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Prenatal Maternal Stress and Neurobehavioral Development of the Neonate

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Prenatal Maternal Stress and Neurobehavioral Development of the Neonate

By

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For Grandma Joyce

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PART I: REVIEW OF THE LITERATURE AND QUESTION FORMULATION

I. INTRODUCTION

Many epidemiologic and clinical studies have identified links between the early environment and later health and disease. From an evolutionary biology perspective, that the environment would influence phenotype is clearly beneficial for future fitness and survival (1). Natural selection acts slowly in a species with a relatively long lifespan, so the ability to incorporate environmental information in order to function most optimally under local conditions is highly adaptive. Building on this premise, long-term health outcomes would seem to depend on how well the environment during gestation or childhood predicts conditions later in life.

There are two commonly cited theories regarding the source of individual differences in health outcomes – the *cumulative exposures model* and the *developmental trajectories model* (2). Central to both of these models is the idea that a person's phenotype is not simply a product of inherited genes, but rather is conditioned by the environment within the uterus, during childhood, and possibly beyond. Adulthood health is no longer assumed to be a genetically preconfigured plan that gradually unfolds over the course of development and aging. Furthermore, genes and environment are no longer considered to be independent or even competing influences on long-term health and disease outcomes.

The hypothesis of *fetal programming* states that there are sensitive periods, such as the prenatal period, during which environmental exposures can have long-term and heritable effects on physical and mental health trajectories (3). Sources of potentially harmful exposures include environmental toxins, infectious diseases, diet, and psychosocial stress that can all manifest as physiologic dysregulation (4; 5).

Another school of thought attributes individual differences in health trajectories not to developmental origins, but rather to an accumulation of adverse social and psychological exposures across the lifespan (6; 7). The concept of *allostasis* describes the body's calibration of regulatory mechanisms to adapt to present environmental conditions, such as an acute stressor, to maintain physiologic equilibrium. When the body's fine-tuning mechanisms are chronically activated, inadequate, prolonged, or unable to habituate, pathophysiologic changes to various body systems may ensue – termed *allostatic load* by McEwen (8).

Barker and colleagues provided some of the first evidence of early environment impacting long-term health trajectories in a maladaptive way. Their studies of cardiovascular and metabolic diseases have shown that fetal undernutrition and altered fetal growth patterns predict higher risk for coronary heart disease and type 2 diabetes (9). Building upon these findings, recent investigation has explored other aspects of the prenatal environment – namely maternal prenatal psychosocial stress. A considerable

body of evidence suggests that a fetus whose mother experienced high levels of prenatal stress may be programmed to become more reactive to stressors, to recover less quickly from a stress response, and to be less sensitive to negative feedback from stress response mediators (10; 11). With more than half of all women reporting symptoms of anxiety and depressed mood during pregnancy (12), the potential negative effects on offspring are widespread and deserve the attention of researchers, public health professionals, and communities.

This paper provides a summary of the literature that culminated in these conclusions about prenatal programming effects of maternal stress on offspring. A discussion of hypothesized mechanisms for the embedding of maternal psychosocial experience into infant physiology follows as well. Finally, this review concludes with a proposal for research designed to satisfy existing gaps in prenatal stress research.

II. VARIABLES DEFINED

Stress

The concept of stress is multifaceted and may be measured in a variety of ways. Some studies reviewed here defined stressors as independent variables (e.g. exposure to a stressful event or to daily hassles). Other studies quantified factors that protect individuals against stressors, such as social support. Finally, an overwhelming majority of studies attempt to quantify the individual's response to stress – either by directly measuring physiologic outcomes (e.g. stress hormone levels) or by administering questionnaires to pregnant women to measure their emotions. These studies draw upon the concept of individual variation in response to potentially identical stressors. In pregnant women there are additional physical and endocrine changes increasing the complexity of individual stress response variation. Studies of the relation between maternal emotion and hypothalamic-pituitary-adrenal (HPA) axis response have shown mixed results, though their findings generally show that the maternal HPA axis becomes desensitized to stressors as the pregnancy progresses, likely due to rising levels of placental corticotropin-releasing hormone (CRH) (13).

Further clarification about the use of the term *stress* in this review is warranted. Many studies of prenatal mental health and infant development use measures of maternal stress and/or anxiety and/or depression as independent variables. These conditions have some unique but predominately overlapping endocrine and autonomic profiles. As an example, highly anxious and highly depressed people both tend to exhibit elevated levels of norepinephrine and cortisol and decreased levels of serotonin; yet anxiety tends to correlate with elevated dopamine, while depression is commonly associated with low dopamine concentrations (14). Findings should therefore be interpreted with caution until there is better understanding of the potentially unique contributions of these hormones and neurotransmitters to infant outcomes.

Furthermore, maternal report of stress, anxiety, and depression have been shown to be highly intercorrelated and stable during pregnancy (15). That said, studies of prenatal maternal mental health rarely tease apart the unique contributions of stress versus depression versus anxiety. This may be attributed to methodological shortcomings or complications (16). For example, in animal research, it may be difficult to design an exposure specific to stress versus anxiety or depression.

Moreover, these psychopathologies have significant rates of comorbidity in pregnant women. Prevalence data for prenatal psychopathology is limited, though a few studies of clinical diagnosis of anxiety and depression have estimated prevalences of 6.6% and 7-20%, respectively (12; 17). Greater than 50% of women diagnosed with anxiety have been shown to also meet criteria for depression (16). However most studies measure maternal report of symptoms of stress, anxiety, or depression rather than clinical diagnostic criteria. The prevalence of anxiety and depressive symptoms in many studies is therefore higher than the previously stated clinical diagnosis prevalence: 54% and 37%, respectively, in a study of 357 pregnant women without any history of psychiatric diagnosis (12). The implication of using reported symptoms versus clinical diagnosis is that effect sizes may be smaller, but also that findings may be more generalizable to healthy pregnant women, rather than only to those with more severe psychiatric illnesses.

