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REVIEW

SPECIAL ISSUE

DEVELOPMENTAL PLASTICITY: FROM MECHANISMS TO EVOLUTIONARY PROCESSES

How is prenatal stress transmitted from the mother to the fetus?

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ABSTRACT

Prenatal stress programmes long-lasting neuroendocrine and behavioural changes in the offspring. Often this programming is maladaptive and sex specific. For example, using a rat model of maternal social stress in late pregnancy, we have demonstrated that adult prenatally stressed male, but not prenatally stressed female offspring display heightened anxiety-like behaviour, whereas both sexes show hyperactive hypothalamo–pituitary–adrenal (HPA) axis responses to stress. Here, we review the current knowledge of the mechanisms underpinning dysregulated HPA axis responses, including evidence supporting a role for reduced neurosteroid-mediated GABAergic inhibitory signalling in the brains of prenatally stressed offspring. How maternal psychosocial stress is signalled from the mother to the fetuses is unclear. Direct transfer of maternal glucocorticoids to the fetuses is often considered to mediate the programming effects of maternal stress on the offspring. However, protective mechanisms including attenuated maternal stress responses and placental 11β-hydroxysteroid dehydrogenase-2 (which inactivates glucocorticoids) should limit materno-fetal glucocorticoid transfer during pregnancy. Moreover, a lack of correlation between maternal stress, circulating maternal glucocorticoid levels and circulating fetal glucocorticoid levels is reported in several studies and across different species. Therefore, here we interrogate the evidence for a role for maternal glucocorticoids in mediating the effects of maternal stress on the offspring and consider the evidence for alternative mechanisms, including an indirect role for glucocorticoids and the contribution of changes in the placenta in signalling the stress status of the mother to the fetus.

KEY WORDS: Anxiety, Fetal programming, Glucocorticoids, 11β-hydroxysteroid dehydrogenase-2, Hypothalamo–pituitary–adrenal (HPA) axis, Placenta

Introduction

It is well established that the environment experienced during early development can influence phenotypic outcomes. This has been amply demonstrated by studies performed in a wide range of wild and captive species from invertebrates to fish, amphibians to reptiles, and birds to mammals (Kofman, 2002; Mueller and Bale, 2008; Brunton and Russell, 2010; Dantzer et al., 2013; Zimmer et al., 2013; Burton and Metcalfe, 2014; Rutherford et al., 2014; Glover, 2015; Eyck et al., 2019; Burton et al., 2021; Crombie et al., 2021a; Palacios et al., 2023).

In mammals, including humans, a key developmental window is gestation. Hence, exposure to a suboptimal *in utero* environment during pregnancy can impact fetal development and have long-lasting effects on an individual's health and susceptibility to disease later in life – a phenomenon known as fetal programming (Glover et al., 2010; Brunton and Russell, 2011; Harris and Seckl, 2011). Research has linked fetal programming to a host of adverse health outcomes, such as cardiovascular disease, metabolic syndrome, psychiatric disorders and cognitive impairments (Harris and Seckl, 2011; Lautarescu et al., 2020). One such factor that can lead to fetal programming is maternal stress exposure during pregnancy. An abundance of evidence over the past three decades, largely from rodent studies, supports the concept that prenatal stress has long-term adverse impacts on the offspring's physiology and behaviour (Bale et al., 2010; Maccari et al., 2014; Brunton, 2015). In particular, the fetal brain is especially 'plastic' during the prenatal period and therefore vulnerable to being shaped by external influences (Tau and Peterson, 2010; Wood and Walker, 2015; Thomason, 2020). Indeed, changes in the offspring's brain likely underpin many of the offspring phenotypes reported following prenatal stress exposure. Here, we will begin by reviewing the impact of maternal stress during pregnancy on two phenotypic outcomes in the offspring – dysregulated stress responses and heightened anxiety-like behaviour – with a focus on findings from rodent studies. Although the impact of maternal stress on the offspring is undisputable, the mechanisms through which the mother's stress status is signalled to the fetus are yet to be fully elucidated. Hence, we will next discuss the different mechanisms proposed and examine the supporting evidence for these.

Impact of maternal stress during pregnancy on offspring phenotypes

Studies in laboratory rodents have linked prenatal stress with adverse pregnancy outcomes such as low birth weight, reduced litter sizes and greater neonatal mortality rates (De Catanzaro, 1988; Pratt and Lisk, 1991; Brunton and Russell, 2010; Paris et al., 2011). Negative effects of maternal stress are also reported in wild animal populations, though effects are evidently greater in viviparous species than in oviparous species (Macleod et al., 2021), suggesting that extended feto-maternal interaction determines the extent of the impact on the offspring. In women, maternal stress is a significant risk factor for preterm birth and low for gestational age birth weight babies (Dunkel Schetter and Tanner, 2012). Later in life, other adverse offspring phenotypes are reported in rodents, including: dysregulated stress responses and increased anxiety- and depression-like or passive stress coping behaviours (Takahashi and Kalin, 1991; Weinstock et al., 1992; Koenig et al., 2005; Fan et al., 2009; Brunton and Russell, 2010; Sheriff et al., 2010); hypertension (Igosheva et al., 2004); impaired glucose regulation, insulin resistance and diet-induced obesity (Nilsson et al., 2001; Tamashiro

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et al., 2009; Brunton et al., 2013); abnormal neural development and cognitive impairments (Lemaire et al., 2000; Paris et al., 2011; Paris and Frye, 2011a); as well as aberrant social and reproductive behaviours (Holson et al., 1995; Frye and Orecki, 2002; Patin et al., 2005; Bosch et al., 2007; Lee et al., 2007; Grundwald et al., 2016). Comparable phenotypes have been described in humans whose mothers experienced stress during pregnancy (Entringer et al., 2008, 2009; King et al., 2012; Sandman et al., 2012; Lautarescu et al., 2020; Van den Bergh et al., 2020).

It has been proposed that at least some of these programmed offspring phenotypes may be adaptations that better prepare the offspring to cope with the predicted post-natal environment (Sheriff and Love, 2013). For example, the fetus of a malnourished pregnancy may develop a 'thrifty phenotype', where their postnatal metabolism is adapted to promote survival in an environment with chronic food shortage (Hales and Barker, 2001). In a similar vein, heightened anxiety behaviour and hyperactive stress responses in the offspring of stressed mothers (Fride and Weinstock, 1988; Henry et al., 1994; Vallee et al., 1997; Brunton and Russell, 2010) may support greater vigilance to environmental threats, which could be beneficial to survival, even if it has a cumulative long-term cost (McEwen, 2008). However, if there is a mismatch between the post-natal environment actually encountered and that forecasted prenatally (i.e. the offspring from a stressed pregnancy reared in an non-stressful post-natal environment, or vice versa), these adaptations can be maladaptive, increasing the susceptibility of the offspring to disease (Godfrey et al., 2007). For instance, an individual that has developed a thrifty phenotype born into an environment where food is plentiful may be more susceptible to developing obesity and type II diabetes (Hales and Barker, 2001). Similarly, prenatally stressed offspring can be hyper-vigilant and hyper-responsive to mild stressors experienced during adulthood, and have a greater predisposition to develop mood disorders (Seckl and Holmes, 2007).

Hypothalamo–pituitary–adrenal axis dysregulation

The hypothalamo–pituitary–adrenal (HPA) axis is the principal neuroendocrine system that responds to stress (Fig. 1). The final output of HPA axis activation is the secretion of glucocorticoids into the blood (e.g. corticosterone in rats, mice and birds; cortisol in guinea pigs, sheep and humans). Glucocorticoids promote energy mobilisation from stores and stimulate gluconeogenesis, thereby increasing glucose availability and ensuring sufficient energy to cope with the stress (e.g. to mount a fight or flight response). Glucocorticoids also aid in the restoration of physiological homeostasis once the stressor no longer poses a threat, through negative feedback regulation of their own secretion at several levels of the system (Fig. 1).

