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Social defeat stress produces prolonged alterations in acoustic startle and body weight gain in male Long Evans rats

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ABSTRACT

Individuals exposed to psychological stressors may experience a long-term resetting of behavioral and neuroendocrine aspects of their "stress response" so that they either hyper or hypo-respond to subsequent stressors. These effects of psychological or traumatic stressors may be mimicked in rats using the resident–intruder model of social defeat.

The social defeat model has been characterized to model aspects of the physiology and behavior associated with anxiety and depression. The objective of this study was to determine if behaviors elicited following repeated social defeat can also reflect aspects of ethologically relevant stresses associated with existing post traumatic stress disorder (PTSD) models. Socially defeated rats displayed weight loss and an enhanced and prolonged response to acoustic startle which was displayed for up to 10 days following repeated social defeat. These data indicate that the severe stress of social defeat can produce physiologic and behavioral outcomes which may reflect aspects of traumatic psychosocial stress.

1. Introduction

Severe psychosocial stressors can precipitate the development of mood disorders such as post traumatic stress disorder (PTSD). PTSD is a mood disorder that can occur following repeated or extreme physical or psychosocial stress (Miller and McEwen, 2006; Yehuda et al., 1998); and most likely interacts with an existing genetic liability (Wakizono et al., 2007; Yehuda and Antelman, 1993). A major feature of this condition is intrusive memories of the traumatic event that are often triggered by sensory input (van der Kolk, 2006). The individual genetic or epigenetic and environmental characteristics of susceptibility to this disorder are not yet clear.

Animal models of traumatic stress are being characterized for behavioral responses which may resemble aspects of PTSD (Lister, 1990; Miller and McEwen, 2006; Yehuda et al., 2006). The complexity of mood disorders like PTSD, however, makes the development of an appropriate animal model for PTSD challenging. In addition, behavioral tests in animal models of anxiety-like behavior cannot directly measure psychological symptoms such as flashbacks and nightmares (Miller and McEwen, 2006). Instead, behavioral testing in animal models of traumatic stress can measure physiological and behavioral endophenotypes associated with PTSD (Cohen et al., 2004; Khan and Liberzon, 2004). Currently, rodent models of PTSD encompass the use of acute or repeated stressors such as immobilization, forced swim (Khan and Liberzon, 2004), underwater trauma, predator exposure (Cohen et al., 2004), or inescapable foot shock (Garrick et al., 2001).

Social defeat is an ethologically relevant stressor which utilizes the natural establishment of social rank in male rodents. During social defeat an aggressive resident male rat fights off an intruding male which has entered his territory. As a consequence of social defeat, the intruder male displays subordinate posturing to prevent further attack from the resident male rat. Following this interaction, subordination is reinforced as the intruder male receives visual, olfactory, and auditory stimuli from the resident male while being separated by a partition (Martinez et al., 2002).

Following social defeat, intruder male rats have been well documented for exhibiting anxiety and depressive-like behaviors (Blanchard et al., 1995, 2001; Koolhaas et al., 1997a; Miczek, 1991). Physiologic effects associated with increased anxiety-like behavior following social defeat have been shown to persist for up to 14 days following social defeat (Koolhaas et al., 1997b). Among the symptoms observed in the subordinate male (intruder) are weight loss, increased heart rate, sleep disturbances, increased body temperature (Koolhaas et al., 1997b) and hypothalamo–pituitary adrenal axis disturbances (Bhatnagar and Vining, 2003).

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Intruder rats may also display anxiety-like behavior when exposed to novel stressors (Frank et al., 2006; Koolhaas et al., 1999; Ruis et al., 1999; Von Frijtag et al., 2000). Therefore, like the ethologically relevant stresses of predator odor and under water trauma, social defeat, may also be an appropriate stressor to investigate for the elicitation of physiological responses and behaviors which can be encompassed by PTSD-associated responses to trauma.

Patients with PTSD can exhibit an enhanced startle response (Grillon and Baas, 2003). In rats, an observed difference in the acoustic startle response is a behavioral measure of anxiety-like behavior (Walker and Davis, 2002). Following exposure to severe stressors, rats do not readily habituate to acoustic startle (Garrick et al., 2001). Although socially defeated rats are known to exhibit an enhanced response to acoustic startle following morphine withdrawal (Miczek, 1991), they have not been observed to exhibit a prolonged enhancement in the startle response without measuring their response to drug withdrawal. In this study, our goal was to determine if rats exposed to repeated social defeat, and absent of drug withdrawal, exhibit an enhanced and prolonged startle response. These data may contain components which can be used to enhance existing PTSD models.

