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Preadolescent Adversity Programs a Disrupted Maternal Stress Reactivity in Humans and Mice

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ABSTRACT

BACKGROUND: Adverse childhood experiences (ACEs) are one of the greatest predictors of affective disorders for women. Periods of dynamic hormonal flux, including pregnancy, exacerbate the risk for affective disturbance and promote hypothalamic-pituitary-adrenal (HPA) axis dysregulation, a key feature of affective disorders. Little is understood as to how stress experienced in late childhood, defined as preadolescence, alters the programming unique to this period of brain maturation and its interaction with the hormonal changes of pregnancy and postpartum.

METHODS: Preadolescent female mice were exposed to chronic stress and examined for changes in their HPA axis during pregnancy and postpartum, including assessment of maternal-specific stress responsiveness and transcriptomics of the paraventricular nucleus of the hypothalamus. Translationally, pregnant women with low or high ACEs were examined for their maternal stress responsiveness.

RESULTS: As predicted, preadolescent stress in mice resulted in a significant blunting of the corticosterone response during pregnancy. Transcriptomic analysis of the paraventricular nucleus revealed widespread changes in expression of immediate early genes and their targets, supporting the likely involvement of an upstream epigenetic mechanism. Critically, in our human studies, the high ACE women showed a significant blunting of the HPA response.

CONCLUSIONS: This unique mouse model recapitulates a clinical outcome of a hyporesponsive HPA stress axis, an important feature of affective disorders, during a dynamic hormonal period, and suggests involvement of transcriptional regulation in the hypothalamus. These studies identify a novel mouse model of female ACEs that can be used to examine how additional life adversity may provoke disease risk or resilience.

Keywords: Adolescence, HPA axis, Paraventricular nucleus, Postpartum, Pregnancy, Stress

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Risk for affective disturbance in the lifetime of females is multifactorial, although how these factors interact is not well understood. One key factor is exposure to adverse childhood experiences (ACEs), which are known to increase affective disorder risk across the lifespan for women (1–5). Furthermore, periods of dynamic hormonal flux, such as pregnancy and postpartum, can exacerbate the risk for affective disturbances and stress dysregulation (6–8). Peripartum depression and anxiety, occurring during pregnancy or shortly following birth, are associated with significant adverse and long-term effects for both mother and baby (9–11). Preclinical animal studies focused on stressors proximal to birth, including prenatal or postpartum social stress or stress hormone exposure, demonstrate significant changes in maternal behavior and offspring outcomes (12–15). However, little is known as to how adversity experienced in late childhood, or preadolescence, may reprogram the female brain to increase risk for such outcomes during and after pregnancy.

A central endophenotype of affective disorders is disruption of the hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for initiating the neuroendocrine response to stressors (16,17). Importantly, the responsiveness of the HPA axis has yet to fully mature in preadolescent animals. In response to a variety of stressors, preadolescent rodents have an HPA axis characterized by a protracted hormonal response compared with neonatal and adult animals, and an insensitivity to factors, such as gonadal hormones, that normally modulate the adult response (18–20). Thus, the preadolescent individual may have an increased risk for adversity to program long-term dysfunction of the HPA axis (21). Indeed, clinical studies show that childhood adversity is associated with HPA axis dysregulation in adult women (22–25). During pregnancy and postpartum, there are dramatic changes in hormone levels. This has important implications for regulation of stress circuitry, as these gonadal hormones and their metabolites have been shown to be potent regulators of the HPA axis (26–29). Critically, as the HPA axis response is highly conserved among vertebrates, it represents a readily translatable outcome for animal models.

We have developed a mouse paradigm to examine the hypothesis that stress experienced during the preadolescent window of brain development would program long-term

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changes in stress pathways that, when interacted with dynamic hormonal changes during pregnancy and postpartum, would produce dysregulation of the stress response. Female mice were exposed to chronic stress during preadolescence and were then examined for changes in HPA stress axis responsiveness during pregnancy. To examine potential mechanisms for programming changes that may have occurred following preadolescent stress (PAS), pregnancy hormones and gene expression changes related to stress circuitry, including transcriptomics of the paraventricular nucleus (PVN), were also measured. To evaluate the translational potential of this model, HPA responsiveness to a maternally relevant stressor was assessed in preadolescent stressed mice and a cohort of women with varying levels of ACEs.