Dysregulation of the HPA axis is a common feature in the offspring of mothers that experienced stress during pregnancy, irrespective of the prenatal stress paradigm employed. Typically, when prenatally stressed offspring themselves are exposed to stress in adulthood, they display adrenocorticotropic hormone (ACTH) and glucocorticoid secretory responses that are greater in amplitude and/or prolonged compared with control offspring (Weinstock et al., 1992; Henry et al., 1994; McCormick et al., 1995; Koenig et al., 2005; Mueller and Bale, 2008; Brunton and Russell, 2010). This has been demonstrated for a range of stressors, and does not appear to depend upon the nature of the stressor, as hyperactive responses have been reported following both physical and psychological stressors (Fride et al., 1986; Henry et al., 1994; Mueller and Bale, 2008; Brunton and Russell, 2010). Given the far-reaching impacts of glucocorticoids on multiple body systems, HPA axis dysregulation, and more specifically glucocorticoid excess, may underlie many of the other adverse phenotypes reported in prenatally stressed offspring, particularly those relating to metabolism, the cardiovascular system, mood and cognition.

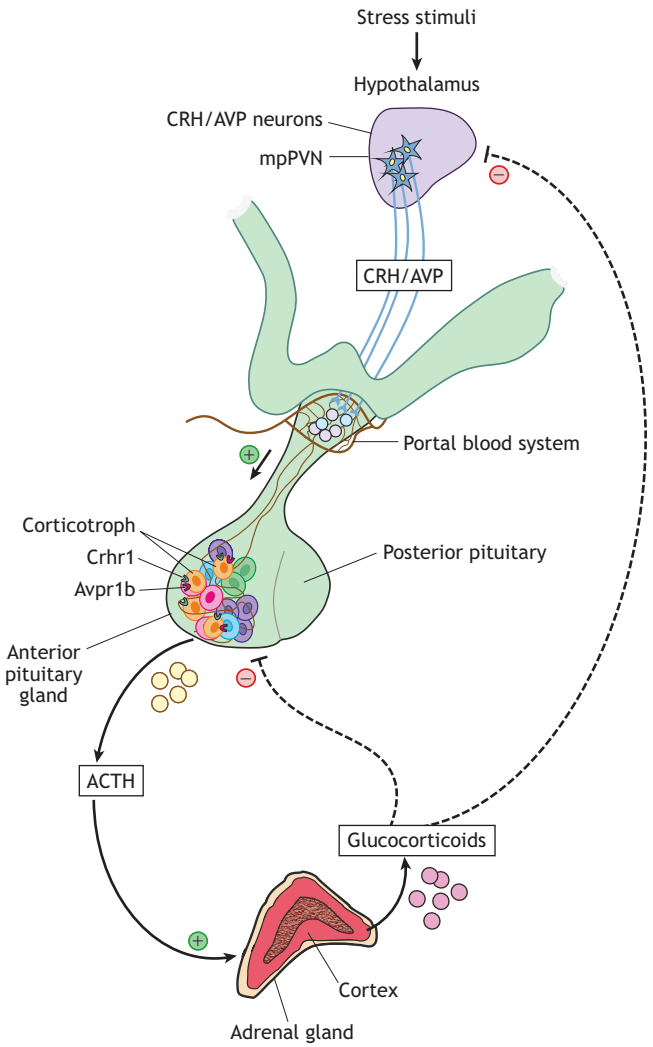

Fig. 1. The hypothalamo–pituitary–adrenal (HPA) axis. Stressful stimuli activate corticotropin-releasing hormone (CRH)/arginine vasopressin (AVP) neurosecretory neurons located in the medial parvocellular paraventricular nucleus (mpPVN) of the hypothalamus. CRH and AVP are released into the hypophysial portal blood vessels at the median eminence and transported to the anterior pituitary gland. CRH and AVP bind to their receptors (Crhr1 and Avpr1b, respectively) on corticotrophs and stimulate adrenocorticotropic hormone (ACTH) secretion into the systemic circulation. ACTH, in turn, triggers the synthesis and secretion of glucocorticoids (i.e. corticosterone/cortisol) from the adrenal cortex. Glucocorticoids regulate their own secretion through a negative feedback loop, acting on mineralocorticoid receptors in the hippocampus and glucocorticoid receptors at various sites, including the anterior pituitary gland, hypothalamus and hippocampus.

We have used a rat model of social stress during the last week of pregnancy (Brunton and Russell, 2010) not only to characterise offspring phenotypes, but also to better understand the mechanisms underlying these phenotypes (Brunton et al., 2011, 2013, 2015; Ashworth et al., 2016; Grundwald et al., 2016; Sze and Brunton, 2021). The mechanisms of HPA axis hyperactivity in response to stress involve adaptations in the brain and a shift in the balance between the excitatory and inhibitory inputs to the corticotropin releasing hormone (CRH) neurones in the medial parvocellular paraventricular nucleus (mpPVN). Augmented ACTH and

corticosterone secretory responses to acute stress in prenatally stressed offspring are driven by increased excitation of the mpPVN CRH neurons (Kapoor et al., 2008; Brunton and Russell, 2010; Zohar and Weinstock, 2011). There is also evidence for reduced glucocorticoid-mediated negative feedback via hippocampal glucocorticoid (Nr3c1) and mineralocorticoid (Nr3c2) receptors, as gene expression for these receptors is significantly lower in prenatally stressed rats compared with controls (Henry et al., 1994; Kapoor et al., 2008; Mueller and Bale, 2008; Brunton and Russell, 2010). Reduced *Nr3c1* expression in the hippocampus of prenatally stressed mice has been linked with hypermethylation of the *Nr3c1* promoter region (Mueller and Bale, 2008), suggesting that epigenetic mechanisms underpin some of the changes in gene expression reported in the brain following prenatal stress exposure. These changes may be present from fetal life, as recent transcriptomic analyses have revealed a modest reduction in *Nr3c1* expression in the prenatally stressed mouse brain at E15.5 (Dong et al., 2023).

GABA signalling

GABA provides the major inhibitory input to the HPA axis. The PVN is innervated by GABAergic neurons, and CRH neurons in the PVN express $GABA_A$ receptors. The number of GABAergic neurons in the prefrontal cortex, amygdala and hippocampus is reduced in prenatally stressed rodents, as is $GABA_A$ receptor expression (Fride et al., 1985; Barros et al., 2006; Laloux et al., 2012; Giovanoli et al., 2014; Uchida et al., 2014; Lussier and Stevens, 2016; Scott et al., 2020), indicating that deficits in central GABAergic signalling could contribute to augmented HPA axis responses in the offspring of stressed mothers. Interestingly, $GABA_A$ receptors are one of the main targets for neuroactive steroids, which are also impacted by maternal stress.

