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Immune activation in lactating dams alters sucklings' brain cytokines and produces non-overlapping behavioral deficits in adult female and male offspring: A novel neurodevelopmental model of sex-specific psychopathology

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ABSTRACT

Early immune activation (IA) in rodents, prenatal through the mother or early postnatal directly to the neonate, is widely used to produce behavioral endophenotypes relevant to schizophrenia and depression. Given that maternal immune response plays a crucial role in the deleterious effects of prenatal IA, and lactation is a critical vehicle of immunological support to the neonate, we predicted that immune activation of the lactating dam will produce long-term abnormalities in the sucklings. Nursing dams were injected on postnatal day 4 with the viral mimic poly-I:C (4 mg/kg) or saline. Cytokine assessment was performed in dams' plasma and milk 2 h, and in the sucklings' hippocampus, 6 h and 24 h following poly-I:C injection. Male and female sucklings were assessed in adulthood for: a) performance on behavioral tasks measuring constructs considered relevant to schizophrenia (selective attention and executive control) and depression (despair and anhedonia); b) response to relevant pharmacological treatments; c) brain structural changes. Maternal poly-I:C injection caused cytokine alterations in the dams' plasma and milk, as well as in the sucklings' hippocampus. Lactational poly-I:C exposure led to sex-dimorphic (non-overlapping) behavioral abnormalities in the adult offspring, with male but not female offspring exhibiting attentional and executive function abnormalities (manifested in persistent latent inhibition and slow reversal) and hypodopaminergia, and female but not male offspring exhibiting despair and anhedonia (manifested in increased immobility in the forced swim test and reduced saccharine preference) and hyperdopaminergia, mimicking the known sex-bias in schizophrenia and depression. The behavioral double-dissociation predicted distinct pharmacological profiles, recapitulating the pharmacology of negative/cognitive symptoms and depression. In-vivo imaging revealed hippocampal and striatal volume reductions in both sexes, as found in both disorders. This is the first evidence for the emergence of long-term behavioral and brain abnormalities after lactational exposure to an inflammatory agent, supporting a causal link between early immune activation and disrupted neuropsychodevelopment. That such exposure produces schizophrenia- or depression-like phenotype depending on sex, resonates with notions that risk factors are transdiagnostic, and that sex is a susceptibility factor for neurodevelopmental psychopathologies.

1. Introduction

Epidemiologic studies show that early exposure to environmental adversities such as infection increases the risk for many adult-onset psychopathologies including schizophrenia and depression (Bale et al., 2010; Brown, 2011; Goodwin, 2011; Kneeland and Fatemi, 2013; Koponen et al., 2004; Machon et al., 1997). The

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epidemiological association in humans has been supported by animal studies demonstrating that early-life infection/immune activation, either prenatal (corresponding to first and second trimester of human pregnancy (Clancy et al., 2007; Semple et al., 2013)) or early postnatal (corresponding to the third trimester of human pregnancy (Clancy et al., 2007; Semple et al., 2013)) produces in the offspring long-term behavioral, brain, and immune abnormalities relevant to schizophrenia and depression (Abazyan et al., 2010; Bilbo and Schwarz, 2012; Doosti et al., 2013; Fatemi et al., 2009; Garay et al., 2013; Harvey and Boksa, 2012; Khan et al., 2014; Meyer and Feldon, 2009; Meyer et al., 2005; Nawa and Takei, 2006; Piontkewitz et al., 2011a; Pletnikov et al., 2002; Rana et al., 2012; Romero et al., 2007; Zuckerman et al., 2003).

Studies of prenatal exposure have illuminated the crucial role of maternal immune response in the deleterious effects of infection/immune activation by demonstrating that induction of maternal cytokines leads to increased cytokine levels in the placenta/amniotic fluid and the fetal brain, where it likely interferes with normal development (Arrode-Bruses and Bruses, 2012; Bilbo and Schwarz, 2012; Garay et al., 2013; Gilmore and Jarskog, 1997; Gilmore et al., 2005; Kneeland and Fatemi, 2013; Meyer, 2013; Meyer et al., 2006; Urakubo et al., 2001). Surprisingly, although the mammary glands/milk link between mother and neonate in a similar manner to the placental link between mother and fetus, and lactation is a critical vehicle of immunological support to the developing neonate (Brandtzaeg, 2003; Field, 2005; Hosea Blewett et al., 2008; Lepage and Van de Perre, 2012), the effects of maternal immune activation in lactation have never been tested, and models of neonatal immune activation use exclusively direct activation in the neonate.