In summary, due to overlapping features and challenges or differences in measurement, the term *stress* will be used generally in this review, encompassing the closely associated psychological constructs of perceived stress, anxiety and depression.

Neurodevelopment

The literature reviewed here includes studies using an outcome measure of neurodevelopment. The study of neurodevelopment aims to understand the relationship between the functional development of the brain and peripheral nervous system and increased risk of later pathophysiology, including psychopathology. One of the most studied examples is schizophrenia, for which there is extensive evidence that prenatal and perinatal characteristics contribute to an increased risk (18). Retrospective studies of schizophrenics have identified subtle aberrations in early cognition and motor function. While this literature review is not specifically focused on schizophrenia, it reviews studies that aim to tease apart subtle early life differences in neurologic function.

There are a variety of methods for measuring neurologic function in neonates and older infants. Biologic measures used include salivary stress hormone levels (basal and reactivity to a stressor) and clinical neurologic examination. Even more widely used than biologic measures are neurobehavioral assessments (e.g. Brazelton Neonatal Behavioral Assessment Scale (19)), and with older infants, language and cognitive measures (e.g. Bayley Scales of Infant Development (20)). Measures of

neurobehavioral outcomes have been shown to be stable within individuals and predictive of later neural function (3). As an example, fetal reactivity (movements, heart rate) is related to motor function in infancy (3), which is predictive of executive function during adulthood (21). Biologic and neurobehavioral measures both provide meaningful information about infant development. However, neither has been shown to be superior in prediction of neurobiology or response to stress later in life. Hence this review is inclusive of these disparate measures of infant neurodevelopment.

III. STRESS PHYSIOLOGY

Before delving into the literature on infant sequelae of maternal prenatal stress, it is important to understand how the body turns psychosocial experience into a physiologic response. The stress response involves many body systems, including neuroendocrine, immune and inflammatory, and autonomic/vascular. The focus of this section will be on neuroendocrine and autonomic aspects of the stress response, since they are both hypothesized to mediate prenatal programming effects of maternal stress (10).

Hypothalamic-pituitary-adrenal Axis

The most immediate response to perception of an acute stressor is the secretion (or cessation of secretion) of several hormones: the sympathetic nervous system secretes catecholamines (epinephrine and norepinephrine), the hypothalamus secretes corticotropin-releasing hormone (CRH), and within seconds of catecholamine and CRH release, the hypothalamus reduces secretion of gonadotropin-releasing hormone (GnRH). The pituitary responds to hypothalamic CRH by increasing secretion of adrenocorticotropic hormone (ACTH) and to reduced GnRH by reducing secretion or pituitary gonadotropins [luteinizing hormone (LH) and follicular stimulating hormone (FSH)], prolactin, and growth hormone. Finally, ACTH and the gonadotropins exert their effects on target organs, the adrenal glands and gonads, respectively. While hypothalamic-pituitary-gonadal axis dysregulation has important implications for reproductive physiology, the focus in this review will be on the HPA axis as it pertains to pregnancy and fetal development (8).

The above description of the initial acute stress response included ACTH secretion by the hypothalamus. ACTH upregulates the synthesis and secretion of glucocorticoids by the adrenal glands. The function of this endocrine activity is to mobilize stored energy and to divert it to vital organs (i.e. skeletal muscle, heart, and brain), to increase immune function, and to inhibit processes that are not immediately essential (e.g. digestion, appetite, and reproduction) (22). The HPA axis is regulated by negative feedback from the glucocorticoid, cortisol, which inhibits secretion of hypothalamic CRH and pituitary ACTH.

HPA Axis and the Placenta

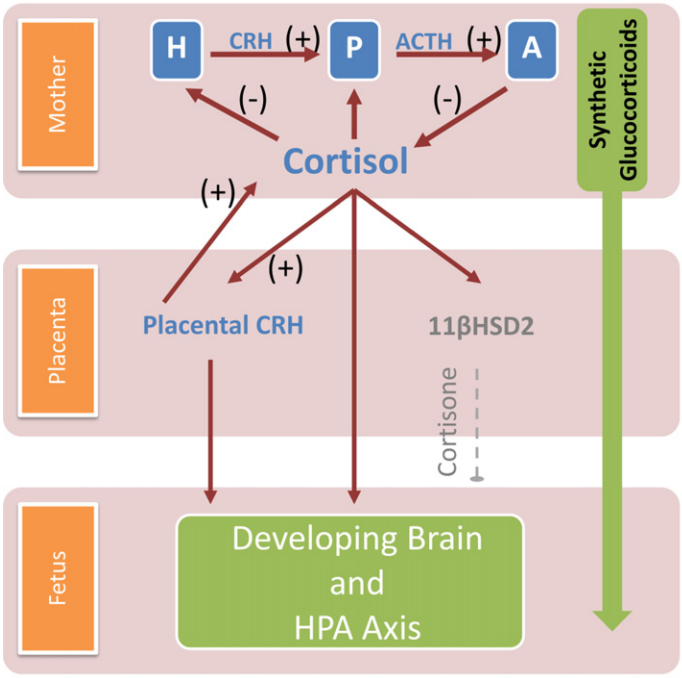

Image credit: Waffarn and Davis 2012

While cortisol exerts *negative* feedback on the hypothalamus, inhibiting secretion of CRH, the placenta reacts oppositely by *increasing* CRH production when stimulated by cortisol (see image to the right). For this reason, maternal serum glucocorticoids rise gradually throughout pregnancy, a process implicated in the initiation of labor. Likely a result of elevated levels of glucocorticoids, the HPA axis of the pregnant woman becomes increasingly desensitized to stressors later in pregnancy, blunting cortisol secretion in response to a stressor. This may explain why studies have shown gradual weakening of the association between maternal report of stress and maternal plasma cortisol over the course of pregnancy (23).

The enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which catalyzes the conversion of cortisol into inactive cortisone, provides a partial barrier between the fetus and rising maternal levels of cortisol. This cortisol inactivation serves an important protective function for the fetus. Despite the partial barrier that placental 11β-HSD2 provides, maternal cortisol levels are nonetheless highly correlated with fetal cortisol levels under stressful circumstances (24). Gitau et al investigated whether fetal cortisol levels were correlated with maternal levels due to placental transmission *or* due to placental CRH stimulation of both maternal and fetal HPA axes. They demonstrated that 80-90% of maternal cortisol was metabolized by the placenta, allowing 10-20% to reach the fetus unchanged. This 10-20% of maternal cortisol accounted for about a third of the variance in fetal cortisol levels. Therefore fetuses did mount an independent stress response as well as receive cortisol directly from maternal circulation.

Gitau et al. (2001) additionally investigated the timing of HPA function in fetuses and found some evidence of HPA function as early as seven weeks. After eighteen weeks, fetuses could mount a stress response that resulted in β-endorphin secretion (an opioid agonist secreted by the pituitary as part of the normal stress response), and after twenty weeks, there was evidence of independent cortisol secretion (25). In summary, fetal exposure to stress hormones early in pregnancy results principally from maternal circulation. In the latter half of pregnancy, maternal cortisol continues to reach the fetus

directly, though some is inactivated by 11β-HSD2. The fetus is exposed to additional cortisol synthesized in its own adrenal glands in response to placental CRH (secreted in response to stimulation from maternal cortisol) (25) or to intrauterine conditions such as hypoxia or undernutrition (26).

Fetal exposure to glucocorticoids of maternal and fetal origin is significant due to the critical role glucocorticoids play in normal fetal brain maturation. Fetal exposure to excess glucocorticoids has been shown to impair growth and brain development, alter the HPA axis response to stress, as well as dysregulate metabolic and cardiovascular function (11). Either elevated or suppressed glucocorticoids can alter the maturation of neurons, synapse formation, myelination, glia, and CNS vasculature (27). Additionally, in rats, prenatal stress reduces the number of hippocampal glucocorticoid receptors (28), reducing sensitivity to negative feedback and thereby increasing basal or stress-induced secretion of glucocorticoids. Increases in HPA activity have been shown to persist into adulthood in animal studies, and as late as ten years of age in human studies (Lupien et al., 2009).

Autonomic Nervous System

Another aspect of the stress response with important relevance to fetal neurodevelopment is the sympathetic branch of the autonomic nervous system. Catecholamines mediate the sympathetic stress response, exerting both central and peripheral actions. A key outcome of sympathetic activation is reduced perfusion of non-vital organs, including the placenta. This effect is mediated directly by catecholamines and also indirectly by glucocorticoids. Peripherally, catecholamines cause vasoconstriction. Findings from studies in pregnant sheep and guinea pigs showed that the uterine artery is particularly sensitive to the vasoconstrictive effects of norepinephrine. The authors commented that evolutionarily this may be beneficial – to spare the mother and even her less vital organs at the expense of the fetus (29). Glucocorticoids augment adrenergic functions by (1) inducing phenylalanine-n-methlytransferase (PNMT), the rate-limiting enzyme in epinephrine synthesis, (2) enhancing affinity of uterine artery β-adrenergic receptors for catecholamines, and (3) increasing synaptic catecholamine concentrations by inhibiting catecholamine reuptake and degradation by monoamine oxidase (MAO-A) and catechol-O-methyltransferase (COMT) (22). In summary, the autonomic response to stress includes vasoconstriction mediated by catecholamines. Uterine artery constriction as a possible mechanism for prenatal programming effects is discussed further below.

To conclude, maternal psychosocial stress evokes two physiologic reactions hypothesized to have effects on fetuses: increased activation of the HPA axis and of the sympathetic nervous system. Studies investigating these mechanisms will be described further below, after a discussion of stress during pregnancy as a predictor of infant neurodevelopmental outcomes.

IV. MATERNAL STRESS DURING PREGNANCY

Psychologic, sociologic, and physiologic factors exist that make stress during pregnancy unique. Below is a discussion of stressors afflicting women during the prenatal period.

Stressors Affecting Pregnant Women

There are well-studied unique pregnancy-specific stressors – for example, unplanned or unwanted pregnancy, fear of negative pregnancy outcomes, fear of childbirth, or insecurity about parenting. Findings from one study showed that only 17% of the variance in pregnancy-specific anxiety could be explained by trait anxiety, suggesting that stress and anxiety in the prenatal period are distinctive (30). Women with a history of current medical comorbidities or with a history of negative reproductive outcomes (e.g. miscarriage, stillbirth, preterm labor) have reported higher levels of pregnancy-specific anxiety (31). Some studies have found that pregnancy-specific stress and anxiety are stronger predictors of negative pregnancy and offspring health outcomes than are other sources of life stress (26; 32; 33).

Aside from the abovementioned pregnancy-specific stress, common stressors such as economic or relationship stress or daily hassles may be heightened during pregnancy. While all women potentially experience stress from these sources, some women are especially vulnerable to these stressors, namely, teenage pregnant women, women of low socioeconomic status, and women with a pre-pregnancy history of mental health problems (34).

