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Short Communication

Chronic unpredictable stress during adolescence causes long-term anxiety

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HIGHLIGHTS

- Stress during adolescence causes a long-term increase in anxiety.
- Increased hyponeophagia is evident 196 days after exposure to unpredictable stress.
- Behavioral changes are not mediated by altered basal corticoid "stress" hormones.

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ABSTRACT

Exposure to stress during adolescence can cause long-term changes in behavior and cognition. Anxiety diagnoses rise during adolescence and are increased by adverse experiences. Currently, it is unknown how long stress during adolescence alters anxiety in adulthood. We found that rats exposed to chronic unpredictable stress during adolescence expressed altered behavior 6.5 months later; showing increased anxiety in a feeding test in a novel environment. Although behavioral changes indicative of anxiety were detected in late adulthood, the basal levels of fecal corticoid metabolites in prior-stressed rats did not differ from unstressed, control rats.

Both laboratory and clinical studies indicate that adolescence is a stage of particular vulnerability to stress exposure [1,2]. Trauma during adolescence appears to increase anxiety rates more than other forms of mental illness, making anxiety an important target for research [3,4]. Anxiety diagnoses increase during adolescence, and can be amplified by adverse conditions [2]. For example, adolescent survivors of the shipwreck of "Jupiter" in 1988 in Greek waters had a 40.7% chance of developing an anxiety disorder, compared to only 18.4% of a demographic-matched control population [3].

In addition to increased psychological vulnerability, laboratory studies with rodents suggest that adolescents are more physiologically vulnerable to stress [5]. During adolescence, the hypothalamic-pituitary-adrenal (HPA) axis that regulates the hormonal stress response is still immature [5,6]. Compared to adults given the same aversive stimuli, adolescents produce glucocorticoid "stress" hormones for a longer duration, thus increasing the adolescent's overall exposure to glucocorticoids [1]. Chronic exposure to glucocorticoids can cause changes in both the brain and behavior that may persist for several months, including altered neural development and modified dendritic branching [5,7]. Currently, however, it is unclear how long changes in anxiety persist after animals experience stress during adolescence (see S1 for a summary of current studies). This ambiguity is due, in part, to the challenge of defining the adolescent phase in rodents; studies vary in timing and duration of stress exposure, making cross-study

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Table 1
Chronic unpredictable stressor descriptions.

Physical stressors	
Smaller cage	Rat pairs were housed for 4 h in a cage 25% smaller than their home cage [15].
Damp bedding	Rat pairs were housed for 6 h with 200 ml of water mixed into 2/3 of the bedding of their home cage [16].
Cage tilt	Home cages were tilted at a 30° angle for 6 h [16].
Social stressors	
Isolation	Rats were housed individually for 1 h in a clean cage with a 7.6 cm diameter PVC tube and a 2.5 cm × 2.5 cm × 8 cm pine wood block [6].
Crowding	Two pairs of rats were combined in one clean cage (20 cm × 45 cm) for 4 h [15,16].
Foreign bedding	Rat pairs were housed in the soiled home cage of older conspecifics for 12 h [16].
Predation stressors	
Taxidermied bobcat	An adult male taxidermied bobcat was placed on a wheeled cart and pushed in front of rat home cages for 30 min [17].
Fox urine	Tink's Red Fox-P® was sprayed onto cotton balls, concealed in plastic container with 6 small holes for air flow, and placed into the rat home cages for 30 min [18].
Cat fur	*Felis catus* fur, inside of mesh, was placed into the rat home cages for 30 min [19].
Feline vocalizations	Bobcat (*Lynx rufus*), mountain lion (*Puma concolor*), domestic cat (*Felis catus*), lion (*Panthera leo*), and tiger (*Panthera tigris*) vocalizations were played for 30 min.

comparisons difficult [8]. Additionally, there are numerous laboratory measures of anxiety that vary in face validity, cost, and ease-of-execution, again making direct comparisons of such assays challenging [9].

The current study investigated behaviors in a novelty suppressed feeding test, which has been used as an assay for anxiety-like behavior in animals for more than seven decades [10]. This test was selected because it has better face validity than other rodent behavioral assays; at least 2 weeks of daily selective serotonin reuptake inhibitor (SSRIs) treatment are required to alter behavior in the novelty suppressed feeding test, which is more consistent with timelines of SSRI effects and human therapies, whereas a single exposure to SSRIs has been shown to alter behavior in forced swim or tail suspension tests [10,11]. Additionally, novelty suppressed feeding tests are based on natural rodent behavior, potentially making their interpretation more reliable [12].

