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Research report

Prenatal Enriched Environment improves emotional and attentional reactivity to adulthood stress

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HIGHLIGHTS

- Wister pregnant dams housed in standard or enriched cages.
- Offspring were exposed to adulthood stress according to experimental groups.
- In offspring, prenatal EE had deteriorating emotional and attentional effects.
- In offspring, prenatal EE followed by adulthood stress resulted in beneficial emotional, attentional and hormonal effects.

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ABSTRACT

Environmental factors seem to play a key role in brain and behavioral development, both in humans and animals. Different environmental manipulations, either pre- or post-natal, have been shown to exert long-term physiological and behavioral effects. While studies in the field of Enriched Environment mainly focus on the post weaning period and provide enrichment as a post adverse-experience manipulation, the preceding effects of prenatal Enriched Environment have rarely been investigated. In this study, we investigated the effects of prenatal Enriched Environment (through the entire pregnancy) followed by adulthood acute stress. In the prenatal Enriched Environment offspring, we found anxiety and depressive-like behaviors with poor attentional performance. Surprisingly, when prenatal Enriched Environment was followed by adulthood stress, we observed a dramatic restoration of these behavioral deficits. Our results suggest that prenatal Enriched Environment may substrate resiliency to adulthood stress.

1. Introduction

The concept of Enriched Environment (EE) was first described by Hebb [1]. Over the years data have accumulated which indicate experience dependent changes in both behavioral and physiological dimensions. The aforementioned changes suggest an active interaction between the animal and its environment [2].

Environmental enrichment involves alterations to the animal's home cage or secondary exploratory area which provide enhanced sensory, motor, cognitive and potentially social opportunities. Although different protocols exist, they all share the same concept of novelty and complexity of the environment. A large space within which the animal experiences exploration and introduction to a variety of objects, varying in shape, size, weight, smell and texture, renders stimulation of visual, somatosensory, and olfactory systems [3].

Many studies in the field of EE investigated the rehabilitative or protective effects of EE in different animal models and multiple implementations. Specifically, EE treatment was found to counteract neurophysiological and behavioral deficits, induced by pharmacological or environmental manipulations [4–6]. Moreover, EE was found to rescue abnormal behaviors, such as emotional reactivity and spatial learning, as well as motor skills deficits, induced by prenatal stress [7–9]. In addition, it was shown that high secretion of corticosterone in response to stress, in the prenatal stressed animals, can be reversed by postnatal EE treatment [8,10]. Furthermore, the exposure to a stressful experience was observed to disrupt sensorimotor gating, often considered a crucial component of normal information processing [11–13]. On the other hand, the exposure to EE, at different ages, has been shown to have a beneficial effect on spontaneously hypertensive rats' performance in

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attention tasks [14] and prevent the degradation of attention performance detected in aged rats [15].

An extensively discussed topic of *in utero* early programming in animal models, revealed the long lasting impact of various fetal manipulations (e.g. maternal stress; exposure to synthetic glucocorticoid, etc.) on different developmental axes such as the neuroendocrine system and its associated behaviors [16]. Given the rehabilitative effects of post-natal (and later) EE and the reported long lasting changes of the various pre-natal manipulations, a question may be raised as for the behavioral and hormonal consequences of implementing a pre-natal EE manipulation. Specifically, how this manipulation may affect the offspring's ability to cope with a stressful experience after birth.

Only a few studies have investigated the effects of prenatal EE. These reports provide evidence of beneficial effects on offspring's behavioral and cognitive performance, specifically on learning and memory [17–19], with indications of brain structural changes [19]. A recently published study has shown that the exposure to prenatal EE has long-term emotional and hormonal effects [20].

In the current study, we aimed to further explore the long-term effects of prenatal EE on both behavioral and hormonal manifestations. We concentrated on better understanding the potential predispositional effect of prenatal EE followed by adulthood stress and the process of prenatal adaptation, by which offspring are perhaps prepared to the environment into which they are to be born. Thus, we investigated rats' hormonal, emotional and attentional reactivity to acute stress in adulthood, following a prenatal EE manipulation.

2. Materials and methods

2.1. Animals

Twenty male rats (PND 22) and 10 pregnant female Wistar rats (at the first week of pregnancy weighting between 260–290 gr) were purchased from Harlan (Harlan, Israel). Male rats were housed 4 per cage in standard plastic cages (30 cm × 30 cm × 18 cm) with sterilized sawdust bedding. In order to estimate a possible litter effect, we examined the behavioral heterogeneity between the subjects in each group. Indeed, a considerable standard error of the means (S.E.M.) in the groups and normal distribution suggests a minimal litter effect. Female pregnant rats were housed in an EE cage (see procedure). Dams were examined at least twice a day for parturition. Once a delivery was observed, each dam was carefully relocated (within several hours after parturition) with her offspring to a standard cage. At PND 21, offspring were weaned and relocated without dams and only male rats were included in the experiment (rats were housed 4 per cage). It should be emphasized that in order to maintain genetic variability: (i) two male siblings were assigned, one to the pEE ($N = 10$) and the other to the pEE & Stress ($N = 10$) group; (ii) the additional 20 male rats that were purchased, were randomly assigned to the control and adulthood-stress groups. Room temperature maintained at 23 ± 1 °C with 67% humidity at 12:12 day/night cycle (lights on at 07:00). Food and water access allowed ad libitum. Animals were weighted twice a week. This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering.

