Psychoneuroendocrinology 62 (2015) 204–216

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

Prenatal stress programs neuroendocrine stress responses and affective behaviors in second generation rats in a sex-dependent manner

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ARTICLE INFO

Article history:
Received 18 June 2015
Received in revised form 5 August 2015
Accepted 13 August 2015

Keywords:
Anxiety
Glucocorticoids
HPA axis
Prenatal stress
Sex differences
Trans-generational

ABSTRACT

An adverse environment in early life is often associated with dysregulation of the hypothalamo-pituitary-adrenal (HPA) axis and higher rates of mood disorders in adulthood. In rats, exposure to social stress during pregnancy results in hyperactive HPA axis responses to stress in the adult offspring and heightened anxiety behavior in the males, but not the females. Here we tested whether, without further intervention, the effects of prenatal stress (PNS) in the first filial generation (F1) are transmitted to the F2 generation via the maternal line. F1 control and PNS female rats were mated with control males and housed under non-stress conditions throughout pregnancy. HPA axis responses to acute stress, anxiety- and depressive-like behavior were assessed in the adult F2 offspring.

ACTH and corticosterone responses to an acute stressor were markedly enhanced in F2 PNS females compared with controls. This was associated with greater corticotropin releasing hormone (*Crh*) mRNA expression in the paraventricular nucleus and reduced hippocampal glucocorticoid (*Gr*) and mineralocorticoid receptor (*Mr*) mRNA expression. Conversely, in the F2 PNS males, HPA axis responses to acute stress were attenuated and hippocampal *Gr* mRNA expression was greater compared with controls.

F2 PNS males exhibited heightened anxiety-like behavior (light-dark box and elevated plus maze) compared with F2 control males. Anxiety-like behavior did not differ between F2 control and PNS females during metestrus/diestrus, however at proestrus/estrus, F2 control females displayed a reduction in anxiety-like behavior, but this effect was not observed in the F2 PNS females. Heightened anxiety in the F2 PNS males was associated with greater *Crh* mRNA expression in the central nucleus of the amygdala compared with controls. Moreover, Crh receptor-1 (*Crhr1*) mRNA expression was significantly increased, whereas *Crhr2* mRNA was significantly decreased in discrete regions of the amygdala in F2 PNS males compared with controls, with no differences in the F2 females. No differences in depressive-like behavior (sucrose preference or forced swim test) were observed in either sex. In conclusion, the effects of maternal stress during pregnancy on HPA axis regulation and anxiety-like behavior can be transmitted to future generations in a sex-dependent manner. These data have implications for human neuropsychiatric disorders with developmental origins.

1. Introduction

Maternal exposure to stress during pregnancy can have wide-ranging and long-lasting effects on the offspring's brain and behavior. A growing body of evidence supports the hypothesis that some psychiatric and behavioral disorders in humans have developmental origins (Glover, 2015; King et al., 2012). The phenomenon of 'fetal programming' of the brain by prenatal stress is well established in rodents and is generally associated with heightened anxiety- and depressive-like behaviors and augmented stress responses (Abe et al., 2007; Brunton and Russell, 2010; Mueller and Bale, 2008; Vallee et al., 1997), though some effects are evidently sex-dependent (for review see (Brunton, 2010; Weinstock, 2007)). The neuroendocrine stress axis, the hypothalamo-pituitary-adrenal (HPA) axis, is particularly susceptible to fetal programming by prenatal stress (Maccari et al., 2014) and the resultant HPA axis dysfunction may underpin altered affective traits and an increased propensity for developing psychiatric disorders (Wingenfeld and Wolf, 2011).

Using an ethologically relevant rodent model of social stress (i.e. resident-intruder test) in pregnancy, we have previously shown that both male and female prenatally stressed offspring

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http://dx.doi.org/10.1016/j.psyneuen.2015.08.010

display markedly enhanced HPA axis responses to acute physical and psychological stressors in later life (Brunton and Russell, 2010), reflected by greater stress-induced ACTH and corticosterone secretion and greater levels of corticotropin-releasing hormone (*Crh*) mRNA expression in the medial parvocellular division of the paraventricular nucleus (mpPVN) (Brunton and Russell, 2010). Impaired central glucocorticoid negative feedback regulation of the HPA axis may explain enhanced HPA axis responses to stress in prenatally stressed offspring and is supported by findings of reduced hippocampal expression of mRNA for mineralocorticoid receptor (*Mr*) in our model or both glucocorticoid receptor (*Gr*) and *Mr* in other models of prenatal stress (Maccari et al., 2014).

In addition, we have demonstrated heightened anxiety-like behavior on the elevated plus maze in the adult male, but not the female prenatally stressed offspring (Brunton and Russell, 2010). The central CRH system plays a major role in the regulation of anxiety-like behavior, particularly at the level of the amygdala (Schulkin, 2006). In rodent models, increased anxiety-related behavior is associated with greater *Crh* gene expression in the amygdala and central administration of CRH is able to induce anxiety-like behavior in rats (Dunn and Berridge, 1990; Schulkin, 2006). Moreover in prenatally stressed rats, the anxious phenotype is associated with greater *Crh* mRNA expression, CRH content and CRH release in the amygdala (Brunton and Russell, 2010; Cratty et al., 1995; Zohar and Weinstock, 2011).

