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Maternal high fructose diet and neonatal immune challenge alter offspring anxiety-like behavior and inflammation across the lifespan

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ABSTRACT

Aims: This study examined the interaction between maternal high fructose diet and neonatal inflammation in neonates (P7), juveniles (P26–34) and adults on measures of anxiety-like behavior and cognition. The study aimed to assess the potential synergistic effects of these two forms of early-life inflammation.

Main methods: We fed Sprague-Dawley dams with high fructose (60%) diet or normal chow. Each litter was treated with either saline or lipopolysaccharide (LPS) on postnatal day (P)3 and P5 and two pups were tested for USVs after maternal separation on P7. Post-weaning, juveniles were tested on the elevated zero maze (EZM) and in a context-object discrimination (COD) task prior to tissue harvest. Adults were tested on the EZM and the COD task as well. Immunohistochemistry and ELISA were used to assess molecular and cellular changes in the offspring.

Key findings: This study demonstrates that maternal diet and neonatal inflammation altered peripheral inflammation in neonates, altered anxiety-like behavior in juveniles, and altered anxiety-like behavior in adulthood. Maternal diet and sex increased juvenile peripheral inflammation and altered memory on the context-discrimination task.

Significance: Maternal diet has a profound impact on fetal and neonatal development, especially as obesity rates are on the rise worldwide. Together, these findings reveal enduring effects of maternal diet on offspring, support the findings on the effects of neonatal inflammation on anxiety-like behaviors in later-life periods, and add to the complex relationship between gestational and neonatal inflammation and anxiety.

1. Introduction

Gestational diabetes, characterized by a state of hyperglycemia during the gestational period, is increasingly common in the US [12]. Both the mother and fetus experience harmful conditions as maternal insulin efficacy is reduced [1,16], allowing glucose to persist in the mother's blood, increasing chances for both birth defects and late-onset diseases. Including metabolic and cardiovascular diseases, these disorders co-occur alongside the nervous system's development, which may be disrupted by the release of pro-inflammatory cytokines, triggered by gestational diabetes [26]. Recent rodent research suggests that exposure to gestational diabetes promotes overactive microglia in brain areas relevant to learning and memory tasks [34]. The release of pro-inflammatory cytokines during development can lead to adult anxiety-like behavior and an increased reactivity to stress through alteration of the hypothalamic-pituitary-adrenal axis [6,15,18]. Similar disruptions in behavior, mediated by neuroinflammation, are caused by the activation of the immune system during developmental critical periods [31].

In rodents, lipopolysaccharide (LPS) treatment during early life leads to the activation of the hypothalamic-pituitary-adrenal (HPA) axis, and results in an HPA hyper-responsiveness in adulthood and an altered glucocorticoid responsiveness to stress. In humans, this dysfunctional glucocorticoid response is linked to anxiety disorders, depression, schizophrenia and other neuropsychiatric diseases [2]. In rodents, models of both neonatal inflammation and maternal immune activation result in abnormal behavior across the lifespan [9,14,18].

Anxiety disorders are the most common neuropsychiatric disorders within the United States and gestational diabetes is increasingly common. Therefore, modeling a combined immune system disruption alongside gestational diabetes is critical given prior evidence suggesting a role of neuroinflammation in determining anxiety-like behavior throughout the lifespan. We used pregnant rats fed a fructose enriched (60%) diet, and then induced neonatal inflammation. The diet (adapted from [30]) was used to create a state of overnutrition for the extent of the gestational and postnatal period, which mirrors the course of

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gestational diabetes within a pregnant human.

Following neonatal immune challenges, we tested for changes in anxiety-like and learning behaviors. We assessed ultrasonic vocalizations (USVs) to test anxiety-like behavior of neonates [8]. In juvenile and adult rats, the Elevated Zero Maze (EZM) paradigm was used to assess anxiety-like behaviors [7]. To test effects on learning and memory, both juveniles and adults were tested on a context-object discrimination (COD) task. The COD paradigm is dependent upon hippocampal memory processes, linking differing contexts and the objects within them. Systemic neuroinflammation interferes with hippocampal-dependent memory processes through the release of inflammatory cytokines. As a result, performance in this task is impaired. [11,37,38].

In this study, we examined if neuroinflammation caused by a neonatal immune challenge interacts with or augments the negative phenotypes caused by gestational diabetes. We hypothesized that the increase in neuroinflammation would affect anxiety-like behavior and impair cognitive function throughout development and into adulthood. We also expected to observe molecular changes in the central and peripheral immune systems, especially in cytokines such as IL-1β, TNFα and IL-10, and microglial morphology and activation as a result of neuroinflammation. Increased understanding of the maternal and neonatal inflammatory mechanisms of early-life immune challenges and gestational diabetes will help shape predictions for human health and behavior.

