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Neonatal lipopolysaccharide exposure impairs sexual development and reproductive success in the Wistar rat

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ABSTRACT

We investigated, in rats, whether neonatal exposure to bacterial lipopolysaccharide (LPS) impairs sexual development, sexual decline, and reproductive behaviour in later life. Rats were administered either LPS (*Salmonella enterica*, serotype enteritidis, 0.05 mg/kg, ip) or saline (equivolume) on days 3 and 5 postpartum. The immediate and long-term effect of treatment on HPA and HPG hormones, testicular morphology, and mating behaviour was assessed. Neonatal LPS exposure induced a significant increase in corticosterone compared to controls, as well as reduced testosterone and LH in males and LH in females immediately following neonatal drug exposure. Neonatal LPS exposure disrupted the normal weight-to-age ratio of puberty onset in males and females, and impaired sexual performance in adulthood. Reproductive function was reflected in significantly diminished sperm presence in rats that had received neonatal LPS. LPS-treated females exhibited LH suppression during puberty, and males demonstrated testosterone suppression in late adulthood. Testosterone and LH surges during mating were significantly reduced in adult offspring treated with LPS as neonates. Furthermore, animals exposed to neonatal LPS and subsequent stress in adulthood, exhibited significantly blunted corticosterone responses. Morphometric assessment of testes taken from neonates revealed reduced gonocyte genesis immediately following LPS exposure and increased seminiferous disorganisation of the epithelium in these animals in adulthood. This research demonstrates the long-term impact of neonatal bacterial exposure on reproductive success given that early life exposure to bacteria disrupted puberty onset and sexual performance. Associated changes in neuroendocrine functioning suggest a possible mechanism through which a subfertile phenotype may arise.

1. Introduction

Reproductive dysfunction is a commonly occurring phenomenon (Davies and Norman, 2002), and often the underlying cause of such dysfunction is unknown (Hull et al., 1985). While it is accepted that teratogenic insults affect fertility, the ever-growing increase in unexplained infertility and subfertility has led to suggestions that perhaps subtle, developmental factors may contribute to limiting the reproductive success of some individuals. Identifying potential perinatal events, which may shift the functional outcome of bodily systems, and hence contribute to later life health has been referred to as *perinatal programming* (Hodgson and Coe, 2006).

Research investigating this phenomenon has demonstrated long-term changes in immune (Shanks et al., 1995; Hodgson et al., 2001), metabolic (Lindsay et al., 1996; Walker et al., 2006), and neuroendocrine function (Matthews, 2002; Walker et al., 2008, 2010), as well as behaviour (Breivik et al., 2002; Walker et al., 2004, 2009) following perinatal stress. Such findings suggest that these programming outcomes may, in some cases, increase disease susceptibility (Hodgson and Coe, 2006). Indeed, various perinatal stressors have been shown to increase susceptibility to diabetes (Freimanis et al., 2003; McPherson et al., 2009), cardiovascular disease (Gluckman et al., 2008), infection (Breivik et al., 2002; Boisse et al., 2004), tumour metastasis (Hodgson et al., 2001), and cognitive and behavioural disturbances (Widom, 1999; Walker et al., 2004, 2009; Gibb et al., 2007; Bilbo, 2010).

Given the body of research indicating that exposure to perinatal environmental insults may result in a 'suboptimal' phenotype predisposing to increased health risks, it is meaningful to view such susceptibilities within an evolutionary setting. An understanding of how environmental insults during this period may alter

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reproductive fitness is particularly important, and assists the emerging field of perinatal programming by elucidating how long-term changes in physiology and behaviour may affect genetic succession to subsequent generations. Thus particular programmed phenotypes may become more or less robust within a population. Of interest in the current study is the way in which neonatal exposure to a bacterial mimetic, lipopolysaccharide (LPS), can alter sexual development and reproductive fitness throughout the lifespan.

To date, few studies have examined the influence of postnatal immune activation on hypothalamic–pituitary–gonadal (HPG) outcomes (Li et al., 2007; Iwasa et al., 2009a,b) and none have examined sexual behaviour concurrently. The scarcity of such research is surprising given that bacterial infection is a common occurrence during early postnatal life, and HPG activity is strongly upregulated during this period (Main et al., 2000). Furthermore, given the trend for couples to delay their child-bearing in recent years (Lutz et al., 2003), environmental factors, such as bacterial exposures, that may increase subfertility, and thus alter puberty onset and HPG function have become increasingly relevant for conception in the modern backdrop. Hence, understanding the role that the early microbial environment plays in influencing reproductive development may elucidate potential pathways through which subfertility can arise. The current study, therefore, utilised a rodent model to understand how neonatal exposure to bacteria may alter HPG outcomes, including sexual behaviour and puberty onset. HPG endocrine function was assessed throughout development and sexual behaviour, including receptive and rejection behaviours, was monitored in adulthood. Estrous cyclicity and success of mating were similarly determined.

