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Research report

Short- and long-term consequences of stressor controllability in adolescent rats

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HIGHLIGHTS

- A rat model to study the effects of controllable stress in adolescence was tested.
- Adolescent subjects were sensitive to the behavioral control dimension.
- Adolescent controllable stress blunted the effects of adult uncontrollable stress.
- Stressor control during adolescence decreased DRN 5-HT activity after stress.
- The mPFC is critical for the acute effects of an adolescent controllable stressor.

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ABSTRACT

Adolescence is a developmental period in which brain structures involved with stress responses, such as the medial pre-frontal cortex (mPFC), mature. Therefore, exposure to a stressor at this time may have effects that endure the lifespan. The goal of the present study was to determine whether behavioral control over an adolescent stressor mitigates the behavioral and neurochemical consequences of the stressor as occurs in adult rats. Adolescent rats (post natal day 35) were exposed to either inescapable (IS) or escapable tailshocks (ES). As in adults we observed a "stressor controllability effect"; IS reduced social exploration and activated the serotonergic dorsal raphé nucleus while ES did not. Excitotoxic lesions of the medial prefrontal cortex prevented the stressor controllability effect. We also demonstrate that a controllable adolescent stress prevents the behavioral and neurochemical consequences of IS in adulthood. Thus, the controllability of a stressor during adolescence is an important psychological factor.

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1. Introduction

Adolescence is a key developmental period during which adult-like behaviors are established and organisms gain independence. Although the adolescent period is not marked by a specific event or age, it is a period of transition from childhood to adulthood marked by many changes, including an increase in social interaction, novelty seeking and risk-taking behaviors [1–3]. Adolescence is also characterized by cortical development, particularly in areas involved in emotional processing and learning, such as the medial pre-frontal cortex (mPFC), which undergoes vast remodeling throughout adolescence [1,4]. Accordingly, exposure to stressors during adolescence increases risk for psychiatric disease in adulthood [5,6]. Thus, it is important to understand how stressors influence behavioral and neural development.

Control, or perception of control, over stressor onset or offset has been identified in both humans and rats to be an important predictor of many stressor consequences [7–10]. In adult rats exposure to inescapable tail shock (IS) produces numerous behavioral (e.g. exaggerated fear conditioning, poor shuttle box escape learning, and decreased social interaction) and neural (e.g. induction of Fos in numerous brainstem and limbic regions and downregulation of serotonin (5-HT) 1A receptors) consequences that do not occur after equal amounts of escapable tail shock (ES) [11–14]. IS and ES differ only in that with ES the subject has control over one aspect of the shocks, their time of termination, whereas IS subjects do not. The effects of IS on later behavior depend upon stress-induced activation of 5-HT containing neurons in the dorsal raphé nucleus (DRN: for review see [15]). Specifically, IS leads to sensitization of the DRN so that it becomes hyper responsive to anxiogenic stimuli [16], with the sensitization being dependent upon downregulation of the 5-HT1A auto receptor [17,18]. ES prevents the behavioral consequences of the stressor by blunting DRN activation [19] and 5-HT1A downregulation [17] with this blunting depending on an

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intact mPFC [13]. All of the aforementioned research was carried out in adult subjects.

Recently, Leussis and Andersen [20] demonstrated an effect of control in adolescent rats given ES at post-natal day (PND) 35. As in adults, adolescent rats that received IS, but not ES, exhibited learning deficits in a shuttle box shortly thereafter [20]. Taking this finding as a base, the present study had three aims. The first was to determine the acute and long term behavioral effects of IS and ES in adolescence on a putative measure of anxiety, social exploration [21]. Since experiential factors have been shown to alter an organism's vulnerability to subsequent aversive experiences [9], the behavioral impact of adolescent stressor controllability on an uncontrollable stressor in adulthood was assessed. For the second aim, Fos immunohisochemistry (IHC) was used to assess the impact of the stressor within serotonergic neurons of the DRN. This was measured shortly after both adolescent stress and adult stress. Because the mPFC is necessary for the effects of stressor control in adults [13], the final aim was to assess the role of the mPFC in the effects of an adolescent controllable stressor.

