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Prenatal stress decreases spatial learning and memory retrieval of the adult male offspring of rats

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HIGHLIGHTS

- Heterogeneous sequential stress (HSS) used as a model for prenatal stress (PS)
- PS applied before pregnancy (BPS), during first (PS1) and second (PS2) half of it
- HSS increased serum corticosterone of BPS and PS1 adult offspring and PS2 mothers.
- Latency and distance of BPS and PS1 increased in acquisition and retrieval tests.
- HSS decreased learning and memory of adult offspring in BPS and PS1, prominently.

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ABSTRACT

Introduction: Early life or prenatal stress induces many lifelong, mostly cognitive, homeostatic alterations in the behavior of the offspring.
Purpose: We investigated the effect of heterogeneous sequential stress (HSS) at three separate periods, before and during the first and second half of pregnancies on spatial learning and memory retrieval of adult male offspring.
Method: HSS is composed of several stressors, each in a day, during nine consecutive days including; restraint, swimming, isolation, and water and food deprivation on Wistar rats. The offspring were studied in a Morris water maze (MWM) apparatus to explore the latency, distance, proximity and target to opposite area as measures of learning and memory. Serum corticosterone was measured as a criterion of stress application.
Results: HSS increased blood corticosterone in dams of PS2 (Pregnancy Stress second half), and also in adult male offspring from BPS (Before Pregnancy Stress) and PS1 (Pregnancy Stress first half) groups. The weight of the offspring decreased in the PS1 and PS2 groups. While distance traveled and latency to locate the hidden platform were increased in BPS and PS1 acquisition trials, swimming speed was unchanged during the acquisition and retrieval tests. Moreover, time to platform location was increased in BPS and PS1 during retention tests. While control rats spent more time in the target quadrant, stressed animals spent a longer duration in the opposite quadrant. Furthermore, proximity measure was increased in all stress treated rats.
Conclusion: It is concluded that prenatal stress, around the beginning of the pregnancy, increases corticosterone in adult male offspring, which might be the basis for spatial learning and memory retrieval deficits in this study.

1. Introduction

Prenatal stress (PS), or adverse life events, during gestation have many deleterious effects on the development and behavior of the offspring [1]. PS may result in adverse effects on social and sexual behaviors and may increase the risk for anxiogenic and depressive-like behaviors, as well as some schizophrenic features. Earthquakes, as natural source of stressors during pregnancy displayed a more depressive state in newborns [2]. Furthermore, PS along with other uncontrolled and prolonged stress, raises the HPA (hypothalamic–pituitary–adrenal axis) activities and leads to higher cortisol or corticosterone in mother's blood [3–7]. In this regard, maternal corticosterone can cross the placental barrier and influence the fetal brain [8], as HPA develops toward the end of pregnancy. However, fetal corticosterone alterations following a mother's response to stress depend on the time that stress is applied during pregnancy [9].

Abbreviations: HPA, hypothalamic–pituitary–adrenal; MWM, Morris water maze; HSS, heterogeneous sequential stress; BPS, Before Pregnancy Stress; PS1, Pregnancy Stress first half; PS2, Pregnancy Stress second half.

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The timing of stress experience is an important factor determining the severity of structural and functional outcomes [10]. Most studies show PS induced learning and memory deficits during the second half of pregnancy [11–14]. For instance, prenatal stress during the second half, decreased spatial learning which may be the consequence of a deficit in neurogenesis correlated with dysfunction of the HPA axis [15]. On the other hand, there are a number of human studies highlighting the effect of stress on the early days of pregnancy specifically during the early gestational period, which leads to lower birth weight, preterm birth labors and more severe motor impairments [1,16]. Moreover, stress before or around pregnancy severely affects the future developmental fate of a fetus [16]. For example, Schachar-Dadon et al. showed that unpredictable variable stress before conception alters affective and social behavior in the adult offspring [17].

PS imposes some behavioral and neurobiological effects related to hippocampal function [18]. As such, deficits in learning and memory of adult offspring are important consequences of stress experience [19]. For instance, Sahu et al. showed that PS from gestation day 11 until delivery increased escape latency in water maze test [20]. Similarly, daily varied gestational stress on gestational days (GD) 17–21 [14] and also GD 15–19 [7] impaired spatial learning in the MWM [7,14]; however there are few reports of enhancing learning capability due to PS [21,22], which indicates the controversy of the stress effect during gestation. Since PS has short and long term effects on learning and memory, and stress during or before pregnancy has developmental effects on HPA axis and other neurodevelopmental cognitive outcomes, it seems likely that prenatal or preconception stress will affect learning and memory in the adult offspring. Thus, in the current study, we are investigating the effect of PS on spatial learning and memory retrieval of offspring, and how corticosterone changes concomitantly after chronic PS application in three time periods; before pregnancy (BPS), during the first half (PS1) and second half of pregnancy (PS2). This will help determine the role of gestational corticosterone on learning and memory changes in adult offspring.

