Prenatal Stress, Glucocorticoids, and Developmental Programming of the Stress Response

Patrick O. McGowan[1,2,3,4] and Stephen G. Matthews[4,5,6]

[1]Department of Biological Sciences and Center for Environmental Epigenetics and Development, University of Toronto, Scarborough Campus, Toronto, Ontario M1C 1A4, Canada; [2]Department of Cell and Systems Biology, University of Toronto, Toronto, Ontario M5S 3G5, Canada; [3]Department of Psychology, University of Toronto, Toronto, Ontario M5S 3G5, Canada; [4]Department of Physiology, University of Toronto, Toronto, Ontario M5S 1A8, Canada; [5]Departments of Obstetrics & Gynaecology and Medicine, University of Toronto, Toronto, Ontario M5G 1E2, Canada; and [6]Lunenfeld Tanenbaum Research Institute, Sinai Health System, Toronto, Ontario M5G 1X5, Canada

The early environment has a major impact on the developing embryo, fetus, and infant. Parental adversity (maternal and paternal) and glucocorticoid exposure before conception and during pregnancy have profound effects on the development and subsequent function of the hypothalamic-pituitary-adrenal axis and related behaviors. These effects are species-, sex-, and age-specific and depend on the timing and duration of exposure. The impact of these early exposures can extend across multiple generations, via both the maternal and paternal lineage, and recent studies have begun to determine the mechanisms by which this occurs. Improved knowledge of the mechanisms by which adversity and glucocorticoids program stress systems will allow development of strategies to ameliorate and/or reverse these long-term effects. (*Endocrinology* 159: 69–82, 2018)

There is now extensive evidence from both human and animal studies that maternal adversity during pregnancy can lead to long-term physiological and pathophysiological outcomes in offspring. There has also been considerable research focus placed on determining the routes and mechanisms by which maternal adversity mediates effects in the developing fetus. In this context, maternal adversity (stress, anxiety, and depression) has been associated with increased maternal and fetal glucocorticoid concentrations. The role of glucocorticoids in mediating the long-term developmental programming has also been of particular interest because synthetic glucocorticoids are administered in human pregnancy, in both the management of congenital adrenal hyperplasia and threatened preterm birth, with the latter occurring in >10% of all pregnancies (1). Although increased fetal and placental glucocorticoid exposure represents an important route of transmission of the effects of maternal adversity to the fetus, there are likely a number of other mediating factors (2). The maternal and uterine

environments are critical in modulating fetal development and long-term outcomes; however, more recent research has highlighted an important role of paternal adversity before conception in modulating endocrine, metabolic, and neurodevelopmental outcomes in offspring. Although the field of adversity- and glucocorticoid-mediated developmental programming has been reviewed extensively in recent years, a number of important findings pertaining to long-term consequences of parental adversity and fetal glucocorticoid exposure on health across the life course are emerging. These include major sex differences in outcomes, the transgenerational nature of perinatal programming, the role of the father, and the genetic/epigenetic mechanisms involved. This review will focus on these areas as well as the most recent publications in the field (past 5 years).

The Hypothalamic-Pituitary-Adrenal Axis

The hypothalamic-pituitary-adrenal (HPA) axis plays a key role in the regulation homeostasis and the response to

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Abbreviations: ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; gd, gestational day; HPA, hypothalamic-pituitary-adrenal; miRNA, microRNA; mRNA, messenger RNA; PTSD, posttraumatic stress disorder; PVN, paraventricular nucleus; sGC, synthetic glucocorticoid; TSST, Trier Social Stress Test.

stress. It also plays a role in the modulation of cardio-vascular, metabolic, reproductive, and neurologic function (3–5). During the stress response, the hypothalamic paraventricular nucleus (PVN) initiates an endocrine cascade with the release of corticotropin-releasing hormone (CRH) and arginine vasopressin that trigger the synthesis of Pro-opiomelanocortin [the precursor of adrenocorticotropic hormone (ACTH)] (Fig. 1). ACTH is released from the anterior pituitary into the peripheral circulation. The adrenal cortex responds to ACTH with the release of glucocorticoids [e.g., cortisol (humans), corticosterone (rats)] that act on glucocorticoid receptors and mineralocorticoid receptor at a number of levels within the axis. Activation of the hippocampus inhibits this endocrine cascade, whereas activation of the amygdala

enhances the HPA response. In this manner, glucocorticoid-sensitive brain regions refine challenges to homeostasis and adaptive responses to stress.

