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Prenatal alcohol exposure and adolescent stress increase sensitivity to stress and gonadal hormone influences on cognition in adult female rats

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HIGHLIGHTS

- Unique metabolic effects of prenatal treatment on postnatal weight gain
- Show novel interactions of HPA, HPG and cognitive function in PAE animals alone
- Interactions were differently impacted by the adolescent environment.
- Illustrates sexually dimorphic outcomes as a consequence of environmental factors

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ABSTRACT

Abnormal activity of stress hormone (hypothalamic–pituitary–adrenal [HPA]), and gonadal hormone (hypothalamic–pituitary–gonadal [HPG]) systems is reported following prenatal alcohol exposure (PAE). PAE increases vulnerability of brain regions involved in regulation of these systems to stressors or challenges during sensitive periods of development, such as adolescence. In addition, HPA and HPG functions are linked to higher order functions such as executive function (EF), with dysregulation of either system adversely affecting EF processes, including attention and response inhibition, that influence cognition. However, how HPA and HPG systems interact to influence cognitive performance in individuals with an FASD is not fully understood. To investigate, we used a rat model of moderate PAE. Adolescent female PAE and control offspring were exposed to 10 days of chronic mild stress (CMS) and cognitive function was assessed on the radial arm maze (RAM) in adulthood. On the final test day, animals were sacrificed, with blood collected for hormone analyses, and vaginal smears taken to assess estrus stage at the time of termination. Analyses showed that adolescent CMS significantly increased levels of CORT and RAM errors during proestrus in adult PAE but not control females. Moreover, CORT levels were correlated with estradiol levels and with RAM errors, but only in PAE females, with outcome dependent on adolescent CMS condition. These results suggest that PAE increases sensitivity to the influences of stress and gonadal hormones on cognition, and thus, in turn, that HPA and HPG dysregulation may underlie some of the deficits in executive function described previously in PAE females.

1. Introduction

Prenatal alcohol exposure (PAE) produces a range of cognitive, behavioral, and physiological abnormalities, which fall under the umbrella term of fetal alcohol spectrum disorder (FASD). Many of the cognitive abnormalities in children with FASD are caused by disturbances in executive function (EF), which includes deficits in attention, planning, inhibition and appropriate behaviors that could contribute to impairments in learning and memory [54,56]. Deficits vary considerably among individuals, even if timing and extent of alcohol exposure are taken into account.

In addition to cognitive deficits, results of both human studies and animal models indicate that PAE-related abnormalities in physiological function may also occur, including alterations in hypothalamic–pituitary–adrenal (HPA) and hypothalamic–pituitary–gonadal (HPG) activity and regulation. Dysregulation of the HPA axis following PAE is well documented in both the clinical [25,32,49] and the animal [30,38,57,66,68] literature. Furthermore, children with an FASD are more likely than their non-exposed counterparts to experience adverse conditions in early life (for review see [41]). Exposure to stressors during vulnerable periods of development may not only exacerbate PAE-related dysregulation of the HPA axis [29,50,68], but also result in synergistic effects with PAE, at least during the adolescent period [31]. As excessive levels of glucocorticoid hormones (cortisol in humans, corticosterone, CORT, in most rodents) are often detrimental to cognition [11,12], enhanced stress responsiveness could further potentiate PAE-related

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cognitive deficits. Furthermore, as the HPA axis appears to show increased plasticity during postnatal development, especially during adolescence [3,22,43] adverse experiences during this period may have significant consequences for functioning later in life [1,44,70].

Sex and gender effects add to the variability in outcome following PAE. Cognitive functions are known to be altered by ovarian hormones, especially estradiol (E_2), although whether its influence is positive or negative appears less clear [7,14,34]. In rats, estradiol can enhance cognitive performance under certain conditions [9,15], but also enhance activity of the HPA axis [6], which could lead to impairments in cognition [12]. Indeed, the function of the HPA axis is highly influenced by the activity of the HPG system. Estrogens appear to have an overall stimulatory effect on HPA function, as they can disrupt negative feedback and directly stimulate production of glucocorticoids, amplifying hormonal responses to stress [42,51,69]. In turn, the HPA hormones can modulate activity of the HPG axis. Of importance, PAE is known to alter both HPG and HPA activity and regulation as well as interactions between these systems [28,37,68]. For example, we have shown previously that PAE-induced changes in HPG and HPA activity may be estrous phase-specific [36]. PAE, but not control females had higher basal and stress E_2 levels in proestrus (when E_2 levels are normally high) compared to other phases of the cycle, and greater variation in LH than control females across the cycle. As well, both basal and stress CORT levels and overall ACTH levels were greater in PAE than control females in proestrus. These data suggest that altered HPA–HPG interactions may differentially affect sensitivity to ovarian steroids in PAE compared to control females.

