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Maternal immune activation affects litter success, size and neuroendocrine responses related to behavior in adult offspring

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HIGHLIGHTS

- We quantified sickness during pregnancy in hamsters given different doses of LPS.
- We assessed the resulting effects on litter success, size, and offspring development.
- Pregnancy success decreased and litter size was reduced with increasing doses of LPS.
- Offspring from LPS treated dams showed greater cortisol responses to stress.
- Cortisol levels in both sexes of offspring were related to defensive behaviors.

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ABSTRACT

It is increasingly evident that influences other than genetics can contribute to offspring phenotype. In particular, maternal influences are an important contributing factor to offspring survival, development, physiology and behavior. Common environmental pathogens such as viral or bacterial microorganisms can induce maternal immune responses, which have the potential to alter the prenatal environment via multiple independent pathways. The effects of maternal immune activation on endocrine responses and behavior are less well studied and provide the basis for the current study. Our approach in the current study was two-pronged: 1) quantify sickness responses during pregnancy in adult female hamsters experiencing varying severity of immune responsiveness (i.e., differing doses of lipopolysaccharide [LPS]), and 2) assess the effects of maternal immune activation on offspring development, immunocompetence, hormone profiles, and social behavior during adulthood. Pregnancy success decreased with increasing doses of LPS, and litter size was reduced in LPS dams that managed to successfully reproduce. Unexpectedly, pregnant females treated with LPS showed a hypothermic response in addition to the more typical anorexic and body mass changes associated with sickness. Significant endocrine changes related to behavior were observed in the offspring of LPS-treated dams; these effects were apparent in adulthood. Specifically, offspring from LPS treated dams showed significantly greater cortisol responses to stressful resident–intruder encounters compared with offspring from control dams. Post-behavior cortisol was elevated in male LPS offspring relative to the offspring of control dams, and was positively correlated with the frequency of bites during agonistic interactions, and cortisol levels in both sexes were related to defensive behaviors, suggesting that changes in hypothalamo–pituitary–adrenal axis responsiveness may play a regulatory role in the observed behavioral differences. Overall, the results of this study provide evidence that maternal immune activation can exert marked effects on offspring physiology and behavior.

1. Introduction

Although genetic influences on a wide range of behaviors are well documented, it is becoming increasingly evident that non-genetic (e.g., environmental) influences also contribute to offspring phenotype, including behavior. While the role of environmental factors has been traditionally under-appreciated, recent studies have highlighted the importance of epigenetic (i.e., "above the genome") effects on an organisms' phenotype. In particular, maternal influences such as energy/nutrient availability, oxygen levels, and hormone concentrations are important contributing factors to offspring survival, development, and potentially behavior (reviewed in: [1,2]).

Pregnancy in eutherian mammals presents a life history stage characterized by prolonged physiological association between mother and offspring. Long gestational periods result in extended prenatal exposure to maternal influences. Further, placental buffering capabilities change throughout gestation varying embryo/fetal susceptibility temporally to maternal influences, such as maternal immune system activation [3]. Whereas some influences, such as morphological abnormalities, are apparent in utero, increasing evidence suggests that

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certain physiological and behavioral maternal effects may not manifest until later in development [4,5]. For example, exposure to prenatal stress can alter stress responsiveness in mammalian offspring [6]. Further, prenatal exposure to maternal stress, food restriction, or elevated glucocorticoid levels impairs male sexual behavior in rats [4]. Therefore environmental perturbations that influence the mother during pregnancy and lactation, can in turn affect offspring development.

In particular, activation of the maternal hypothalamo–pituitary–adrenal axis (HPA) during pregnancy can result in profound effects on offspring development, including altered gonadal steroid production, stress responsiveness, growth rate, and immunocompetence of offspring from both live-bearing and egg-laying species [7–12]. For example, prenatal stress in albino rats (Ola strain) results in decreased body mass and growth rates from birth [13]. There are many common environmentally relevant phenomena that have the capacity to activate the HPA axis and thus affect physiology and behavior. For example, predation attempts, resource limitation, social competition, and certain immunological responses to pathogens or parasites can all alter HPA axis activity (reviewed in: [14–16]). Therefore, immune-related maternal effects on offspring are common within an organism's natural environment and an important contributor to individual phenotype, including behavior.

