Brain, Behavior, and Immunity xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Maternal immune activation transgenerationally modulates maternal care and offspring depression-like behavior

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ARTICLE INFO

Article history:
Received 19 July 2016
Received in revised form 29 September 2016
Accepted 17 October 2016
Available online xxxx

Keywords:
Maternal immune activation
Depression
Hippocampus
Maternal care behavior
Transgenerational
Glucocorticoid receptor
Mineralocorticoid receptor
Oxytocin receptor
Epigenetics

ABSTRACT

Gestational infection is increasingly being recognized for its involvement as causative mechanism in severe developmental brain abnormalities and its contribution to the pathogenesis of psychopathologies later in life. First observations in the widely accepted maternal immune activation (MIA) model based upon the systemic administration of the viral mimetic Polyinosinic:polycytidylic acid (poly(I:C)) have recently suggested a transmission of behavioral and transcriptional traits across generations. Although maternal care behavior (MCB) is known as essential mediator of the transgenerational effects of environmental challenges on offspring brain function and behavior, the possible propagation of alterations of MCB resulting from MIA to following generations has not yet been examined. Here we show that poly (I:C) stimulation at embryonic day 12.5 (E12.5) leads to aberrant MCB and that this effect is transmitted to the female F1 offspring. The transgenerational effects on MCB are paralleled by enhanced depression-like behavior in the second generation F2 offspring with contributions of both maternal and paternal heritages. Examination of offspring hippocampal expression of genes known as targets of MCB and relevant for ensuing non-genetic transmission of altered brain function and behavior revealed transgenerationally conserved and modified expressional patterns in the F1 and F2 generation.

Collectively these data firstly demonstrate the transgenerational transmission of the impact of gestational immune activation on the reproductive care behavior of the mother. Behavioral and molecular characteristics of first and second generation offspring suggest transgenerationally imprinted consequences of gestational infection on psychopathological traits related to mood disorders which remain to be examined in future cross-fostering experiments.

1. Introduction

Early life adversity including exposure to intrauterine immune challenges resulting of infection during pregnancy is a known risk factor for the development of various psychopathologies later in life (Cowan et al., 2016; Groger et al., 1996; McEwen, 2003; Meyer et al., 2008). Epidemiological studies demonstrate a link between maternal immune activation (MIA) during pregnancy and the development of severe psychiatric disorders, including autism, personality and affective disorders (Knuesel and et al., 2014; Reisinger et al., 2015; Ronovsky et al., 2015). Excellent animal models of MIA have been generated as tools for the study of the causal relationship between MIA and offspring behavioral and emotional disturbances and to examine the relevant neurobiological mechanisms involved (Meyer et al., 2009). Among those is administration of the viral mimetic Polyinosinic:polycytidylic acid (poly(I:C)) to pregnant rodents, which causes activation of the maternal innate host defense mechanism against viruses via the toll-like receptor 3 (TLR3) pathway (Reisinger et al., 2015). Major advances in the understanding of the pathological events underlying fetal brain damage and the ensuing behavioral consequences have been made using the poly(I:C) MIA model (Labouesse et al., 2015; Meyer, 2014; Meyer and Feldon, 2012, 2009; Meyer et al., 2007). Among these, accumulating evidences for long-lasting effects of MIA are demonstrated by reports from our laboratory linking poly(I:C)-induced MIA at embryonic day (E) 12.5 with a depression-like phenotype in adult offspring (Khan and et al., 2014; Reisinger et al., 2016) at the behavioral, morphological and electrophysiological levels.

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http://dx.doi.org/10.1016/j.bbi.2016.10.016
0889-1591/

Interestingly, a seminal study recently proposed transgenerational, non-genetic effects of MIA on offspring behavior extending beyond the first generation (F1) to the second (F2) and even third generation, without repetition of immune stimulation. Unique and shared patterns of gene expression changes have been identified in the offspring F1 and F2 amygdala in this experimental setting (Weber-Stadlbauer et al., 2016).

A prime candidate for mediating behavioral and transcriptional alterations across generations is maternal care behavior (MCB) (Champagne and Curley, 2009; Gudsnuk and Champagne, 2012, 2011; Monk et al., 2012) and MCB has been shown to be sensitive to early life adversities (Meek et al., 2001). Specifically, deficits in MCB have been suggested to promote a modulatory impact on hypothalamic-pituitaryadrenal (HPA) axis function. Glucocorticoid receptors, the high-affinity mineralocorticoid receptor and the low-affinity glucocorticoid receptor play a central role in the long-term, compensatory processes resulting from chronic stress exposure with relevance for the regulation of emotional, cognitive and neuroendocrine responses to stress and the susceptibility to the development of severe psychopathologies, including depression (de Kloet and et al., 2016; Farrell and O'Keane, 2016). The effects of MCB on the HPA axis can be propagated via epigenetic mechanisms (Meaney, 2001) - mediators of changes in gene expression without alterations of DNA sequences - to subsequent generations.