2. Materials and methods

2.1. Subjects

Male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were housed 4/cage in plastic tubs with free access to food and water on a 12 h light/dark cycle (lights on at 7:00 A.M. and off at 7:00 P.M.). Group housing was used to negate the effect of social isolation at this time point and was shown to be necessary for the effect of controllability in adolescence [20]. Cagemates were randomly assigned to groups and exposed to the same experimental conditions. Experiments were conducted during the light phase and all procedures were approved by the University of Colorado Institutional Animal Care and Use Committee.

2.2. Procedures

2.2.1. Escapable and inescapable tailshock procedure

ES and IS were administered in 14 cm × 11 cm × 17 cm acrylic wheel turn boxes enclosed in sound-attenuating chambers. Electric shock was delivered by a Precision Regulated Animal Shocker (Coulbourn Instruments, Allentown, PA, USA) through copper electrodes augmented by electrode paste attached 2–4 cm from the base of the tail. Eighty tailshocks were administered on a variable interval schedule with a range of 20–100 s and an average inter-shock interval of 60 s (VI-60). Shock intensity was 0.8 mA for the first 26 trials, 1.0 mA for the following 27 trials, and 1.2 mA for the remaining 27 trials. As with adult rats, turning a wheel at the front of the chamber terminated each tailshock for rats in the ES condition [13]. The escape response requirement began with 1/4 of a full turn of the wheel. If the rat performed the response requirement within 5 s, the response doubled on the next trial until a maximum of 4 full wheel turns was reached. If the rat did not escape within 30 s the shock was terminated and the response requirement reset to 1/4 turn. These parameters help to insure that the rat learns an operant response contingency and does not simply perform a reflexive behavior. IS animals were yoked to animals receiving ES so that they received the same number and duration of shocks. Animals were returned to the colony room immediately following the tail-shock procedure. Stress naïve control rats remained in their home cages (HC).

To examine the long-term "immunizing" stress protective effects of ES, a separate set of rats received ES or IS on PND 35 and then 100 trials of IS 35 days later. For this second stressor rats were placed in a clear acrylic restraint tube that measured 17 cm in length and 7 cm in diameter. The restraint tube contained a small platform extending from the rear to which the tail was fixed with cloth tape and electrodes. Here IS consisted of 100, 5 s shocks on a VI-60 schedule at an intensity of 1.0 mA for the first 10 shocks, 1.3 mA for the second 10 shocks and 1.6 mA for all subsequent trials as previously reported [22,16].

2.2.2. Social exploration test

Social exploration tests were conducted exactly as described previously [14]. Each experimental rat was allocated a separate transparent plastic cage with shaved wood bedding and a wire lid located in a brightly lit testing room; food and water were not available in the testing cages. After 60 min a stimulus rat (PND 28) was added to the cage. Investigative behaviors, including sniffing, pinning, and allogrooming, initiated by the experimental rat were timed by an observer who was blind to group membership. After 3 min, the juvenile was removed and the experimental animal was returned to the homecage. As in our published methods [16], juvenile stimulus rats were used multiple times during a single social exploration test.

2.2.3. Dorsal raphé nucleus serotonin and Fos immunohistochemistry

Rats were deeply anesthetized with sodium pentobarbital approximately 2 h after the last tailshock and perfused with 50 ml of cold physiological saline and 200 ml of 4% paraformaldehyde in 0.1 M phosphate buffer. Extracted brains were post-fixed in the same 4% paraformaldehyde overnight and then transferred to 30% sucrose until sectioning. Sections measuring 30 μm were obtained in a cryostat and were stored at 4C in cryoprotectant.

Serial immunohistochemical staining for Fos and 5-HT was conducted as described previously [13]. Briefly, staining for Fos was conducted first using rabbit polyclonal Fos primary antibody (1:15,000; Santa Cruz Biotechnology, Santa Cruz, CA) and biotinylated goat anti-rabbit secondary antibody (Jackson ImmunoResearch, West Grove, PA) and visualized with avidin-biotin horseradish peroxidase (ABC Kit, Vector Labs) and nickel enhanced diaminobenzidine (DAB). 5-HT containing neurons were identified with rabbit 5-HT primary antibody (1:10,000; ImmunoStar, Hudson, WI) and nonbiotinylated goat anti-rabbit IgG (Jackson ImmunoResearch) secondary antibody and were visualized with peroxidase antiperoxidase and DAB to produce a brown cytoplasmic precipitate. After staining, sections were floated onto glass slides in .15% gelatin, dehydrated, defatted in Histoclear (Fisher Scientific), and cover-slipped with Permount (Fisher Scientific).

