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Glucocorticoids and the prenatal programming of neurodevelopmental disorders.

Jessy Cartier¹, Yan Zeng¹, Amanda J Drake¹

¹University/BHF Centre for Cardiovascular Science, University of Edinburgh. Queen’s Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK

Corresponding author:
Dr Amanda J Drake
University/BHF Centre for Cardiovascular Science,
Queen’s Medical Research Institute,
47 Little France Crescent,
Edinburgh EH16 4TJ,
UK
Email: mandy.drake@ed.ac.uk
Tel: 0044 131 2426748
Abstract

Synthetic glucocorticoids are frequently used antenatally in order to reduce morbidity and mortality in babies born preterm and have been used in the management of fetuses known to be at risk of congenital adrenal hyperplasia. Although such treatment has short term advantages, evidence suggests that it can affect health in later life. Several studies have reported negative consequences of prenatal exposure to the synthetic glucocorticoid dexamethasone on offspring behaviour in humans and in animal models, in association with changes in brain structure, hypothalamic-pituitary-adrenal axis function, neurotransmitter pathways, gene transcription and epigenetic regulation. These studies also highlight the importance of timing and tissue/organ- and sex-specific effects of prenatal glucocorticoid exposure. Here we review the evidence from human and animal studies that links prenatal synthetic glucocorticoid exposure with an increased risk for neurodevelopmental disorders.
Introduction

The concept of ‘early life programming’ is used to describe the association between environmental factors that occur during early life (pre- or postnatal) and an increased risk of diseases in later life [1]. According to this theory, environmental insults occurring at critical developmental periods can permanently alter the physiology and function of developing organs, resulting in lifelong consequences for health [2]. Such developmental plasticity has been proposed to help the unborn animal to optimize its body systems for better survival in a predicted adverse postnatal environment, however if the expected environment does not match the real postnatal environment, it may lead to malfunction and disease [3].

Physiological responses to environmental adversities are mediated by the hypothalamic-pituitary-adrenal (HPA) axis, which controls circulating glucocorticoid (GC) concentrations. Dysregulation of the HPA axis is associated with several neurodevelopmental disorders including autism [4] and schizophrenia [5]. Circulating GC levels are lower in the fetus compared to its mother as a consequence of the action of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β- HSD2) at the fetoplacental barrier, which catalyses the conversion of active GC (e.g. cortisol in humans and corticosterone in rodents) into their inactive forms (cortisone and 11-dehydrocorticosterone), and thus protects the fetus. Nevertheless, this barrier is not complete, and high levels of maternal GC can overcome this enzymatic protection [6]; indeed, fetal exposure to elevated levels of maternal GC, as a consequence of maternal stress during pregnancy, may impact on the developing brain and result in permanent changes in brain function [7].

In addition to exposure to increased endogenous GC levels following maternal stress, fetuses can also be exposed to high levels of synthetic GC (sGC), e.g. dexamethasone (DEX) or betamethasone, which are frequently administrated to mothers at risk of preterm delivery because of the clear benefits of GC on organ maturation, particularly the fetal lung [8]. sGC are additionally used in the management of fetuses at risk of (or diagnosed with) congenital adrenal hyperplasia to suppress fetal androgen production [9]. Such sGC are poor substrates for the 11β-HSD2 enzyme, and can readily cross the placental barrier. In this short review, we focus on the effects of prenatal sGC exposure on the brain, including effects on brain function and the epigenome.
**Effects of prenatal glucocorticoid exposure on behaviour**

Although prenatal DEX exposure has been associated with a decrease in the rate of cerebral palsy in preterm infants [10], it has also been associated with an increased risk of anxiety, hyperactivity and distractibility in preterm and term born children [10–12], with females being more susceptible to stress than males [11]. Nevertheless, reports on the outcomes of GC overexposure during early life are sometimes contradictory; for example, a long-term follow-up study of Swedish children who were prenatally treated with DEX showed no changes in psychopathology, behavioral problems or adaptive functioning, on the contrary they were described by their parents as being more sociable than the controls [13].

The difficulties in undertaking long-term follow-up studies in humans have led to the development of animal models for the study of effects of prenatal exposure to sGC. The majority of these models use DEX administration to pregnant dams, however the programmed phenotypes depend critically on the timing of exposure. In marmoset monkeys, juveniles exposed to early DEX (gestation day 42-48; gestation length ~148 days) were less sociable and more motivated to obtain a palatable reward, whereas later DEX treatment (from Day 90-96 of gestation) enhanced reversal learning of stimulus association. Offspring of both treatment groups showed a deficit in skilled motor reaching, however the effect was stronger with late DEX treatment [14].

