibility, which is expressed as the interaction among numerous factors, including trauma intensity and frequency, as well as intrinsic factors, such as genetics, gender, early life experience, hormones, cognitive strategies, neurobiology, culture and personality (Daskalakis et al., 2013b; Zoladz and Diamond, 2013). There is a vast literature on risk factors associated with the development of PTSD, with strong evidence to indicate that perceived social support, or the lack thereof, is a powerful influence on whether PTSD will develop. Thus, insufficient social support and an unstable social life increase susceptibility to develop the disorder (Brewin et al., 2000; Ozer et al., 2003; Sippel et al., 2015). Therefore, the final component of our PTSD model was daily social instability. Beginning with the day of the first cat exposure, the stressed rats were exposed to unstable housing conditions for each of the next 31 days. The rats were pair-housed, and every day, their cohort pair combination was randomly changed. We reported that the combination of social instability with predator exposure is an essential feature of the model, as either predator exposure or social instability, in isolation, did not produce PTSD-like changes in rat behavior (Zoladz et al., 2008a).

5. PPS-induced physiological and behavioral changes comparable to PTSD

Our psychosocial predator–based animal model of PTSD has been shown to generate a broad range of physiological and behavioral abnormalities which are quite similar to those observed in people with PTSD. For example, 3 weeks after the second predator exposure, stressed rats exhibited increased anxiety, an exaggerated startle response, impaired memory for newly learned information, greater cardiovascular and hormonal (corticosterone) reactivity to an acute stressor and an exaggerated physiological and behavioral response to yohimbine, an α2-adrenergic receptor antagonist (Zoladz et al., 2008a).

Our group then assessed the persistence of the PTSD-like sequelae in our psychosocial model. In recent work, we demonstrated that the two cat exposures, occurring 10 days apart, resulted in intact fear-conditioned memory, heightened anxiety, increased blood pressure and impaired cognition 4 months after the first cat exposure (Zoladz et al., 2015). This work also demonstrated that the addition of a third cat exposure (administered 21 days after the second) enhanced the magnitude of predator-based contextual fear memory assessed at the 4-month time point. These findings further validated our predator-based psychosocial model of PTSD as a means with which to assess the mechanisms involved in persistent trauma-induced changes in brain functioning and as a model for the development of novel therapeutic strategies for alleviating the persistent PTSD symptoms.

In work conducted over the past several years, our group has collaborated with investigators at Louisiana State University (LSU), which has extended the clinical relevance of our psychosocial stress model of PTSD. The group at LSU, led by Joseph Francis and Brad Wilson, has deployed the PPS model to conduct important studies on the effects of psychosocial stress on physiological measures, as well as to assess the effects of pharmacological treatments on behavior and brain neurotransmitter levels. They have demonstrated that psychosocial stress produces neurotransmitter changes which are similar to those seen in human patients with PTSD. Specifically, following 31 days of psychosocial stress, 5-HT decreased and NE increased in the hippocampus and PFC (Wilson et al., 2014a). These changes in neurotransmitter levels might help explain some of the behavioral effects observed in our model, such as impaired memory. In other research, these investigators demonstrated that psychosocial stress produced increased measures of oxidative stress and inflammation in the brain, adrenal glands and systemic circulation (Wilson et al., 2013), all of which may play a critical role in the development of psychiatric, as well as somatic, symptoms of PTSD.
6. “Traumatic” memory expression in the PPS model

A pathologically intense memory of the trauma is a hallmark feature of PTSD. Therefore, it was important to include a measure of the rat’s memory for the cat exposure experiences. To accomplish this goal, we measured a rat’s memory for trauma indirectly by placing the rat in a distinct chamber (which never contained the cat) immediately prior to each of the two cat exposures (Zoladz et al., 2012a). The strategy behind this manipulation was to use a form of classical conditioning to demonstrate that the rats have a fear-conditioned memory of a cue which was associated with each of the two cat exposures. Specifically, the rats were left in the neutral chamber for 3 min, with tone delivery occurring during the last 30 s of each chamber exposure. They were then removed from the chamber, immediately immobilized and brought to another room, where they were given the 1-h cat exposure. This situation can be considered analogous to the panic response people with PTSD exhibit when they experience a cue, such as an odor or a sound, which reminds them of their traumatic experience. Our test of the rats’ memory of the cat was confirmed with the finding that psychosocially stressed rats exhibited significant immobility (fear memory-induced freezing) in response to being returned to the original chamber (contextual fear conditioning) or when they were exposed to the tone that was paired with the cat exposures (cued fear conditioning) (Zoladz et al., 2012a).

