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

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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.

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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.

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,

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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 α_2-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 naïve 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) fear conditioning] 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 [10,12]. 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 DecapiCones (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 DecapiCones 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