In rats, DEX administration during the last week of gestation increased acoustic startle responses (ASR) in animals which had undergone prior blood sampling [15], suggesting that DEX offspring are more susceptible to anxiety [15]. Other studies also show DEX-exposed rats spend less time on the open arms of an elevated plus maze (EPM) [16,17] and spend less time in the central area of an open field test (OFT) with increased defecation and decreased exploratory activity [16,18,19], consistent with a phenotype of hyperanxiety. However, experimental findings vary in this field. Very recently, we have shown that DEX-exposed male rats demonstrate altered cognition in the Morris water maze [20], but no behavioural differences on an EPM or OFT (Table 1).

Overall, these studies suggest that prenatal DEX exposure programs an anxious phenotype, however the timing and dose of the DEX administered to the pregnant dams and the age and sex of the offspring may be crucial in determining any later effects.
Prenatal sGC exposure and brain development

sGC can exert effects by binding to the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR), although they have a higher affinity for GR. Glucocorticoid receptors (GR) are widely expressed in the brain from early in prenatal development [21,22], especially in the hippocampus [21], explaining why prenatal exposure to sGC could have a significant impact on brain development [23]. Indeed, prenatal or postnatal sGC administration in rodents and humans significantly reduces brain weight [24,25] and produces structural changes in brain regions including the prefrontal cortex (PFC) [26], amygdala [27], hippocampus [28] and striatum [29] (Figure 1).

The hippocampus may be particularly vulnerable to prenatal sGC exposure: several studies report that prenatal DEX exposure increases apoptosis in hippocampal structures (Dentate Gyrus and Cornu Ammonal (CA)) in rats [29,30]. In mice prenatal DEX exposure reduces hippocampal volume and increases apoptosis, however these effects do not persist until adulthood, whereas it induces a permanent proliferation deficit in the adult hippocampus [28]. In rats, prenatal, but not postnatal DEX increases cleaved caspase-3, a marker of apoptosis, in the CA1 and CA3 regions of the hippocampus, particularly in females [27].

Some studies suggest that the effects of prenatal DEX exposure can be both detrimental and protective within the same organism. For example, one study reports prenatal exposure to DEX prevents the increased vascularization observed in the amygdala after chronic stress exposure but exacerbates the retraction of vascularization in the hippocampus [19]. In another study, fetal exposure to DEX in mice resulted in a decrease in blood vessel density and impaired blood-brain barrier in the hypothalamus, whereas it enhances the barrier integrity in the cortex [31].

Furthermore, prenatal exposure to DEX in rats correlates with an increased volume and increased dendritic length of the bed nucleus of the stria terminalis (BNST), which is involved in fear responses. In contrast, prenatal exposure to DEX results in reduced volume of the amygdala due to dendritic length diminution [17]. In addition, prenatal DEX exposure in rats is also associated with a smaller volume and reduced cell number in the nucleus accumbens (NAcc), a heterogenous structure belonging to the striatum which is involved in the “reward pathway” and drug addiction [32]. Interestingly, this effect is stronger in males than females, which may be relevant to the increased vulnerability of males to drug addiction.
Thus, prenatal DEX exposure affects the development of the brain, and the effect can be negative or positive depending on the specific region of the brain.

**Prenatal sGC exposure and HPA axis regulation**

Several studies suggest that prenatal DEX exposure alters GR expression in the brain, in association with changes in HPA axis regulation [18]. In marmosets, late gestation DEX exposure leads to a decrease in GR mRNA expression but no effect on MR expression in the PFC, both in neonates and in adulthood [34]. In rats, prenatal DEX decreases GR expression in the hippocampus [35], whereas in guinea pigs GR expression is decreased [36]. In addition, following a 1 hour-recovery from a restraint stress, prenatally DEX-exposed rats demonstrate reduced GR mRNA expression in the pituitary gland, whilst control rats have increased GR mRNA expression [37]. Moreover, in rats and guinea pigs, offspring of DEX-treated dams have increased mRNA levels of the corticotrophin-releasing hormone (CRH), a critical coordinator of the HPA axis, in the PVN of the hypothalamus [35–37], indicating that prenatal DEX leads to persistent changes in HPA axis regulation. CRH is normally released by the hypothalamus following a stressor, and acts synergistically with arginine vasopressin (AVP) to induce the production of adrenocorticotropic hormone (ACTH) by the pituitary. ACTH then stimulates the production of corticosterone by the adrenal gland, inducing the negative feedback loop (Figure 1).

In rats, maternal adrenalectomy, which removes endogenous glucocorticoids, induces a depression-like phenotype in the offspring when assessed using the OFT and FST and is associated with increased GR expression in the hippocampus and decreased GR expression in the hypothalamus [38]. Whilst supplementation with high-dose corticosterone (CORT) following adrenalectomy during pregnancy restores normal GR expression in the hippocampus, it still results in HPA axis dysregulation. Interestingly, there is a sex-specific effect in that although both sexes had an exaggerated plasma ACTH response to stress, only females show a reversal of the effect following high dose CORT substitution [38].