Socioeconomic Status and Stress

Individuals of lower socio-economic status (SES) bear an increased burden of psychosocial stress (17; 35). There is certainly a relation between SES and birth outcomes such as small for gestational age (SGA) or preterm birth (36), though health disparities in offspring may be independent of birth outcomes.

There is strong evidence that people of lower SES have higher basal catecholamine and cortisol levels, indicating that they have chronically elevated sympathetic nervous system and HPA axis activity (37). This association is independent of race, gender, age, body mass, and smoking. The effect size was reduced, but remained significant, when accounting for health practices and psychosocial factors. The relevance of this research to prenatal stress is that the two systems hypothesized as mediators for prenatal stress programming effects are the HPA axis and the sympathetic nervous system. If the association between low SES and elevated stress hormones and sympathetic nervous system activity applies to pregnant women as well, then the offspring of lower SES women are at higher risk of prenatal stress programming effects than those of middle or high SES. Thus, not only do women of lower SES experience increased stress, they may also have an increased propensity to transmit biologic effects of stress to their

offspring. It is therefore imperative that economically diverse samples be included in future research (including interventional studies) of prenatal stress.

Pregnancy Outcomes

The two best-documented pregnancy outcomes of adverse prenatal psychosocial factors are preterm birth and low birth weight, both of which are risk factors for later cognitive and social impairment (10).

Women with higher reported pregnancy-specific anxiety, negative life events, and perceived racial discrimination during pregnancy have been shown to be at higher risk for preterm birth. Results from a prospective cohort study of ethnically diverse women demonstrated that those who reported significant depressive symptoms in their first trimester were 60% more likely to deliver preterm, and women reporting severe depressive symptoms were more than twice as likely to deliver a baby preterm (38). These effects of stress on birth outcomes have been shown to occur independent of the level of perceived social support received by the women (31). Not only are poverty and chronic stress predictors of adverse birth outcomes, but acute, unexpected stress can also have effects in an otherwise healthy sample. For example, women who were pregnant and in close proximity to the World Trade Center during the September 11 attacks delivered their infants on average one week earlier. Even more significant was that those infants were twice as likely to be lower than tenth percentile for birth weight given their gestational age (39).

Infants who were growth restricted *in utero* or who were born preterm are at elevated risk for mental and behavioral problems, including psychopathologies such as ADHD and schizophrenia (40). Birth weight and length of gestation must therefore be included in statistical models assessing postnatal associations with prenatal stress and anxiety. However these birth outcomes will not be discussed further in this review, the focus of which is neurodevelopmental outcomes predicted by maternal prenatal stress *independent* of birth characteristics.

V. ASSOCIATIONS BETWEEN MATERNAL PRENATAL STRESS AND INFANT NEURODEVELOPMENTAL OUTCOMES

Recent reviews of human and animal research examining prenatal maternal stress and anxiety have found that, overall, evidence suggests an independent association with offspring's mental (10; 41) and physical health outcomes (41). Following is a review of human studies of pregnant women with prenatal anxiety, elevated stress, or depression whose infants' neurobehavioral or neuroendocrine function was assessed in the first year of life. In all studies reviewed here, the aim was to investigate effects of the prenatal environment on neurologic outcomes independent of any neurodysregulation that may occur as the result of complicated pregnancy, intrauterine growth restriction, or

preterm delivery. These studies therefore included only generally healthy, singleton pregnancies and excluded data from subjects who delivered preterm.

Studies that Assessed Infant Behavior, Emotion or Cognition

Earlier investigations of the effects of the prenatal environment collected maternal report of stress and anxiety and used primarily observation to assess infants. Lou et al. published the first study examining neonate neurologic outcomes of maternal prenatal stress (42). The mothers in the stressed group had experienced stressful events during their pregnancies, for example, marital separation, job loss, or death of a spouse. Neurologic function of neonates was assessed using Prechtl's Assessment of General Movements, a measure of neurologic optimality in newborns that examines sleep-wake state, spontaneous motility, neonatal reflexes, muscle tone, and deep tendon reflexes (43). The findings showed that infants of stressed mothers scored more poorly on the Prechtl. An additional outcome measure examined because of its ability to predict long-term neurologic and cognitive development, neonate head circumference, was shown to be smaller in the stressed group independent of birth weight. More recent studies assessing infant behavioral outcomes have opted to use standardized assessments such as the Neonatal Behavioral Assessment Scale (NBAS) (19) for infants up to two months, or the Bayley Scales of Infant Development for infants 1-42 months (20).

Field et al. studied 166 pregnant women and their neonates to examine associations between maternal anxiety and infant neurodevelopment (14). The mothers were classified as either high anxiety or low anxiety, based on the study sample's median score on the Trait Anxiety Inventory. Infants of high anxiety mothers scored less favorably on the Brazelton NBAS subscales of motor function, autonomic stability, and withdrawal symptoms, which was consistent with the authors' previous data on neonates of depressed and angry mothers (44). Hypothesizing placental transmission of stress hormones as the operating mechanism, the authors examined maternal urine at 20 weeks gestation and the first neonate urine for catecholamines (norepinephrine, NE) and cortisol. They found that high-anxiety mothers had significantly higher NE than low-anxiety mothers, which is consistent with literature on NE levels in anxious people. The neonates' hormone profile matched a profile typically found in depressed people, with elevated NE and cortisol and low dopamine and serotonin. The researchers replicated these biochemical findings in another sample that included pregnant women with depression, rather than anxiety, and their neonates (45).