Neuroactive steroids (also neurosteroids) are steroids that act in the brain via membrane-bound receptors to exert rapid non-genomic effects on neuronal excitability (Paul and Purdy, 1992). The term ‘neuroactive steroid’ typically refers to steroids synthesised in peripheral endocrine tissues that enter the brain to modulate neural activity, whereas neurosteroids are generated locally in the brain, either *de novo* from cholesterol or by conversion of steroid precursors that enter the brain from the periphery, by centrally expressed steroidogenic enzymes (Brunton et al., 2009; Giatti et al., 2019). Some neurosteroids act as positive allosteric modulators of the $GABA_A$ receptor, enhancing GABA-mediated inhibitory neurotransmission, and among many other actions, exert stress-suppressing and anxiolytic effects (Patchev et al., 1994, 1996; Brunton et al., 2009, 2015; Brunton, 2016). Major examples of such neurosteroids include allopregnanolone (3α-hydroxy-5α-pregnan-20-one), 3α-androstandiol (5α-androstane-3α,17β-diol) and tetrahydrodeoxycorticosterone (THDOC; 5α,3α-tetrahydrodeoxycorticosterone). These neurosteroids are synthesised from progesterone, testosterone and corticosterone, respectively, through the sequential actions of 5α-reductase (Srd5a1) and 3α-hydroxysteroid dehydrogenase (Akr1c4), both of which are expressed in the brain (Baulieu, 1991; Agis-Balboa et al., 2006). In rats, acute stress upregulates *Srd5a1* expression in the brain and evokes a rapid increase in allopregnanolone and THDOC levels in the brain and blood (Purdy et al., 1991; Sanchez et al., 2008; Sze et al., 2018). This stress-induced increase in neurosteroid production in the brain is considered important in facilitating cessation of the stress response by potentiating GABA action. Indeed, both allopregnanolone and THDOC suppress stress-induced HPA axis activity in rats (Owens et al., 1992; Patchev et al., 1996), and allopregnanolone strongly inhibits mpPVN CRH neuronal firing in mice (Gunn et al., 2013). Hence reduced production and/or action of these neurosteroids would be expected to lead to a loss of inhibitory tone, and consequently enhanced and/or protracted HPA axis responses to stress. Together, these findings led to the hypothesis that deficits in neurosteroidogenesis or neurosteroid action may contribute to HPA axis dysregulation in prenatally stressed offspring.

Prenatal stress and neurosteroid signalling

As mentioned above, expression of the main target of 5α-reduced neurosteroids, the $GABA_A$ receptor, is reduced in the brains of prenatally stressed offspring (Laloux et al., 2012; Scott et al., 2020; Crombie et al., 2022). Moreover, we and others have demonstrated that maternal stress exposure during pregnancy negatively impacts the capacity for neurosteroidogenesis in the offspring brain (Brunton et al., 2015; Hirst et al., 2016). The enzyme Srd5a1 seems to be particularly vulnerable to the effects of maternal stress. Studies have reported reduced Srd5a1 activity and/or gene expression in the brains of fetuses, and juvenile and adult offspring of stressed dams, including in regions known to influence HPA axis activity, such as the prefrontal cortex, hypothalamus, hippocampus and brainstem (Ordyan and Pivina, 2005; Paris et al., 2011; Paris and Frye, 2011a,b; Walf and Frye, 2012; Brunton et al., 2015). The mechanism by which prenatal stress decreases *Srd5a1* expression in the offspring’s brain is not clear. However, similar findings are reported in guinea pigs following maternal administration of synthetic glucocorticoids during pregnancy (McKendry et al., 2010), suggesting a possible role for glucocorticoids in regulating the expression of *Srd5a1*. It is not known whether epigenetic mechanisms contribute to downregulation of central *Srd5a1* expression in prenatally stressed offspring.

Other evidence also supports the hypothesis of reduced neurosteroidogenesis in prenatally stressed offspring. Upregulating *Srd5a1* and *Akr1c4* expression in the brainstem using adenoviral gene transfection normalises hyperactive HPA axis responses to an acute immune stressor in prenatally stressed rats (Brunton et al., 2015). Likewise, administration of exogenous allopregnanolone to prenatally stressed rat offspring prior to acute stress exposure has similar effects (Brunton et al., 2015). Together, these data suggest that prenatally stressed offspring have deficient neurosteroid generation in the brain and this contributes to enhanced HPA axis responses to stress. However, until recently, stress-induced neurosteroid concentrations had not been directly quantified in the brains of prenatally offspring. Despite allopregnanolone administration ameliorating HPA axis hyperactivity in prenatally stressed rats (Brunton et al., 2015), it is likely that this effect occurs independent of deficits in endogenous allopregnanolone levels, as we found no deficit in stress-induced allopregnanolone production in the brains of male and female prenatally stressed rats (Sze and Brunton, 2021). Nevertheless, we did find that acute stress failed to evoke a significant increase in THDOC concentrations in several brain regions in both male and female prenatally stressed offspring, in contrast to control offspring (Sze and Brunton, 2021). As THDOC also acts as a positive allosteric modulator at $GABA_A$ receptors (Reddy, 2006), this finding supports the concept that neurosteroid modulation of GABA-mediated inhibitory signalling may be disrupted in prenatally stressed offspring, though further studies are necessary to test this.

Anxiety-like behaviour

Increased expression of anxiety-like behaviour is frequently reported in the offspring of rodent prenatal stress models (Fride and Weinstock, 1988; Vallee et al., 1997; Kapoor and Matthews,

2008; Zuena et al., 2008; Brunton and Russell, 2010; Bennett et al., 2015). Often these behaviours are sex dependent, with one sex more affected than the other, or one sex not affected at all. For example, using our prenatal social stress model, we have found that the male, but not the female offspring exhibit greater levels of anxiety-like behaviour than control offspring from unstressed pregnancies (Brunton and Russell, 2010). This finding is consistent with those of other studies using different prenatal stress paradigms in rats and guinea pigs (Emack et al., 2008; Kapoor and Matthews, 2008; Wang et al., 2013), whereas others report an increase in anxiety-like behaviour in both sexes (Zohar and Weinstock, 2011). CRH neurones in the central nucleus of the amygdala (CeA) play a key role in mediating anxiety-like behaviour (Dunn and Berridge, 1990). Accordingly, CRH concentrations and gene expression in the amygdala is greater in the offspring of rats, mice and pigs exposed to gestational stress (Jarvis et al., 2005; Mueller and Bale, 2008; Brunton and Russell, 2010; Zohar and Weinstock, 2011). CRH action is mediated through two distinct receptors. CRH binding to the type 1 receptor (Crhr1) typically promotes anxiety-like behaviours, whereas activation of type 2 receptor (Crhr2) is generally anxiolytic (Bale and Vale, 2004). *Crhr1* expression is significantly upregulated in the amygdala of male offspring of mothers exposed to social stress during pregnancy, which show an anxious phenotype, compared with control male offspring that do not (Brunton and Russell, 2010; Brunton et al., 2011). Moreover, this effect is not observed in the female prenatally stressed offspring, which also do not show an anxious phenotype (Brunton and Russell, 2010; Brunton et al., 2011). Again, this change in gene expression may result from epigenetic mechanisms, as DNA methylation is reduced at several CpG islands in the *Crhr1* promoter in the hypothalamus of male, but not female offspring, exposed to gestational hypoxia, where only the male offspring display an anxious phenotype (Wang et al., 2013). Similar findings have been found in a prenatal stress model where pregnant mice are exposed to fear conditioning. The male offspring display increased anxiety-like behaviour and this is associated with increased hippocampal Crhr1 expression and hypomethylation of the Crhr1 promoter (Golub et al., 2016; Plank et al., 2021). In contrast to *Crhr1*, expression of *Crhr2* in the amygdala is downregulated in the male, but not female offspring of a prenatal stress paradigm (maternal social stress) where only the males develop an anxious phenotype (Brunton et al., 2011), whereas it is reduced in both males and females using a different prenatal stress paradigm (chronic variable stress), where both sexes display heightened anxiety-like behaviour (Zohar and Weinstock, 2011). It has not been tested whether this is a result of hypermethylation of the *Crhr2* promoter; however, this seems a reasonable proposition.

There is also evidence, albeit indirect, that anxiety-like behaviours in prenatally stressed rodents are linked with deficits in neurosteroid production in the brains of these animals. Prenatal administration of allopregnanolone to pregnant rats in parallel with maternal stress exposure during pregnancy ameliorates anxiety-like behaviour in the offspring as neonates and in adulthood (Zimmerberg and Blaskey, 1998), whereas postnatal administration of ganaxolone, a synthetic analogue of allopregnanolone, reverses the anxious phenotype induced by prenatal stress in a guinea pig model (Crombie et al., 2021b). In accordance, reduced neuroactive steroid concentrations are reported in people with affective disorders and interestingly antidepressant treatment normalises this disequilibrium in neuroactive steroids (Romeo et al., 1998). Hence, reduced neurosteroidogenesis and reduced targets for neurosteroid action in the brain (i.e. $GABA_A$ receptors) are both expected to contribute to enhanced anxiety and HPA axis reactivity in prenatally stressed offspring.