2. Materials and methods

2.1. Animals

Experimental subjects were male Long Evans rats obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Rats in all experiments were maintained on a 12:12 h light:dark cycle (lights on at 0700 h) with food and water freely available. A normal schedule of care and cage cleaning was performed by the animal facility staff.

Male resident Long Evans rats weighing 400–500 g were paired for one week with 3 month old virgin females weighing (300 g) in (54 cm × 25 cm × 22 cm) polycarbonate cages to establish territory of the resident. Male intruders weighing 325–350 g were housed individually for one week before each experiment in (32 cm × 22 cm × 20 cm) polycarbonate cages under similar conditions. All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Emory University.

2.2. The social defeat model

The social defeat model used in this study was adapted from experiments by others (Nikulina et al., 2004). One week after pairing, male resident rats with female rats, the male residents were trained to attack novel intruder male opponents twice a day for 4 days. Once the female was removed, a novel intruder male was placed in the cage of the resident male. Training consisted of measuring the attack latency of the resident male to novel intruder male opponents. Attack latency of male residents was scored and only those resident males with an attack latency of <60 s were used in the study (Buwalda et al., 2001). Females were re-introduced into the cage following the training period.

During the social defeat procedure, the female was removed from the resident's cage and a naïve intruder male was placed into the resident's cage behind a wire mesh partition for 5 min. Following this 5 min period, the partition was removed and the resident–intruder interaction occurred for a maximum of 5 min or until the intruder displayed 5 s of a continuous submissive supine posture. Following the resident–intruder interaction, the resident, and intruder were again separated by a partition for 60 min (Covington and Miczek, 2001). During this period the intruder experienced psychosocial stress via exposure to visual, olfactory and auditory cues from the resident. The intruder was returned to its home cage after 60 min. This procedure was repeated every third day (i.e., days 1, 4, 7, and 10) for a total of four social defeat episodes (Covington and Miczek, 2001; Miczek and Mutschler, 1996).

A cage-transfer control group (CTC) was used. The CTC group consisted of naïve male rats which were placed into a new empty cage with the same dimensions as its home cage. The CTC group and the social defeat (SoD) group were placed in a cage for the same duration of time, but the SoD group animals were placed in the cage of a male resident. The CTC group was used as a control for handling, cage transfer, and exposure to a novel cage. All animals were also weighed before the SoD occurred (day1), and again on days 10 and 22. For this experiment, ($n = 10$) for the CTC and SoD treatment groups.

2.3. The response to acoustic startle following SoD

Next, we assessed the acoustic startle response in another set of socially defeated animals to determine if this response was still enhanced 10 days following the last SoD. Methods for measuring acoustic startle were adapted from experiments performed by others (Chabot and Taylor, 1992; Walker and Davis, 2002). On day 7 following SoD, all animals received a 5 min acclimation period to the startle chamber in the presence of 65 dB background noise. The next day, each animal was placed in a startle chamber with lights off. Startle responses were measured using the SR Lab startle reflex system (San Diego Instruments, San Diego, CA). In individual chambers, animals were exposed to 5 min of 65 dB background noise followed by a series of 30 startle-eliciting noise bursts (10 each at 95, 110, and 125 dB; 50 ms pulse duration). The three noise bursts were presented at an interstimulus interval of 30 s in a pseudorandom order; startle noise bursts were presented with the constraint that each noise burst, was presented once within a block of the three different noise bursts. The following day, the startle response was measured in the same manner, but with the chamber lights on. The standard lighting of the chamber was 420 lux and was at a constant level when the lights were turned on. One week later, the startle response measurement was repeated at the same light intensity. For the SoD and CTC treatment groups, ($n = 11$) animals.

2.4. Statistics

Body weight in both the CTC and SoD groups was analyzed. A two-way repeated measures ANOVA (body weight × day) was conducted with Bonferroni post hoc comparisons when appropriate using graphpad Prism 3.0 statistical software.

In an analysis of the overall acoustic startle amplitude displayed between treatment groups, the mean of the startle amplitudes at 95, 110, and 125 dB was compared across all experimental days of acoustic startle. A paired t-test comparing the CTC and SoD groups was then performed using Prism 3.0 statistical software.

Next, the overall effect of experimental day between the CTC and SoD groups was analyzed. To determine the overall effect of the experimental day, the mean of the startle amplitudes at 95, 110, and 125 dB was compared between experimental days, using paired t-tests with graphpad Prism 3.0 statistical software.

An analysis of the effect of treatment, noise level, and treatment day was conducted using a three-way repeated measures ANOVA (treatment × day × noise level) using SPSS 15.0 statistical software. A test for sphericity was conducted. Where sphericity was not met, a Greenhouse–Geisser correction test was performed. To determine the effects of the noise levels during the days of startle testing, paired t-tests were conducted using graphpad Prism 3.0 statistical software.