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Research report

An investigation into the effects of antenatal stressors on the postpartum neuroimmune profile and depressive-like behaviors

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HIGHLIGHTS

- Inflammatory cytokines are differentially expressed in the brain postpartum.
- Pregnancy modulates the neuroimmune response to immune activation.
- Pregnancy and sub-chronic stress both induce anhedonia immediately postpartum.

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ABSTRACT

Postpartum depression is a specific type of depression that affects approximately 10–15% of mothers [28]. While many have attributed the etiology of postpartum depression to the dramatic change in hormone levels that occurs immediately postpartum, the exact causes are not well-understood. It is well-known, however, that pregnancy induces a number of dramatic changes in the peripheral immune system that foster the development of the growing fetus. It is also well-known that changes in immune function, specifically within the brain, have been linked to several neuropsychiatric disorders including depression. Thus, we sought to determine whether pregnancy induces significant neuroimmune changes postpartum and whether stress or immune activation during pregnancy induce a unique neuroimmune profile that may be associated with depressive-like behaviors postpartum. We used late-gestation sub-chronic stress and late-gestation acute immune activation to examine the postpartum expression of depressive-like behaviors, microglial activation markers, and inflammatory cytokines within the medial prefrontal cortex (mPFC) and the hippocampus (HP). The expression of many immune molecules was significantly altered in the brain postpartum, and postpartum females also showed significant anhedonia, both independently of stress. Following late-gestation immune activation, we found a unique set of changes in neuroimmune gene expression immediately postpartum. Thus, our data indicate that even in the absence of additional stressors, postpartum females exhibit significant changes in the expression of cytokines within the brain that are associated with depressive-like behavior. Additionally, different forms of antenatal stress produce varying profiles of postpartum neuroimmune gene expression and associated depressive-like behaviors.

1. Introduction

Postpartum depression is a specific type of major depression outlined by the National Institute of Mental Health that affects approximately 10–15% of mothers after birth [28,18]. Approximately 30% of women will experience minor symptoms of anxiety and sadness, called “baby blues”, immediately postpartum [9–11]; however, these symptoms typically last only one to two weeks. The much more severe symptoms of postpartum depression including erratic mood swings, anhedonia, increased anxiety, insomnia, and social withdrawal may continue for weeks, months, or even years after giving birth [16,6]. Pregnancy and parturition are associated with dramatic changes in hormone levels [10], which are believed to be the cause of these postpartum mood disorders. However, little research has examined whether other potential mechanisms contribute to the development or etiology of postpartum mood changes and depressive-like behaviors.

Pregnancy significantly alters peripheral immune function in order to accommodate and foster the growth of the developing fetus [12,25]. As a result of these changes in peripheral immune function, the induction of classic Th1/M1 pro-inflammatory

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immune molecules, such as IL-1β, are decreased over the course of gestation while a unique composition of Th2/M2 immune molecules are expressed in response to immune challenges (e.g., IL-4, Arginase 1, and IL-10). The difference between the Th1/M1 and Th2/M2 phenotypes is striking and results in very distinct profiles of immune activation in response to a challenge. To date, these changes have been observed and measured in the periphery of pregnant females [15]. Very few animal studies have examined the expression of cytokines in the brain of either late-gestation or immediately postpartum females to determine if changes in central cytokine expression mimic peripheral changes, particularly in response to an immune challenge.

Thus in our first experiment, we implemented a modified protocol of stress-induced depression using a sub-chronic forced swim stressor in late-gestation females in order to determine the effect of this stressor on pro-inflammatory cytokine expression in the brain and depressive-like behaviors immediately postpartum. Our second experiment used acute immune activation via lipopolysaccharide (LPS) during late gestation to determine potential neuroimmune and behavioral changes in the early postpartum period. We predicted that physiological stress or immune activation during late gestation may increase the risk for developing postpartum depression by triggering a unique neuroinflammatory state that would be evident in the immediate postpartum period.

2. Materials and methods

2.1. Animals

All experiments used female Sprague–Dawley rats ordered from Harlan Laboratories (Indianapolis, IN). Rats were housed in clear polypropylene cages with ad libitum access to food and water in rooms under a 12:12-h light:dark cycle and maintained temperature and humidity. All experiments were performed in accordance with the Institutional Animal Care and Use Committee of the University of Delaware and under the *Guide for the Care and Use of Laboratory Animals* of the National Institute of Health.

Each experiment used both pregnant and non-pregnant females. Day of conception was determined by the presence of a sperm plug and assigned as Embryonic (E) day 1, and day of birth (typically E23) was assigned as Postnatal (P) day 0. All experimental manipulations in non-pregnant females were time-matched to their pregnant counterparts. Neither stress nor immune activation during late gestation produced any adverse outcomes on gestation length.

