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Research report

Exposure to variable prenatal stress in rats: Effects on anxiety-related behaviors, innate and contextual fear, and fear extinction

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HIGHLIGHTS

- Prenatal stress leads to decreased innate fear responses to predator odor.
- Prenatal stress leads to impaired fear extinction.
- Prenatal stress leads to increased locomotor activity and stereotypy.
- Prenatal stress was not associated with increased anxiety-like behaviors.

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ABSTRACT

Rats repeatedly exposed to variable prenatal stress (PNS) exhibit behavioral features often observed in neuropsychiatric disorders including elevated sensitivity to stimulants and impairments of attention, inhibitory control and memory-related task performance. However, to date there have been relatively few studies designed to assess the effects of PNS on anxiety, stress and fear responses, or the function of the hypothalamic-pituitary-adrenal (HPA) axis (a system clearly linked to stress and fear-related responses as well as neuropsychiatric disorders). In the current study, rats exposed to variable PNS were evaluated for anxiety-related behaviors in open field, elevated plus maze, and light/dark preference tasks. Innate fear responses were assessed using a predatory odor task and learned fear and extinction were assessed with a contextual fear conditioning task. As an indicator of HPA axis function, serum corticosterone levels were determined by enzyme immunoassay at various time points. The results indicated that PNS resulted in several behavioral anomalies including decreased innate fear responses to predator odor, impaired fear extinction, increased locomotor activity and stereotypic-like behaviors. Baseline levels of corticosterone in PNS subjects were similar to non-stressed controls; however, when exposed to acute stress, they exhibited an increase in corticosterone that was greater in magnitude. PNS was not associated with increased anxiety-like behaviors or deficits in learning or retention during contextual fear conditioning. Collectivity, these data support the argument that variable PNS in rats is a valid model system for studying some behavioral components of neuropsychiatric disorders as well as the influence of stress hormones.

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1. Introduction

During the prenatal period in mammalian species, the rapid growth of the central nervous system makes the fetus particularly vulnerable to insults [30]. This phenomenon is evident from the results of several independent (and prospective) human studies which indicate that maternal stress during pregnancy is associated with adverse neurodevelopmental outcomes in the child later in life, including attention-deficit/hyperactivity disorder (ADHD), autism, schizophrenia, and anxiety disorders [2,12,20,25,27,43,49,51]. However, there are many aspects of this relationship (i.e., prenatal stress to neuropsychiatric disorders) that are unclear and the development of appropriate animal models for the purpose of elucidating this relationship as well as for evaluating novel therapeutic interventions is greatly needed [29,55]. It has been suggested that repeated variable prenatal stress in rodents (henceforth referred to as PNS) might be an etiologically appropriate neurodevelopmental model for some components of schizophrenia [22]. Exposure to variable PNS was previously found to result in social withdrawal, elevated amphetamine-induced locomotor activity, and deficits in sensory-motor gating; behavioral

Abbreviations: PNS, prenatal stress; ADHD, attention deficit/hyperactivity disorder; PTSD, post traumatic stress disorder; HPA, hypothalamic-pituitary-adrenal; EPM, elevated plus maze; CFC, contextual fear conditioning; GD, gestational day; PND, postnatal day; ED, extinction day.

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characteristics commonly associated with a schizophrenia-related phenotype [22,24]. Further, PNS subjects were also found to exhibit impairments in spatial and recognition memory-related tasks, attention, and inhibitory control [31,52].