Despite the wealth of information supporting the concept of fetal programming by maternal stress exposure in pregnancy, what remains unclear are the mechanisms through which maternal stress is transmitted from the mother to her offspring. More specifically, what factor(s) are responsible for signalling to the fetus that its mother is experiencing stress?

Putative mechanisms involved in signalling stress from mother to fetus

Several mechanisms have been proposed to play a role in mediating the effects of prenatal stress. In human pregnancy, prenatal exposure to increased levels of placental CRH (and/or glucocorticoids) has been linked with aberrant neurodevelopment, altered behaviour and altered metabolic phenotypes in the infants/children (Sandman, 2015; Kassotaki et al., 2021). In contrast, the placenta does not produce CRH in rodents (Sasaki et al., 1990; Muglia et al., 1994); however, other mechanisms including activation of the maternal HPA axis and hence exposure to excessive levels of maternal glucocorticoids (Barbazanges et al., 1996; Cottrell and Seckl, 2009), activation of the maternal sympathetic–adrenomedullary system (Sandler et al., 1963; Morgan et al., 1972; Sodha et al., 1984), activation of the fetal HPA axis (Ohkawa et al., 1991; Williams et al., 1999; Fujioka et al., 2003), an increase in placental oxidative stress and altered placental function (Bronson and Bale, 2016; Phillips et al., 2017; Scott et al., 2020; Lamothe et al., 2021) and/or stress-induced alterations in maternal behaviour after delivery (Leonhardt et al., 2007; Golub et al., 2016) have been proposed. Next, we discuss the evidence for some of these putative mechanisms, focusing on maternal–fetal glucocorticoid transfer and adaptations in the placenta.

Maternal glucocorticoids and prenatal programming

For some time, it has been widely assumed that maternal glucocorticoids are the factor responsible for fetal programming of the offspring following maternal stress exposure. However, direct evidence to support this theory is somewhat limited and is increasingly the subject of debate (Montano et al., 1993; Barbazanges et al., 1996; Holmes et al., 2006; Meise et al., 2016; Wieczorek et al., 2019; Westrick et al., 2021; Sze et al., 2022) for several reasons that will be discussed next.

Protective mechanisms in pregnancy limit fetal exposure to maternal glucocorticoids

During a normal pregnancy, there are innate protective mechanisms that are considered to play important roles in minimising fetal exposure to excessive levels of maternal glucocorticoids. Firstly, stress-induced activation of the maternal HPA axis is restrained in pregnancy, meaning the glucocorticoid secretory responses to stressful stimuli are substantially attenuated, compared with the non-pregnant state (Table 1). This has been repeatedly demonstrated in response to acute stress exposure, irrespective of whether the stress in psychological or physical in nature (Table 1).

Adaptations primarily in the hypothalamus, but also in limbic brain regions, as well as the anterior pituitary gland, contribute to suppressed HPA axis stress responses in late pregnancy (Brunton et al., 2008). The mechanism responsible for limiting HPA axis responses in pregnancy involves induction of an inhibitory endogenous opioid mechanism by the increased levels of allopregnanolone in pregnancy, and has been reviewed in detail elsewhere (Brunton and Russell, 2008; Brunton, 2016). This restraint of HPA axis activity in pregnancy is considered important

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Table 1. Corticosterone secretory responses to different stressors in virgin and late pregnant rats

	Fold-increase from basal			
Stressor	Virgin	Pregnant (GD20/21)	Increase (% virgin)	Reference
Elevated plus maze (5 min)	8.8	3.3	45	Neumann et al. (1998)
Forced swimming (90 s)	13.1	5.3	54	Neumann et al. (1998)
Interleukin-1β (iv)	2.3	1.1	7.4	Brunton et al. (2005)
Neuropeptide Y (icv)	4.3	1.2	39.5	Brunton et al. (2006)
Orexin-A (icv)	3.0	1.4	28	Brunton and Russell (2003)
Social stress (30 min)	1.6	1.0	3.4	Brunton et al. (2003)
Social stress (10 min×5 days)	2.4	1.1	17	Brunton and Russell (2010)

The increase in plasma corticosterone concentrations at the peak of the response in virgin female and late pregnant (GD20/21) rats, expressed as a fold-increase from basal levels. Also shown is the increase in corticosterone secretion in late pregnant rats, expressed as a percentage of the increase in corticosterone secretion in virgin female rats. GD, gestational day; icv, intracerebroventicular; iv, intravenous.

not only to promote anabolic adaptations in the mother, necessary for a successful pregnancy outcome, but also to protect the fetuses from exposure to excessive levels of maternal glucocorticoids (Brunton and Russell, 2008).

The second mechanism presumed to protect the fetuses from excessive maternal glucocorticoid exposure is placental expression of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (Hsd11b2). Hsd11b2 oxidises active glucocorticoids such as corticosterone and cortisol into their inactive metabolites, 11-dehydrocorticosterone and cortisone, respectively (Fig. 2), serving as a functional barrier. Hence, Hsd11b2 protects the placenta from the growth-inhibiting effects of glucocorticoids and controls the transfer of endogenous glucocorticoids from the maternal circulation into the fetal compartment (Seckl and Holmes, 2007).

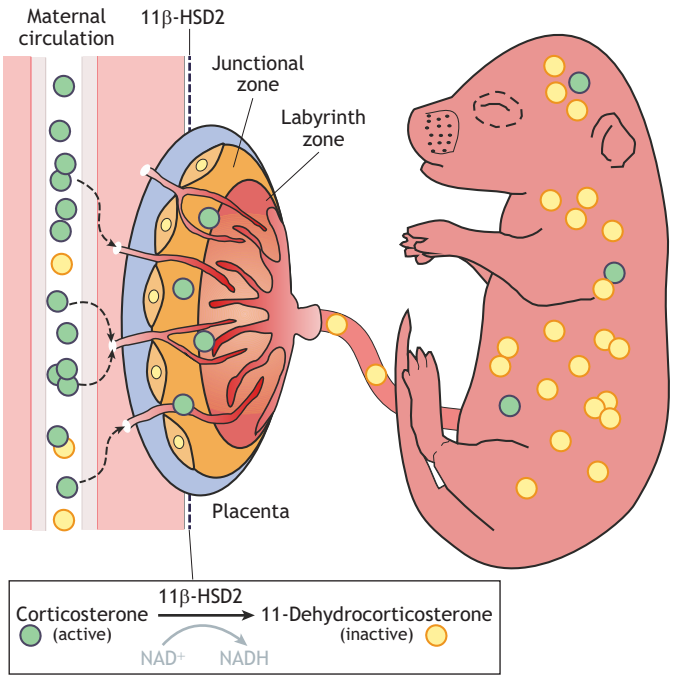

Fig. 2. Placental 11β-hydroxysteroid dehydrogenase type 2. The enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) is expressed in the placenta. 11β-HSD2 converts active glucocorticoids entering the placenta in the maternal blood (i.e. corticosterone in rats/mice or cortisol in sheep/humans) into their inert metabolites (i.e. 11-dehydrocorticosterone in rats/mice or cortisone in sheep/humans). The reaction is nicotinamide adenine dinucleotide (NAD+)-dependent, with NAD+ reduced to nicotinamide adenine dinucleotide+hydrogen (NADH). The inert metabolites cannot be regenerated in the fetus, as it lacks the reductase enzyme 11β-HSD1 (which catalyses the reverse reaction) until near term. Hence, the major source of active cortisol in the fetus is its own adrenal glands.

A role for maternal glucocorticoids in fetal programming?