HPA function: prenatal adversity

The effect of maternal adversity on HPA function in offspring, from human and animal studies, has been reviewed in detail previously (6, 7). Maternal adversity during pregnancy arising from acute/chronic stress, anxiety, and depression can result in increased levels of maternal glucocorticoid and a subsequent increase in fetal exposure. Although some studies have shown maternal depression to be associated with increased reactivity of the HPA axis (8), the association between maternal adversity and fetal glucocorticoid exposure is
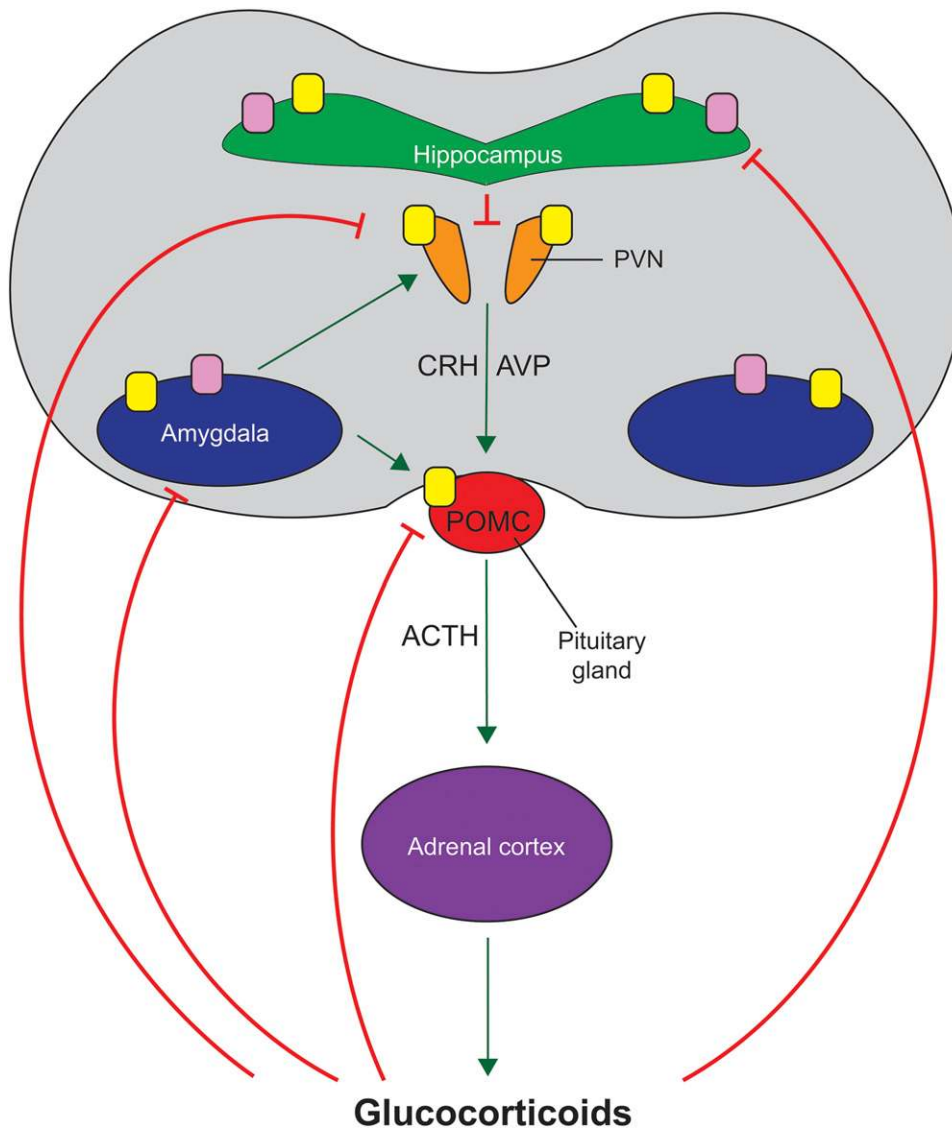
Figure 1. The HPA axis plays a key role in the regulation homeostasis and the response to stress. During stress, the hypothalamic PVN initiates an endocrine cascade with the release of CRH and arginine vasopressin (AVP) that trigger the synthesis of pro-opiomelanocortin (POMC; the precursor of ACTH). ACTH is released from the anterior pituitary into the peripheral circulation. The adrenal cortex responds to ACTH with the release of glucocorticoids, which act on glucocorticoid receptors (GRs; yellow boxes) and mineralocorticoid receptors (MRs; pink boxes). Activation of the hippocampus inhibits this endocrine cascade, whereas activation of the amygdala enhances the HPA response.

complex, nonlinear, and likely dependent on a number of variables. In human studies, elevated maternal cortisol during the late second and third trimesters was associated with an increased response to stress (heelstick procedure) in infants 24 hours after birth. However, in this study, there was no association between maternal anxiety and depression scores and maternal cortisol (9). Another study in infants (12 months) associated maternal anxiety with an increased cortisol response to bathing, but a reduced response to immunization and maternal separation (10). More recently, maternal depression was linked to elevated salivary cortisol responses to stress (Still Face Paradigm) at 4 months of age (11). Further, high levels of maternal subjective stress during the 2008 Iowa floods were associated with increased cortisol responses to stress in children (2.5 years); effects were confined to females and were greater if distress was experienced later in pregnancy (12). Interestingly, maternal anxiety and depression was also associated with an increase in the sympathetic nervous system response to stress, an effect that was confined to boys (13). In contrast, other studies have demonstrated that maternal anxiety in late gestation is associated with reduced HPA function in children and adolescents (13, 14). As such, it appears that the effects of maternal anxiety and depression in pregnancy on HPA function and stress responsiveness in children likely depends on; the stage of pregnancy when stress occurred; infant/child age and sex; and the context of the stressor used to activate the HPA axis.

A large number of animal studies have linked prenatal stress to subsequent HPA function in offspring. These have improved understanding of the relationship between maternal stress during pregnancy, maternal glucocorticoid concentrations, HPA function, and behaviors in offspring, as well as determining the mechanisms involved. However, there has been considerable variability in the results of these studies that likely has arisen from outcomes that are highly dependent on the nature of the exposure (type of stress, duration, time in gestation), the timing of assessment (prepubertal, peripubertal, adult, aged), sex of the offspring, and, in females, the time of the reproductive cycle when testing was undertaken.