Importantly, the timing of the bacterial exposure in our model coincides with the early postnatal surge of LH, follicle stimulating hormone (FSH), oestrogens and androgens responsible for the differentiation of Leydig cells and spermatogenesis in the testes (Sharpe et al., 2003). Therefore, we also examined testicular morphology during neonatal life and in adulthood to determine whether hormonal perturbations brought about by neonatal LPS exposure coincided with changes in testicular formation.

2. Materials and methods

2.1. Animals and neonatal drug administration

Twelve 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 130 (65 males, 65 females) offspring, which were used in this study. At birth (postnatal day [PND] 1), litters were randomly allocated into either LPS or saline control conditions. No significant difference in litter size was observed between litters allocated to LPS (M = 11.5 pups, SD = 3.12) or saline (M = 13.3 pups, SD = 3.21). No difference in the percentage of males and females per litter were observed (all litters $\approx$ 50% males). On PND 3 and 5, animals were administered 0.05 mg/kg LPS (*Salmonella enterica*, serotype enteritidis; Sigma–Aldrich Chemical Co., USA, dissolved in sterile pyrogen-free saline) or an equivolume of non-pyrogenic saline (Livingstone International, Australia) via intraperitoneal microinjection. This dosage and timing of LPS exposure has previously been found to be critical in producing long-term reproductive changes (Knox et al., 2009). Drug administration procedures and housing conditions were identical to those previously described (Walker et al., 2009). Animals were randomly allocated to either (1) the developmental study (n = 25 for each sex) or (2) the adult sexual behaviour study (n = 40 for each sex). Apart from a subset of animals sacrificed to assess the immediate effect of treatment on HPA and HPG function, animals underwent daily assessment of pubertal markers from weaning. Animals were maintained under a 12 h light/dark schedule (lights on 06:00 h for animals in the developmental study, and lights on 02:00 h for animals in the sex behaviour study as copulation typically occurs nocturnally). Weekly weights were collected from weaning (i.e. PND 22, 29, 36, 43, 50, 57, 64, 71, 78, 85). All experimentation occurred in accordance with the 2004 NH&MRC Australian Code of Practice for the care and use of animals for scientific practice.

2.2. Neonatal blood and testicle collection

A subgroup of rats was sacrificed during neonatal life in order to assess plasma corticosterone, testosterone and LH responses to treatment, as well as for morphometric assessment of testes. Four hours following injection on PND 5, trunk blood and testes were collected. This time point was chosen as we have previously demonstrated neuroendocrine activation 4 h following neonatal LPS administration (Walker et al., 2009, 2010). Collection of trunk blood occurred via rapid decapitation into EDTA-coated tubes (Livingstone International, Australia) and testes were excised, excess connective tissue was removed, and placed immediately into Bouin's Solution (Sigma–Aldrich, Australia) for fixation.

2.3. Sexual developmental cohort

Animals were monitored daily following weaning for physical markers of puberty, such as preputial separation for males and vaginal opening for females. Once vaginal opening occurred, estrous cyclicity was monitored using a Rat Vaginal Impedance Checker (Muromachi Kikai, Tokyo, Osaka; described in Walker et al., 2010) according to the manufacturer's instructions, and proestrous was confirmed using vaginal smears.

Blood was collected via the saphenous vein into EDTA-coated tubes (Livingstone International, Australia) over three time points during adolescence to assess LH and testosterone in males (PND 43, 46, 49), and to assess LH in females (PND 33, 36, 39). These time points were chosen given that the hormonal surges necessary for the attainment of puberty have been shown to typically occur during these time periods (Goldman et al., 2000; Zapatero-Caballero et al., 2003). These hormones were again assessed in late adulthood to determine sexual decline. At 9, 10, 11, and 12 months of age blood was collected via the saphenous vein from males and weights were taken. Females were monitored for cyclicity at these ages and blood was collected during proestrus.

2.4. Behavioural testing in adulthood

2.4.1. Three-day stress protocol

Prior to sexual behaviour testing, half of the animals underwent an additional "restraint and isolation stress" protocol as previously described (Walker et al., 2009) given that the impact of early life LPS exposure has been shown to be amplified in the presence of a subsequent stressor in adulthood (Walker et al., 2009, 2010). Animals allocated to sexual behaviour testing in adulthood (PND 85) were randomly divided into either a 3-day "stress", or "no stress" condition, following which animals were exposed to sexual behaviour testing.

2.4.2. Behavioural procedures

All behavioural testing was conducted in adulthood (PND 85) under infrared light between 16:00 h and 18:00 h during the dark phase. During the 15 min provided for experimental animals to habituate to the mating arena prior to the introduction of the untreated conspecific, anxiety-like behaviours were assessed using the arena as an adapted Hide Box/Open Field. A description of