METHODS AND MATERIALS

Full details of experimental procedures and analyses are provided in the Supplement.

Animals

All mice bred were virgin, in-house mixed C57BL/6:129 mice (30–33). All procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Preadolescent Stress

Administration of PAS was performed as described previously (33). Female mice underwent 14 days of chronic variable stress starting on postnatal day (PN) 21, during which one stressor was administered per day. Animals in the PAS group were weaned into singly housed cages at the beginning of stress, and were pair-housed with a same-sex, same-stress cage mate at the end of stress. Control individuals remained with the dam until they were weaned at PN28 into pair-housed cages.

Breeding Scheme

At 8 to 10 weeks of age, female mice were bred with naïve male mice for 1 to 3 nights. Upon confirmation of a copulation plug, female mice were established as pregnant and were immediately removed to their own cage. Female mice were left undisturbed until testing.

HPA Axis Responsiveness

Female mice that were nulliparous, in the early stage of pregnancy (7.5 days postconception [dpc]), in the late stage of pregnancy (17.5dpc), or postpartum (PN4) were tested for HPA axis responsiveness to a 15-minute restraint stress as previously (34,35). Each group represents a different set of subjects, such that female mice were not tested more than once.

Activation of the HPA axis by adrenocorticotropic hormone (ACTH) in the absence of any additional stressor was examined. Plasma corticosterone was measured in late pregnancy (17.5dpc) female mice as previously, except that animals were injected with 50 μg/kg ACTH (Sigma-Aldrich, St. Louis, MO) at time 0 minutes and were not restrained.

Light-Dark Box

To assess anxiety-like behavior, late pregnancy (17.5dpc) female mice were tested in light-dark box and analysis was performed as previously (35,36).

Postpartum Pup Separation

Female mice (PN7) were tested for behavioral and HPA axis responsiveness to a 15-minute separation from pups. Four pups (2/sex) were placed in a novel cage 5 minutes prior to the start of testing. The arena was outfitted with a plastic mesh gate that hemisected the cage. This allowed the dam to see, smell, and hear the pups, but not come into physical contact with them. Dams were placed in the side of the arena opposite of the pups and behavior was recorded from above. Distance traveled was quantified using ANY-maze software (version 4.75; Stoelting, Kiel, WI). Following the separation, the dam was removed and tail blood was collected. The dam and pups were returned to the home cage, after which tail blood was collected from the dam at 30 and 120 minutes following the start of the separation test. Blood was not collected at the start of test so as to not interfere with maternal behavior during the separation.

Pup Retrieval

To assess maternal care, female mice (PN2) underwent a pup retrieval test. The dam was removed from the cage, and two pups (1/sex) were placed each in the two corners of the cage opposite to that of the nest. The dam was placed back in the cage, and latency to retrieve each of the four pups was recorded.

Human Infant Separation Test

Subjects. Pregnant women (age range 19–35 years) were recruited to an ongoing study focusing on the role of maternal life stress on pregnancy and infant outcomes conducted at the Penn Center for Women's Behavioral Wellness. See Human Studies in the Supplement for a full description of the cohort. The study was approved by the Perelman School of Medicine at the University of Pennsylvania Institutional Review Board, and all adult participants provided written informed consent.

Assessment of Preadolescent Adverse Experience. Upon enrollment, women were given the ACE questionnaire, a 10-item self-report that assesses exposure to abuse, neglect, and household adversity from birth to 18 years of age (Supplemental Table S2) (37). An item was considered to be a preadolescent ACE if the experience was reported to have first occurred at least 2 years prior to reported age of menarche. Participants were separated into low (0 ACE) and high (2+ ACEs) preadolescent ACE categories.

Infant Separation Test. Mothers underwent a maternal-specific laboratory stressor that consisted of exposure to infant separation, during which the infant (6 months old) experienced 3 bursts of 90 dB sound (30-s intervals), followed by a 2-minute restraint stressor (38). Mothers were asked to sit