2.2. Procedure

The experiment included 4 groups: Control ($N = 10$), prenatal Enriched Environment (pEE; $N = 10$), adulthood stress with no preceding pEE (Stress; $N = 10$), pEE and adulthood stress (pEE & Stress; $N = 10$). Control and adulthood stressed rats were housed in standard conditions, during the entire experiment. Pregnant female rats were housed in EE cages. At PND 21 male offspring were separated from their dams and reared in standard conditions. At PND 80-82, stress procedure was executed for the relevant groups (i.e. adulthood stress; pEE & Stress). Starting at PND 83, rats were tested in various behavioral tools. We began with the Sucrose Preference Test (SPT; until PND90). Twenty-four hours later, rats were tested in the Object Recognition (OR; PND91-3). Finally, at PND 96 rats were examined in the freezing test and at PND 97 in the Pre-Pulse Inhibition (PPI) test. All behavioral procedure and tests were carried in a dimly lit room (50 lx). All behavioral tests were conducted between 1000 and 1400.

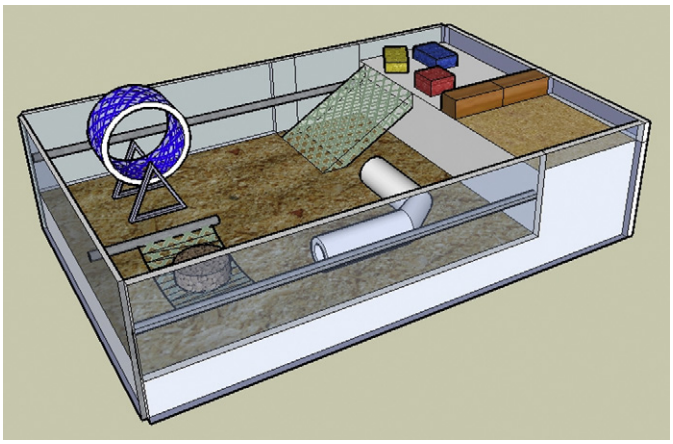

Fig. 1. A schematic description of the enriched environment cage. Enriched cage (100 cm L × 50 cm W × 50 cm H) consisted of various objects such as: running wheel, stairs, tubes, lego, wood parts, an extra elevated (25 cm) surface (20 cm L × 50 cm W) that was accessible via stairs, in order to diverse the cage texture.

2.2.1. Prenatal Enriched Environment (pEE)

Ten pregnant rats were placed in a customized enriched environment cage (100 cm L × 50 cm W × 50 cm H; Fig. 1) for approximately 3 weeks, until delivery day (in order to encompass the entire gestational period). Cage consisted of various objects (e.g. running wheel, stairs, tubes, lego, wood parts) which were weekly replaced and cleaned. The cage also consisted of an extra elevated (25 cm) surface (20 cm L × 50 cm W) that was accessible via stairs. In addition, a sandbox (20 cm L × 10 cm W) was included in the main floor; in order to diverse the cage texture. In order to maintain social enrichment, without over- crowding, 5 pregnant rats were housed in each EE cage. During their stay in the cage, rats had free access to food and water. Cages were located in a temperature and humidity controlled room, under day/night cycle (lights on between 07:00 and 19:00).

2.2.2. Adulthood stress

Adulthood stress was comprised of three separated procedures delivered randomly on 3 consecutive days at PND 80-82 (one procedure each day).

Acute swim. At PND 80, rats were allowed 15 min to swim in a squared water tank: 38 cm × 30 cm, water depth: 60 cm. Water temperature maintained at 23 ± 1 °C [21,22].

Elevated Platform. At PND 81, rats were placed on a 50 cm high, 10 cm diameter platform, three times for 30 min each time with 1 h spent in a resting cage between periods on the platform [21,22].

Restraint stress. At PND 82, rats were placed in a radial-shaped metal restrainer with (6 cm height, for animals that weigh 250 ± 50 g), which allowed a small amount of lateral movement. The exposure regime was according to that of the elevated platform [22].

2.3. Behavioral tests

2.3.1. Sucrose preference test (SPT)

During the acclimatization period (1 week), rats are allowed to consume 10% (w/v) sucrose/water solution or tap water, in order to overcome neophobia. Moreover, they are under water limitation (access allowed for 4 h a day). On the 6th day the total amount of liquid consumption is assessed (over a period of 4 h), to detect general differences in liquid consumption. Following acclimation period, on the 8th day, the test is carried out individually for each rat: following a 16 h of water deprivation, two drinking bottles, identical to the home cage water bottles, are inserted into the cage through the metal mesh top cover. The bottles, one containing a 10% sucrose solution and the other water, are weighed just before the test and immediately following its completion, after 4 h. The relative positioning of the bottles providing sucrose and water is reversed after 2 h, in order to prevent the development of side preference. The initial relative positioning of the bottles is counterbalanced between groups [23].

2.3.2. Object recognition

The test is taking place in a black lusterless Perspex box (50 cm × 50 cm). Rats are acclimated to the test arena for 10 min per day during two successive days, i.e.; they are allowed to explore the arena without any objects. At the beginning, the rat is placed in the distal part of the box, facing the wall. On the third day, rats are allowed to explore the arena for 10 min, with the present of two identical objects. Following the first exploration session, rats are removed and placed in home cages. Subsequently, after 2 h rats are placed back in the arena (which is being cleaned) for the test trial for 10 min divided into 5 min of habituation (no objects) and 5 min with two unidentical objects (before locating the objects, the rat is removed and