Although these protective mechanisms during pregnancy should minimise fetal exposure to excessive levels of maternal glucocorticoids, it has been suggested that these mechanisms may only mitigate the impact of acute/mild stressors, and may not be sufficiently protective in cases of excessive or chronic stress. Even with a hyporeactive HPA axis, maternal glucocorticoids may still increase to a level that could be detrimental to the developing offspring (Duthie and Reynolds, 2013). In our model of repeated prenatal social stress, corticosterone levels in the pregnant dams after the stressful stimuli are significantly greater than those in unstressed dams; however, they only reach around 30% of the levels measured in non-pregnant females (Brunton and Russell, 2010; Sze et al., 2022). These data indicate that attenuated HPA axis responses persist in late pregnant rats even in the face of repeated stress. This raises questions about whether these suppressed responses adequately limit fetal glucocorticoid exposure, and indeed, whether these diminished glucocorticoid responses are sufficient to program the offspring. As described above, despite the attenuated glucocorticoid response in the mother, the offspring do exhibit a 'programmed' phenotype (Brunton and Russell, 2010; Brunton et al., 2011, 2015; Grundwald et al., 2016). This elicits some uncertainty around whether maternal glucocorticoids are the factor that directly mediates the programming effects of maternal stress on the fetus, especially given that the stress-induced corticosterone levels reported in these pregnant rats are lower than those measured at the diurnal peak in late pregnancy (Atkinson and Waddell, 1995; Brunton and Russell, 2010; Sze et al., 2022). Nevertheless, the timing of elevated glucocorticoids may be important. For example, an unexpected peak in glucocorticoid secretion during the trough phase of the circadian rhythm could potentially contribute to fetal programming. Indeed, placental Hsd11b2 activity displays circadian variation in women, with the highest activity correlating with the circadian peak in cortisol secretion (Lamadé et al., 2021). This may represent a mechanism that protects the fetuses from high levels of maternal glucocorticoids that occur daily at the onset of the active period (i.e. the morning in humans, the evening in rats and mice). Hence, one could argue that unexpected increases in maternal glucocorticoid secretion during the day at a time when Hsd11b2 activity is at its lowest could potentially mean greater transplacental transfer.

The majority of evidence supporting a role for maternal glucocorticoids in prenatal programming of negative offspring phenotypes comes from studies where exogenous glucocorticoids (such as dexamethasone or betamethasone) are administered to pregnant rodents, resulting in offspring phenotypes reminiscent of those observed following prenatal stress (Levitt et al., 1996; Welberg et al., 2001; Dunn et al., 2010). However, there is a caveat

that should be taken into account when interpreting these data – unlike endogenous glucocorticoids, synthetic glucocorticoids are poor substrates for Hsd11b2, meaning they readily cross the placenta and enter fetal tissues (Smith et al., 1988; Ballard and Ballard, 1995; Romero et al., 2000). Moreover, *in vivo* experiments suggest that although biological and synthetic glucocorticoids can exert similar long-term effects on certain aspects of offspring physiology, for some phenotypes the outcomes differ or are more pronounced following synthetic glucocorticoid overexposure (Fowden and Forhead, 2015). For example, in rats, prenatal dexamethasone results in hypertensive offspring, whereas antenatal treatment with a natural glucocorticoid (which is metabolised in the placenta) does not (Celsi et al., 1998). Similarly, in sheep, maternal betamethasone administration in pregnancy is associated with fetal growth restriction and lung maturation, whereas maternal cortisol administration has no apparent effect on these parameters (Jobe et al., 2003). These differences may be because synthetic glucocorticoids bind predominantly to glucocorticoid receptors, whereas endogenous glucocorticoids bind to both glucocorticoid and mineralocorticoid receptors (Reul et al., 1987), and because of their differing pharmacokinetics (Czock et al., 2005). Notwithstanding, at the cellular/molecular level, both endogenous and synthetic glucocorticoids have significant effects on developing neurons. Exposure of *in vitro* neuronal models to glucocorticoids demonstrates negative impacts on neurogenesis, synaptic plasticity and glucocorticoid receptor signalling, and induces epigenetic changes that can prime future stress responses (Provencal et al., 2020; Bassil et al., 2023), supporting the idea that inappropriate activation of downstream targets following glucocorticoid receptor activation is detrimental to the offspring.

One early study showed that the prenatally stressed offspring of adrenalectomised (hence stress-induced corticosterone secretion is prevented) pregnant rat dams do not exhibit the typical prolonged HPA axis response to stress (Barbazanges et al., 1996). Although it is important to acknowledge that glucocorticoids are not the sole factor secreted by the adrenal glands in response to stress [e.g. catecholamines, dehydroepiandrosterone (DHEA) are also secreted], dysfunctional HPA axis responses in the offspring were reinstated by maternal corticosterone replacement at the time of the maternal stress (Barbazanges et al., 1996). Hence, these data imply that the negative effects of prenatal stress are dependent on an increase in corticosterone in the maternal compartment. In a more recent study, when the corticosterone synthesis inhibitor, metyrapone, was given prior to stress exposure in pregnant mice, abnormalities in HPA axis function and firing patterns of hippocampal dentate gyrus cells in the offspring were abrogated (Liu et al., 2023), again suggesting that the production of maternal glucocorticoids is necessary for mediating the adverse effects observed in the offspring.

As such, there is evidence to support the 'glucocorticoid hypothesis' – that excessive glucocorticoid production following maternal stress is detrimental and a key contributor to prenatal programming. However, despite three decades of research, key questions remain unanswered – are maternal glucocorticoids directly involved in fetal programming of the brain or is their role indirect? If it is the former, then how are excessive levels of glucocorticoids in the maternal compartment transmitted to the fetus following stress (Seckl and Holmes, 2007)? The prevailing theories in the field propose that maternal stress reduces the expression of placental Hsd11b2, leading to the protective barrier being 'leaky' and increased transplacental crossover of glucocorticoids, thereby affecting all aspects of intrauterine development, including fetal brain development (Chapman et al., 2013; Fowden and Forhead, 2015). However, recent studies discussed below reveal that this explanation is too simplistic, and cannot fully account for the modest increase in fetal corticosterone levels following maternal stress.

Fetal corticosterone levels increase modestly despite larger increases in the maternal compartment

To clarify whether there is transplacental passage of corticosterone, an understanding of circulating fetal corticosterone levels relative to maternal corticosterone levels needs to be established. Most studies reviewed here report a significant increase in fetal plasma corticosterone concentrations, concomitant with the rise in circulating maternal corticosterone concentrations induced by maternal stress exposure or exogenous corticosterone administration (Table 2); however, others report no difference in plasma corticosterone or corticosterone binding globulin (CBG) levels between fetuses of control and stressed pregnancies (Mairesse et al., 2007). Although there are methodological variations in terms of the stressor type and sample collection times, leading to variability in absolute values, fold changes in the mothers always tend to be greater when compared with those observed in the fetuses (Table 2). For example, in our prenatal social stress model, there was a 3.7-fold increase in maternal circulatory corticosterone concentrations, whereas fetal plasma corticosterone concentrations in the stressed group was only 1.3-fold greater than the control group (Sze et al., 2022). Moreover, this effect was only observed in the female fetuses, and not in the males (Sze et al., 2022), indicating sex differences in placental transfer of glucocorticoids from the maternal blood to the fetal compartment, consistent with previous reports (Montano et al., 1993; Wieczorek et al., 2019). Nonetheless, as the concentrations of corticosterone in the fetal brain do not increase with maternal stress or in parallel with maternal circulating corticosterone levels (Sze et al., 2022), it seems unlikely that corticosterone is acting as the 'programming mediator' directly in the fetal brain. Whether there is an indirect role for maternal glucocorticoids in signalling maternal stress to the fetus, for example by altering placental function or triggering a cascade of downstream events, remains to be elucidated.