2. Methods

2.1. Animals

Virgin female Wistar (Iran Pasteur Institute) rats (16 dams) weighing 200–220 g at the beginning of experiments were used and paired randomly with male Wistar rats. To ensure pregnancy, vaginal smears were checked on a slide every day at 7:00 am. The day of sperm detection was considered GD 0, males were taken out of the breeding cage and delivery was expected 21 days later. Each dam plus its litter was maintained in the home cage on wood flaked bedding in standard lab conditions, until weaning at 30 days postnatal. Then, male pups were selected (two male pups per mother to avoid litter effect), and grown 5 per cage normally until 2 months old, when they were used for water maze procedure. All experiments were done in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 23-80, revised 1996) and were approved by the research ethical standards for the care and use of animals in Damghan University. Rats were kept 5 per cage, except pregnant dams which were kept alone in the cage; all were under normal 12–12 light–dark cycle, with lights on at 07:00 am, and food and water ad libitum.

2.2. Experimental groups

Female rats were randomly assorted into four experimental groups (8 animals in each group): (1) Control; (2) Before Pregnancy Stress (BPS); (3) First half Pregnancy Stress (PS1); and (4) Second half Pregnancy Stress (PS2). Adult male offspring of these control and stressed mothers were used to investigate spatial learning and memory retrieval in water maze task. The timeline of experiments is illustrated in Fig. 1.

2.3. Stress protocol

We used the heterogeneous sequential stress (HSS) model to investigate learning and memory changes in stressed mother's offspring.

HSS was applied in 9 days [23] and included several daily stress paradigms as follows:

1. Forced swimming (22 ± 2 °C) for 10 min.
2. Restraint stress for 3 h,
3. Water deprivation for 24 h,
4. Restraint at 4 °C for 1.5 h,
5. Isolation from others for 24 h,
6. Food deprivation for 24 h,
7. Water deprivation for 24 h,
8. Restraint at 4 °C for 2 h,
9. Food deprivation for 24 h [23].

The timing of HSS is outlined below:

Treatment	Start of treatment
BPS	10 days before mating
PS1	GD 0
PS2	GD 11

The rate of successful mating was 7 out of 10 and stressed female rats with unsuccessful mating for 48 h were excluded from the study. Neither the mothers nor the pups received stress in the control non-stressed group and they were left undisturbed in the colony cage until MWM test.

2.4. Corticosterone assay

Blood samples were taken from mothers one day after the termination of HSS application and from adult offspring after finalizing MWM task, all through the tail lateral vein in conscious restrained animal by an expert lab technician. To avoid stress to neonates, sampling was performed from its male sibling from home cage on the second day of delivery (2 days old) by heart puncture. Sampling from the control dams was similar to the PS2 group. All of the blood samples were transferred to an anticoagulant coated tube using sodium citrate. After mixing, the specimen was centrifuged in 10,000 rpm for 15 min and the resulted plasma was stored in −80 °C freezers until assay. Concentration of corticosterone in plasma was measured using a corticosterone EIA competitive assay kit (Cayman Chemical; cat, 500655). On the testing day, supernatants (10 μl from 100 μl) were incubated with reaction mix solution for 30 min at 37 °C. Absorption was determined at 450 nm using a spectrophotometer and amounts of corticosterone were determined based on the standard curve. The sensitivity of the kit was 150 pg/ml. Intra-assay and inter-assay coefficients of variation were 6.6% and 5.7%, respectively.

2.5. Water maze test

A circular tank (140 cm in diameter, 60 cm high) filled with water at a temperature of 24 ± 2 °C to depth of 30 cm, was used for the procedure. An escape invisible platform (15 cm in diameter) was placed 2 cm below the water surface, midway between the center and rim of the pool in the same quadrant throughout the task with the spatial cues available around the pool room. One day before the behavioral testing, each animal was habituated for 1 min on the platform to explore the pool. During the learning task, animals had to navigate the hidden platform, given four trials a day for 5 days, while the starting positions changed randomly over trials. Each trial began with the animal in the pool facing the sidewalls and ended either after 60 s of swimming or reaching the platform. The rat was then allowed to remain on the platform for 10 s after each trial. A probe task was performed 24 h