In juvenile rhesus monkeys, maternal stress increased basal morning cortisol and decreased cortisol inhibition following dexamethasone suppression (15). In a recent study in wild chimpanzees, male, but not female offspring, of low-ranking mothers exhibited lower fecal glucocorticoid metabolite levels; effects that increased with age (16). In guinea pigs, prenatal stress in late gestation [gestational day (gd) 50 or gd60] resulted in adult male offspring that exhibited increased basal and ACTH-stimulated cortisol levels (17). In females,

prenatal stress on gd50, but not gd60, led to elevated basal cortisol levels (18). In addition, in adult females, prenatal stress resulted in a reduction in the adrenocortical response to stress in estrous, but not luteal phases of the reproductive cycle (18). In rats, daily prenatal stress over the last week of gestation led to elevated basal and activated corticosteroid responses in offspring (19–21).

HPA function: prenatal glucocorticoid exposure

There has been considerable interest in the effects of synthetic glucocorticoid (sGC) on HPA function and behaviors from their widespread use in cases of suspected congenital adrenal hyperplasia and threatened preterm birth (1). Maternal treatment with sGC effectively decreases the incidence of neonatal respiratory morbidity and mortality. Given the difficulty in predicting preterm delivery, a considerable proportion (~30%) of women who receive a single course of sGC do not deliver within 7 days (22). This led to the routine use of weekly (multiple) courses of sGC at many centers in the late 1990s/early 2000s. At this time, and because of emergence of studies indicating potential longer term effects on other organs, particularly the brain, a National Institutes of Health update recommended restriction to a single course of sGC, except in ongoing trials (23). A very recent American College of Obstetricians and Gynecologists update recommends a single course of sGC for women presenting at risk for preterm birth between 24 and 34 weeks' gestation, and that regularly scheduled repeat courses (>two courses) are not recommended (24).

The impact of early exposure to sGC (primarily betamethasone and dexamethasone) on endocrine and neurodevelopmental outcomes has been investigated extensively in many species including humans (7). As for the situation with early adversity, effects are highly sex-, age-, and species-specific, as well as being dependent on the time in development when exposure occurs. Studies investigating the effects of prenatal sGC exposure on HPA function in humans have been significantly confounded by the presence of preterm birth (and its varied etiologies) in study populations, variability in the latency between treatment and delivery, and variability in the timing and number of doses administered. These have led to considerable variability in outcomes reported.

Human infants who received a single exposure to sGC as fetuses, but were born at term (reducing the confounding effects of prematurity), exhibited normal baseline cortisol but an increased response to stress (heelstick procedure) 24 hours after birth (25). In preterm delivered infants (~29 weeks), there was no association between a single course of sGC exposure and resting cortisol at 3, 8, and 18 months (26). However, two

studies undertaken in children born at normal term indicated long-term effects on stress responsiveness. Single-course sGC resulted in children (ages 6 to 11 years) that exhibited elevated cortisol responsiveness during the Trier Social Stress Test (TSST); an effect that was most prominent in girls (27). In the other study, children (age 10 years) born at term also exhibited an increased cortisol response to the TSST (28). In adults that were born preterm (<32 weeks) and that had received a single course of sGC, there was no difference in basal HPA function compared with adults (age 19 years; men and women) born preterm that had not received sGC, although sGC treatment was associated with increased plasma dehydroepiandrosterone and androstenedione (29). In another study in adults (age 30 years; men and women), single-course sGC did not affect basal cortisol levels, although those exposed to sGC did exhibit early markers of insulin resistance (30). No studies have investigated HPA responsiveness to challenge in adults exposed prenatally to sGC. It is critical to understand the long-term consequences of prenatal sGC on HPA function in humans born both preterm and at term. The latter group is of importance because almost 30% of pregnant women who are treated with sGC give birth at normal term (22).

A large number of studies have investigated HPA function in animal models following prenatal sGC exposure. When considering these studies, it is important to note that there are very important species differences in glucocorticoid sensitivity. Old World primates (e.g., baboons, rhesus and vervet monkeys) and sheep are relatively glucocorticoid sensitive. Conversely, rats and mice are very glucocorticoid sensitive. Guinea pigs are relatively glucocorticoid resistant, whereas New World primates (e.g., macaques) are extremely glucocorticoid resistant. In Old World primates, treatment with single- and multiple-course sGC resulted in offspring (born at term) that exhibited an increase in basal and activated HPA function as juveniles (31, 32). In contrast, in the New World common marmoset, multiple courses of sGC administered in early or late gestation did not modify basal pituitary-adrenocortical activity during the first year (33). Interestingly, the effects of prenatal sGC on HPA outcomes were similar between juvenile Old World primate offspring and humans. To our knowledge, there have been no studies on the impact of prenatal sGC on HPA function in adult primates.

In guinea pigs, a single course of sGC in late gestation led to a reduced cortisol stress response in young (age 18 days) males and females (34). In contrast, exposure to multiple-course sGC led to increased cortisol responsiveness to stress in female offspring (age 19 days) with a trend toward a reduction in responsiveness in

males (35). Interestingly, in another study, multiple-course sGC decreased HPA responsiveness in infant male (age 10 day), but not female guinea pigs (36). In adult male guinea pigs, multiple-course sGC resulted in a reduction in basal and stress-induced cortisol levels; this was linked to a decrease in Crh messenger RNA (mRNA in the PVN and an increase in Nr3c2 mRNA (encodes mineralocorticoid receptor) in the hippocampus (37). The latter suggests an increase in hippocampal glucocorticoid feedback sensitivity facilitating reduced HPA function (37). In adult females, prenatal sGC exposure resulted in reduced basal HPA activity, although this was only evident in the luteal phase of the reproductive cycle (37, 38). Hippocampal Nr3c1 (encodes GR) and Nr3c2 mRNA was elevated in sGC-exposed animals, suggesting increased glucocorticoid feedback sensitivity at this time (38). These studies suggest that the programming effects of sGC are highly dependent on sex and the age at which HPA outcomes are assessed and that there is interaction between programming and the reproductive cycle in females.