While several domains of cognition have been evaluated in the variable PNS model, relatively little is known about its effects on anxiety, stress, and fear-related responses. Anxiety, maladaptive fear responses, and impaired fear extinction are primary symptoms of a number of neuropsychiatric disorders including post traumatic stress disorder (PTSD) and schizophrenia. An additional question relates to how PNS might affect the function of the hypothalamic-pituitary-adrenal (HPA) axis, a system that is intimately linked to stress, and fear-related responses. It is relatively well established that dysfunction of the HPA axis is a common feature in adults with neuropsychiatric disorders (e.g., schizophrenia, anxiety disorders) [14,28,54], however, it is currently unclear how PNS might alter HPA axis regulation and affect the susceptibility to psychopathology later in life [9,15,19,32]. Interestingly, Koenig and co-workers [21] previously reported that rats exposed to variable PNS have significantly higher plasma levels of the stress hormone corticosterone following exposure to a mild restraint stress than control animals suggesting an altered response of the HPA axis to acute stress. Importantly, corticosterone (in rodents) is known to play a crucial role in anxiety-related behaviors as well cognitive processes including conditioned fear and extinction learning [13,34,45].

There were, therefore, four objectives of the current study: (1) to determine if PNS in rats is associated with anxiety like behaviors in open field, elevated plus maze (EPM), and light/dark preference tasks, (2) to determine if PNS produces impairments in innate fear responses (using cat odor), (3) to determine if PNS is associated with learning, memory, and extinction deficits in a fear conditioning task (CFC), and (4) to determine how PNS affects the function of the HPA (via measurements of serum corticosterone) in the adult.

2. Material and methods

2.1. Animals

Timed pregnant Sprague-Dawley female rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN, USA) arriving on day five of gestation were housed individually in a temperature-controlled (25 °C) and light-controlled (12-h light/dark cycle) facility. Pregnant animals had free access to food (Teklad Rodent Diet 8604 pellets, Harlan, Madison, WI, USA) and water following their arrival. All procedure employed during this study were reviewed and approved by the Georgia Health Sciences University Institutional Animal Care and Use Committee and are consistent with the AAALAC guidelines. Measures were taken to minimize pain or discomfort in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. Animals were handled for one week (beginning on postnatal day, PND 56) prior to all testing and training to reduce human contact-related stress and anxiety-related behaviors. Study subjects were transferred (in their home cages) to the behavioral testing rooms each morning approximately 30 min prior to training and testing. Both male and female rats were tested in each behavioral task and testing began on PND 63.

2.2. Stress paradigm

The repeated variable prenatal stress paradigm used in this study was adapted from Koenig and co-workers [21,22]. Pregnant rats were exposed to the paradigm beginning on day 14 of gestation until delivery of pups on gestational day 22 or 23. The stress paradigm consisted of: (1) restraint in Broome style rodent restrainers (PLAS Labs, Inc.) (1 h); (2) exposure to a cold environment (4 ± 1 °C, 6 h); (3) overnight food deprivation; (4) forced swim in room temperature water (15 min); (5) reversal of the light–dark cycle; and (6) social stress induced by overcrowded housing during the dark phase of the cycle. Stressors were applied in a randomized manner to prevent accommodation with one to three stress sessions per day. Pregnant control rats were exposed to normal animal care and maintenance procedures during this period. Following birth, all dams and pups were left undisturbed until weaning on postnatal day 22. Offspring were double housed with same sex littermate, food and water was allowed ad libitum. Exposure to the prenatal stress procedures did not result in changes to the number of live born pups or the latency to parturition. Further, there were no differences in the number of pups per litter or the ratio of male to female pups between the control groups or those exposed to the paradigm (data not shown).