In Experiment 1, we examined the effects of a sub-chronic forced swim stressor during late gestation in pregnant females compared to time-matched non-pregnant females on the expression of cytokines, microglial activation markers, and depressive-like behavior during the immediate postpartum period. Experiments 1.1, 1.2, and 1.3 used a total of 96 female rats assigned to one of four experimental groups: Postpartum, No Stress ($n = 8$); Postpartum, Stress ($n = 8$); Non-pregnant, No Stress ($n = 8$); or Non-pregnant, Stress ($n = 8$). In this case, all female rats were mated, and rats that did not become pregnant (i.e., no sperm plug was found) were used as the Non-pregnant control rats throughout the duration of the experiments. In Experiment 1.1, female rats were subjected to a forced swimming stressor for seven consecutive days during their last week of gestation (E16–E22) or the time matched equivalent for Non-pregnant females. Rats in the No Stress group remained undisturbed during this time. Prior to the first day of testing (E15) or the time matched equivalent for No Stress and Non-pregnant groups, all females were separated to be housed individually for the remainder of the experiments. On day of birth (E23) or 24 h after the last day of forced swimming in non-pregnant rats, the females were euthanized to examine the expression of cytokines and microglial activation markers in the brain in response to this late gestation sub-chronic stressor. Female rats in Experiment 1.2 underwent the same stress regimen; however, these rats were used to analyze immediate postpartum anhedonia on day of birth (P0) and P1. Females in Experiment 1.3 were also subjected to the forced swimming stressor, and these animals underwent behavioral testing for anhedonia at two time points: P0 and P1, to replicate our findings in Experiment 1.2, and one week after parturition on P7 and P8 in order to better understand the time course for the expression of this behavior in the early postpartum period. See Fig. 1 for a timeline of these experiments.

In Experiment 2, we examined the effects of acute immune activation during late gestation in pregnant females and time-matched non-pregnant females using an intraperitoneal injection of LPS on the expression of cytokines, microglial activation markers, and anhedonia during the immediate postpartum period. Experiments 2.1 and 2.2 used a total of 64 rats assigned to one of four experimental groups: Postpartum, Saline ($n = 8$); Postpartum, LPS ($n = 8$); Non-pregnant, Saline ($n = 8$); or Non-pregnant, LPS ($n = 8$). In this case, all female rats were mated, and rats that did not become pregnant (i.e., no sperm plug was found) were used as the Non-Pregnant control rats throughout the duration of the experiments. Females in Experiment 2.1 were given one injection of either LPS (100 μg/kg) or sterile saline (1 mL/kg) at least 24 h prior to giving birth (approximately E22) or the time matched equivalent for non-pregnant females. Prior to the day of injection (E21, or the time-matched equivalent), all rats were separated to be housed individually for the remainder of the experiments. On the day of birth (P0) or at least 24 h after the injection for non-pregnant animals, females were euthanized to analyze gene expression in response to late gestation immune activation. Females in Experiment 2.2 underwent the same injection procedures, and these animals were used to analyze postpartum anhedonia at two time points: P0 and P1 as in the previous experiment, and one week after parturition on P7 and P8 in order to observe a time course of the expression of this postpartum depressive-like behavior. See Fig. 1 for a timeline of these experiments.

2.2. Forced swim test

In Experiments 1.1, 1.2, and 1.3, separate cohorts of female rats were assigned to the stress condition and underwent the Forced Swim Test (FST) for 7 consecutive days. Rats were forced to swim in a clear cylinder (Stoelting Co., IL) filled with $20 \pm 1\,^{\circ}C$ tap water with no option of rest or escape for five minutes each day for the last week of their gestation (E16–E22) or a time-matched period for non-pregnant rats. Rats were monitored by researchers to prevent accidental drowning, and during the course of this experiment, no rats in any of the treatment groups "gave up" and stopped swimming while in the apparatus. Prior to the first day of testing (E15) or the time matched equivalent for No Stress and Non-pregnant groups, all females were separated to be housed individually for the remainder of the experiments. Unstressed rats remained undisturbed during the time-matched period.

2.3. Lipopolysaccharide

Lipopolysaccharide (LPS) derived from *Escherichia coli* 0111:B4 was obtained from Sigma–Aldrich® (Cat. No. L2630). LPS was diluted with sterile Dulbecco's phosphate buffered saline (DPBS) to a concentration of 100 μg/mL for injections and rats were injected at a volume of 1 mL/kg with a final dose of 100 μg/kg LPS.