In a previous study of the effects of adverse social conditions during adolescence and early adulthood (from 32 to 77 days of age) on novelty suppressed feeding in mice, Sterlemann et al. [13] found increased anxiety-like behavior immediately following social unpredictability, but, 12 months later there was no observable difference from unstressed controls. Using a similar approach, we investigated novelty suppressed feeding in adult rats after exposure to a diverse, chronic unpredictable stress paradigm during adolescence that included social, physical, and predation stress (Table 1). A variation of this chronic unpredictable stress paradigm has previously been shown to induce long-term behavioral changes including enhanced reward loss sensitivity, accelerated decision-making, and a negative cognitive bias [19]. The current stress regimen included physical stress, which appears to induce longer-term changes in behavior and physiology compared with treatments that use only social stress [20], and also included a short period of early adulthood. We assessed anxiety-like behavior 6.5 months after completion of the chronic unpredictable stress paradigm (Fig. 1).

Male Sprague-Dawley rats ($n = 30$) were obtained at 21 days of age from Harlan Laboratory in Fredrick, Maryland, USA. Animals were pair-housed and maintained on a reverse 12/12 light–dark cycle to allow for behavioral testing during the dark phase when rats are most active. Nine days after rats arrived in the lab, a subset of rats ($n = 14$) began the chronic unpredictable stress paradigm that lasted throughout the adolescent period and into early adulthood from 30 to 78 days of age, based on Sterlemann et al. [13]. Stressors were presented at variable times between 06:00 h and 01:00 h for 6 days per week; although presentation order was randomized, on average rats were exposed to each of the three types of stress twice per week. After completion of the chronic stress procedure, all rats were maintained in standard housing with no further exposure to stressors for the remainder of the experiment. The additional 16 rats were maintained in standard pair housing throughout development and served as unstressed controls. All experiments were approved by the Pennsylvania State University IACUC committee, protocol #35761.

At 274 days of age, 196 days after the chronic stress procedure, anxiety levels were assessed using a novelty suppressed feeding test. In this test, rats were exposed to a familiar food reward in a novel environment; a longer latency to consume the reward is indicative of behavioral inhibition and increased anxiety. To familiarize animals with the food reward, an almond slice was placed in a petri dish in their home cage in the same manner in which the animals would encounter the reward in the novel context [13]. Three days later, the rats were tested by placing them in a fixed starting position along the base of a wall in a novel 122 cm × 122 cm × 46 cm opaque Plexiglas arena. The latency of each rat to pick up and consume the almond slice in the center of the arena was measured. The arena and petri dish were cleaned with 70% ethanol between trials.

Ten days after the novelty suppressed feeding test, production of glucocorticoid "stress" hormone (corticosterone) was estimated from fecal corticosterone metabolites at 287 days of age. It has been suggested that long-term behavioral changes could be a result of altered circulating levels of glucocorticoids [13]. Currently, there is conflicting evidence whether long-term changes in circulating corticosterone occur after exposure to adolescent stress [6,7,13,22]. In addressing this question, to our knowledge only plasma-based measures of corticosterone have been used after such a long delay following exposure to adversity. Recently, it was demonstrated that male rats exposed to either novel or no social partners during adolescence exhibited decreased basal corticosterone using a fecal measure at 110 days of age [22]. The use of a fecal measure later in life may shed light on whether stress during adolescence causes long-term changes in glucocorticoid production because fecal measures quantify corticoid metabolites, which represent only free corticoids. Biologically active, free corticoids are the subset of corticoids available to respond to challenge because they are unbound to corticosteroid-binding globulin (CBG) [21]. Plasma measures typically quantify all corticoids both bound and unbound to CBG. Consequently, measures of fecal corticoid metabolites are suggested to more accurately represent the ability of an animal to physiologically respond to challenge than plasma measures [21]. Fecal sample collection is also non-invasive, which may provide a more accurate measure of unstressed, basal corticosterone production [21,23]. It should also be noted, however, that fecal measures often require a greater difference in circulating corticosterone to