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Sickness-induced changes in physiology do not affect fecundity or same-sex behavior

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ABSTRACT

Previous work in our lab has shown that early-life infection affects female reproductive physiology and function (i.e., smaller ovaries, abnormal estrous cycles) and alters investigation and aggression towards male conspecifics in a reproductive context. Although many studies have investigated the effects of postnatal immune challenge on physiological and behavioral development, fewer studies have examined whether these changes have ultimate effects on reproduction. In the current study, we paired Siberian hamsters (*Phodopus sungorus*) and simulated a bacterial infection in early life by administering lipopolysaccharide (LPS) to male and female pups on pnd3 and pnd5. In adulthood, hamsters were paired with novel individuals of the same sex, and we scored an array of social behaviors (e.g., investigation, aggression). We then paired animals with individuals of the opposite sex for 5 consecutive nights, providing them with the opportunity to mate. We found that females exhibited impaired reproductive physiology and function in adulthood (i.e., smaller ovaries and abnormal estrous cycles), similar to our previous work. However, both LPS-treated males and females exhibited similar same-sex social behavior when compared with saline-treated controls, they successfully mated, and there were no significant changes in fecundity. These data suggest that the physiological changes in response to neonatal immune challenge may not have long-term effects on reproductive success in a controlled environment. Collectively, the results of this study are particularly important when investigating the relationships between physiology and behavior within an ultimate context. Animals exposed to early-life stress may in fact be capable of compensating for changes in physiology in order to survive and reproduce in some contexts.

1. Introduction

Early-life stressors (e.g., maternal care, social changes, sickness) can greatly influence physiology and behavior in adulthood (reviewed in Bilbo & Schwarz [1]). It is well-established that the neonatal period is an extremely sensitive time in the life of an individual [3], and infection during this time may increase susceptibility to a range of nervous system disorders, including autism and schizophrenia [4]. Further, infection during the neonatal period may affect the timing of puberty, as well as the development of the reproductive system and the immune system [1,5].

Treatment with lipopolysaccharide (LPS), a cell wall component of gram-negative bacteria, is commonly employed to induce an immune response in animals. LPS administration mimics the actions of a live bacterial infection by binding to toll-like receptor (TLR)-4, which leads to the subsequent release of circulating cortisol and pro-inflammatory mediators in the body [6–8]. Previous work suggests that postnatal LPS treatment not only affects the development of the reproductive axis in males and females (e.g., early puberty, suppression of luteinizing hormone [LH] and testosterone [T]), but LPS treatment also alters reproductive behavior in adulthood [9]. Specifically, LPS-treated male Wistar rats show fewer mounts, and females exhibit more aggression and fewer hops towards male conspecifics [9]. Additionally, LPS-treated males produce lower levels of sperm present in female partners following an interaction, suggesting they may not be able to successfully reproduce. Similarly, recent work from our lab suggests that postnatal LPS affects reproductive physiology and opposite-sex social behavior in a sex-dependent manner as well (see Fig. 1). Specifically, Siberian hamster (*Phodopus sungorus*) females treated with LPS as neonates show no changes in consummatory reproductive behaviors (e.g., lordosis), however, they do show heightened levels of pre-copulatory investigation and aggression when paired with male conspecifics. Interestingly, however, there were no changes in adult male physiology or social behavior following postnatal LPS [10]. Additionally, LPS-

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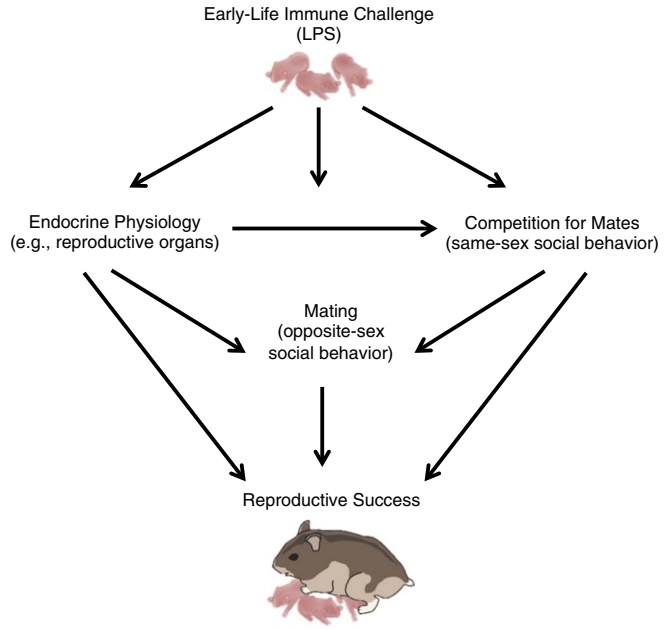

Fig. 1. Graphical depiction of how early-life infection can influence physiology and behavior. Early-life immune activation can affect physiology and behavior in sex-dependent ways. We have previously shown that postnatal LPS affects reproductive physiology and pre-copulatory behavior (e.g., investigation and aggression) in female hamsters, but not in males [10]. In the present study, we investigated whether these changes in physiology consequently affect the ability to interact with same-sex individuals (e.g., investigation, aggression), behavior that is important for finding and attracting a mate, and whether early-life LPS treatment influences mating and reproductive success in males and females in a lab setting. There are complex interactions taking place among the endocrine systems and its effector organs, reproductive function, and behavior that is necessary for fitness and reproduction in various contexts.

treated females exhibited altered estrous cycles and smaller ovaries in adulthood, suggesting that they may not be capable of successfully reproducing [10]. Moreover, LPS-treated female Wistar rats exhibit reduced follicle stimulating hormone (FSH) levels in adolescence, early reproductive maturity, and decreased follicular reserves [11], further suggesting that LPS-treated females of various species may not be capable of successfully reproducing.