CRH exerts its actions through two receptors: CRH-type 1 (CRHR1) and CRH-type 2 (CRHR2), with both receptors present in the amygdala, but with distinct expression profiles (Van Pett et al., 2000). Several lines of evidence from studies using receptor antagonists, antisense oligonucleotides and knockout mice strongly support a role for CRHR1 in mediating the anxiogenic effects of CRH (Liebsch et al., 1995; Muller et al., 2003; Smith et al., 1998; Wang et al., 2012). The role for CRHR2 in regulating anxiety-like behavior is less clear (Bale and Vale, 2004), however several studies support an anxiolytic role for CRHR2 (Bale et al., 2000; Kishimoto et al., 2000; Vetter et al., 2002). In our rat model of prenatal stress, we have demonstrated that increased anxiety-like behavior in the adult male offspring is associated with increased *Crhr1* and decreased *Crhr2* mRNA expression in the amygdala (Brunton et al., 2011). These data are consistent with other rodent models of early life stress where an anxious phenotype has been identified (Wang et al., 2013; Wang et al., 2012; Zohar and Weinstock, 2011).

A large body of data supports the hypothesis that the long-term effects of prenatal stress on the HPA axis and behavior involve stable and persistent changes in gene function. The underlying mechanisms by which an adverse prenatal environment is embedded in the genome have yet to be fully elucidated however epigenetic modifications are likely to play a key role (Bale, 2015; Maccari et al., 2014). This raises the possibility of transmission of prenatal stress effects to future generations. It has become increasingly evident that the adverse effects of a sub-optimal environment in early life can be transmitted to subsequent generations, apparently via non-genomic mechanisms (Bale, 2015). Most extensively studied is maternal malnutrition (under- or over-nutrition) during pregnancy. In guinea pigs, the effects of maternal undernutrition during pregnancy on HPA axis activity in the F1 offspring is transmitted to the F2 generation (Bertram et al., 2008) and in mice, phenotypes resulting from maternal high-fat diet (e.g. insulin insensitivity) can be passed to subsequent generations (Dunn and Bale, 2011).

Studies where pregnant rodents have been administered synthetic glucocorticoids (e.g. dexamethasone or betamethasone) to mimic maternal stress exposure have demonstrated changes in glucose tolerance, HPA axis regulation and anxiety-like behavior in the second generation offspring (Drake et al., 2005; Iqbal et al., 2012), however to date, very few studies have specifically investigated transgenerational inheritance of phenotypes induced by prenatal stress exposure. Thus, the aim of the current study was to investigate whether, without further intervention, the effects of social stress exposure during pregnancy on HPA axis function and related behaviors in the F1 offspring are transmitted to the F2 offspring and whether there were any sex differences in transmission.

2. Materials and methods

2.1. Animals

Sprague-Dawley rats for the parental (P) generation were purchased from Charles River (Margate, Kent, UK). The first (F1) and second (F2) filial generations of control and prenatally stressed (PNS) offspring were bred in-house. Rats were maintained on a 12–12 h light–dark cycle, under controlled temperature ($22 \pm 2^{\circ}C$) and humidity ($55 \pm 5\%$) with free access to standard 14% protein rodent diet (Harlan Teklad). Breeding females were ad libitum fed a 50:50 mixture of 14% and 19% protein diet (Harlan Teklad) throughout pregnancy and lactation. Rats were group housed (4–6 females, 3–5 males) in open-top cages. A maximum of 2 rats/sex from each F2 litter were used/group for each experiment. All experiments were approved by the local Animal Welfare and Ethical Review Body and performed in accordance with the UK Animals (Scientific Procedures) Act 1986 and the European Directive (2010/63/EU).

2.1.1. Mating and pregnancy monitoring

Pairs of rats from the P or F1 generations were mated overnight. The presence of a semen plug in the breeding cage was designated day 1 of pregnancy. All males used for breeding were non-stressed controls. To generate the F1 offspring, pregnant females were either undisturbed throughout pregnancy (producing F1 control offspring) or exposed to repeated social stress (see Section 2.2) to produce PNS F1 offspring. To generate the F2 offspring, adult unmanipulated F1 females born to either control mothers or born to mothers that experienced social stress during pregnancy (see Section 2.2) were mated with control males. All the pregnant F1 rats were undisturbed throughout pregnancy, except for weighing every 4 days to monitor pregnancy progression. Pregnant rats were initially group housed until day 14 (P) or 20 (F1) of pregnancy, after which time they were separated into single cages. Soon after parturition, litter sizes, pup sex and pup weights were recorded. Pups were weighed again on post-natal day (PND) 8. Dams remained with their litters until weaning at PND 23 (F1) or 25 (F2), then offspring were housed in groups by litter and sex under standard conditions (see Section 2.1) until experiments commenced.

2.2. Prenatal stress paradigm

Pregnant rats (10–12 weeks old) forming the P generation ('grandmothers') were exposed to repeated social stress utilizing a resident-intruder paradigm as previously described (Brunton and Russell, 2010). Briefly, pregnant rats were placed in the cage of a different lactating 'resident' rat (days 4–9 of lactation) for 10 min/day on days 16–20 of pregnancy. We have previously demonstrated this social stress paradigm increases corticosterone secretion in the pregnant rats (Brunton and Russell, 2010). Pregnant control females (forming the P1 generation) remained in their home cages throughout gestation except for weighing on days 16 to 20.

2.3. Surgery: Jugular vein cannulation

Adult F2 control and PNS rats (males aged 13–15 weeks and females 16–17 weeks) were fitted with a silicone jugular vein cannula filled with sterile heparinized 0.9% saline (50U heparin/ml)