In sheep, antenatal sGC exposure resulted in altered HPA function in young offspring that was also sex- and age-dependent. Single-course sGC delivered in early gestation led to decreased basal and activated HPA function in females, but elevated HPA activity in male offspring (39). In adult sheep, sGC effects on HPA function are dose-, age-, and sex-specific. Single course sGC treatment (betamethasone) led to elevated basal and stress-activated adrenocortical function in mixed-sex offspring at 12 months of age (40), but there were no differences in HPA function at 24 months (41). In another study, single-course dexamethasone treatment at a similar stage of gestation increased basal cortisol, but reduced the stress-activated cortisol response in older female offspring (30 to 42 months); males were not tested (42). This suggests a strong influence of age on long-term programming outcomes, but also that there may differences between the programming effects of dexamethasone and betamethasone.

In rats, daily administration of antenatal sGC over the last week of gestation led to decreased basal corticosterone in male offspring (4 weeks), with no difference at 7 and 10 weeks of age (43). At 7 and 10 weeks, there was a prolonged corticosterone response to stress in animals prenatally exposed to sGC. In other studies in adult offspring, multiple antenatal sGC treatment resulted in increased basal corticosterone levels in male and female offspring and elevated corticosterone responsiveness to stress in females, but not males (44, 45). A recent study in mice has shown that prenatal corticosterone exposure results in age-dependent dysregulation of adrenal function (including increased basal corticosterone) and altered

adrenal morphology in male offspring, with no effect in females (46).

Prenatal adversity: offspring behavior

Prenatal stress has been linked to increased risk for conduct disorder, anxiety disorders, attention deficit hyperactivity disorder, reduced cognitive performance, and schizophrenia; in many cases, effects are sex-specific (47). In humans, maternal prenatal cortisol concentrations (32 weeks' gestation) can predict infant emotionality in a sex-dependent manner. Female infants (5 weeks) born to mothers with high waking cortisol exhibited negative emotionality; male infants were less affected (48). Another study found that prenatal depressive symptoms associated with increased $Nr3c1$ 1F DNA methylation in boys and decreased brain derived neurotrophic factor IV DNA methylation in boys and girls in buccal samples collected at 2 months of age (49). There was no association between maternal cortisol levels and infant DNA methylation, suggesting that the effect of maternal depression on epigenetic modifications is not mediated directly by glucocorticoids (49). Studies on the Growing Up in Singapore Towards Health Outcomes cohort have demonstrated that maternal anxiety during pregnancy is linked to structural alterations in corticolimbic brain regions associated with anxiety-related phenotypes, including behavioral inhibition (50, 51); interestingly, there was interaction with $COMT$ haplotypes (52).

Several recent studies have identified potential routes by which maternal stress in pregnancy influences neurodevelopmental and behavioral outcomes. Increased neurosteroid levels in late gestation protect the fetal brain from hypoxic insult and promote normal neurodevelopment. Prenatal stress in guinea pigs reduces neurosteroid production and sensitivity, as well as reducing myelination and modifying behavior (53). Other studies in newborn and young lambs (1 month) have shown that prenatal stress increases dendritic spine density in the hippocampus and prefrontal cortex (54, 55), and that this is associated with negative affective state, increased fear reactions and impaired cognition (56).

As alluded to previously, it is important to consider the role of genetic variation in the interaction between early environment and long term outcome. Although maternal prenatal anxiety, depression, and stress results in increased internalizing behaviors, not all children are affected. Different brain derived neurotrophic factor polymorphisms may account for this altered individual vulnerability to prenatal anxiety on internalizing behaviors (57). Further, another recent study linking maternal prenatal anxiety to attention deficit hyperactivity

disorder identified a strong interaction with variation in the $COMT$ gene (58).

Prenatal glucocorticoid exposure: offspring behavior

Antenatal sGC exposure affects neurodevelopmental outcomes and behaviors in animal and human studies (7). As discussed previously, prematurity represents a major risk factor for poor neurodevelopmental outcomes. As such, many of the studies describing the long-term effects of sGC exposure on behaviors in humans are confounded by prematurity. Early studies identified increased risk of behavioral disturbances including attentional problems, hyperactivity, and neurodevelopmental anomalies in children that had been exposed to repeated courses of antenatal sGC (59, 60). Relatively few human studies have considered the impact of sGC exposure in children born at term. In children (ages 6 to 10 years) who were born at term and had been exposed to single-course sGC, there were decreases in thickness of the anterior cingulate cortex, an area known to be involved in affective disorders (61). Interestingly, a subsequent study has shown a substantial interaction between prenatal sGC exposure and postnatal sociodemographic adversity. Children exposed to antenatal sGC (born at term) and postnatal socioeconomic adversity demonstrated impaired memory performance, whereas those that were exposed to sGC only with no postnatal adversity showed no impairment of memory function (62). A recent follow-up from one of the largest clinical trials comparing the effect of single vs multiple course sGC exposure on childhood outcomes, identified increased incidence of neurosensory deficits in children (age 5 years) exposed to multiple sGC in the subgroup (~30%) that were born at normal term (63).