It is also important to note that in human clinical studies, despite maternal plasma cortisol being significantly correlated with amniotic fluid cortisol (Sarkar et al., 2008), maternal anxiety or stress show only weak or no associations at all with maternal and fetal glucocorticoid concentrations (Sarkar et al., 2008; O'Donnell and Meaney, 2017; Vuppaladhadiam et al., 2021), raising questions about the role of the maternal HPA axis in mediating the effects of maternal stress on the fetus. However, studies in humans are often complicated by difficulties in assessing the extent of maternal distress, and also variabilities in terms of the type of sample collected (plasma, amniotic fluid, saliva) and the timing of sample collection. That said, similar findings are reported in sheep exposed to chronic psychosocial stress during gestation, where plasma cortisol concentrations in the fetuses do not correlate with circulating maternal cortisol levels (Dreiling et al., 2018), indicating that the relationship between maternal and fetal glucocorticoid levels is not necessarily linear.

Activation of the fetal HPA axis by maternal stress

In rodent models, fetal plasma is typically collected from systemic blood, thus one cannot rule out the possibility that the fetuses' own adrenal glands contribute to circulating fetal corticosterone concentrations following maternal stress. Indeed, the fetal adrenal glands are capable of secreting corticosterone by day 15–16 of pregnancy in mice and rats, and three-quarters of the active cortisol in the human fetal circulation is reportedly derived from the fetal

Table 2. Maternal and fetal plasma/serum corticosterone concentrations measured in rodents following different types of experimental stressors during pregnancy

				Maternal Cort concentration (ng ml^{-1})			Fetal Cort concentration (ng ml^{-1})			
Species	Method	Stressor	Gestational day (GD)	Non-stressed	Stressed	Fold-change	Non-stressed	Stressed	Fold-change	Reference
Mouse	RIA	Restraint	GD18	∼970	∼1320	1.4 ↑	M: 120 F: 190	M: 120 F: 140	M: ↔ F: 0.7 ↓	Montano et al. (1993)
Mouse	RIA	Tail shock	GD20	94±5	123±8*	1.3 ↑	86±3	117±4*	1.4 ↑	Takahashi and Kalin (1991)
Mouse	RIA	Restraint	GD17	210±45	∼480*	2.3 ↑	∼50	∼100*	2.0 ↑	Williams et al. (1999)
			GD18	241±19	∼380	1.6 ↑	∼200	∼220*	1.1 ↑	
			GD19	156±33	∼350*	2.2 ↑	∼250	∼320*	1.3 ↑	
			GD21	203±17	∼450*	2.2 ↑	∼150	∼180*	1.2 ↑	
Mouse	ELISA	Noise	GD13.5	∼200	∼400*	2.0 ↑	M: ∼10 F: ∼10	M: ∼10 F: ∼12*	M: ↔ F: 1.2 ↑	Wieczorek et al. (2019)
Rat	RIA	Exercise	GD21	∼165	∼655*	4.0 ↑	∼175	∼300	1.7 ↑	Carlberg et al. (1996)
Rat	RIA	Immune stress (LPS)	GD18-19	∼160	∼695*	4.3 ↑	∼210	∼340	1.6 ↑	Cui et al. (2011)
Rat	RIA	Immobilisation	GD20	∼90	∼1030*	11.4 ↑	∼220	∼360*	∼1.6 ↑	Bingham et al. (2013)
Rat	LC-MS	Social stress	GD20	83±8	304±52*	3.7 ↑	M: 270±19 F: 263±24	M: 311±19 F: 350±31*	M: ↔ F: 1.3 ↑	Sze et al. (2022)

ELISA, enzyme-linked immunosorbent assay; F, female; GD, gestational day; LC-MS, liquid chromatography-mass spectrometry; LPS, lipopolysaccharide; M, male; RIA, radioimmunoassay. * indicates a significant difference versus non-stressed group of same sex/gestational stage; ↑ indicates an increase, ↓ indicates a decrease and ↔ indicates no change.

adrenal glands (Beitins et al., 1973). Corticosteroids of fetal origin play an integral role in modulating the HPA axis of the fetus, supported by evidence that fetal mice which lack the ability to produce their own corticosterone show deficits in negative feedback control of their HPA axis, despite there still being considerable amounts of circulating maternal corticosterone (Huang et al., 2012). Together, these observations indicate that the fetus has substantial control over its own glucocorticoid production, especially during the period closer to term, where adrenocortical activity increases dramatically in order to prepare for the maturational changes that accompany birth (Fowden et al., 1998).

The fetal HPA axis is also able to respond to maternal stress during late pregnancy (Ohkawa et al., 1991; Montano et al., 1993; Fujioka et al., 2003). In mice, plasma corticosterone concentrations following maternal stress are similar in fetuses whose mothers underwent adrenalectomy to those in fetuses of intact mothers (Montano et al., 1993). Furthermore, chronic stress in pregnant ewes reduces uterine blood flow and is associated with prolonged plasma cortisol responses in the fetuses that are not correlated with maternal cortisol concentrations (Dreiling et al., 2018). These studies suggest that the source of much of the corticosterone in the fetal circulation following maternal stress is in fact the fetal adrenal glands, rather than transfer from the maternal circulation. With the recent advancement in mass spectrometry techniques, it is now also possible to obtain glucocorticoid measurements (both corticosterone and its inactive metabolite 11-DHC) from tissues such as the placenta, fetal brain and liver, which will provide a fuller picture of glucocorticoid metabolism in the maternal, placental and fetal compartments. Collection of blood directly from the umbilical vein in larger animal models (e.g. sheep) could shed further light on the transplacental passage of glucocorticoids following maternal stress.

Impact of maternal stress on placental 11βHSD2

There is conflicting evidence on whether the placental Hsd11b2 barrier is compromised by maternal stress. Hsd11b2 is undoubtedly necessary for normal offspring development, as placental vascularisation and nutrient transport are disrupted in placental Hsd11b2 knockout mice (Wyrwoll et al., 2009). In addition, *Hsd11b2* global knockout mice (which do not express Hsd11b2 in the labyrinth zone of the placenta and other fetal tissues) exhibit altered cerebellar development as neonates, and increased anxiety- and depressive-like behaviour that persists into adulthood (Holmes et al., 2006). Moreover, inhibition of Hsd11b2 using carbenoxolone in pregnant rats results in offspring of low birth weight that exhibit dysregulated HPA axis function and heightened anxiety- and depression-like behaviour (Welberg et al., 2000), similar to the phenotypes reported in prenatally stressed offspring (Weinstock et al., 1992; Henry et al., 1994; McCormick et al., 1995; Koenig et al., 2005; Mueller and Bale, 2008; Brunton and Russell, 2010). In humans, the children of women who consumed large amounts of liquorice (which contains the Hsd11b2 inhibitor, glycyrrhetinic acid) during pregnancy also exhibit impairments in cognitive development and HPA axis function (Raikkonen et al., 2010). However, evidence linking maternal stress and placental Hsd11b2 are often conflicting. Studies report that maternal stress or glucocorticoid treatment can lead to an increase or a decrease in placental Hsd11b2 expression and activity, or have no impact, and often the effect is sex dependent (Table 3).

Such inconsistencies may arise from the different timing, duration and nature of the stressor. For example, although acute restraint stress increased placental Hsd11b2 activity in rats, a chronic stress regime prior to the acute stressor abolished this increase, indicating that stressor duration and intensity matter (Welberg et al., 2005). However, using a different stress paradigm, we have shown that in pregnant rats, 5 days of chronic social stress increased the expression of placental *Hsd11b2* expression, but only in the males, indicating sex-specific regulation of its expression (Sze et al., 2022). The social status of the dams also affects placental Hsd11b2 expression, with only socially dominant mice showing a stress-induced upregulation of Hsd11b2, despite having similar maternal corticosterone responses to stress as their submissive conspecifics (Gross et al., 2018). Overall, the data suggest that the 'Hsd11b2 glucocorticoid barrier' is not necessarily compromised following stress exposure; indeed, several studies support the concept that there is a compensatory upregulation in placental Hsd11b2 following maternal stress or glucocorticoid exposure (Table 3).

