Psychoneuroendocrinology (2012) 37, 1320—1335

Available online at www.sciencedirect.com

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Transgenerational transmission of anxiety induced by neonatal exposure to lipopolysaccharide: Implications for male and female germ lines

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Received 13 September 2011; received in revised form 6 December 2011; accepted 11 January 2012

KEYWORDS
Transgenerational transmission;
Perinatal programming;
LPS;
Anxiety;
HPA axis;
Maternal care

Summary Neonatal lipopolysaccharide (LPS) exposure increases anxiety-like behaviour and alters neuroendocrine responses to stress in adult rats. The current study assessed whether this anxiety-related phenotype observed in rats neonatally exposed to LPS is transferable to subsequent generations. Wistar rats were exposed to LPS (0.05 mg/kg, *Salmonella enteritidis*) or non-pyrogenic saline (equivolume) on postnatal days 3 and 5. In adulthood, animals were subjected to restraint and isolation stress or no stress, and subsequently evaluated for anxiety-like behaviours on the elevated plus maze, acoustic startle response, and holeboard apparatus. Blood was collected to examine corticosterone responses to stress and behavioural testing in adulthood. Animals from both treatment groups which exhibited the anxiety-like phenotype were bred with untreated partners. Maternal care of the second generation (F2) was monitored over the first week of life. In adulthood, the F2 generation underwent identical testing procedures as the parental (F1) generation. The F2 offspring of females exposed to LPS as neonates exhibited an anxiety-like phenotype in adulthood and a potentiated corticosterone response to stress ($p < .05$). F2 offspring of males exposed to LPS as neonates also exhibited an anxiety-like phenotype ($p < .05$), however, no differences in corticosterone responses were observed. To determine the impact of maternal care on the anxiety-like phenotype, a cross-fostering study was conducted in which offspring of LPS-treated females were fostered to saline-treated mothers and vice versa, which was found to reverse the behavioural and endocrine phenotypes of the F2 generation. These data indicate that a neonatally bacterially induced anxiety phenotype is transferable across generations in both sexes. Maternal care is the mediating mechanism along the maternal line. We suggest that transmission may be dependent upon heritable epigenetic phenomena for the paternal line. The implications of this study apply to potential neuroimmune pathways through which psychopathology may be transmitted along filial lines.

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0306-4530/$ — see front matter.
doi:10.1016/j.psyneuen.2012.01.005

1. Introduction

Psychopathology, including depression, schizophrenia and anxiety disorders, has been well-documented to transmit down filial lines (Baron et al., 1985; Cadoret et al., 1985; Holma et al., 2011; Merikangas and Swendsen, 1997) yet little clear genetic evidence has surfaced to explain this phenomenon. A range of perinatal and infant environmental factors have been reported to increase susceptibility to the onset of later life psychopathology, however, the underlying mechanisms remain largely elusive. It is now widely accepted that a combined effect of nature and nurture is likely to be responsible for this transgenerational phenomenon — a promising direction of which lies in the area of non-genomic modifications, or *epigenetics*.

Epigenetics refers to the modification of gene activation without altering the underlying DNA sequence. Chromatin remodelling and RNA transcripts have been presented as the primary avenues through which epigenetic modifications may arise, with limited evidence indicating that these changes to the epigenome may be heritable (reviewed in Meaney and Ferguson-Smith, 2010; Skinner and Guerrero-Bosagna, 2009). Certainly, environmental programming of biology in one generation has become widely accepted, whereby the perinatal environment acts as a crucial contributor to the long-term functioning of an individual. Environmental stressors during perinatal life, such as malnutrition, gestational stress, and maternal care are known to not only alter the developmental trajectory of physiological systems, but also increase the potential for later health complications (Felitti et al., 1998; Repetti et al., 2002).