**Prenatal sGC exposure and the neurotransmitters**

Several neurotransmitters are important for HPA axis regulation and mood, and are altered following prenatal sGC exposure, including the serotonergic, dopaminergic and GABAergic systems (Figure 2). Prenatal DEX treatment leads to decreased serotonin (5-HT) concentrations in the hippocampus, decreased mRNA expression of the serotonin receptor 5-
HT1A-R in the PFC and decreased protein expression of brain-derived neurotrophic factor (BDNF), which is implicated in the pathogenesis of depression [39], in the PFC and hippocampus. Interestingly, early intervention with fluoxetine reverses the dysregulation of 5-HT signaling as well as the behavioral phenotype in the prenatally DEX-exposed offspring [39]. However, a separate study showed that prenatal DEX induces an increase in 5-HT in the cortex and hippocampus [40]. The discrepancies might be explained by the different timing of DEX exposure between studies, e.g. exposure to DEX daily throughout pregnancy [40] versus exposure only during the final week of gestation [41]. In addition, the depressive-like phenotype induced by prenatal DEX exposure in rats correlates with a decrease in dopamine (DA) in the NAcc and amygdala and this is partially rescued by a treatment with L-3,4-dihydroxyphenylacetic acid (L-DOPA) [42]. Finally, prenatal DEX exposure in rats decreases calretinin expression, a calcium binding protein (CBP) that is expressed in GABAergic neurons, in the amygdala of adult female, but not male offspring [24].

**Epigenetic dysregulation and prenatal programming of the brain**

The mechanisms by which the effects of prenatal exposure to stressors are established and sustained are not well understood. Epigenetic dysregulation may provide a plausible link between early life adversity (including sGC exposure) and sustained alterations in gene expression that lead to adulthood diseases [43]. Recent studies suggest epigenetic mechanisms such as DNA methylation or histone modifications are involved in the development of neuropsychiatric disorders [44,45]. In addition, the developmental period is a time when major epigenetic remodelling events occur [46], especially in the brain [47], which renders it a time of particular susceptibility to epigenetic re-programming.

Animal studies suggest that prenatal GC overexposure can induce epigenetic changes in a number of candidate genes implicated in neuropsychiatric disorders, including the glutamic acid decarboxylase (GAD67), reelin and BDNF genes [48–50]. Prenatal DEX exposure in rats leads to a reduction in the protein and mRNA levels of reelin and GAD67 and associates with an overexpression of DNA methyltransferase 1 (DNMT1) in the hippocampus [49]. Melatonin restores the level of reelin and GAD67 by reducing DNMT1 mRNA expression and thus reducing the binding of DNMT1 and methyl-CpG binding protein 2 (MeCP2) to the reelin promoter. This result is supported by another study showing that an increase in DNMT1 following maternal stress leads to hypermethylation at specific CpG-rich regions of the GAD67 and reelin promoters, associated with decreased expression of both genes [50].
Similar results were obtained in another recent study which reported that prenatal stress leads to decreased cortical expression of reelin, accompanied by hypermethylation at the reelin promoter region. These molecular changes were associated with persistent behavioral consequences in adults, such as increased spontaneous locomotor activity, high anxiety levels and cognitive deficits [48]. sGC treatment during late gestation in guinea pigs induces genome-wide epigenetic changes in promoter methylation and histone acetylation in the fetal hippocampus [51,52]. The same group also reported changes in the developmental trajectory of DNA methylation in several other fetal tissues in prenatally exposed guinea pigs, which has been associated with altered expression of genes involved in the DNA methylation process. Furthermore, these effects persisted into adulthood and were transmitted to the next generation [52].

**Conclusions and suggestions for further work**

Prenatal sGC exposure associates with altered behavior and increased neurodevelopmental disease risk. Importantly, these effects depend highly on the timing of exposure and may be sex-specific [24,32,38] with females being more susceptible to stress and a depression-like phenotype than males [11,53]. Additionally, the discrepancies between studies using the same behavioral tests in animal models suggests that other factors such as animal housing and external stressors can affect the outcome [54,55]. There is increasing evidence suggesting a role for epigenetic dysregulation in prenatal sGC programming; future in vivo studies could be designed to elucidate the consequences of prenatal sGC on the level/activity of epigenetic regulators, e.g. the Dnmts, the ten-eleven-translocase (TET) proteins that are involved in the generation of cytosine 5-hydroxymethylation (5hmC) and the polycomb-group proteins regulating histone marks. Further, studies which delineate the effects on genome wide patterns of 5-methylcytosine, 5hmC and histone modifications in dissected brain regions will help to elucidate the mechanisms by which prenatal sGC exposure induces long-lasting effects on the phenotype.