2.3. Serum corticosterone levels

2.3.1. Serum sample collection

Blood sample collection was performed on both pregnant rats exposed to the variable prenatal stress paradigm and pregnant control rats. Samples were collected on gestational day 13 (baseline levels), day 16 (3rd day of stress paradigm), and day 19 (6th day of stress paradigm). Samples were taken at a specific time point for each animal on all days which correlated with the completion of stress in day 16 and day 19. To minimize additional stress associated with the blood sampling, dams were anesthetized by delivering isoflourane via a Vetroson small animal anesthesia machine (Summit Hill Laboratories, Wavesink, NJ). Animals were under approximately 90 s, during which blood was collected from the tail. Blood sample collection was also performed on both control and prenatally stressed (PNS) pups (i.e., both male and female) on PND 75 with minimal stress and PND 76 following a foot shock (1 mA AC, 1000 ms) 30 min prior to collection. Rats were placed on heating pad (to promote vasodilation) and briefly anesthetized (<90 s) using isofluorane. Blood was taken by using a razor to produce a small tail nick and collected in BD Microtainer® tubes (approx. 100 μl) with serum separator (Becton, Dickinson and Company, Franklin Lakes, NJ). Collected blood was incubated at room temperature for 30 min to allow clotting, followed by centrifugation at 6000 × *g* for 90 sec. Serum samples were stored at −20 °C.

2.3.2. Corticosterone measurements

Serum corticosterone levels were determined using a corticosterone enzyme immunoassay (EIA) kit (Abnova, Taipei City, Taiwan). The EIA was carried out according to the manufacturer's instructions. Absorbance was measured at 450 nm and concentrations were calculated from the measured absorbance values using Gen5 data analysis software (BioTek, Winooski, VT). The intra-assay and inter-assay coefficients of variation ranged between 4.9% and 7.4%.

2.4. Locomotor activity

Rat open field activity monitors (43.2 cm × 43.2 cm, Med Associates, St. Albans, VT, USA) were used to analyze locomotor activity of control and PNS rats in a novel environment. Animals were placed in the middle of the test arena facing the back wall. The following parameters were recorded for the entire 30 min test duration: horizontal activity (horizontal photobeam breaks or counts), number of stereotypical movements (repeated photobeam breaks), and vertical activity (vertical photobeam breaks). Thus, spontaneous locomotor activity, exploratory activity (rearing and sniffing movements) and stereotypical movements were assessed. Time spent in the center and peripheral zone was also recorded.

2.5. Elevated plus maze

To assess the effects of prenatal stress on anxiety, animals were evaluated using an EPM consisting of a plus shaped maze made of opaque Plexiglas with two opposite open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm with 40 cm walls) under low lighting (50 lx, lumen/m^2). The task was initiated by placing the test subject into the center of the maze facing an open arm. Activity was monitored via a mounted overhead camera and video tracking system (Noldus EthoVision® Pro 3.1) for a 10 min period. The total distance traveled, entries into each arm, and the time spent in open arms, closed arms, and center of maze were evaluated. The frequency of head dips (the rat dipping its head into the space below the open arm and observing the environment) was also scored manually from the video files).

2.6. Light/dark preference test

To further assess the effects of prenatal stress on anxiety levels, a light/dark preference test (also referred to as light/dark exploration or emergence neophobia test) was conducted. For these experiments, Med Associates Inc. (St. Albans, VT) rat open field activity monitors (43.2 cm × 43.2 cm) fitted with dark box inserts (which are opaque to visible light) to cover half the open field area, thus separating the apparatus into two zones of equal area (i.e., a brightly lit zone and a darkened zone) were utilized. Lamps were located above the activity monitors to provide an illumination level of approximately 1000 lx in the brightly lit zone, whereas the illumination level in the darkened zone was approximately 5 lx. The task was initiated by placing the test subject into the lighted zone of the activity chamber. Time spent in the light and dark zones of the apparatus was monitored and recorded continuously for 5 min. Latency to entry into dark zone was also recorded.

2.7. Predatory odor avoidance task

A predatory odor avoidance task was used to determine if prenatal stress results in alterations of innate defensive behaviors toward predatory odors [38]. Cat hair (compressed into a ball, 10 cm in diameter) was obtained from a domestic male cat prior to the day of testing and kept in airtight (Zip-Lock) plastic bag until use. Similar textured "fake" hair (polyester fiber filling) was used as a control stimulus. Testing was conducted in a 35 cm × 26 cm × 50 cm box under low lighting (70 lx). The arena was divided into four equal quadrants. Animals were recorded via an overhead camera. The box was cleaned with 10% alcohol solution between each

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