In humans, a common event in foetal and neonatal life is exposure to bacteria. Perinatal exposure to bacteria permanently alters the development of critical physiological systems and programs alterations in neuroendocrine and behavioural responses. In particular, the early microbial environment influences physiological stress response systems, such as the hypothalamic—pituitary—adrenal (HPA) axis, and impacts behavioural outcomes (Bilbo et al., 2005a,b; Breivik et al., 2002; Hodgson et al., 2001; Meyer et al., 2006; Shanks et al., 1995, 2000; Shanks and Meaney, 1994; Walker et al., 2004, 2008, 2009, 2010, 2011). Recent data from this and other laboratories have indicated that exposure to bacteria early in life predisposes rodents to anxiety-like behaviour that persists into adulthood. We and others have employed animal models to investigate the underlying mechanisms of this phenomenon, and have observed robust and replicable increases in anxiety-like behaviour in rats following exposure to a bacterial mimetic, lipopolysaccharide (LPS), during the early postnatal period (Breivik et al., 2002; Shanks et al., 2000; Walker et al., 2004, 2008, 2009). Along with these changes in behaviour are associated perturbations to the HPA axis stress response (Hodgson et al., 2001; Shanks et al., 1995; Walker et al., 2009, 2010). A number of mechanisms have been proposed to account for these functional changes such as microglial priming and activation (Bilbo et al., 2005a, 2008; Bilbo and Schwarz, 2009; Sominsky et al., 2012), central cytokine regulation (Bilbo et al., 2005a; Walker et al., 2010; Kohman et al., 2008; Harre et al., 2008), glucocorticoid receptor density (Shanks et al., 1995) and epigenetic modifications.

As a step towards understanding the epigenetic inheritance of anxiety, we examined the potential for transgenerational inheritance of an anxiety-like phenotype following neonatal LPS administration to the F1 generation. Neonatal LPS exposure was employed to induce the known anxiety-like phenotype in the F1 generation (previously published in Walker et al., 2004, 2008, 2009). LPS and saline-treated males were then bred with untreated partners and the behavioural and neuroendocrine phenotypes of the F2 generation were examined. Cross-fostering was conducted to determine the relative influence of maternal care on the behavioural and endocrine profile of the F2 offspring. Identical anxiety-related variables were assessed for each generation using behavioural testing on the elevated plus maze (EPM), holeboard apparatus, and acoustic startle response (ASR). Schematics of the experimental design are provided in Fig. 1A and B.

2. Methods

2.1. Ethics statement

All experimentation occurred in accordance with the 2004 NH&MRC Australian Code of Practice for the care and use of animals for scientific practice. All efforts to reduce animal suffering, the numbers of animals used, and to utilise alternatives to in vivo techniques, if available, were made. This study was approved by The University of Newcastle Animal Care and Ethics Committee (approval number: ACEC 901).

2.2. F1 generation animals and neonatal treatment

Twenty experimentally naïve female Wistar rats obtained from the University of Newcastle animal house were bred in the University of Newcastle Psychology vivarium resulting in a total of 163 (79 male, 84 female) offspring, which were used in this experiment. At birth (postnatal day [PND] 1), whole litters were randomly assigned to either LPS or saline conditions. On PND 3 and PND 5, animals were briefly removed from their home cages, weighed, and administered either 0.05 mg/kg LPS (*Salmonella enterica*, serotype enteritidis; Sigma—Aldrich Chemical Co., USA) or an equivolume of non-pyrogenic 0.9% saline (Livingstone International, Australia) intraperitoneally. To determine that neonatal LPS administration was effective in activating the neonatal endocrine system, trunk blood was collected from a subgroup of animals (39 males and 44 females) deriving from 6 litters, on PNDs 3 and 5 following neonatal drug administration for assessment of plasma corticosterone concentrations. The remaining litters were left with their dams until weaning (PND 22) when they were separated into same-sex pair housing (41.5 cm × 28.0 cm × 22.0 cm cages; Mascot Wire Works, Sydney). Animals were left undisturbed from weaning until behavioural testing in adulthood (PND 85) except for weekly weights and observation. Housing conditions were identical to those previously reported (Walker et al., 2009).

2.3. Adult testing

2.3.1. Estrous cycle in females

Estrous cyclicity in females was monitored in adulthood using a Rat Vaginal Impedance Checker (Muromachi Kikai, Tokyo, Osaka) according to the manufacturer's instructions and is