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Research report

Two weeks of predatory stress induces anxiety-like behavior with co-morbid depressive-like behavior in adult male mice

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HIGHLIGHTS

- Predatory stress elicits anxiety-like and depressive-like behaviors in male mice.
- Two-weeks of predatory stress induce sustained alterations in behavior.
- Predatory stress is a validated chronic stress paradigm in mice.

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ABSTRACT

Psychological stress can have devastating and lasting effects on a variety of behaviors, especially those associated with mental illnesses such as anxiety and depression. Animal models of chronic stress are frequently used to elucidate the mechanisms underlying the relationship between stress and mental health disorders and to develop improved treatment options. The current study expands upon a novel chronic stress paradigm for mice: predatory stress. The predatory stress model incorporates the natural predator-prey relationship that exists among rats and mice and allows for greater interaction between the animals, in turn increasing the extent of the stressful experience. In this study, we evaluated the behavioral effects of exposure to 15 days of predatory stress on an array of behavioral indices. Up to 2 weeks after the end of stress, adult male mice showed an increase of anxiety-like behaviors as measured by the open field and social interaction tests. Animals also expressed an increase in depressive-like behavior in the sucrose preference test. Notably, performance on the novel object recognition task, a memory test, improved after predatory stress. Taken as a whole, our results indicate that 15 exposures to this innovative predatory stress paradigm are sufficient to elicit robust anxiety-like behaviors with evidence of co-morbid depressive-like behavior, as well as changes in cognitive behavior in male mice.

1. Introduction

Anxiety and depression impact the lives of an estimated 5.2% and 13.3% of adults, respectively [1]. Chronic stress has been implicated in the development and severity of anxiety disorders, as evident in post-traumatic stress disorder [4,5]. In addition, a major stressful life event of an acute nature often precedes a depressive episode and chronic stress increases the overall lifetime risk for developing depression [2,3]. Given the relationship among stress, anxiety, and depression, and the need for additional therapeutic options, chronic stress models are often used to study anxiety-like and depressive-like behaviors in a variety of rodent species, including mice.

Chronic stress paradigms that precipitate lasting effects are limited in adult male animals [6,7]. In addition to behavioral changes, standard effects of chronic stress include changes in corticosterone levels, vasculature, hippocampal volume, amygdaloid plasticity, body weight, and neurochemistry [5,8–12]. A commonly

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used chronic stress paradigm is chronic mild unpredictable stress, which has both face validity in producing symptoms and predictive validity for response to antidepressants in rats [13]; however, the effects of this paradigm in mice have been less consistent [6,7]. Social defeat is another commonly used stress paradigm in rats [11], but a percentage of mice are resistant to the negative effects of chronic social defeat [14–16]. An alternative chronic stress paradigm is the use of predatory stress, which is more potent than exposure to chronic mild unpredictable stress in mice [17].

Predatory aggression, a type of psychological stressor, can be used to test the effects of stress on anxiety-like and depressive-like behaviors in rodents. Predatory scents such as cat odor have been presented to experimental mice as part of the stress paradigm [18]. An alternative source of predatory stress is the use of live predators, usually cats or snakes [19]. Barriers that curb the interaction of the predator with the prey are essential for the safety of the animals, but these safeguards limit the level of stress induced by exposure to a predator the predatory–prey experience. Furthermore, it may not be feasible to bring certain types of predator animals into the laboratory because of the need for specialized facilities that may not be readily available in many settings and potential inadvertent sensory contamination. Rats are natural predators of mice and these species already frequently share colony facilities (although in separate housing rooms). The accessibility of rats in the research setting, paired with the aggression that rats naturally express toward mice, led to the development of a rat-on-mouse predatory chronic stress paradigm [17]. This paradigm protects the mouse from injury by placing it in an activity ball which allows the rat to move the mouse around the cage but prevents direct physical contact. This design exposes the mouse to the sight, sound, smell, and physical effects of one of its natural predators, the rat, in a controlled laboratory environment. Previous use of the predatory stress model demonstrated that a 28-day period initiated during adolescence elicited anxiety-like and depressive-like behaviors in adult mice one week after the end of stress [17].