Recent animal studies have begun to identify routes by which antenatal sGC affect neurodevelopmental outcomes and behaviors. Maternal sGC exposure decreased the neurosteroid allopregnanolone and myelination in the fetal sheep brain (64, 65). In the fetal rat brain, sGC exposure resulted in decreased PVN volume and cell number, although only female fetuses were assessed (66). Other recent studies have shown antenatal sGC exposure to increase expression of drug transporters and tight junction proteins in endothelial cells of the developing blood-brain barrier in the guinea pig (67, 68). The latter will directly affect the transport of factors across the fetal blood-brain barrier, which may in turn affect brain development.

In adult male rat offspring, prenatal sGC exposure reduced the length of astroglial processes in the hippocampus (69) as well as decreasing dendritic outgrowth in dentate granule cells, impairing spatial memory and increasing anxiety-like behaviors in male rat offspring

(70, 71). In contrast, in other studies, prenatal sGC altered hippocampal morphology and reduced levels of hippocampal *reelin* (*Reln*) and *glutamate decarboxylase 1* (*Gad1*) mRNA (72), but did not affect spatial memory and anxiety-like behavior; again, only males were investigated (72, 73). Indeed, in one study, prenatal sGC exposure was associated with an increase in cognitive flexibility and adaptability (73). These studies highlight the variable behavioral outcomes associated with prenatal sGC exposure, likely a result of different treatment and testing paradigms. However, they also highlight that the majority of studies have only considered outcomes in male offspring. In this regard, a recent study demonstrated that antenatal sGC increased anxiety and depressive-like behaviors in females but not in males, and that these effects were associated with altered function of the central serotonin system (74). Hence, it is critical to consider the effect of sex in these studies.

Transgenerational Influences of Stress and Glucocorticoids on HPA Function and Behaviors

Recent focus has been placed on the potential transgenerational influences of early adversity and sGC on HPA function and stress-related behaviors. Studies have investigated the effects of maternal exposures during pregnancy on outcomes across multiple generations as well as maternal and paternal exposures before pregnancy on HPA function and related behaviors in the next generation. Other studies have focused on transgenerational influences of other environmental challenges including undernutrition, overnutrition, and endocrine disruptors, but these are outside the scope of the current review.

Transgenerational outcomes: maternal exposures

In rats, social stress during pregnancy resulted in adult F2 female offspring that exhibited increased HPA responses to stress following maternal transmission (75). These changes were associated with an increase in *Crh* mRNA in the PVN and decreased *Nr3c1* and *Nr3c2* mRNA in the hippocampus, suggesting reduced glucocorticoid feedback sensitivity. In contrast, in males, HPA responses to acute stress were attenuated and this was linked to elevated hippocampal *Nr3c1* mRNA (75).

In the guinea pig, antenatal sGC exposure was associated with decreased stress-activated HPA function and modified glucocorticoid sensitivity in F2 juvenile and adult offspring following maternal transmission (76). Decreased HPA responsiveness was associated

with a reduction in anterior pituitary *Pomc* mRNA levels and ACTH content, together with decreased *Crhr1* mRNA; the latter suggests reduced pituitary sensitivity to CRH (76). Interestingly, molecular effects in the pituitary were greater in females than males. In another study in sheep, single-course sGC resulted in increased basal HPA function but a reduction in stimulated HPA function in F2 female offspring following maternal transmission; male offspring were not investigated (42). In a recent study in guinea pigs, antenatal treatment with multiple courses of sGC led to transgenerational effects on HPA function across three generations via both maternal and paternal transmission (35). This was associated with extensive transgenerational changes in gene expression in the hypothalamic PVN, including gene networks linked diabetes, thermoregulation, and collagen formation; transmission was sex- and generation-dependent (35).

Several studies have described effects of environmental exposures on transgenerational behavioral outcomes; the majority has been undertaken in rats (77). A recent study has shown that prenatal stress results in increased anxiety-like behavior in adult F2 male offspring following maternal transmission, with little effect in females (75). Heightened anxiety in the F2 males born to prenatally stressed grandmothers was associated with increased *Crh* mRNA in the amygdala as well as modified *Crhr1* and *Crhr2* mRNA (75). In this study, there was no effect of grandmaternal social stress on depressive-like behaviors. A recent study has shown that multiple courses of sGC lead to profound effects on open-field locomotor activity in juvenile preweanling guinea pig offspring across multiple generations (35). Importantly, effects were confined to F2 and F3 female offspring following paternal transmission; there were no transgenerational effects of sGC on locomotor activity in male offspring or in male and female offspring following maternal transmission. The same study demonstrated a reduction prepulse inhibition (sensorimotor gating, and an indicator of attention) in prepubertal female offspring after prenatal sGC, but again only after paternal transmission (35). It is clear that endocrine and behavioral outcomes in offspring following maternal prenatal stress and antenatal sGC exposure can pass across multiple generations. Further, the paternal route of transmission, following the initial maternal exposure, can result in stronger phenotypes than maternal transmission.

Stress exposure before pregnancy may also have long-term consequences in offspring. Chronic stress in preadolescent females resulted in a substantial blunting of the corticosterone response to restraint stress during pregnancy (78). In another study, maternal stress before

pregnancy modified spine number and dendritic length in the anterior cingulate and prelimbic/infralimbic regions in rat offspring. The nature and extent of effects was dependent on the temporal proximity of adversity to pregnancy and was sex-specific (79). In the former study, modified HPA responsivity to stress during pregnancy likely results in altered fetal exposure to glucocorticoid and may indirectly influence development of the fetal HPA axis, stress responsiveness, and related behaviors.