The number of 5-HT-stained cells and the number of cells double-labeled for both 5-HT and Fos were quantified by an observer naïve to experimental treatment. Fos-stained nuclei were identified by black ovoid particles. Larger reddish-brown particles, with or without Fos-stained nuclei, were counted as 5-HT-positive cells. Caudal DRN sections were taken at approximately −8.3 mm from bregma. Three sections from this caudal position were taken and averaged for each rat for a cumulative mean score of double-labeled cells.

2.2.4. Excitotoxic mPFC lesions

All surgeries were conducted under inhalational anesthesia with 2–3% isoflurane in oxygen. A rat was placed in a stereotaxic frame (Kopf Instruments) with a bite bar and nose-cone for gas delivery. The skull was adjusted so that bregma and lambda were in the same horizontal plane. Each rat received an injection of 0.5 μl of 5% NMDA (Sigma, St Louis, MO) through a 25 μl Hamilton syringe into the border between infralimbic and prelimbic cortices (AP +2.5, DV −2.9). The Hamilton syringe remained in place for 5 min after the injection to permit diffusion. After surgery each rat received prophylactic antibiotic, 25 ml Twin-Pen (AgriLabs). At the end of each experiment rats were overdosed with sodium pentobarbital (60 mg/kg i.p.) and brains were removed and flash frozen in −60 °C isopentane. Frozen sections (30 μm) were cut in a −20 °C cryostat, mounted onto glass slides and stained for cresyl violet in order to verify lesion location.

2.3. Experimental procedures

2.3.1. Experiments 1 and 2: The short and long term effects of stressor controllability during adolescence on later social exploration

The first set of experiments assessed the short and long-term effects of adolescent stressor controllability on social exploration. Animals arrived to the vivarium at post-natal day (PND) 26 and received HC, ES or IS treatment at PND 35. Social exploration tests were conducted on PND 36. The long-term effect of adolescent stressor controllability was assessed using a separate set of animals that received either HC, ES, or IS at PND 35 and remained undisturbed until social exploration tests on PND 70. PND 70 was used because it is well outside the range known to be adolescence (weaning to PND 60) [1] and comparable to prior research using these methods [20]. To assess whether adolescent ES produces a long lasting resistance to later IS animals received ES, IS, or HC at PND 35. ES, IS and half of the HC subjects then received IS on PND 70, while the other half of the HC group received HC again to provide a baseline. Social exploration tests were conducted 24 h later.

2.3.2. Experiments 3 and 4: The effect of adolescent stressor controllability on DRN 5-HT activation

As in experiment 1, animals were received at PND 26 and given either HC, ES, or IS at PND 35. In experiment 3, rats were sacrificed 2 h after the end of stress for 5-HT and Fos analysis. In experiment 4, rats returned to the vivarium and were sacrificed on PND 70 2 h after IS. Fos and 5-HT immunohistochemistry was performed as described by Grahn et al., 1999 [23].

2.3.3. Experiment 5: The role of the mPFC in the behavioral effects of adolescent stressor controllability

To assess whether the mPFC is necessary for the short-term effects of ES, excitotoxic lesions were made prior to adolescent stress. Lesion was used rather than muscimol microinjection because of the difficulty inherent in canulation at PND 35 while the skull continues to grow rapidly. Rats arrived on PND 24 and received NMDA lesions on PND 28 followed by either ES, IS, or HC at PND35 and were tested in a social exploration test 24 h later.

2.4. Statistical analyses

Wheel-turn escape data was analyzed by repeated-measures ANOVA. One-way ANOVA tests were run for all experiments except for the lesion experiment in which a multi-way ANOVA was used. Significant main effects and interactions