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Research report

Parents' adulthood stress induces behavioral and hormonal alterations in male rat offspring

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HIGHLIGHTS

- We examined the effect of parents' adulthood stress on their offspring.
- Chronic stress enhanced anxiety and serum corticosterone levels in adult male and female rats.
- Frequency of maternal licking/grooming decreased among the stressed mothers.
- Parents' stress can induce behavioral and hormonal alterations in male pups through improper maternal care.

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ABSTRACT

Exposure to stress can influence hypothalamo-pituitary-adrenal (HPA) axis in mammals and impair their behavioral/hormonal development. Stress during fetal or early life may have wide range effects on the offspring phenotype in rodents. Since the role of parents' adulthood stress before mating is not fully understood yet, we investigated the effects of parents' adulthood stress on behavioral and hormonal parameters in 10- and 30-day-old male offspring. To induce stress in the adult male and female rats, a repeated forced swimming paradigm was employed daily over the course of 21 days. Then, they were categorized into four parental breeding groups: stressed parents (SP), stressed mother (SM), stressed father (SF) and non-stressed parents (NSP). Anxiety-like behavior was tested in adult rats and 30-day-old male pups, using the elevated plus maze (EPM). The level of serum corticosterone was measured by ELISA in all groups. Stressed adult rats showed enhanced serum corticosterone concentration and anxiety-like behavior. Serum corticosterone level of the 10- and 30-day-old pups of the SP, SM and SF groups was significantly higher than pups from the non-stressed group. Furthermore, 30-day-old pups of the SP, SM and SF groups had lower time spent in the open arms compared to the control group, but stress had no significant effects on the percent of entries into the open arms. In addition, serum corticosterone level in 30-day-old pups were raised by a stressed mother was markedly more than 10-day-old pups. These findings revealed that parents' adulthood stress have negative impacts on behavioral and hormonal responses of their male offspring.

1. Introduction

In recent years, many people have been affected by various stressful situations. The stress response is a normal reflection of the nervous system that generates an alarm signal about a dangerous condition, which is essential for adaptation, but chronic stress and maladaptive response to stress can induce stress related disorders [1]. The vulnerability to stress can be transferred to the next generation. The heritability of stress effects has been investigated in the behavioral, genetic and epigenetic aspects in human and animal models. Not only genetic factors, but also behavior and hormone are essential for the normal development of offspring [2]. Stress has a major effect on the HPA axis function, which is closely correlated with cognition and behavioral responses, and stimulates synthesis and release of corticotropin-releasing factor (CRF) from the hypothalamus [3–5]. CRF increases adrenocorticotropin hormone (ACTH) synthesis and secretion from the anterior pituitary, and leads to glucocorticoids (GCs) production. The main circulating

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glucocorticoid in humans is cortisol, but it is corticosterone in rodents. In animal models, prolonged stress can cause strong HPA axis activation, sustained GCs level elevation, and finally hippocampal structure and function damage [6–8]. Decrement in inhibitory control of the hippocampus on the HPA axis causes a further increase in the circulating GCs levels, subsequent hippocampal damage and behavioral changes [9]. As a result, plasma corticosterone elevation can be considered as a marker of stress vulnerability during the exposure to a stressor. If these alterations find the capacity to transfer to the next generation, offspring will be more sensitive in response to stress. Abnormal mother–offspring interaction can cause HPA reactivity alteration and anxiety-like behavior [10]. In rodents, extending research has investigated the effects of maternal stressor exposure during pregnancy on behavior and endocrine pathologies in offspring [11,12]. In addition, repeated early life stress and improper maternal care can produce anxiety-like behavior in the adult mice offspring in the open-field and EPM tests [13–15]. In human, studies have mainly focused on the maternal stress during pregnancy or postnatal period and have shown that behavioral development of children can be influenced by this issue [16–18]. Furthermore, there are few reports regarding the father's role on offspring's anxiety-like behavior in rodents and humans [2,19]. Based on the mentioned data, it seems necessary to investigate the role of stressful adulthood life of mothers and/or fathers on the offspring's behavior. In this regard, we exposed male and female rats to a stressful condition before mating, using a repeated forced swimming model; and we then analyzed the behavioral and hormonal changes in their male offspring.

2. Methods and materials

2.1. Animals

Male and female Wistar rats aged three months (200–250 g) were obtained from the Pasteur Institute of Iran and housed for one week before the experiment under a steady temperature (21 ± 1 °C) and standard light/dark cycle (light on from 07:00 to 19:00 h). In this experiment, male and female rats were randomly divided into two groups: the repeated swim stress (RSS) group (n = 20 male and 40 female rats); and control group (n = 20 male and 40 female rats). This study was approved by Ethical Committee of Tehran University of Medical Sciences based on National Institutes of Health Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1985).