2. Materials and methods

2.1. Animals

Adult female Sprague-Dawley rats were singly housed in transparent plastic cages (45 cm L × 25 cm W × 15 cm H) in a temperature-controlled colony room with a 12 h light: 12 h dark cycle (0700 lights on). Female rats were divided into experimental cohorts, and given ad libitum access to water and either fructose diet (Envigo Teklad Diet 89,247) or normal chow diet (Envigo Teklad Diet 7012). After 1 week, male rats were placed in the cages for a period of 7 days for mating to induce pregnancy. Male rats were then removed, and female rats were allowed to carry to full term. Lactating mothers were also maintained on these diets following delivery up to postnatal day (PD) 12 in order to mimic the duration and physiological effects of gestational diabetes as seen in previous studies [24], and to overlap high fructose diet exposure with hippocampal development during the early lactation period for Sprague-Dawleys [13]. On postnatal day (PD) 12, all rats were returned to normal chow diet. Male and female juvenile rats from the resulting litters were weaned on PD25. During the juvenile period, rats were housed three-to-a-cage in same-sex groupings in the same colony room with ad libitum access to food and water. On P40, all rats were pair-housed. The Institutional Animal Care and Use Committee at Williams College approved all procedures.

2.2. Diet

The rats were allowed to acclimatize to the high-fructose diet for a period of a week before pregnancy was induced. The normal chow diet (Envigo Teklad Diet 7012) contained 44.3% carbohydrate, 19.1% protein, 13.7% neutral detergent fiber, 5.8% fat, and 4.6% crude fiber. The fructose-enriched diet (Envigo Teklad Diet 89247) contained 60% fructose, 21% casein, 8% fiber, 5% lard, and approximately 7% of a mineral and vitamin mix. The diet regimen was maintained through pregnancy until pups reached P12 to expose pups to the diet during gestation and lactation [35,40]. All rats were fed normal chow (Diet 7012) after P12 until weaning and thereafter.

2.3. Neonatal treatments

During the neonatal period, litters on both diets were treated with either saline or lipopolysaccharide (LPS, membrane component of Gram negative bacteria) on P3 and P5. For injections, all pups in a given litter were separated from the dam and placed in a plastic cage (18.4 cm × 29.2 cm × 12.7 cm) with bedding. To minimize heat loss, each cage was rested on a heating pad during the separation from the dam. All injections were done between 10:00 and 14:00. Each pup received a subcutaneous injection of either endotoxin-free saline (SAL) or *E. coli*–derived LPS (strain 0111-B4, Sigma-Aldrich, St. Louis, MO) dissolved in endotoxin-free saline. All pups in a given litter were given the same treatment and each litter was randomly assigned to a condition. Pups in the SAL group received 0.1 mL of saline, while pups in the LPS group received a dose of 50 μg/kg of LPS. Following injections, all pups were returned to the dams.

2.4. Ultrasonic vocalization (USV) testing and tissue harvest

On P7, 1 female and 1 male pup were randomly chosen from each litter and placed into a plastic cage with bedding, separated from each other. The pups were transported into the habituation room, where they habituated for 10 min on a heating pad set to low. After this brief maternal separation, pups were individually placed in a darkened chamber housing a circular glass dish (20 cm D × 10 cm H), with no bedding. This glass dish was located beneath an S-25 ultrasound bat detector (Ultra Sound Advice, London) set to detect signals at 50 ± 5 kHz. The ultrasonic vocalizations (USVs) for each pup were recorded for two minutes by listening through headphones attached to the detector and every individual vocalization was counted using LabChart to produce a total number of vocalizations for each pup (n = 42).

Pups were returned to small plastic containers on the heating pad until tissue collection that followed USV testing. Following rapid decapitation, trunk blood was collected in microcentrifuge tubes at the time of decapitation, spun down at 16.1g for 10 min, and then serum was collected and frozen at −20 °C.

2.5. Elevated zero maze

The elevated zero maze (EZM) behavioral testing apparatus is a black wooden ring, raised to 0.51 m above the ground with two opposite with tall walls (the closed portion), and two opposite quarters with low walls (the open portion) (Fig. 1D). During their respective testing, juvenile and adult rats were individually transported in a transfer cage to the EZM testing room, which was illuminated by two lights aimed away from the testing apparatus. At the time of testing, rats were placed in the center of the open arm, and the experimenter left the testing room to allow the rat to freely explore the maze. Test sessions lasted 5 min. Following testing, the rat was placed back in the transfer cage and returned to the colony room. The maze was cleaned and disinfected with Quatricide between animals. All behavior was automatically and immediately scored using AnyMaze software (Wood Dale, IL, USA). Amount of time spent in the closed arms of the maze is correlated with anxiety-like behavior in Sprague-Dawley rats [7].

2.6. Context-object discrimination

Both juvenile and adult rats underwent the COD memory task following five days of handling (2 min per day). This paradigm consists of two training days, during which the animal experiences each of two context-object sets, and one test day, on which an animal is exposed to a familiar context containing one in-context object and one out-of-context object (image and schematic of testing, Fig. 1A–C).

COD involves two apparatuses, identified as Context A and Context B. For juveniles, Context A is a white plastic cylinder, measuring 48 cm