7. HPA axis alterations in the PPS model

PTSD is characterized by an aberrant biological profile in multiple physiological systems. One of the most extensively researched physiological systems in people with PTSD is the HPA axis. Empirical investigations of the adrenal hormone, cortisol, have reported abnormally low baseline levels of cortisol in PTSD patients (Daskalakis et al., 2013a; Gill et al., 2008; Pervanidou and Chrousos, 2012; Yehuda, 2002, 2005). One explanation for the presence of low baseline cortisol levels in people with PTSD is that the disorder is associated with enhanced negative feedback inhibition of the HPA axis. Studies have reported that people with PTSD display an increased number and sensitivity of glucocorticoid receptors and an increased suppression of cortisol and adrenocorticotrophic hormone (ACTH) following the administration of dexamethasone, a synthetic glucocorticoid (Duval et al., 2004; Yehuda et al., 2002, 2004). Studies have also employed the dexamethasone–corticotropin releasing hormone (CRH) challenge paradigm to study abnormal HPA axis functioning in people with PTSD. This approach has reported reduced ACTH levels in dexamethasone-treated PTSD patients who were subsequently administered CRH (Strohle et al., 2008). Therefore, we examined the effects of our psychosocial stress model on rat corticosterone levels at baseline and following dexamethasone administration (Zoladz et al., 2012a). We found that, at baseline, stressed animals exhibited significantly lower corticosterone levels than non-stressed rats, which replicates the clinical PTSD condition. Additionally, after dexamethasone administration, psychosocially stressed rats displayed a blunted increase in corticosterone levels and a more rapid recovery of those levels following exposure to acute stress. These findings demonstrate that our PPS regimen produces HPA axis abnormalities in common with those found in people with PTSD.

8. Epigenetic modification of the BDNF gene in the PPS model

One of the most promising areas of PTSD research is the study of the interaction of environmental stressors with genetic and epigenetic expression (Boks et al., 2015; Kwapis and Wood, 2014; Radley et al., 2011; Sipahi et al., 2014; Trollope et al., 2012; Vukovic et al., 2014; Zannas and West, 2013). For example, epigenetic alterations of the brain-derived neurotrophic factor (BDNF) gene have been linked to impaired brain functioning, memory, stress, and neuropsychiatric disorders (Andro et al., 2014; Fuchikami et al., 2010; Ikegame et al., 2013).

Therefore, we examined whether there was evidence of epigenetic alterations of the BDNF gene in our PTSD model. In a collaborative project with Tania Roth from the University of Delaware, we found that rats administered PPS exhibited robust and selective hypermethylation of the BDNF gene in the dorsal CA1 of the hippocampus and reduced hippocampal BDNF mRNA, with no evidence of hypermethylation in the ventral CA1, amygdala or prefrontal cortex (Roth et al., 2011). These results are consistent with those reported in work from the Cohen laboratory, in which predator scent stress resulted in a significant down-regulation of BDNF mRNA in the CA1 region of the hippocampus, particularly in stressed rats exhibiting an “extreme behavioral response” (i.e., more susceptible to stress effects) (Kozlovsky et al., 2007). Our findings also provided evidence that traumatic stress occurring in adulthood can induce CNS gene methylation, and specifically, support the hypothesis that epigenetic marking of the BDNF gene may underlie hippocampal dysfunction in response to traumatic stress. Furthermore, this work provides support for the speculative notion that altered hippocampal BDNF DNA methylation is a cellular mechanism underlying the persistent hippocampus-specific cognitive deficits which are prominent features of the pathophysiology of PTSD.

It is potentially of great importance that our epigenetic work demonstrated a selective hypermethylation of the BDNF gene in the dorsal hippocampus, but not amygdala. If we consider BDNF production as an important component of local synaptic structure, plasticity and overall functioning, then the imbalance of BDNF production between the hippocampus and amygdala would be consistent with our recent speculation regarding PTSD as an imbalance between amygdala and prefrontal cortical functioning (Diamond and Zoladz, 2016). We hypothesized that the hyper-functional status of the amygdala in PTSD is adaptive, from an evolutionary perspective, as a means with which to maintain heightened vigilance in a potentially hostile environment. The resistance of the amygdala to exhibit hypermethylation of the BDNF gene, and therefore to generate normal levels of BDNF, would enable this structure to continue to serve the function of maximizing one's vigilance, and to maintain memories of the trauma in an active state, following exposure to a life-threatening event. The differential expression of epigenetic modification to the hippocampus and prefrontal cortex versus amygdala may underlie the combination of amygdala-based hypervigilance and intrusive memories of trauma in conjunction with impaired hippocampal–prefrontal based processing of new information in people with PTSD (Diamond and Zoladz, 2016; Samuelson, 2011; Zoladz and Diamond, 2013).