Here we expand the use of this predatory stress model to determine if the robust behavioral effects previously reported can be produced in adult male mice with a shorter duration of exposure to the stress paradigm (15 days). In addition, we assessed the behavioral effects of the paradigm as much as two weeks after the conclusion of the stress exposure and demonstrate lasting effects of the predatory stress exposure on behavior. Collectively, the data presented here demonstrate that the rat-on-mouse predatory stress paradigm causes lasting changes in behavior of adult male mice following a 15 day exposure to the chronic stress paradigm.

2. Materials and methods

2.1. Animals

Adult male C57/Bl6 mice (Stress $n = 10$; Controls $n = 8$) were single-housed and maintained on a reverse 12:12 h light:dark cycle in a temperature and humidity controlled environment in an AAALAC-approved facility. Mice were given *ad libitum* food and water throughout the experiment, and were housed in a room separate from the rats. Twenty adult Long Evans rats were used as the predatory animals. These rats were pair-housed and maintained on a 12:12 h light:dark cycle. Rats had access to *ad libitum* water throughout the experiment. All experiments were performed in accordance with the Institutional Animal Care and Use Committee of Emory University and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

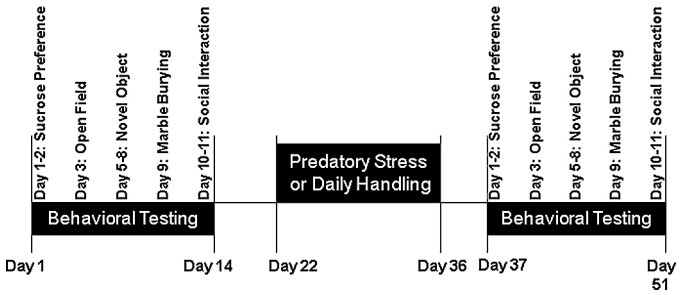

Fig. 1. Timeline of behavioral testing and predatory stress. Behavioral testing was conducted prior and following chronic stress in order to measure the effects of the stress paradigm on anxiety- and depressive-like behaviors, as well as on cognition. Behaviors were completed in the following order before and after stress: sucrose preference, open field, novel object, marble burying, and social interaction. Predatory stress lasted 15 days and post-behavioral testing began the day after the end of stress.

2.2. Predatory stress

The predatory stress paradigm consisted of placing a mouse in a 5″ diameter plastic hamster ball (Super Pet, Elk Grove Village, IL; material # 1000079348). Each ball was then placed in the cage of two Long Evans predator rats that were fed a restricted diet of 8 standard lab chow pellets per day. For 30 min on 15 consecutive days, the rats were allowed to freely agitate the hamster balls. To ensure the safety of the mice, the lids were secured for the duration of the stress period. Mice in the control condition were exposed to daily cage transport and handling for 15 consecutive days.

2.3. Behavioral tests

We used a variety of established behavioral tests to measure patterns in anxiety-like and depressive-like behaviors in these mice. Behavioral tests were performed in the following order before and after stress: sucrose preference, open field, novel object, marble burying, and social interaction (see Fig. 1). Control mice were exposed to the same sequence of behavioral tests with a 21-day break between exposures to control for the duration of the stress exposure and waiting period.

2.3.1. Affective-like behaviors

Open field: The open field test is used as a measure for anxiety-like behavior and general locomotor activity [20–22]. Mice were placed in the center of a 45 cm × 45 cm square box and allowed to explore for 10 min. Mice were videotaped and scored using Cleversys, Inc. behavioral tracking software (Reston, VA). *Social interaction*: The social interaction test is a measure of anxiety-like behavior in mice [23,24]. Mice were placed in the open field box for 5 min along with a stimulus male mouse of similar size. The total time the experimental mouse spent in active contact with the stimulus animal was measured using Cleversys, Inc. behavioral tracking software. *Marble burying:* The marble burying test is used as a measure of anxiety-like behavior [25]. Mice were placed in a standard rat cage containing 5 inches of bedding and 20 black marbles arranged in 4 columns, and allowed to roam freely for 30 min. A marble was considered buried if 50% or less of the marble was visible after 30 min. *Sucrose preference:* The sucrose preference test is traditionally an assessment of anhedonia, a central symptom of depression in humans [26,27]. We used a highly palatable food paradigm in this study, in which mice were exposed to sucrose enriched pellets and their standard diet for 1 h on two consecutive days [28]. Pellets were weighed before and after the 1-h testing period and the weights were averaged together. The mice were also weighed in order to control for body mass.