Rieger et al. (2004) conducted a similar study in a smaller sample of 87 pregnant women and neonates, with results supporting those in the study by Field et al. (2003) – that high-chronic stress mothers had infants who performed less well on the NBAS. However the two studies' positive findings were in different dimensions of the NBAS. While Field et al. found that maternal anxiety significantly predicted poorer infant motor and autonomic function and withdrawal symptoms, Rieger et al. found that it significantly predicted poorer infant orientation, state regulation, and robustness (46). Field et al.

compared the high- and low-anxiety groups based on a median split, and Rieger et al. compared the most stressed quartile with the least stressed quartile. Another important distinction between these two studies is demographics: the sample in the study by Field et al. was more diverse (48% Hispanic, 29% white, 23% African American), while the study by Reiger et al. was conducted in a homogenous, predominately white European sample. To date, there is insufficient existing literature on maternal prenatal stress in different populations to make further conclusions about why the NBAS outcomes differed in these two studies.

In a larger sample (N=170), Huizink et al. collected detailed information on maternal prenatal stress and anxiety and later assessed infants at three and eight months of age using the Bayley Scales (47). The Bayley was specifically used to evaluate infant attention regulation, difficult behavior, and adaptability to novelty, all of which were additionally assessed by maternal report and by independent observation of infant behavioral response to a mildly challenging, novel environment. Study results showed that after adjusting for maternal education and postnatal stress and depression, elevated pregnancy-specific anxiety and perceived stress in early pregnancy together accounted for 5% of the variance in poorer infant attention regulation at three and eight months. Increased perceived stress also predicted difficult behavior at three and eight months, accounting for 8.2% and 2% of variance, respectively. The authors found no association between maternal daily hassles and infant outcomes, although this sample had a low average number of daily hassles, so findings may not be generalizable to pregnant populations with higher reported daily hassles.

These study findings show independent associations between maternal report of prenatal stress and anxiety and infant neurobehavioral outcomes. More recent studies have included biologic markers of maternal stress, as well as of the infant stress response.

Human Studies that Measured Biologic Markers of Stress

The studies outlined above used measures of perceived maternal prenatal psychosocial experience as predictor variables. In contrast, Huizink et al. measured in the same sample as the previously described study serum cortisol levels during each trimester of pregnancy (2003). Higher maternal early morning cortisol levels in late pregnancy were associated with poorer infant mental and motor development at 3 months, and poorer motor development at 8 months of age (30).

Continuing the use of physiologic markers, Davis et al. (2011a) and Tollenaar et al. (2011) investigated cortisol reactivity in infants instead of the neurobehavioral measures used in most of the early studies in this field. They hypothesized that maternal psychosocial stress would prenatally program a dysregulated physiologic response to stress in offspring.

In the study by Tollenaar et al., maternal self-reported pregnancy-specific stress and was mildly associated with infant increased cortisol reactivity to bathing at five weeks, decreased cortisol reactivity to vaccination at eight weeks, and decreased cortisol reactivity to stressful infant-mother separation assessments twelve months of age (48). This study showed that there was both HPA hypo-and hyperreactivity in infants whose mothers were more stressed during pregnancy, and that the direction of change in cortisol secretion depended on infant age and/or the stressor. This study was conducted in a homogenous Dutch, highly educated, healthy sample, so the findings may not be generalizable to a more diverse population.

Davis et al. found that increased maternal plasma cortisol in the second and third trimesters (collected at multiple time points in each trimester) of pregnancy predicted increased neonate cortisol reactivity to a painful heel-stick at 24-36 hours of life (15). This is consistent with previous findings that maternal prenatal cortisol in mid- and late pregnancy predicts infant behavioral outcomes (30), though the behavioral outcomes in the study by Davis et al. consisted of behavioral state changes in response to a challenge, which is difficult to compare with the mental and motor development outcomes in the study by Huizink et al. Behavioral recovery from the heel-stick was predicted not by maternal cortisol, but rather by maternal report of psychosocial stress during pregnancy. Of note, in this study by Davis et al., as well as in another study (49), maternal psychosocial stress, anxiety, and depression were not significantly associated with cortisol measurements collected at the same time points, indicating that maternal reports of psychosocial stress and HPA function may uniquely shape offspring response to stressors. Possible mechanisms for early psychosocial stress effects on infant behavior include the timing of placental development or the timing of the formation of associations between the brainstem, limbic structures, and cortical regions – areas that together regulate behavior. The timing of fetal cortisol receptor (both glucocorticoid and mineralocorticoid) development in areas such as the hypothalamus, hippocampus and amygdala coincides with middle and late pregnancy, possibly explaining why Davis et al. found that measures of maternal cortisol during second third trimesters predicted neonate cortisol reactivity.

The study by Davis et al. (2011) is unique amongst others cited here in that infant assessments were conducted early after birth, which allowed the researchers to infer prenatal programming effects independent of postpartum confounders such as parenting or postpartum maternal mood. Bergman et al. also investigated fetal exposure to cortisol as a possible mechanism for prenatal programming, though they measured it even more directly by collecting amniotic fluid of 125 pregnant women undergoing amniocentesis (49). They found that elevated amniotic fluid cortisol at 17 weeks predicted lower Bayley Cognitive Development scores at 17 months, a time point later than outcomes in other studies reviewed here. However this study was included here because it answers an important question posed by this field of study – how persistent are prenatal programming effects in a given postnatal environment? Are the consequences of maternal stress during gestation moderated by the postnatal

environment, as is suggested by animal models (50)? Bergman et al. assessed postnatal attachment using Ainsworth's Strange Situation, a behavioral observation of parent-child separation and reunion that is considered a relatively stable and validated measure of early life caregiving. Child-parent attachment indeed moderated prenatal cortisol prediction: in infants with insecure attachments, higher amniotic fluid cortisol strongly predicted poorer cognitive development, but in infants with secure attachments, there was almost no correlation between cortisol and Bayley scores.