9. Assessment of different therapeutic approaches in the PPS model

Another goal of our research has been to assess strategies with which to block stress-induced abnormalities in the PPS model. In an exploratory pharmacotherapeutic approach, we examined the effectiveness of amitriptyline (tricyclic antidepressant), clonidine (noradrenergic antagonist) and tianeptine (glutamate modulator antidepressant) in blocking physiological and behavioral sequelae manifested in our PPS model (Zoladz et al., 2013), with treatment beginning 1 day after the first cat exposure. Treatment beginning 24 h after exposing rats to an intense stressor is potentially relevant to treatment applications initiated in people within a day of a traumatic experience occurring.

Tianeptine is a well-established and effective antidepressant (Brink et al., 2006; Kasper and McEwen, 2008), which has been shown to block the adverse effects of stress on memory and brain functioning (McEwen et al., 2010; Zoladz et al., 2008b), and in a pilot study produced salutary effects in the treatment of PTSD (Franciskovic et al., 2011). We found that whereas amitriptyline and clonidine produced therapeutic effects in a subset of measures in our model, tianeptine was the only agent to block the effects of psychosocial stress in our entire battery of physiological and behavioral endpoints (Zoladz et al., 2013). Specifically, tianeptine blocked the expression of predator-
based fear-conditioned memory and prevented the effects of psychosocial stress on anxiety, startle response and cardiovascular reactivity. Importantly, these beneficial effects of tianeptine occurred in the absence of adverse side effects. This strategy may highlight the importance of beginning a treatment regimen as soon as possible after a person experiences trauma, as well as the potential value of tianeptine as a treatment for PTSD.

Francis, Wilson and their co-workers have investigated pharmacotherapy in the PPS model with daily treatment of rats with valproic acid (VA), a histone deacetylase (HDAC) inhibitor. HDAC treatment is relevant to persistent PTSD effects, as it can modify genetic transcription and diminish oxidative stress and levels of pro-inflammatory cytokines. These investigators reported that VA reversed the psychosocial stress-induced increase in anxiety-like behavior and restored the stress-induced alterations of neurotransmitter levels, oxidative stress and inflammation to control values (Wilson et al., 2014c). Particularly important is that the reversal of these changes occurred in the hippocampus and PFC, emphasizing that the neurochemical alterations in these brain regions might underlie the anxiogenic and cognitive effects of the PPS model.

In an extension of their previous work, this group also reported that 5-HT levels were normalized with chronic treatment of psychosocially stressed rats with a selective serotonin reuptake inhibitor (SSRI) (Wilson et al., 2014b). Perhaps most important, NE levels remained significantly increased in stressed rats treated with sertraline, which may explain why sertraline provided no benefit in relation to anxiety-like behavior in the stressed rats (Wilson et al., 2014b). The relative ineffectiveness of SSRIs, in general, and sertraline, in particular, as a treatment for some forms of PTSD (Davis et al., 2001; Robb et al., 2010; Stein et al., 2002; Stroddard et al., 2011) may occur because sertraline normalizes 5-HT levels, but the hyper-vigilance produced by elevated NE levels contributes to PTSD symptoms. Interestingly, similar work with predator scent stress revealed that immediate, but not delayed, treatment with sertraline prevented the development of anxiety-like behavior and an exaggerated startle response in stressed rats (Matar et al., 2006). Therefore, the timing of SSRI administration may also be crucial in its ability to ameliorate PTSD-like effects in rodents.

In recent work we developed a clinically based treatment strategy in the PPS model, but in this case we deployed a non-pharmacological approach designed to block the development of the PTSD-like effects (Seetharaman et al., 2016). As noted above, predator exposure, alone, did not produce significant effects on behavior in our test battery; the expression of PTSD-like effects required that the acute episodes of predator exposure occur in conjunction with daily social instability (Zoladz et al., 2008a). This finding provided strong support for the hypothesis that for rats, as well as people, the social context is an important influence on whether acute trauma results in resilience or susceptibility to develop PTSD-like effects. In the latest work, we extended our program on social factors as an influence on the expression of PTSD-like effects in rats. We assessed the hypothesis that daily social interactions, on a background of two cat exposures and daily housing instability, would block the development of PTSD-like effects on behavioral and physiological measures. We found that daily social stimulation, composed of 8 rats spending 2 h of time together each day in an enriched environment (in addition to daily social instability), blocked the development of predator-based fear memory, anxiety-like behavior and startle response (Seetharaman et al., 2016). These findings in rats are consistent with clinical evidence that social support is a critical factor in enabling traumatized people to exhibit resilience against the development of PTSD. This finding, in particular, illustrates the great importance in considering how crucial the social environment is in facilitating a complete recovery of function by individuals following traumatic stress exposure.