Although many studies have investigated the effects of postnatal LPS on physiological and behavioral development (reviewed in Bilbo & Schwarz [2]), fewer studies have investigated whether these developmental changes have ultimate (i.e., fitness) effects on reproduction. In one study, researchers found that female rats postnatally treated with LPS exhibited increased corticosterone concentrations in the juvenile, adolescent, and adult stages, suggesting a heightened hypothalamo-pituitary-adrenal (HPA) axis response [12]. Further, they found that when males were given the opportunity to mate with both LPS-treated and saline-treated females (in the same cage), novel males showed no preference for mating. Specifically, LPS- and saline-treated females did not differ in fecundity rate after being paired with a stud male for two weeks in adulthood, however, offspring born to LPS-treated females showed higher rates of mortality. The mating effects of LPS treatment on males, however, were not investigated in the study [12]. In a subsequent study, immediately following postnatal immune challenge, ovaries showed a significant up-regulation in genes important for immune cell signaling and inflammation, as well as reproductive development, suggesting that early-life immune activation may have severe implications for ovarian development and reproduction [13].

In order to investigate whether early-life immune activation affects the functioning of the hypothalamo-pituitary-gonadal (HPG) axis in adult males, we stimulated HPG activity using the RFamide peptide, kisspeptin. Kisspeptin is one of the primary regulators of gonadotropin releasing hormone (GnRH) neurons at the hypothalamus and ultimately serves as a crucial regulator of the entire HPG axis, including the onset of puberty and fertility [14]. Work in our lab has shown that male and female Siberian hamsters' reproductive axes are reactive to exogenous kisspeptin at different stages of development. Specifically, males and females exhibit increased LH in response to exogenous kisspeptin, and males show increased testosterone in response to kisspeptin injection. Exogenous kisspeptin, however, does not affect normal seasonal changes in body mass or food intake [15,16].

While our previous findings and those of others have allowed an understanding of how an early-life immune challenge affects reproductive physiology and behavior, our work here provides insight into whether or not males and females from the same litters treated with postnatal LPS exhibit altered same-sex social behavior, an important aspect of finding potential mates. Further, we investigate if postnatal LPS treatment affects the ability for males and females to successfully mate when paired alone with a novel individual in the lab. As a result of the adverse effects of postnatal LPS on reproductive physiology that we found in our previous study, in this investigation, we hypothesized that females, but not males treated with postnatal LPS, would exhibit decreased mating success and reduced fecundity in adulthood.

2. Materials and methods

2.1. Animal housing and immune challenge

Adult male and female hamsters were paired (n = 13 pairs) and housed in a 16:8 light:dark photoperiod, in polypropylene cages (28 × 17 × 12 cm). Ambient temperature was maintained at 20 ± 2 °C, and relative humidity was maintained at 55 ± 5%. Hamsters were given ad libitum access to tap water and standard laboratory rodent chow (Lab Diet 5001, PMI Nutrition) throughout the experiment. Pups remained in their litters until weaning (postnatal day 24), when they were individually housed for the remainder of the study. On postnatal day (pnd) 3, approximately half of the litters were randomly assigned to either a treatment group, in which pups were given a single intraperitoneal (i.p.) injection (100 μL) of 50 μg/kg of lipopolysaccharide (LPS, from *Salmonella enterica* serotype typhimurium, Sigma-Aldrich, St. Louis, MO, USA), suspended in 0.9% sterile saline (n = 7 litters) or a control group, in which litters received i.p. injections of 0.9% sterile saline (n = 6 litters). All pups received a second injection of LPS or saline on pnd5 according to a previously validated protocol, as there is heightened sensitivity of the GnRH pulse generator at these time points [5,10]. All pups in an individual litter received the same treatment (LPS or saline). Once injected, pups were monitored throughout the study, and all animals were weighed weekly for the remainder of experimentation. At the conclusion of the study, all animals were euthanized and organs were weighed. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Bloomington Institutional Animal Care and Use Committee (BIACUC) at Indiana University.

2.2. Reproductive physiology

2.2.1. Estimated testis volume

Beginning at pnd25 and once per week thereafter until reproductive maturity males (n = 16 saline; n = 10 LPS) were lightly anesthetized with isoflurane, and the length and width of the left testis was measured externally (± 0.1 mm) with calipers, as a proxy for reproductive maturity [10,17]. Estimated testis volume (ETV) was calculated as the length × width^2, which is directly correlated with testis mass and spermatogenesis [17,18]. An ETV of 400mm^3 indicates a mass of approximately 200 mg, which is correlated with the critical mass for production of viable spermatids [17,18].