Studies of Exogenous Glucocorticoids

Since controlled studies of maternal prenatal stress hormones and infant outcomes are limited, many studies have investigated possible effects of exogenous glucocorticoids. Glucocorticoids such as betamethasone and dexamethasone are routinely used as prenatal treatment to accelerate fetal lung maturation when there is a risk for preterm labor. Synthetic glucocorticoids cross the placenta and the fetal blood-brain barrier and interact with fetal glucocorticoid receptors. They therefore have potential for interfering with fetal HPA regulation and neurodevelopment in the same manner as endogenous glucocorticoids.

Studies of exogenous glucocorticoid exposure during gestation certainly inform hypotheses about chronic exposure to stress hormones, though results should be interpreted with caution, for exogenous glucocorticoids do not exactly mimic the actions of endogenous glucocorticoids. One distinction is that placental 11β-HSD2 converts cortisol into inactive cortisone, while synthetic glucocorticoids dexamethasone and betamethasone are unaffected by 11β-HSD2 (51). Therefore a larger proportion of exogenous glucocorticoids circulating in maternal plasma reach the fetus as compared to endogenous glucocorticoids. Additionally, exogenous and endogenous glucocorticoids have diverse affinities for glucocorticoid and mineralocorticoid receptor subtypes, which signal distinct functions in responses to stress and maintenance of homeostasis and may thus exert disparate influences on prenatal programming.

In a study of antenatal betamethasone exposure, there was no difference in baseline cortisol levels between treatment and comparison groups, however term infants prenatally exposed to exogenous glucocorticoids secreted more cortisol in response to a painful heel-stick at 24-36 hours of life than controls who were matched for sex and gestational age (52).

In monkeys, antenatal administration of dexamethasone predicted poorer infant scores of motor function (53). Animal studies investigating the mechanisms for the negative effects of antenatal corticosteroids on infant neurodevelopment have found a reduction in the number of glucocorticoid receptors in the fetal prefrontal cortex, hippocampus, amygdala, and pituitary. The number of receptors present in the brain has profound implications for the activation and regulation of the stress response (51). Fewer receptors in these brain regions could lead to less sensitivity to the negative feedback of

circulating stress hormones, thus increasing the magnitude or duration of infant HPA activation in response to stress.

The studies reviewed above employed somewhat distinctive measures of exposures as well as outcomes assessed. A recent publication of more than two decades of fetal programming research in rhesus monkeys provides a rare opportunity to compare effects from a variety of prenatal exposures, including unhandled controls (N=177), maternal handling (N=35), maternal stress (N=100), ACTH-stimulated (N=13), corticosteroid-treated (N=16), and others, all in healthy, term pregnancies (53). All infant monkeys were assessed two weeks postnatally using a modified version of the Brazelton NBAS. Findings showed that daily ACTH-stimulation of the maternal HPA axis in late pregnancy was the strongest predictor of poorer infant behavior outcomes, specifically in the areas of orientation, motor, and sensory function. This association is consistent with human research showing that increased antenatal HPA activity predicts lower scores on neurodevelopmental assessments (30). The effect size from maternal stress (daily relocation to a dark space with an acoustic startle paradigm) was smaller but still a significant predictor of poorer infant performance on motor and sensory items, results which are not surprising given the abundance of studies showing negative associations between maternal stress and offspring neurobehavioral assessments (10).

VI. MECHANISMS OF TRANSMISSION OF STRESS FROM MOTHER TO BABY

Understanding the mechanism involved in prenatal programming effects is essential for identifying modifiable prenatal factors that can be targeted by prevention efforts. The HPA axis and the autonomic nervous system are key players in the stress response, and both have been implicated in the transmission of stress biology from mother to fetus. Much of the mechanistic research has been conducted using animal models due to methodological barriers to studying maternal prenatal stress in humans in a controlled manner. Animal studies provide the opportunity to standardize the exposure to stress and to isolate stress relative to other physical exposures that commonly coexist with stress in human experience. However in interpreting the results of animal studies, care must be taken to evaluate to what extent findings are relevant to humans.

A variety of prenatal maternal stress experiments have been conducted in non-human primates and in rodents (and a few other species such as goats, sheep, and pigs). The most common exposures used to induce acute or chronic stress include physical restraint and exposure to loud noise (10). Maternal exposure to stressful stimuli during pregnancy provokes a response by the HPA axis, which has been shown in animal experiments to predict offspring HPA function (i.e. elevated basal function, hyperarousal), poorer behavioral, and cognitive function (10).

Endocrine Mechanism: Transplacental Transport of Maternal Stress Hormones

The most commonly-hypothesized mechanism for the transmission of maternal psychosocial experience into infant biology is the passage of maternal stress hormones across the placental barrier (10). Non-human primate and rodent study findings have shown that administration of ACTH and glucocorticoids during pregnancy produces a similar phenotype as infants whose mothers experienced psychosocial stress during pregnancy, with delayed motor development, reduced exploration and adaptive behavior, increased emotional and anxious reactions to unfamiliar environments, and impaired attention and learning (26; 53). These parallels to the impaired development, stress response, and cognitive function in human offspring of stressed mothers suggest that the HPA axis plays a mediating role between prenatal stress and infant outcomes (26; 54).

The transplacental transport of cortisol is unique amongst stress hormones. Maternal and fetal serum cortisol are strongly correlated, while other stress hormones, such as catecholamines and β-endorphin, are not correlated (55). Stress hormone mediators are metabolized by various placental enzymes (e.g. cortisol by 11β-HSD2 and norephinephrine by MAO). The effect of maternal stress on placental enzymatic function is incompletely understood. The relationship between maternal stress and cortisol metabolism is the most studied of the stress response mediators.