The first aim of this study was therefore to test the hypothesis that immune activation of the *lactating dam* will have a long-term impact on the sucklings' brain and behavior. The second aim was to test whether such long-term outcomes depended on the sex of the neonates. Sex bias is a ubiquitous characteristic of adult onset neuropsychiatric disorders, suggesting that it may be an important susceptibility factor (Abel et al., 2010; Bale et al., 2010; Essau et al., 2010; Legato, 2010; McCarthy et al., 2012; McGlashan and Bardenstein, 1990). Specifically, during reproductive years, the incidence of depression is two times higher in women than in men (Gobinath et al., 2014; Goldstein et al., 2014), whereas the incidence of schizophrenia is approximately 1.4–1.5 times higher for men than for women (Abel et al., 2010; Hayes et al., 2012; McGrath et al., 2008). However, little research has addressed the effects of early life immune activation on brain and behavior in both sexes (Rana et al., 2012). The third aim was to assess two factors potentially mediating between immune activation in lactating dams and abnormal neurodevelopment in the sucklings: 1. alteration of cytokine levels in milk and neonatal brains as has been shown in placenta and fetal brains; 2. alteration of maternal behavior, long-postulated to underlie the effects of early manipulations on the pup (Smotherman et al., 1977).

Hence, here we exposed both female and male neonates to immune activation either through their nursing mothers or directly, and assessed both sexes in adulthood for the presence of depression- and schizophrenia-like behavioral and brain volumetric abnormalities. We show that a single exposure of lactating mothers to the viral mimic polyriboinosinic-polyribocytidylic acid (poly-I:C) alters cytokine levels in dams' milk and in sucklings' brain, and produces long-term behavioral and brain abnormalities in the sucklings. Furthermore, the behavioral consequences are sexually dimorphic and pharmacologically distinct, consistent with the known sex-bias in schizophrenia and depression as well their clinical pharmacology.

2. Methods and materials

2.1. Animals and neonatal treatments

All experimental protocols conformed to the guidelines of the Institutional Animal Care and Use Committee of Tel Aviv University and NIH. Wistar rats (Tel-Aviv University Medical School) were kept under reversed cycle lighting (lights on: 1900–0700 h) with ad lib food and water. At about 3 months of age, female rats were extensively handled for 5 days and mated. Pregnant dams were housed individually, and the day of birth was defined as day zero. On postnatal day (PND) 4, whole litters were removed from the dams and placed in cages with clean bedding warmed with hot water bottles. While dams' weight was recorded, their litters were culled to ten, composed of five females and five males when possible. Poly-I:C dissolved in saline (4 mg/kg/1 ml) or saline were injected intraperitoneally to the dams (lactational poly-IC; LPIC) or subcutaneously (using a Hamilton syringe [10 μL; 30G needle], Hamilton Inc., Reno, NV) to pups (to whole litters; neonate poly-I:C; NPIC), and whole litters were returned to the mother. All injections were given between 15:00 and 17:00 h. Time away from the mother was less than 3 min per litter. On PND 21 pups were weaned and housed 2–4 to a cage by sex and litter, and maintained undisturbed until behavioral testing in adulthood at which time they were assigned to the experimental groups.

2.2. Maternal-neonatal interface

2.2.1. Maternal behavior

Dams and their pups were observed in their home cages on PNDs 2–7 for 60 min 4 times daily (06:00, 11:00, 16:00, 20:00). PNDs 2–7 were chosen because sickness behavior induced by systemic poly-I:C doses similar to that used in this study, lasts between 24 and 48 h (Cunningham et al., 2007; Gibney et al., 2013), so the observations span 3 periods, before, during, and after poly-:C exposure. The observations were conducted in the vivarium by a "blind" observer who was able to view the rats from several angles without disturbing them. The following behaviors were scored: (1) mother off pups, (2) mother grooming/licking any pup or pups, (3) mother nursing pups in an arched-back posture, (4) mother nursing pups in a "blanket" posture in which the mother lays over the pups, (5) mother nursing pups in a passive posture in which the mother is lying either on her back or side while the pups nurse. The ongoing behavior of each dam was observed every 3 min for 5 s (20 observations per period X 4 periods a day = 80 observations per mother per day) and ticked on a checklist, and the total number of occurrences of each behavior were calculated (Caldji et al., 1998; Champagne et al., 2003; Myers et al., 1989)

2.2.2. Cytokine and corticosterone assessment in maternal milk and plasma

Two hours after poly-I:C/saline injection, dams were anesthetized [isoflurane (2.5% in 97.5% O2) followed by ketamine (100 mg/ml, i.p.) and xylazine (20 mg/ml, i.p.)] and injected s.c. with 4 IU oxytocin (Sigma, Israel) to stimulate milk flow. In order to minimize stress, pups were not separated from dams prior to milking resulting in a small amount of milk sufficient for the testing of no more than three substances. We chose corticosterone, and the cytokines IL-1β and IL-6, based on the known effects of poly-I:C (Cunningham et al., 2007; Gandhi et al., 2007; Kimura et al., 1994; Meyer et al., 2006), and their involvement in schizophrenia and depression (Dowlati et al., 2010; Khandaker et al., 2015).

Milking was done manually. Milk samples were kept on ice until centrifuged at 14,000 rpm for 30 min at 4 °C. Immediately after milking, two ml of blood were withdrawn by cardiac puncture using