2.2. Stress model

Animals were swum in 25 °C water in a 70 cm × 40 cm × 80 cm Plexiglas aquarium (35 cm depth of water) for 10 min daily at 08:30 h for 21 consecutive days. In the control group, unstressed rats were handled daily [20].

2.3. Elevated plus maze

EPM is a validated apparatus for measuring the anxiety-like behavior in rodents. The EPM apparatus is made of the four elevated arms above the floor, arranged in two opposite closed arms, two opposite open arms, and a center area. Rats were placed in the intersection of the four arms of the EPM and their behaviors were recorded for 5 min. To measure anxiety-related behavior, all stressed male and female animals were tested in the EPM on day 0 (before), day 11, and day 21 of the forced swimming test. Unstressed rats were also tested in the EPM in the control group. The percent of open arm entries (100 × open/total entries) and time spent in the open arms (100 × open/open + enclosed) were calculated to measure anxiety-like behavior.

2.4. Corticosterone assay

24 h after the final swim stress, blood samples were obtained from all rats (stressed and unstressed) to assess the corticosterone level. This timing was kept consistent in all animals and blood sample was collected under resting condition. Samples were collected by intracardiac puncture between 10:00 and 11:00 h and were centrifuged at 4 °C. Serum samples were stored at −20 °C until being assayed for corticosterone. Corticosterone levels were measured by ELISA kit (Alpco Diagnostics, USA) up to one week after blood collection. Sensitivity of the corticosterone immunoassay was 4.1 ng/ml. The intra-assay coefficient of variation was 4.7% and inter-assay coefficient of variation was 6.5%.

2.5. Breeding and experimental design

After recovery period (one week after the final swim), parental breeding groups of one male and two female rats were formed a group in which both male (n = 10) and female animals (n = 20) were stressed (SP group), a group of stressed females (n = 20) and standard treatment males (n = 10) (SM group), a group of stressed males (n = 10) and standard treatment females (n = 20) (SF group), and a group in which both males (n = 10) and females (n = 20) received standard no-stress treatment (NSP group). After the mating period (10–15 consecutive days) males were removed from the breeding cage and the pregnant females were housed until parturition. Each female rat was housed singly near the labor time. Duration of time after the final swim which breeding took place varied from 38 to 45 days (1 week before mating, 10–15 days prior to conception and 21–23 days for the duration of pregnancy). To achieve litter-size standardization, we selected dams with approximately equal number of pups. In each group, dams with 9–11 pups constituted the highest percentage of dams with equal number of pups. Final sample size per each of the breeding groups was 8 dams. Pups were weaned from the dams after four weeks. Fathers never had any contact with the offspring.

2.6. Observations of maternal behaviors

The behaviors of postnatal dams from each of the four breeding groups were observed three times a day at: 08:30, 13:30, and 18:30 h. This allowed for two observations during the day (lights on) and one at night (lights off). An observer watched and recorded dams' activities at each time point for 75 min. Maternal behavior was observed for seven consecutive days following parturition. Within each observation period, the behavior of each mother was considered every three min (25 observations per period × 3 periods per day = 75 observations/dam/day). We focused on the following activities: dams' licking and grooming (LG) any pup and arched-back nursing (ABN) [21,22].

2.7. Behavioral and hormonal assessment of offspring

One male pup from each dam (8 male pups from each breeding group) was subjected to blood sampling for corticosterone measurement on postnatal days 10 and 30. Pups were also tested in the EPM for behavioral effects on postnatal day 30.

2.8. Statistical analysis

All results are presented as mean ± SEM. Student's t-test was used to assess serum corticosterone data of adult male and female rats. One-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison test was performed for the EPM test of adult rats, maternal observation, litter parameter and susceptibility of offspring. P-Values less than 0.05 were considered statistically significant.

3. Results

3.1. Effect of stress on adult rats in the EPM

In the EPM, The percent of open arm entries and time spent were measured and considered as anxiety-like behavior. In the stressed male and female groups, EPM test at 11 and 21 days of the swimming stress was compared with the self- control (day 0). Since there are controversial reports about the effect of prior exposure to the EPM in rodents' behavior in the open arms [23–26], control male and female animals were also tested in the EPM on day 0, day 11 and day 21 over the course of 21 days. In addition, the maze was placed in a novel situation in each day of the experiment [27,28]. One-way ANOVA revealed that this stress paradigm strikingly increased anxiety in the animals. Subsequent 11 days of the swimming test, the percent of the entries and time spent in the open arms in males (n = 20, P < 0.05, P < 0.01) and females (n = 40, P < 0.001, P < 0.001) significantly decreased compared to the day 0. At the end of the stress phase, male and female rats had lower entries (P < 0.001) and time spent (P < 0.001) in the open arms compared to the self-control groups, while repeated testing in the EPM could not produce such behavioral effects in the control group (Fig. 1A and B).

3.2. Effect of stress on the corticosterone levels in adult rats

After 21 days of repeated swim stress, the level of serum corticosterone was measured by ELISA in male and female rats. According