Transgenerational outcomes: paternal exposures

Recent studies have investigated the effects of paternal stress prior to breeding on HPA function and related behaviors in offspring (6). Exposure of male mice to chronic stress (6 weeks) during the peripubertal period, or in adulthood, resulted in offspring with a reduced HPA response to acute stress; adult male and female offspring were affected (80). Gene set enrichment analysis following RNA-sequencing in the hypothalamic PVN of offspring revealed substantial changes in gene transcription, including increased expression of glucocorticoid sensitivity genes (80). Little is known concerning the roles of paternal stress on HPA outcomes in other species. In the rhesus macaque, early separation of fathers in juvenile life resulted in mixed sex infant offspring (3 to 4 months) that exhibited increased stress-activated cortisol secretion and increased emotionality (81). A single study in humans found no association between paternal prenatal anxiety and adolescent cortisol levels (14). However, follow-up of children of Holocaust survivors with posttraumatic stress disorder (PTSD) revealed reduced basal cortisol levels and increased glucocorticoid sensitivity (82, 83).

Paternal stress and glucocorticoid exposure have also been shown to impact behaviors and related brain structures. Males subjected to postnatal traumatic stress sire F1 offspring that exhibit impaired long-term memory and altered synaptic plasticity (84), but improved behavioral flexibility (85). In contrast, exposure of male mice to chronic stress (6 weeks) during the peripubertal period, or in adulthood, did not affect behavior in F1 offspring (prepulse inhibition, tail suspension test, Barnes maze, and light-dark box) (80). Treatment of adult mice with corticosterone (4 weeks) before mating resulted in male F1 offspring that exhibited hyperactivity and increased anxiety-like behavior and female F1 offspring that exhibited impaired memory retention and altered fear extinction (86, 87). In F2, both male and female offspring displayed reduced anxiety-like behavior and males exhibited a depression-like phenotype (87). Together, these studies indicate an interaction between the paternal environment and offspring HPA function and behaviors across multiple generations.

Epigenetic Mechanisms of Developmental Programming

Epigenetic modifications are associated with the effects of prenatal stress, glucocorticoids, and the developmental programing of HPA function, although our understanding of the mechanisms involved remains limited. Emerging areas of research include genome-wide analyses of DNA methylation modifications (conventionally referred to as DNA methylation) and small noncoding RNAs as vectors for intergenerational and transgenerational transmission of epigenetic effects. Epigenetic signatures are to some extent tissue-specific, and only a few studies to date have examined the correspondence between central and peripheral signatures as a function of maternal adversity and glucocorticoid exposure (88–91).

Prenatal adversity: epigenetic mechanisms

Since earlier reports implicating DNA methylation of the *Nr3c1* gene in the impacts of maternal mood on offspring cortisol (92) and childhood adversity on *Nr3c1* 1F promoter methylation in the brain (93), several studies have reported increased DNA methylation of *Nr3c1* promoter variants in maternal stress in humans (94–98). Prenatal stress exposure as a result of chronic or war trauma stress was associated with differential DNA methylation in a number of genes within the HPA axis, including *Crh*, *Crhbp*, *Nr3c1*, and *FKBP5* in placenta and *Crh* and *Nr3c1* in cord blood (99). Exposure to war trauma leading to PTSD during pregnancy was associated with lower cortisol and *Nr3c1* levels and higher DNA methylation in the *Nr3c1* 1F promoter in peripheral blood of their children who were examined in adolescence (100). The offspring of survivors of the Holocaust born after World War II (examined at a mean age of 57.2) showed a moderation of PTSD effects depending on the affected parent, with lower *Nr3c1* 1F promoter methylation in offspring with both maternal and paternal PTSD and higher *Nr3c1* 1F promoter methylation with paternal-only PTSD (98). A meta-analysis combining data from 977 individuals found a substantial correlation between prenatal stress and the methylation status of *Nr3c1* 1F, supporting the association between prenatal stress and the methylation status of specific CpG sites within the *Nr3c1* promoter (101). These data support several studies in animal models indicating prenatal stress effects on *Nr3c1* promoter DNA methylation (102).

A few genome-wide analyses of maternal stress effects on DNA methylation have been performed in humans. A recent study examining transgenerational transmission of epigenetic effects found that the methylation of 5 CpG sites in saliva from grandchildren associated with exposure of the grandmother to community or domestic

violence during pregnancy (103). A series of recent studies has explored DNA methylome modifications in response to a traumatic event experienced during pregnancy (caused by the 1998 Quebec Ice Storm) (104). T cells isolated from early adolescent children of stressed mothers showed DNA methylation modifications in hundreds of genes associated with objective and maternal cognitive appraisal of the event, which were enriched predominantly in immune annotations. Differential DNA methylation of 33 genes in T cells was also enriched in genes associated with immune system function in neonates and hippocampi of adult males exposed to nonmedicated maternal depression (88). Similarly, nonmedicated maternal anxiety/depression was associated with differential DNA methylation in 42 CpG sites relative to controls in cord blood (105). However, data combining two large independent population-based samples from the Generation R Study and the Avon Longitudinal Study of Parents and Children (n = 1740) revealed no major CpGs associated with a normative range of prenatal stressors (*i.e.*, nontraumatic) in cord blood. It appears likely that the type, severity, and timing of exposure are crucial factors determining the degree of epigenetic plasticity associated with prenatal stress (106). To our knowledge, no genome-wide epigenetic study of maternal stress has been performed in animal models.