### 10. Exposure to the PPS model exacerbates ischemic heart damage

Individuals with PTSD exhibit numerous risk factors for cardiovascular disease (CVD) (Boscarino, 2011; Buckley et al., 2013; Coughlin, 2011; Edmundson et al., 2013; Edmundson and Cohen, 2013), including elevated heart rate, blood pressure and noradrenergic activity at baseline (Strawn and Geraciotti, 2008). They also have lower heart rate variability, reduced baroreflex sensitivity and increased QT variability (Cohen et al., 1998, 2000a; Rozanski et al., 2005), each of which has been linked to greater CVD incidence or cardiovascular-related mortality (Bigger et al., 1992; Piccirillo et al., 2007; Rozanski et al., 2005). CVD has been described as a disease of inflammation (Libby et al., 2009), and, consistent with this approach, PTSD patients exhibit elevated levels of pro-inflammatory cytokines, such as tumor necrosis factor α and IL-1β (Von Kanel et al., 2007, 2010), as well as increased incidence of autoimmune diseases (O’Donovan et al., 2015).

Because previous work with our model of PTSD revealed long-term adverse effects on cardiovascular reactivity (e.g., hypertension), as well as increased levels of inflammation, we explored whether the model would exacerbate myocardial damage following experimentally induced ischemia (Rorabaugh et al., 2015). After the 31-day stress paradigm, rats were anesthetized, and their hearts were removed and placed in a Langendorff isolated heart system. The hearts were perfused with Krebs solution and then exposed to 20-min ischemia, followed by 2 h reperfusion. Throughout this time frame, myocardial contractile function was measured continuously. After reperfusion, the hearts were stained to examine the amount of infarction. This study demonstrated that the hearts from rats exposed to our model of PTSD exhibited significantly larger infarct sizes than was found in hearts from non-stressed rats. The stressed animals also displayed reduced recovery of contractile function, as evidenced by a significantly reduced rate pressure product and a significantly elevated end diastolic pressure.

Another aspect of this study is that it was the first to examine physiological and behavioral effects of our PTSD model in females. This was an important issue as there is strong evidence that PTSD is a risk factor for the development of cardiovascular disorders in women (Sumner et al., 2015). Interestingly, while psychosocially stressed males exhibited anxiety-like behavior and the myocardial effects described above, psychosocially stressed females displayed neither. This is not completely surprising, considering the robust influence of gonadal hormones on female behavior and evidence from previous work that female rodents are more resilient than males in response to stress (Cohen and Yehuda, 2011). Nevertheless, epidemiological data regarding gender differences in PTSD suggest that females are more likely than males to develop the disorder (Tolin and Foa, 2006) (however, see Zoladz and Diamond, 2013 for an alternative perspective).

The influence of gender in stress responses and PTSD susceptibility is a complex issue. For example, we have shown previously that male and female rats exhibit a robust stress and cognitive impairing response to cat exposure (Park et al., 2008), and further, a reminder of predator exposure can impair hippocampus-dependent memory in females, but not males (Burke et al., 2013). Because it is important to establish a model of cardiovascular risk as a component of PTSD in females, resolution of the apparent contradictions in these findings warrants further investigation. For example, it is well-established that PTSD is comorbid with metabolic syndrome, particularly obesity, in men and women (Boscarino, 2008; Dobie et al., 2004; Pagoto et al., 2012; Violanti et al., 2006). Therefore, investigation of PPS effects in females should take into account how interactions between dietary factors and traumatic stress increase cardiovascular disease risk (Rasmussen et al., 2010).

Despite the absence of effects of PPS in females in our first study, the findings do indicate that if a myocardial infarction (i.e., cardiac ischemia) were to occur in conjunction with psychosocial stress, at least in males, it would result in greater damage to the heart, and potentially, reduced likelihood of survival. Overall, the finding that chronic psychosocial stress increased myocardial sensitivity to ischemic injury suggests...
that our model can enhance our understanding of the adverse cardiovascular consequences of traumatic stress and, potentially, the abnormalities observed in people with PTSD.

Using our model of PTSD to study stress effects on the heart provides important advantages over studying the impact of PTSD on myocardial infarction in a clinical setting. One advantage is that our model avoids factors that are commonly comorbid with PTSD (e.g., depression, smoking, drug and alcohol abuse, dietary factors), all of which can independently affect risk of cardiovascular disease (Breslau et al., 2003; Dobie et al., 2004; Nunnink et al., 2010; Vin-Raviv et al., 2014; Violanti et al., 2006). In addition, ischemic conditions in patients who experience heart attacks are heterogeneous. Our approach provides a strategy with which to compare the extent of myocardial injury under controlled conditions in which the parameters of the ischemic insult are held constant between groups.