Animal studies have found reduced expression of placental 11β-HSD2 in highly stressed mothers. (11) Since 11β-HSD2 acts as a partial barrier protecting fetuses from maternal glucocorticoids, fetuses of stressed mothers are hypothetically exposed to increased concentrations of glucocorticoids. A recent human study found a similar pattern when examining associations between prenatal anxiety and depression and 11β-HSD2 mRNA in their placentas one hour postpartum. Women in the highest tertile of anxiety scores, according to the Spielberger State-Trait Anxiety Scale, had thirty percent less placental 11β-HSD2 mRNA than the least anxious tertile (56). Norepinephrine (57) and pro-inflammatory cytokines (58; 59) were cited as possible mechanisms for the association since both have been shown to depress 11β-HSD2 *in vitro* (2). Although another study that used anxiety diagnosis as a categorical predictor (based on structured clinical interviews) did not find a significant association with placental 11β-HSD2 mRNA (60).

While these human study findings show mixed results, evidence from animal studies points strongly to 11β-HSD2 playing a significant role in the fetal programming of the maternal environment. Adult rats who were administered carbenoxolone, a derivative of licorice that inhibits 11β-HSD2, showed permanent increased basal HPA function and altered patterns of CNS glucocorticoid receptor expression, as well as increased anxiety-like behavior (61). An alternative approach employed to investigate the role of 11β-HSD2 is to cross wild-type with 11β-HSD2-deficient mice to create mice that are heterozygotes for the 11β-HSD2 gene. This allows for a female heterozygote to give

birth to wild-type, heterozygotes, and 11β-HSD2 knock-outs within the same litter. This experiment found that the mice with absent 11β-HSD2 showed increased anxiety-like behavior relative to their wild-type littermates (62). As expected, intermediate levels of anxiety-like behavior were demonstrated by heterozygotes that possessed reduced levels of 11β-HSD2 as compared to wild-types. These results were achieved without exposing the mice to any prenatal stressors. The finding that non-stressed 11β-HSD2 knock-out mice behave similarly to the offspring of prenatally stressed mice adds to the body of evidence suggesting a central role of 11β-HSD2 in prenatal programming effects.

The prefrontal cortex, as a regulator of behavior and emotion, is an important input to the HPA axis through association with limbic structures (63). Therefore the *in utero* development of the fetal prefrontal cortex and related structures under conditions of maternal stress is of great import. Exposure to teratogens during certain points in neurogenesis alters the expression of neurotransmitters, neuropeptides, and their receptors in various regions of the brain. Several studies have found that alterations in this delicate balance modify the pattern of neuronal projection outgrowth, synapse formation, and the pattern of excitatory and inhibitory signals (13). Importantly, teratogens are not limited to substances, pollutants, and infectious agents, but also include social and behavioral factors such as excessive exercise or maternal mental illness (64). Research targeting the teratogenic effects of stress have investigated the role of glucocorticoids on the developing brain and have found that they impact neurogenesis particularly in the prefrontal cortex (13). In summary, psychosocial stress influences the timing and structural development of the fetal prefrontal cortex, thus programming future limbic-HPA axis integration.

Autonomic Mechanism: Reduced Placental Perfusion

A review of prenatal stress and anxiety and neurodevelopment literature offered an alternative mechanism: impaired uterine blood flow (13). As described earlier, an important component of the maternal stress response is reduced perfusion of non-vital organs, including the placenta. Catecholamines directly cause peripheral vasoconstriction, an effect that is indirectly intensified by glucocorticoids.

Resistance index (RI) quantifies vascular resistance to uteroplacental blood flow using color Doppler studies. An increased resistance index is associated with placental ischemia, meaning less blood and therefore less oxygen and nutrients are delivered to the fetus (65). Several studies have found that an increased uterine artery RI predicts negative obstetric outcomes such as preeclampsia and intrauterine growth restriction (66). Maternal prenatal anxiety has been shown to be associated with increased RI in the uterine artery, demonstrating that fetuses whose mothers are more anxious may receive less oxygen and nutrition (29).

Hypotheses of HPA axis and autonomic function are not mutually exclusive. As an example, catecholamines, which reduce placental perfusion, also have been shown *in vitro* to down-regulate placental 11β-HSD2 mRNA (11). Furthermore, it is unlikely that a single mechanism explains all of the behavioral and neurodevelopmental outcomes associated with prenatal stress (40).

Epigenetic Processes as Mediators of Endocrine and Autonomic Mechanisms

The processes described above outline mechanisms for relatively stable, long-term alterations to offspring stress neurobiology. In several animal studies, such alterations have been shown to be long-term not only for the individual exposed but heritable for up to three generations (11). These changes are not the result of mutations of the actual DNA sequence, but rather epigenetic modifications influencing gene expression. Epigenetics describes patterns of DNA methylation and histone modification that turn gene expression on or off. The epigenome is sensitive to environmental cues, allowing species early on (e.g. during gestation) to adapt to current conditions, theoretically improving chances for survival and reproduction. However in some cases, adaptation to the prenatal environment places offspring at increased risk for poor physical and psychological health outcomes. The most well-studied example of this is fetal metabolic and cardiovascular changes in the face of maternal undernutrition, predisposing to diabetes and cardiovascular disease (67).