Table 3. Changes in placental 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) expression/activity following experimental stressors or manipulation of glucocorticoid levels

Experimental model	Manipulation	Changes in placenta Hsd11b2 expression/activity	Reference
Mouse	Maternal corticosterone	Increased 11βHSD2 mRNA expression at E14.5, but decreased at E17.5, males only	Cuffe et al. (2012)
	Restraint stress	Increased 11βHSD2 protein expression, only in socially dominant dams	Gross et al. (2018)
	Maternal betamethasone	Increased 11βHSD2 protein expression	Ni et al. (2018)
	Sound stress	Increased 11βHSD2 expression in males only	Wieczorek et al. (2019)
Rat	Restraint stress	Acute stress increases 11βHSD2 activity, chronic stress prevents increase	Welberg et al. (2005)
	Restraint stress	Decreased 11βHSD2 mRNA expression and activity	Mairesse et al. (2007)
	Restraint stress	Decreased 11βHSD2 mRNA expression	Jensen Peña et al. (2012)
	Maternal betamethasone Maternal dexamethasone	Increased 11βHSD2 mRNA expression (betamethasone only)	Vackova et al. (2009)
	Social stress	Increased 11βHSD2 mRNA expression in males only	Sze et al. (2022)
	Mild chronic variable stress	No change in 11βHSD2 protein levels in either sex	Lan et al. (2017)
Sheep	Maternal dexamethasone	Decreased 11βHSD2 mRNA expression	Kerzner et al. (2002)
Baboon	Maternal betamethasone	Increased 11βHSD2 mRNA and protein expression	Ma et al. (2003)
Human placental trophoblasts	Corticosterone application	Increased 11βHSD2 activity and mRNA expression	Van Beek et al. (2004)
Clinical/human	Maternal betamethasone	Increased 11βHSD2 activity in females only	Stark et al. (2009)
	Maternal distress	Decreased 11βHSD2 activity in females only	Mina et al. (2015)
	Maternal anxiety	Decreased 11βHSD2 activity	O'Donnell et al. (2012)

However, this seems dependent on the context of the stressor, the timing of the exposure and/or fetal sex. These studies highlight the importance of adequate reporting of experimental conditions, but also the need for more systematic approaches in the field.

Moreover, although changes in Hsd11b2 occur in response to synthetic glucocorticoid administration, the direction of the changes is inconsistent across the different compounds used and the species tested (Table 3). Importantly, there is no known glucocorticoid response element in the promoter region of the *Hsd11b2* gene that is directly targeted by glucocorticoids, indicating that regulatory mechanisms controlling Hsd11b2 expression respond to glucocorticoid signalling through indirect mechanisms. Although synthetic glucocorticoids are not a substrate for Hsd11b2, and therefore are not inactivated by this enzyme (Romero et al., 2000), the placenta does express glucocorticoid receptors to which they may bind (Mark et al., 2009; Saif et al., 2015; Sze et al., 2022). As well as affecting overall placental function, placental glucocorticoid receptor activation can also influence the methylation patterns of genes such as *Hsd11b2* (Zhu et al., 2019), where hypermethylation of the *Hsd11b2* promoter reduces its expression (Togher et al., 2014) and hypomethylation increases *Hsd11b2* expression (Alikhani-Koopaei et al., 2004). Furthermore, when cortisol is administered directly into the fetal compartment in sheep, rather than the maternal compartment, *Hsd11b2* expression is downregulated, implying that *Hsd11b2* expression is responsive not only to a rise in maternal glucocorticoids, but also to changes in response to glucocorticoids of fetal origin (Clarke et al., 2002).

Studies utilising *ex vivo* placental perfusion have further revealed that the kinetics of glucocorticoid diffusion appear to be more complicated than previously thought. In the late pregnant (GD21) rat, placental Hsd11b2 is capable of metabolising both maternal and fetal corticosterone with similar potency; however, the conversion capacity of Hsd11b2 (for both materno-fetal and feto-maternal passage) decreases with increasing corticosterone concentrations (Staud et al., 2006). In human placentae, even when Hsd11b2 activity is completely inhibited using carbenoxolone, less than 8% of the total injected glucocorticoid crosses the placenta into the fetal compartment (Stirrat et al., 2018), indicating that other mechanisms, besides glucocorticoid inactivation by Hsd11b2, control the crossover of glucocorticoids. These mechanisms may include placental vascularity and the rate of blood flow in the maternal and fetal vessels, or the role of efflux transporters that regulate active transport of cortisol [e.g. ATP-binding cassette (ABC) transporters or P-glycoprotein]. For example, P-glycoprotein restricts access of natural and synthetic glucocorticoids to the glucocorticoid receptor in a human placental cell line (Mark and Waddell, 2006), and inhibition of P-glycoprotein in pregnant rats and mice increases glucocorticoid concentrations in the fetal plasma, concomitant with an increase in the maternal compartment (Ge et al., 2021). Together, the data presented above make it difficult to draw conclusions about the extent of glucocorticoid transperfusion from mother to fetus based solely on placental Hsd11b2 expression or activity. It seems that regulation of maternal–fetal glucocorticoid transfer is contingent upon several factors and complicated by the difficulty in differentiating glucocorticoids of maternal versus fetal origin. Moreover, it seems likely that in addition to Hsd11b2, other placental mechanisms participate in regulating glucocorticoid transfer across the placenta.

The placenta as a mediator of prenatal programming

Beyond Hsd11b2, the placenta, as the interface between the mother and fetus, has several other functions that could make it a central player in mediating the effects of maternal stress. The placenta integrates and communicates environmental information such as the nutritional and stress status of the mother to the developing fetus by altering its vasculature to affect nutrient transport (Bronson and Bale, 2016). Other than controlling the passage of substances between the mother and fetus, the placenta also actively produces factors (e.g. neurotransmitters, lactogens, growth factors) that may directly affect fetal growth and development. There is evidence that prenatal stress can disrupt some of these functions, while recent studies that experimentally manipulate genes that are involved in these functions recapitulate the phenotypes observed in prenatally stressed offspring (e.g. Bronson and Bale, 2016).

Firstly, models of prenatal stress in rodents report changes in placental function associated with nutrient transport. For instance,

maternal chronic restraint stress alters placental glucose transporter (GLUT) expression, where GLUT1 expression is decreased, and GLUT3 and GLUT4 expression is increased (Mairesse et al., 2007). Considering these fetuses also have lower plasma glucose levels, it suggests lower transplacental glucose transfer in prenatally stressed fetuses, though there may be possible compensatory changes in other GLUT subtypes. In another study, Howerton et al. (2013) showed that maternal chronic stress suppressed the expression of placenta O-GlcNAc transferase (OGT), which is a nutrient sensitive protein that functions to posttranslationally modify proteins associated with histone remodelling, with broad functions in brain development. More significantly, the offspring of a mouse model that lacks placental OGT completely recapitulated the prenatal stress phenotype, further cementing the role of OGT in modulating the effects of prenatal stress. As an X-linked protein, sex differences were also noted, where there is a more dramatic decrease in OGT levels in the male placenta, which may account for the vulnerability to prenatal stress often reported in male offspring (Howerton and Bale, 2014).

