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Juvenile stress affects anxiety-like behavior and limbic monoamines in adult rats

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HIGHLIGHTS

- Juvenile stress increased anxiety-like behaviors in the OF and EPM in adult rats.
- Juvenile stress failed to affect adult depressive-like behaviors, including anhedonia and cognition.
- Juvenile stress only altered mPFC DA and 5-HIAA levels, and AMY NA levels of adult rats.
- Anxiety-like behaviors in the OF induced by juvenile stress were correlated with mPFC DA levels.
- Juvenile stress induced long-term changes in the expression of anxiety-like behaviors and limbic monoamines.

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ABSTRACT

Epidemiological evidence suggests that childhood and adolescent maltreatment is a major risk factor for mood disorders in adulthood. However, the mechanisms underlying the manifestation of mental disorders during adulthood are not well understood. Using a recently developed rat model for assessing chronic variable stress (CVS) during early adolescence (juvenility), we investigated the long-term effects of juvenile CVS on emotional and cognitive function and on monoaminergic activities in the limbic areas. During juvenility (postnatal days 27–33), rats in the stress group were exposed to variable stressors every other day for a week. Four weeks later, anhedonia was tested in the sucrose test, anxiety-like behaviors were assessed in the elevated plus-maze (EPM) and open field (OF) tests, and cortically mediated cognitive function was evaluated during an attentional set-shifting task (AST). After the behavioral tests, the rats were decapitated to determine limbic monoamine and metabolite levels. Adult rats stressed during juvenility exhibited higher anxiety-like behaviors, as evidenced by reduced locomotion and rearing behavior in the OF and fewer entries into the open arms in the EPM. There were no differences between the stressed rats and the controls in depressive-like anhedonia during the sucrose preference test or in cognitive function during the AST test in adulthood. In addition, the previously stressed rats exhibited increased dopamine (DA) and decreased 5-HIAA in the medial prefrontal cortex (mPFC) and decreased noradrenaline in the amygdala compared with controls. Furthermore, DA levels in the mPFC were correlated with adult anxious behaviors in the OF. These results suggest that juvenile stress induces long-term changes in the expression of anxiety-like behaviors and limbic monoaminergic activity in adult rats.

1. Introduction

Epidemiological studies indicate that psychological and/or physical trauma during childhood is a major risk factor for the subsequent onset and development of mood disorders [1–5]. However, how childhood trauma affects behavior and the underlying neural and molecular mechanisms are not yet fully understood. To model the detrimental effects of childhood trauma observed in humans, rats were exposed to relatively brief but significant, variable stress (with mixed physical and psychological adverse stimuli) during juvenility (a period suggested to be relevant to human childhood) [6–8]. Long-term effects on anxiety-like behaviors have been reported in this "juvenile stress" model. For example, juvenile emotional trauma (exposure to predator odor followed by placement on an elevated platform during postnatal day (PND) 28–30) or psychological and physical trauma (compound stressors including restraint, elevated-platform stress, and foot shocks

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delivered in an unpredictable manner during PND 21–32, or 27–29) increases anxiety-like behaviors in adult rats, including impaired the extinction of fear memory in adult rats [9], an enhanced acoustic startle response [10], and anxiety-like behaviors in the open field (OF) and elevated plus-maze (EPM) [11,12]. Juvenile stress was also reported to induce learned helplessness-like behaviors (a type of depression phenotype) in 2-way avoidance task in adult rats [11,12]. However, the ability of juvenile stress to produce other depressive-like behaviors has not been adequately addressed. Anhedonia is one of the core symptoms observed in depressive patients and animals and can be ameliorated with antidepressant treatment [13]. In addition to emotional bias, the impairment of executive function associated with the prefrontal lobe dysfunction is increasingly recognized as a major component of depression [14,15]. The attentional set-shifting test (AST) is a newly developed prefrontal cortex-dependent cognitive rodents task that was adapted from a human-based cognitive task called the Wisconsin Card Sorting Task (WCST) [16,17]. In this study, we will further profile the long-term behavioral consequences of juvenile stress in this model by examining whether juvenile stress can alter the above depressive-like behaviors.

Exposure to juvenile stress has also been found to induce lasting alterations in the limbic brain areas involved in both emotional and cognitive processes, such as alterations in the structure and function of the prefrontal cortex (PFC), the hippocampus (HIP), and the amygdala (AMY) [18–22]: alterations in the expression of cell adhesion molecules and GABA((ϒ-aminobutyric acid) ergic system in limbic areas; and alterations in levels of circulating corticosterone [23]. The central monoaminergic system (including serotonin (5-hydroxytryptamine, 5-HT), noradrenaline (NA) and DA) has been widely implicated in the pathophysiology and therapeutic strategies for emotional disorders [24–26]. In adult rats, chronic variable stress (CVS) causes complex and inconsistent alterations in DAergic, 5-HTergic and NAergic activities in different brain areas depending on the type, duration, and intensity of the stressor and the testing time after the cessation of stress [27–30]. During juvenility, the monoaminergic system, including the output level and the density of transporters and receptors, undergoes rapid and significant development [31–34]. It is unknown if brief CVS during this specific stage has long-term effects on limbic monoamine content during adulthood.