Finally, one fundamental difference between our psychosocial stress paradigm and a heart attack in naturalistic conditions is that atherosclerotic blockage of coronary arteries can develop over a lifetime and may be accompanied by the formation of collateral vessels or other myocardial compensatory changes as ischemic heart disease progresses. In contrast, the fact that our psychosocial stress manipulation exacerbates myocardial ischemia through a mechanism that is independent of atherosclerotic coronary occlusion represents an important distinction from the clinical scenario. Hence, this paradigm can distinguish the stress component, per se, from long-term factors that contribute to the development of coronary heart disease. It is also important to note that the model ultimately provides the advantage to explore, under controlled conditions, the interaction of psychosocial stress with lifestyle factors, e.g., diet and exercise, on the susceptibility of the cardiovascular system to develop pathology.

11. Summary: progress toward a clinically relevant animal model of PTSD

We have taken clinical risk factors for PTSD into account in developing our animal model of PTSD, factors that maximize the likelihood of expressing stress-induced psychopathology in people. Specifically, our model involves exposing immobilized rats to a predator to provide them with an inescapable, life-threatening experience. This experience to the rat, in theory, is analogous to the fear and horror experienced by traumatized individuals. However, despite the fact that this experience provokes a powerful stress response in rats, as well as a fear-conditioned memory of the experience, cat exposure or social instability, alone, does not produce persistent PTSD-like abnormalities in behavior. It was only when we combined acute episodes of predator exposure with chronic social instability that we observed persistent behavioral and physiological abnormalities in the stressed rats which are comparable to those found in people diagnosed with PTSD. The cluster of symptoms we observed in the psychosocially stressed rats include a strong memory of the trauma, increased anxiety, exaggerated startle, impaired memory for new information, increased cardiovascular and hormonal reactivity to an acute stressor, abnormally low basal corticosterone levels, increased sensitivity to dexamethasone and an exaggerated physiological and behavioral response to the α₂-adrenergic receptor antagonist, yohimbine (Summarized in Table 1). Moreover, we have provided guidance for clinical research on PTSD with the finding of epigenetic modifications (methylation) of the BDNF gene in the hippocampus, which may provide the basis for impaired cognitive functioning in traumatized people.

In addition, our work has identified two approaches which may be of value in PTSD treatment. First, we showed that daily post-trauma treatment with the antidepressant tianeptine was effective in blocking the expression of the entire cluster of symptoms in the PPS model. Second, we showed that by inserting daily social interactions to the model we blocked the expression of the fear memory and hypervigilance, two measures that are hallmark features of PTSD. This level of analysis in an animal model serves to underscore the importance of clinical research addressing social factors which mitigate risk factors for PTSD, as well as non-pharmacological treatments for the disorder.

Finally, we have summarized recent work which has served as an extension of the original PTSD model by our collaborators at LSU who generated novel findings on neurotransmitter and neuroinflammatory abnormalities that develop in psychosocially stressed rats, with insight into how pharmacotherapies affect brain and behavior. We have also more recently reported that our model of PTSD increases myocardial sensitivity to ischemic injury, which may afford investigators a strategy with which to examine how traumatic emotional stress can exacerbate cardiovascular damage following an ischemic event. Overall, the integration of clinically relevant risk factors for people with PTSD with a reductionist approach in animal work provides an ideal strategy for generating translational research into the etiology and treatment of PTSD.

References


Adamec, R.E., 1998. Evidence that NMDA-dependent limbic neural plasticity in the right hemisphere mediates pharmacological stressor (FG-7142)-induced lasting increases in anxiety-like behavior. Study 1—role of NMDA receptors in efferent transmission from the cat amygdala. J. Psychopharmacol. 12, 122–128.

Table 1

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<th>PTSD phenotype</th>
<th>Comparable effects observed in psychosocially stressed rats</th>
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<td>Traumatic memory</td>
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<td>Exaggerated startle response</td>
<td>Greater startle reflex to auditory stimuli</td>
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<td>Zoladz et al., 2008a; Zoladz et al., 2015</td>
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<td>Enhanced sensitivity to yohimbine (e.g., panic attacks)</td>
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<td>Greater levels of norepinephrine in the hippocampus and prefrontal cortex</td>
<td>Elevated levels of norepinephrine in the hippocampus and prefrontal cortex</td>
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