Apart from changes in genes affecting nutrient transfer, we have found that there is an increase in oxidative stress markers in the placenta following 5 days of chronic social stress (Scott et al., 2020). Reactive oxygen species (ROS) are produced in cells during normal metabolic processes; however, an imbalance between the production of ROS and antioxidants results in oxidative stress that can damage cells, proteins, lipids and DNA (Allen et al., 2021). The placenta, especially, is in a constant state of mild oxidative stress owing to its high metabolic demands; hence any additional stress may tip it towards dysfunction (Wu et al., 2016). Crucially, blocking oxidative stress with a placenta-targeted antioxidant prevents the anxious phenotype in male prenatally stressed offspring, as well as the stress-induced reduction in markers of GABAergic signalling in the brain (Scott et al., 2020). Furthermore, when placental-conditioned media from stressed pregnancies (with increased ROS) was applied to embryonic cortical neuronal cultures, it led to a reduction in the expression of GABA receptors in the neurons, mimicking the changes observed in the juvenile offspring's brains. Again, this was not observed if dams were treated with an antioxidant, indicating a putative role for oxidative stress in mediating the effects of maternal psychosocial stress on GABA signalling in the offspring's brain (Scott et al., 2020). The mechanisms through which oxidative stress regulates gene transcription are not known, though may involve modulation of transcription factor function and/or activity (Kunsch and Medford, 1999).

We also identified several differentially abundant extracellular microRNAs in the placental-conditioned media from stressed placentae and the predicted targets of these were enriched for risk genes for several psychiatric disorders, including anxiety (Scott et al., 2020). This suggests that microRNAs released from the placenta into the fetal circulation following maternal stress could target and modulate the expression of key developmental genes in the fetal brain. Whether increased secretion of glucocorticoids from the adrenal glands is necessary for the increase in placental oxidative stress following maternal psychosocial stress still needs to be determined; however, there is evidence that maternal glucocorticoid administration alters markers of oxidative stress and antioxidant function in the placenta in a sex-specific manner (Bartho et al., 2019).

In addition to microRNAs, there may be other changes in the secretion of other placental factors that can affect neurodevelopment. Neurotransmitters are one such candidate, as placental-conditioned media from dams exposed to other forms of maternal stress (e.g. hypoxic stress) have higher levels of glutamate and tryptophan (Curtis et al., 2014). Placental-derived serotonin is important for fetal brain development, and alterations in serotonin-related genes in the placenta have been associated with maternal distress in humans, though this has not been thoroughly investigated in rodent models (St-Pierre et al., 2016).

Recently, other studies have convincingly demonstrated that manipulating the expression of placental genes can recapitulate the prenatal stress phenotype. Firstly, deletion of insulin-like growth factor 2 (Igf2) in mice from the placenta and fetus recapitulates the anxious phenotype observed in adult prenatally stressed offspring (Mikaelsson et al., 2013). As well as altered Igf signalling in these mice, there are also sex-dependent knock-on effects on steroid and placental lactogen hormone production (Aykroyd et al., 2020). Secondly, the placenta plays a key role in maintaining allopregnanolone concentrations in the fetal circulation and brain during pregnancy, providing a mechanism to protect the developing brain from excitotoxicity and neurodevelopmental disorders (Brunton et al., 2014). However, the capacity of the placenta to produce allopregnanolone is compromised in cases of maternal stress or repeated synthetic glucocorticoid exposure (Hirst et al., 2016). Indeed, it was recently demonstrated that placental allopregnanolone insufficiency caused by placental deletion of *Akr1c14* (the gene encoding one of the enzymes involved in allopregnanolone synthesis) in mice leads to social behaviour deficits in the male offspring (Vacher et al., 2021). Thirdly, overexpression of Pleckstrin homology-like domain family A member 2 (*Phlda2*) in the placenta, which regulates nutrient allocation, results in increased anxiety-like behaviours, cognitive deficits and abnormal social behaviours in the offspring, with the males more severely impacted (Harrison et al., 2021). In recent years, there have been more genome-wide screens that reveal associations between placental genes and offspring traits (Bhattacharya et al., 2022), and sex differences in placental DNA methylation have also been characterised in humans (Andrews et al., 2022). It is likely that following the initial genome-wide screening carried out in the stressed mouse placenta in Howerton et al. (2013), there will be more studies that aim to identify potential stress responsive genes in rodent models that can be experimentally manipulated and investigated in the future.

Lastly, it is worth noting that the placenta is a major target of glucocorticoids, and all of the above placental functions can therefore be influenced by changes in glucocorticoid action, most likely through the glucocorticoid receptors expressed in the placenta (Saif et al., 2014). *Nr3c1* signalling plays an essential role in shaping the DNA methylation profile of the fetus, and deficient *Nr3c1* signalling in a mouse model leads to broad downstream changes in target genes, and anxiety-like behaviour in adulthood (Schmidt et al., 2019). A large body of evidence also shows that synthetic glucocorticoids can alter placental vascularisation and nutrient transfer (Braun et al., 2013). It is therefore plausible that rather than having direct effects by crossing over to the fetus, excess maternal glucocorticoids act in an indirect manner, targeting placenta nutrient transfer and secretion, to alter fetal outcomes.

Concluding remarks

In summary, maternal stress exposure during pregnancy programs adverse offspring phenotypic outcomes. The fetal brain is particularly vulnerable to the effects of maternal stress, and dysregulated HPA axis stress responses and heightened anxiety-like behaviour are frequently reported in prenatally stressed offspring. The evidence supports a role for deficits in inhibitory signalling mechanisms in the

brains of prenatally stressed offspring, including impaired glucocorticoid negative feedback control of the HPA axis, as well as deficits in GABA-mediated inhibitory signalling. This is important, as dysfunction in both systems is implicated in the pathophysiology of psychiatric disorders, for which prenatal stress increases risk. Compromised neurosteroid action via $GABA_A$ receptors evidently contributes to both hyperactive HPA axis responses and the greater expression of anxiety behaviour in prenatally stressed rodents. The central expression of a key enzyme (Srd5a1) in neurosteroidogenesis and the expression of $GABA_A$ receptors (through which neurosteroids exert their effects) are reduced in the offspring of stressed mothers. However, whether epigenetic mechanisms, such as DNA hypermethylation, underlie these gene expression changes in prenatally stressed offspring, as has been demonstrated for other genes, remains to be established.

Less is understood about how the effects of prenatal stress are signalled from the mother to the fetus during pregnancy. Here, we considered the evidence for transplacental passage of maternal glucocorticoids, activation of the fetal HPA axis and altered placental function triggered by maternal stress. In pregnancy, in-built protective mechanisms, namely, attenuated maternal glucocorticoid responses to stress and placental Hsd11b2 expression, should buffer the impact of maternal stress on the fetus. The evidence presented supports the idea that the placenta is able to limit materno-fetal glucocorticoid transfer, even in the face of maternal stress, though it seems unlikely this is solely down to the actions of Hsd11b2, and other mechanisms are probably also at play. Given the lack of correlation between maternal and fetal glucocorticoid concentrations reported in some studies, the smaller magnitude of the stress-induced increase in glucocorticoids in the fetal circulation compared with the maternal circulation, and the absence of an increase in glucocorticoid concentrations in the fetal brain following maternal stress, we conclude that the programming effects of maternal stress during pregnancy are unlikely to be a result of direct actions of excessive maternal glucocorticoids acting on the fetal brain following transplacental crossover to the fetus. However, based on the studies reviewed here, it does seem likely that there is a role for glucocorticoids in signalling the stress status of the mother to the fetus. Whether that role involves inducing oxidative stress in the placenta and/or altering placental function remains to be elucidated. There is a growing body of evidence from animal models and clinical studies indicating that maternal stress and mood alter the methylation status, miRNA signature and hence the expression of several placental genes, including those enriched for neuronal development and glucocorticoid signalling (Gheorghe et al., 2010; Babenko et al., 2015; Durbagula et al., 2022). Hence, moving forward, it will be important to establish the stress-associated factor(s) that induce such dysregulation of the placental epigenome. Moreover, it is critical to determine to what extent alterations in placental gene expression influence neurodevelopment and gene expression in the fetal brain. We propose that fetal programming is probably not the consequence of a single factor, but rather is more likely mediated by multiple mechanisms. Future work in this area will hopefully enhance our understanding of these mechanisms and may help identify potential candidates for intervention to prevent fetal programming of adulthood disease.

Competing interests

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