Therefore, the present study aimed to characterize the long-term behavioral and neurochemical effects of juvenile CVS. Furthermore, a correlation analysis was conducted to assess the relationship between monoaminergic activities and patterns of individual behavioral changes. We hypothesized that adverse juvenile experiences would result in changes in the expression of emotion, cognition, and limbic monoamines in adult rats.

2. Materials and methods

2.1. Animals

Twenty male Wistar rats (postnatal day 22, PND22) were obtained from the Academy of Chinese Military Medical Science (Beijing, China) and were acclimated for 5 days prior to the experiments. The animals were housed in groups of 3 or 4 per cage and maintained at 22 ºC on a 12-h light/dark cycle (lights on at 07:00) with free access to food and water except during the sucrose preference test and the AST. All animals were weighed weekly starting at the initiation of the experiment and ending 4 weeks after the completion of the stress procedure. The experimental procedures were approved by the Institutional Review Board of the Institute of Psychology of the Chinese Academy of Sciences.

2.2. Juvenile stress procedure

The juvenile stress procedure was adapted from previous studies [11,12]. The animals were assigned to 2 groups: the juvenile stress group (stress, n = 10) and a control group (control, n = 10). At PND 27, the rats in the stress group were exposed in tandem to different stressors every other day for 1 week (PND 27–33) as detailed below. All stress procedures were performed in a separate room to eliminate the possible effects of vocalization or pheromones on the control rats. The controls were left undisturbed in their room under the previously described maintenance conditions.

Restraint stress (PND 27): The rats were restrained in opaque conical plastic tubes (18.0-cm length and 4-cm internal diameter) for 2 h starting at 08:00. The nose of the tube was cut open to allow the rat to breathe freely. The tubes were secured in polystyrene holders. The rats were not physically squeezed and could rotate from the supine to the prone position, but they could not turn head to tail.

Elevated platform (PND 29 and PND 33): Two 30-min trials were performed: one in the morning (08:00–12:00) and one in the afternoon (12:00–16:00). The rats were returned to their home cages for a 3-h inter-trial interval. For each 30-min trial, the individual rats in the stress group were placed on an elevated platform (12 cm × 12 cm) in the middle of a water pool (180-cm diameter), with the top of the platform 20 cm above the water surface.

Foot shock (PND 31): During this 20-min session, stimulation consisting of electric foot shocks (5 s, 0.9 mA) was delivered 40 times, with a 25-s interval between shocks. The foot shock was performed in a small cube-like chamber (31 cm × 31 cm × 31 cm) with a metal-grid floor that was connected to a computer-controlled electrical shocker device (Med Associates Inc., USA).

2.3. Assessment of emotional and cognitive behaviors in adulthood

On PND 58, which is characterized as early adulthood [6–8], the rats in the stress and control groups were transferred to individual cages. Beginning on PND 61, the following behavioral and cognitive assessments were performed.

2.3.1. Emotional behavior tests

The sucrose preference test is the most commonly used test to measure hedonic alteration, which is the core symptom of depression [35]. The OF and EPM tests have been used to measure anxiety-like behaviors in rodents [36,37].

The sucrose preference test: This test was performed on PND 61 as described in a previous study [38]. The stressed and control groups were deprived of water and food starting at 17:00 h. After 18 h of food and water deprivation, the rats were given a 2-h sucrose preference test (13:00–15:00, PND 62). The rats were given 2 bottles that contained either a 1% sucrose solution or tap water. The amount of liquid intake from each bottle was determined by comparing the bottle weights before and after the 2-h testing window. Sucrose preference was calculated as the percentage of the sucrose intake to the total liquid intake. A low sucrose preference score reflects anhedonia, a core symptom of depression.

OF test: This test was performed between 13:00 and 16:00 on PND 63. The apparatus was a circular arena with a diameter of 180 cm and a 50-cm wall. The test room was dimly illuminated (40 lux) to decrease the averse nature of the test [39]. A rat was placed in the center of the field, and each rat's behavior was recorded for 5 min and analyzed using a computer-based system (Med Associates Inc., USA) that measured the distance traveled and the number of rearings. The OF was cleaned after each test.

EPM test: This test was performed after the OF test as described in a previous study [38]. The maze consisted of 2 opposite open arms (50 cm × 10 cm × 1.3 cm) and 2 opposite closed arms (50 cm × 10 cm × 40 cm). The arms were connected by a central zone (10 cm × 10 cm). The apparatus was 72 cm above the floor and was exposed to dim illumination (40 lux). The rat was placed in the center of the maze facing an open arm. The rat's behavior was recorded for 5 min and analyzed using a computer-based system (Med Associates Inc., USA). The number of entries into the open and closed arms and