However, the particular involvement of MCB in the transgenerational effects of MIA on offspring depression-like behavior has not been examined so far. Here we employed a standard protocol for poly(I:C)-based MIA to investigate the effects of immune activation during pregnancy on MCB, its potential transmission to F1 generation females and depression-like behavior in F2 female offspring along the maternal and paternal lineages. Expressional changes of genes known as candidates for epigenetic modifications by MCB and relevant for the pathophysiology of depression were interrogated as potential molecular correlates in the F1 and F2 generations.

2. Materials and methods

2.1. Animals

C57Bl6/N mice were used for all experiments. For initial breeding animals were purchased from Charles River (Sulzfeld, Germany) at 6–8 weeks of age. All animals were housed under standard conditions in a temperature controlled colony room (22 ± 1 °C) with a 12 h light/dark cycle and food and water *ad libitum* unless otherwise stated. The light intensity was 5–10 lux inside the cages. All animal experiments were carried out in accordance with the ARRIVE guidelines and the U.K. Animals (Scientific Procedures Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments). Animal experiments described in this study were approved by the national ethical committee on animal care and use (BMWF-66.009/0200-WF/V/3b/2016; Bundesministerium für Wissenschaft und Forschung).

2.2. Timed mating

For breeding of the F1 and F2 generations, a timed mating procedure was employed as previously described (Khan and et al., 2014). Briefly, custom-made cages with a central division by a plexiglas wall allowing for odor exchange were used in order to induce the "Whitten Effect" (stimulation of the estrus in females by male pheromones) prior to mating. 3–4 females were put together on one side of the cage wall and one male on the other side for 60 h. Animals were mated overnight starting from 7 pm by placing one female into a male cage. The following day (9 am), the presence of vaginal plugs (considered as E 0.5) together with the body weight was recorded and males were transferred into a new cage.

2.3. MIA and breeding scheme

Poly(I:C) (Sigma, Vienna, Austria) was dissolved in 0.9% NaCl at a final concentration of 2 mg/ml (calculated based upon the weight of poly(I:C) in the mixture itself). Pregnant females were randomly divided into two groups, for MIA and control treatment, respectively. On E12.5, MIA dams received poly(I:C) (20 mg/kg, intraperitoneal (i.p.)) and controls were applied 0.9% NaCl (i.p.), both at 10 ml/ kg injection volume. The dosage regime and time point were chosen based upon previous studies demonstrating enhanced depression-like behavior in adult offspring after MIA on E12.5 (Khan and et al., 2014; Reisinger et al., 2016). MCB was recorded in P0 dams from postnatal day (PD) 1–6. All pups (F1 offspring) were weaned on postnatal day 21, separated by sex and group-housed until adulthood (8 weeks of age). Adult F1 offspring were separated in two cohorts which were used for hippocampal gene expression or breeding of F2 offspring respectively. The mating scheme for the generation of F1 and F2 offspring is illustrated in Fig. 1. At the age of 8 weeks mice were considered adult and were used in a timed mating procedure or for behavioral analyses. Behaviorally naïve mice were always used for breeding and gene expression analyses in order to exclude the possibility of effects of testing history and their interaction with the experimental manipulations on maternal behavior and/or transcriptional changes. In order to control for potential litter-specific effects, experimental groups using F1 and F2 offspring were always composed by selecting corresponding numbers of representative animals from different litters. The numbers of animals/samples used in each experiment is summarized in Supplementary Table 1.

2.4. Behavioral analysis

2.4.1. Maternal care behavior

P0 dams and F1 dams were assessed for MCB after birth of the F1 and F2 generations, respectively. The analysis of MCB followed a published protocol with the day of birth considered as PD 0 (Franks and B., 2011). From PD 1 to PD 6 maternal behavior was recorded daily from 11 am to 1 pm and from 3 pm to 5 pm with commercially available webcams (Logitech C525 HD Webcam, Microsoft LifeCam HD-3000, Trust Widescreen HD-Webcam, Creative LIVE! Cam Chat HD USB-Webcam). Behaviors were video-scored every 3 min by an experimenter blind to the experimental conditions. Parameters evaluated consisted of pup licking/grooming, nest-building, nursing and non-pup relevant parameters (eating/sleeping, self-grooming).

2.4.2. Sucrose preference test (SPT)

Sucrose preference in the SPT was tested in female F2 offspring. The SPT was conducted as previously described (Savalli and et al., 2015). Briefly, mice were deprived of food and water 18 h before the test where they were given the choice to consume liquid from two equal bottles, one containing a 2% sucrose solution and one filled with regular drinking water. The volumes of sucrose solution and drinking water consumed during the SPT were determined by weighing the bottles before and after the 3 h testing period and used for the calculation of the percentage of sucrose preference.

2.4.3. Forced swim test (FST)

Time spent immobile in the FST was assessed in female F2 offspring. The FST was carried out as described earlier (Monje and

Please cite this article in press as: Ronovsky, M., et al. Maternal immune activation transgenerationally modulates maternal care and offspring depression-like behavior. Brain Behav. Immun. (2016), http://dx.doi.org/10.1016/j.bbi.2016.10.016