While various environmental perturbations can alter the prenatal environment and thus contribute to the development of offspring behavior, the specific physiological mechanisms underlying these effects are unclear. Accumulating evidence suggests that neonatal or early-life exposure to elevated cytokines during an immune response can result in prolonged neural and behavioral abnormalities (reviewed in: [17,18]). Further, emerging evidence suggests that elevated cytokine concentrations during pregnancy cause marked changes to fetal brain development and therefore likely downstream changes in offspring behavior [5,19]. In fact, maternal immune activation may contribute to several psychiatric conditions including autism, and proinflammatory cytokines (i.e., interleukin [IL]-6, tumor necrosis factor [TNF]-α) play an important role in the regulation of social behavior such as aggression [20,21]. Activation of an innate immune response results in a cascade of molecular and biochemical events that involve activation of specific immune cells (e.g., neutrophils, macrophages), mobilization of complement proteins and production of cytokines, soluble molecules that aid in the targeting and destruction of the pathogen. Furthermore, this immune response can trigger the HPA, axis resulting in elevated glucocorticoids, which could in turn affect offspring [12,22]. Therefore, common environmental pathogens such as viral or bacterial organisms can induce maternal immune responses which have the potential to immensely alter the embryonic/fetal environment via multiple pathways [12,23,24]. It is also apparent that maternal immune activation can induce offspring brain pathology, having direct implications for behavior, and in extreme cases, miscarriage [5,25,26].

Although much progress has been made over the last decade in our understanding of the effects of maternal immune activation on neuroendocrine and behavioral responses, considerable gaps in our knowledge persist. For example, much of the work examining experimentally-induced immune activation has focused on biochemical and physiological changes occurring within the organism [27]. Substantially less is known regarding how specific behavioral phenotypes, particularly social behaviors, are affected by immune activation. In the current study, we examined the effects of maternal immune activation on reproductive success and offspring endocrine and behavioral development using the Siberian hamster (*Phodopus sungorus*) as a model system. To induce an immune response we utilized lipopolysaccharide (LPS), a molecule present on the outer coat of Gram-negative bacteria, which acts on Toll-like receptors (TLR) present on immune cells (specifically TLR4) [28]. LPS elicits an inflammatory and pyrogenic response without actually exposing the animal to an infectious agent [29,30].

Prenatal infection may enhance offspring immunity according to the transgenerational priming of immunity theory, which may be adaptive because the offspring are likely to encounter similar infectious agents [31,32]. Therefore, fetal exposure to a maternal innate immune response may increase the ability of the offspring to respond to an innate immune challenge through altered circulating immune components. We tested the immunocompetence of the offspring in adulthood with a bacterial killing assay as a functional measure of innate immunity. We also tested the ability of the animals to respond to an antigenic challenge. Testing both the innate and humoral arms of the immune system provides a broader understanding of the immune system and how it was altered during development.

The approach of the current study was two-pronged: 1) quantify sickness response during pregnancy in adult female hamsters in response to varying levels of immune responsiveness (i.e., varying doses of LPS), 2) measure the effects of maternal immune activation on pregnancy success and litter size, and 3) assess the effects of maternal immune activation during pregnancy on offspring development, endocrine responses, and behavior during adulthood. Specifically, we assessed the sickness response of pregnant females exposed to relatively low and high doses of LPS, measuring food intake, body mass and temperature, and pregnancy success. We subsequently assessed offspring initial birth mass, growth rate, and adult immunocompetence (i.e., bactericidal ability and KLH-antibody response), resident–intruder aggression test and associated steroid hormone concentrations (i.e., cortisol, testosterone), all factors known to be associated with maternal stress and potentially immune activation.

2. Materials and methods

2.1. Experiment 1A and B: effects of immune activation on maternal physiology and behavior

2.1.1. Animals and housing

Sixty-nine adult nulliparous female Siberian hamsters were obtained from our breeding colony at Indiana University. All animals were initially group-housed (2–4 per cage with same sex siblings upon weaning at 21 days of age). Ten days before the start of the experiment, animals were housed individually in polypropylene cages (28 × 17 × 12 cm). Conditions were maintained at 16:8 (light:dark) hour photoperiod, temperature (20 ± 2 °C), and humidity (50 ± 10%). All animals were given ad libitum access to food (Purina rat chow, St. Louis, MO) and water throughout the study. All animals were treated in accordance with the Bloomington Institutional Animal Care and Use Committee (BIACUC).

2.1.2. Maternal immune activation

To examine the effects of mounting a costly immune response on offspring investment, adult female hamsters were randomly assigned to one of two treatment groups in each of two separate studies. A) In the first experiment animals were injected on gestational day (GD) 11 (±2 days; i.e., GD 10–13) with either high dose of LPS 0.7 mg/kg (high dose, n = 12) or saline vehicle 0 mg/kg (control, n = 15). Because of the low pregnancy success and the desire to measure offspring effects a second iteration of the experiment was run using a lower dose of LPS. B) In the second experiment animals were injected on GD 11 (±2 days) with either low dose of LPS 0.07 mg/kg (low dose, n = 12) or saline vehicle 0 mg/kg (control, n = 15). Gestational day was assigned with the second day of pairing the male and female as GD 0. Males and females were paired for 4 days in the cage of the female, after which the male was removed. All pairs were given a cotton nestlet to create a nest. Variation arose due to the fact that hamsters do not have regular estrous cycles, which introduces some uncertainty in the date of conception. Injections occurred on GD 11 (±2 days) because this time point is late enough to reduce the risk of spontaneous abortion and early enough to affect brain development during a period