Despite data showing that HPA and HPG systems appear especially vulnerable to challenges during sensitive periods of development, that HPA and HPG functions are linked to higher order functions such as executive function (EF), and that PAE alters both HPA and HPG systems, how HPA and HPG systems interact to influence EF in individuals with FASD, and particularly in females, is not fully understood. We hypothesized that PAE would produce an increased sensitivity to the physiological and behavioral effects of CORT and E_2, which would be further impacted by a period of chronic stress during adolescence, a sensitive period of development, translating into altered cognitive function in adulthood. To investigate, we used our well-established rat model of moderate PAE. Female offspring were exposed to a 10-day period of chronic mild stress (CMS) during adolescence, followed by a battery of behavioral tests, the last of which was the radial arm maze (RAM), a task that assesses aspects of EF such as working memory. Animals were sacrificed on the final RAM test day and we assessed the possibility that PAE females would exhibit altered sensitivity to the influence of stress and sex hormones on cognitive function in adulthood.

2. Material and methods

2.1. Animals and prenatal treatments

This study utilized 102 female offspring obtained from 66 litters (24 PAE, 19 pair-fed, 23 ad libitum-fed control). Sprague Dawley rats were obtained from Charles River Laboratories Inc., St. Constant, Quebec. Animals were pair-housed in standard Plexiglas cages on a ventilated rack with same sex cage mates for a 1–2 week adaptation period, provided with laboratory chow and water ad libitum, and maintained on a 12:12 light–dark cycle. All experimental protocols were carried out in accordance with the Canadian Council on Animal Care guidelines and the Guide for the Care and Use of Laboratory Animals [8].

At the start of breeding, females were individually paired with a single male just prior to lights-off. Each morning thereafter, vaginal smears were taken shortly after lights-on to check for the presence of sperm, which confirmed pregnancy and was denoted as gestation day (GD) 1. At this time, the dam was removed from the male, singly housed on a separate ventilated rack and randomly assigned to one of three prenatal treatment groups: Prenatal alcohol exposure (PAE; ad libitum access to a liquid ethanol diet, 36% ethanol-derived calories); pair-fed (PF; yoked to a PAE dam and provided liquid control liquid diet (maltose–dextrin isocalorically substituted for ethanol) in the amount consumed by their PAE partner (g/kg/body weight/gestation day)); or control (Con; pelleted control diet ad libitum). Experimental diets were prepared by Dyets Inc. Bethlehem, PA (Weinberg/Keiver High Protein Experimental Diet #710324, and Control Diet #710109) and formulated to provide optimal nutrition, regardless of ethanol intake. Food consumption was recorded daily and fresh diet presented approximately 1 h before lights-off, as studies have shown that under restricted feeding conditions, such as those of the pair-fed group, circadian rhythms re-entrain to the feeding time rather than the light cycle. Feeding at lights off, toward the peak of the CORT circadian rhythm, results in normal circadian elevations, comparable to those in control animals [18,61]. On GD 21, experimental diets were replaced with laboratory rat chow (19% Protein Extruded Rodent Diet, #2019, Teklad Global). Throughout, dams were maintained in a controlled environment (21 °C) on a 12/12 light/dark cycle (lights on 0800–2000 h), with ad libitum access to water.

On postnatal day (PND) 1, offspring and dams were weighed and litters culled to ten (five females, five males when possible), and the dam and litter were moved to standard Plexiglas cages with environment controlled filtered lids. Cages were changed weekly thereafter at which time dam and offspring weights were recorded. On PND 22, pups were weaned, ear notched for identification purposes, and housed with same-sex littermates. Only the results from female offspring were assessed in this study. At PND 30, two females from each litter (with a few exceptions in which a single female was taken from a given dam) were pair-housed with same-sex, same prenatal treatment, non-littermate cage partners. Female cage-mates were then assigned together to either the CMS or Non-CMS experimental condition, and remained housed together throughout testing until the end of the study. Animals in the CMS and Non-CMS conditions were housed in separate colony rooms.

2.2. Chronic mild stress

Animals in the CMS condition were exposed to mild stressors twice a day for ten consecutive days, on PNDs 31–41, during the peri-pubertal period, as defined by vaginal opening [71]. Morning stressors took place between 0700 and 1100 h, and afternoon stressors took place between 1300 and 1830 h. Animals were assigned to cohorts by age so that their entrance into the CMS condition could be coordinated. All animals received the same number of each stressor over the 10 days of CMS, but the randomized order and timing of stressors varied by cohort. This CMS regimen was designed to be as unpredictable as possible, as research has shown that female rats do not habituate to unpredictable stressors in the same manner as they do predictable ones [47,48,65].

The stressors utilized included: soiled cage (another animal's soiled bedding), 60 min; cage tilt on a 30° angle, 120 min; elevated platform, 10 min; restraint stress in a PVC tube (tube varied with animal size to ensure a snug fit without physical pressure on the animal), 30 min; cage with novel bedding, 60 min; overnight social isolation, immediately followed by a period of water deprivation in the home cage in the presence of an empty water bottle, 60 min. All stressors, with the exception of the water deprivation, took place in a test room separate from the colony room.

All animals (CMS and Non-CMS) were weighed on the first and fifth days of CMS, as well as on the morning following the last day of CMS exposure.

In addition, basal blood samples were taken by tail nick on the first day of CMS and again the morning following the last day of the CMS period. Blood collection occurred in an adjacent procedure room within 1 h of lights-on and prior to stress exposure to obtain measures of basal corticosterone levels at the trough of the circadian rhythm. Samples