Prenatal glucocorticoid exposure: epigenetic mechanisms

To date, no study has examined the impact of sGC on DNA methylation modifications in humans; however, recent studies in animal models have moved beyond candidate genes to examine epigenome-wide responses to prenatal sGC exposure. A series of studies has examined the effects of multiple course prenatal exposure to sGC in guinea pigs on epigenetic modifications and gene regulation. Global levels of DNA methylation assessed 14 days after the final treatment (gd65) varied by tissue type, but all tissues examined (liver, adrenal, kidney, and cerebellum) were hypomethylated in F1 and F2 adults. At each time point examined, the magnitude of the effect of sGC varied by tissue type and was associated with the differential expression of epigenetic regulators (107). In the hippocampus, sGC exposure altered DNA methylation in hundreds of gene promoters at gd65 (108, 109). However, different sets of genes showed epigenetic alterations acutely after the final exposure, indicating a protracted time course of modifications possibly related to dynamic feedback activity among genes initially affected and their downstream targets. In these studies, only male offspring were examined. In a recent report using RNA-sequencing, prenatal glucocorticoid exposure altered gene expression in the PVN through the F3

generation together with HPA and hyperactivity consistent with developmental programing by sGC (35). Female offspring were more sensitive than males to the programming by sGC, with transmission occurring through the paternal line.

Paternal glucocorticoids and prenatal stress: mechanisms

Given the accumulating data reviewed indicating a paternal impact on offspring stress vulnerability, there has been great interest in elucidating mechanisms that may convey intergenerational and transgenerational inheritance through the paternal germline [as opposed to transgenerational transmission of epigenetic effects (110)]. Some investigations have examined the potential involvement of DNA methylation in sperm, including at specific genes underlying stress-related odor conditioning (111). Sensitivity to stress in adulthood in a model of maternal separation and maternal stress was associated with decreased DNA methylation of the hippocampal *Nr3c1* promoter in F1 offspring, and methylation in some CpGs normalized after environmental enrichment in the sperm of male offspring and the hippocampus of F2 males (112). Exposure to sGC in adult male mice was associated with increased global DNA methylation 60 days later in sperm (113). Their male offspring also showed a selective decrease in methylation in regulatory regions of the promoters of *Nr3c2*, *Nr3c1*, and *Esr1* (encodes estrogen receptor a) in kidney at postnatal day 50. Precisely how epigenetic information may be transferred via DNA methylation modifications and maintained in the face of global demethylation of the male pronucleus, which occurs shortly after fertilization, is not well understood (114). Imprinted genes, which escape reprogramming and show parent-of-origin effects on transcription are rare; however, recent DNA motif analysis and analyses of allele-specific methylation patterns has indicated that many more showing monoallelic DNA methylation patterns appear to exist, particularly in brain (115).

Another recent focus is on small noncoding RNAs, which are abundant in mature sperm and play a role in posttranscriptional regulation of gene expression. Male offspring in a mouse model of maternal separation and maternal unpredictable stress showed differential microRNA (miRNA) expression in sperm (116). Importantly, injection of sperm RNAs from the stressed males was sufficient to reproduce metabolic and behavioral outcomes associated with stress. These data indicate that small noncoding RNAs are sensitive to early traumatic stress. The expression of nine miRNAs was increased in sperm after 6 weeks of chronic stress before breeding, with offspring showing altered transcription in PVN and the bed nucleus of stria terminalis (80).

Microinjection of zygotes with these nine miRNAs recapitulated the effects of paternal stress, long-term programming of transcription in the hypothalamus, and a blunted HPA response to stress (117). These studies implicate small noncoding RNAs in behavioral and physiological changes related to prenatal stress, likely as a result of altered neurodevelopment during embryogenesis.

Mechanisms of Programming: Caveats and Perspectives

The primary focus of experiments to date, particularly in animal models, has been analysis of long-term changes in HPA responses and associated behavior with developmental programming, with offspring typically examined in adulthood; however, the studies reviewed here underline the importance of understanding the relationship between initial phenotypes and later life phenotypes. Examining epigenetic mechanisms proximal to the time of initial exposure and across developmental milestones has important implications for interpretation of later molecular phenotypes for at least two reasons: 1) the initial insult may induce pathophysiological outcomes that impair HPA function as early as the time of exposure and/or 2) the initial exposures may lead to cellular reprogramming (presumably via epigenetic mechanisms) that "prime" differential responses to the same environmental conditions later on that *then* lead to pathology

(Fig. 2). Hence, examining both early and later life time points are needed to disambiguate these alternatives. This issue highlights the importance of animal models in developing hypotheses that can be examined in humans. At the same time, it will be critically important that information from studies in humans is used to assess the extent to which animal models provide useful phenotypes that recapitulate not only phenotypic outcomes but epigenetic signatures. As such, human data can be used to model specific outcomes that can then be examined in animal models to assess their external validity.