**Acknowledgements**

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References


11* Study looking at the long term consequences of prenatal exposure to glucocorticoids in term-born children

12* Recent study presenting the long-term and adverse effects of prenatal glucocorticoids on mental health in children in the Northern Finland Birth Cohort 1986.


28* Demonstration of the transient effect of a single dose of glucocorticoids at E15.5 during pregnancy in mice on hippocampal volume, in association with increased apoptosis and decreased proliferating cells. The cells start to proliferate again after birth but stop in adulthood, showing the long-term consequences of prenatal Dex exposure.


31** Paper showing the differential effect of dexamethasone exposure during fetal development on the blood brain barrier depending on the brain region. The study showed that postnatally, the barrier integrity was enhanced in the cortex while decreased in the hypothalamus.


42** Study showing that in utero dex-exposed animals present altered social behavior associated with a reduction in dopamine signalling and that a restoration of the dopamine level by oral administration of L-3,4-dihydroxyphenylacetic acid (L-DOPA) coincides with amelioration of several aspects of social behaviours.


48* Recent study showing the impact of prenatal restraint stress on the regulation of a neuronal gene by DNA methylation.


49 * Results showing the beneficial effect of postnatal administration of melatonin in rats exposed in utero to dexamethasone in restoring the level of reelin and gad 67 in the GABAergic neurons of the hippocampus through the reduction of the level and the binding of DNMT1 to the reelin promoter.


50* Follow-up study showing the association between 5-methylcytosine and 5-hydroxymethylcytosine levels in the promoter of reelin and gad67 with a behavioral phenotype following prenatal stress in mice.

Interesting paper showing that fetuses exposed to synthetic glucocorticoid presented differences in DNA methylation and H3K9acetylation patterns in specific gene promoters that did not persist 14 days after the injection, whereas other promoters became affected later indicating that prenatal exposure to glucocorticoids alters the epigenetic landscape.


Study showing genome-wide alteration in transcription, GR binding and in DNA methylation profile following prenatal treatment with glucocorticoid in the hippocampus. The work also highlighted that the modifications observed were dynamic and continued to evolve during adulthood.


Figure 1: **The different components of the brain and their roles in behaviour and the HPA axis negative feed-back loop (in italics).** CRH: Corticotrophin Realease Hormone, ACTH: AdrenoCorticoTropic Hormone, CORT: corticosterone, PFC: Pre-frontal cortex, NAcc: Nucleus Accumbens.

Figure 2: **Neurotransmitter pathways implicated in mood balance and dysregulation in the offspring from DEX-treated mothers (black arrow).**
Table 1: Behavioural tests and the consequences of prenatal DEX exposure

<table>
<thead>
<tr>
<th>Test</th>
<th>Measure</th>
<th>Description</th>
<th>Prenatal DEX effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open Field Test</td>
<td>Anxiety</td>
<td>Large open area with wall where the locomotion and the willingness of the animal to explore is measured</td>
<td>decreased locomotion and exploration activity</td>
<td>17.18.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>no differences</td>
<td>Zeng et al</td>
</tr>
<tr>
<td>Elevated plus maze</td>
<td>Anxiety</td>
<td>The test setting consists of a plus-shaped apparatus with two open and two enclosed arms, each with an open roof, elevated 40–70 cm from the floor. The model is based on rodents’ aversion of open spaces.</td>
<td>Animals spend less time in the open arms</td>
<td>16.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>no differences</td>
<td>Zeng et al</td>
</tr>
<tr>
<td>Morris water maze</td>
<td>spatial learning and memory</td>
<td>The rat is placed in a large circular pool and is supposed to find an invisible or visible platform that allows it to escape the water by using various cues</td>
<td>animal slower the first day of trial then faster to locate the platform</td>
<td>Zeng et al</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>no differences</td>
<td>18</td>
</tr>
<tr>
<td>Force Swim Test</td>
<td>depression</td>
<td>Animals are forced to swim in an acrylic glass cylinder fill with water several times.</td>
<td>increased immobility</td>
<td>15.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>no differences</td>
<td>17</td>
</tr>
</tbody>
</table>
PFC
- Working memory
- Decision-making
- Inhibitory response control
- Attentional set-shifting

NAcc
- Motivation
- Reward
- Addiction
- Reinforcement learning

Thalamus

Cerebellum

Hippocampus
- Spatial learning
- Memory

Figure 1
Dopamine
- Reward
- Motivation

Serotonin
- Obsession
- Compulsion
- Memory

Mood balance

GABA
- Relaxation

Figure 2