Given this, it is important to identify developmental programming effects on hormone activity, post-translational modifications to nonhistone proteins, and cell proportion changes, which may not reflect true epigenetic reprogramming events (118). With respect to investigations of transgenerational gametic transmission, the possible role of genetic selection, or the behavioral transmission of epigenetic effects, including of paternal stress effects in rodents (119), should not be discounted. Longitudinal analyses can help determine the extent to which exposures lead to permanent epigenetic modifications, the stability of those modifications over time, and the extent to which these modifications arise later on, which can prime differential responses to later exposures. For example, the mediating effects of parental care also lead to differential response to later life stress (120). Other factors that should be considered include possible
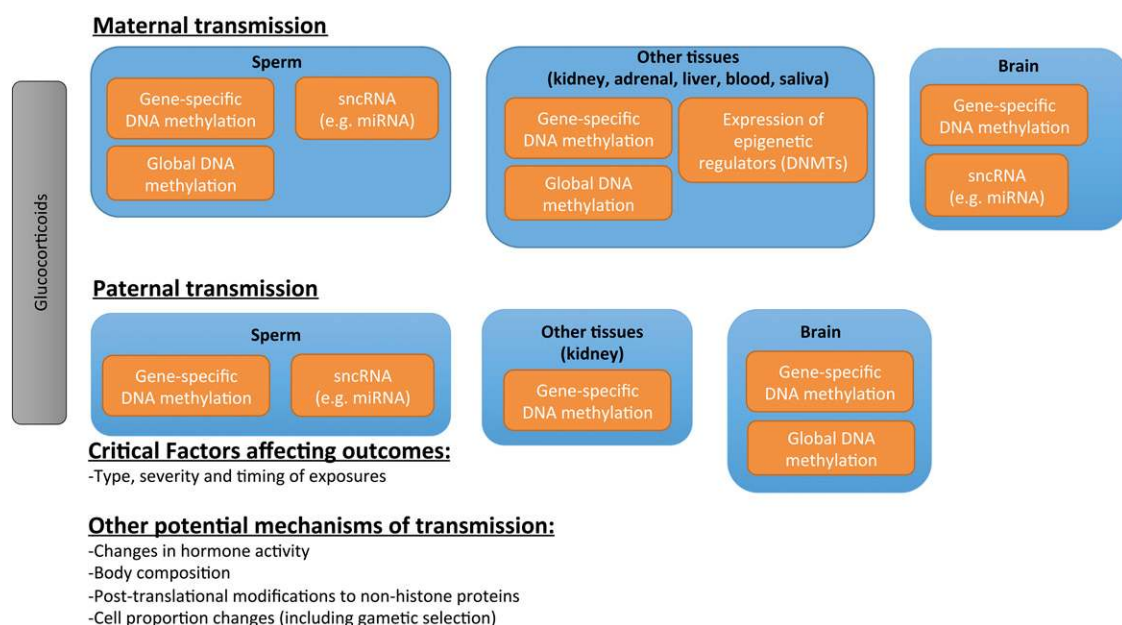

Figure 2. Mechanisms of programming by prenatal glucocorticoid exposure discussed in this review. Blue boxes refer to offspring tissues affected by parental exposures transmitted along the maternal or paternal line. Recent studies have reported epigenetic modifications in offspring with both maternal and paternal exposures to glucocorticoids in the (grand)parent generation. Notably, the specific genes affected are highly sex-specific, yet modifications to similar classes of epigenetic mechanisms in offspring [DNA methylation, small noncoding RNA (sncRNA); orange boxes] have been reported via both maternal and paternal transmission, affecting gametes, peripheral tissues, and the central nervous system of offspring. Factors affecting specific outcomes of prenatal glucocorticoid exposure are listed, as are potential alternative explanations for outcomes that may not involve direct effects of epigenetic reprogramming. DNMT, DNA methyltransferase.

role of microbiota in modifying trajectories of stress exposures (121) and, relatedly, the role of diet and body composition that interact with stress physiology (122, 123). In considering the relationship between epigenetic modifications and functional outcomes, epigenetic modifications such as DNA methylation are not only associated with active functional changes in gene expression but, perhaps more commonly, lead to genes *poised* for differential transcription (124). Histone modifications affecting DNA accessibility and nucleosome positioning may also be involved. Examining epigenetic modifications and gene expression in the context of challenge conditions (*e.g.*, the TSST described previously) can be informative in elucidating the association between epigenetic modifications and functional outcomes. In this sense, repeated stress responses may potentiate deleterious outcomes, whereas interventions that buffer stress, such as environmental enrichment, may mitigate them (112); the threshold may depend upon epigenetic potentiation. Ultimately, detailed analyses of the binding of transcription factors, the drivers of transcriptional regulation, will be needed for a mechanistic understanding of the role of epigenetic modifications.

Conclusions

Understanding the long-term consequences of parental (maternal and paternal) adversity and glucocorticoid exposure on stress endocrinology and related behaviors in offspring is critical. Parental depression and anxiety are prevalent and use of sGC in the management of preterm birth will likely increase with adoption of recent guidelines focused around decreasing infant morbidity and mortality (24). Development is a continuum and it is becoming clear that an early exposure can lead to an altered developmental trajectory that, in turn, influences interactions between the individual and the environment after birth and indeed throughout life. To date, by far the majority of studies in this field have confined follow-up analysis to adult male offspring; however, there are major sex and age differences in outcomes, and these need to be carefully addressed in future studies. Emerging evidence suggests that the impact of early exposures is transmitted across multiple generations via both the maternal and paternal lineage. The mechanisms by which this occurs represents a major ongoing research focus. The physiological consequences of such transmission and implications for long-term population health are of considerable importance. Recent studies also suggest that paternal preconception exposures may be as effective as antenatal exposures in programming endocrine function and behaviors in offspring. Improved knowledge of the mechanisms by which adversity and glucocorticoid program

the fetus and neonate will allow development of strategies to ameliorate and/or reverse these effects and thus prevent long-term poor health outcomes. Such knowledge will also potentially allow the identification of individuals at risk for poor developmental outcomes for whom early intervention is most effective.

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