Research in rats has shown that maternal stress during pregnancy induces both increased and decreased expression of hundreds of genes encoding hippocampal axon growth, ion and transport channel regulation, synaptic vesicle transport and release of neurotransmitters (68), indicating a non-genomic mechanism for prenatal stress effects on offspring neurologic function. Cross-fostering studies in mice have provided further evidence that the gestational period significantly influences later infant gene expression and behavioral phenotype. Mice with a one genotype (called B6) who were prenatally cross-fostered and reared by dams with a different genotype (called BALB) exhibited the behavioral phenotype of BALB mice, exhibiting less exploratory and more anxiety-related behavior. Genetically B6 mice gestated by B6 dams and reared by BALB dams did not exhibit the BALB phenotype (69). The prenatal environment was therefore a key determinant of offspring postnatal behavior, and differences in behavioral phenotype could not be attributed to DNA sequence.

In conclusion, *in utero* exposure to stress response mediators, including excess glucocorticoids, catecholamines, or hypoxia, programs CNS and peripheral tissue gene expression, effects which are hypothesized to be mediated by epigenome modification. The fact that epigenetic modifications are heritable means that adverse effects of maternal prenatal stress persist in children, grandchildren, and possibly beyond. This fact also poses a great opportunity for public health prevention efforts. Preventing adverse effects of maternal prenatal stress becomes increasingly important and

valuable given the large number of individuals impacted intergenerationally by an individual pregnant woman's experience of pregnancy.

VII. SUMMARY

Overall, evidence from the human and animal studies reviewed here suggests that maternal prenatal stress and stress hormones exert a programming effect on offspring, resulting in altered patterns of infant HPA function (i.e. hyperarousal and increased reactivity) and poorer neurologic (cognitive, behavioral and motor) development. The long-term significance of such changes noted during infancy has yet to be elucidated by large, controlled, longitudinal studies. However, there is evidence of independent associations between maternal mental health and maladaptive offspring stress response and psychopathology later in childhood and adulthood (10).

While prenatal stress research continues to fill gaps in knowledge about when and by what molecular mechanisms prenatal programming takes place, many researchers are shifting focus to prevention efforts – attempting to answer the question "to what extent are the effects of prenatal maternal stress modifiable?" Interventions during pregnancy deem maternal stress a modifiable risk factor for offspring psychopathology (41). An additional opportunity for intervention can be gleaned from the results of animal studies. If animal findings that an enriched postnatal environment modifies effects of prenatal stress (50) are indeed applicable to humans, future research must investigate the efficacy and feasibility of both prenatal and postnatal interventions for maximal benefit to health outcomes across the lifespan.

Additionally, as was discussed earlier, pregnant women of low SES are especially vulnerable to poor infant neurodevelopmental outcomes that may result from a stressful prenatal environment. Therefore studies of prenatal programming effects in low-income women constitute an important future direction. The prevention of adverse effects and intergenerational transmission of poverty and health disparities serve as important motivation for the continuation of this work.

VIII. PROPOSED RESEARCH

The literature reviewed in this paper demonstrates a consistent association between maternal psychosocial experience and infant neurodevelopment – specifically HPA function and cognition. The proposed research will build upon this body of literature by investigating in sixty ethnically diverse, low-to-middle income mother-infant dyads the relation between maternal psychosocial stress and newborn behavior and neurologic function.

The unique contribution of the proposed study is twofold. First, previously published studies of prenatal stress and infant development have largely been conducted in white, middle SES samples, with the exception of two conducted in a sample with nearly half

of participants identifying as Hispanic (14; 52). The proposed study targets an ethnically and economically diverse sample to investigate whether the findings in white, middle-income samples are generalizable to a more diverse population. Secondly, the proposed study will investigate birth characteristics, such as mode of delivery and maternal childbirth experience, as potentially meaningful covariates. This research will additionally serve as a foundation for a subsequent series of analyses on the full study sample (N=180) and with biologic markers such as salivary cortisol. Measuring and analyzing a variety of maternal and infant variables may help clarify the mechanisms responsible for prenatal programming effects – and assist in creating targeted interventions that improve health trajectories.

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PART II: ORIGINAL RESEARCH

Maternal Prenatal Stress Predicts State Regulation in Neonates

I. ABSTRACT

Although a growing body of evidence demonstrates that maternal prenatal psychosocial stress programs fetal neurodevelopment (1; 2), there is little study of this effect in ethnically and socioeconomically diverse samples. The study presented here investigates in a predominately minority, low-to-middle-income sample (n=50) whether maternal perceived stress during pregnancy predicts offspring neurobehavioral competence. We expected that infants of mothers who experienced higher levels of stress would demonstrate less optimal neurobehavioral competence than infants of mothers who perceived lower levels of prenatal stress. To test this hypothesis, maternal perceived stress was measured during the second trimester of pregnancy. Approximately four weeks after birth, neonate neurodevelopment was measured by an independent observer using a standardized neurobehavioral assessment tool. Results showed that higher maternal prenatal stress predicted less optimal state organization in infants. There were no significant differences detected in motor function or responsivity between the infants of high- and low-stress mothers. The relation between maternal stress and infant state organization could not be explained by race, socioeconomic status, maternal or infant age, parity, birth weight, infant sex, or mode of delivery, suggesting an independent association. These data provide further evidence for prenatal programming of offspring neurodevelopment. Early childhood self-regulation, in particular, is an important predictor of psychological and behavioral development later in childhood and is therefore a meaningful indicator of a child's long-term health trajectory (3; 4).

II. INTRODUCTION

Many epidemiologic and clinical studies have identified links between the fetal environment and health and disease later in childhood and adulthood (1; 2; 5; 6). An evolutionary biology perspective proposes that the influence of the environment on phenotype is beneficial for future fitness and survival (3; 4; 7). Natural selection acts slowly in a species with a relatively long lifespan, so the ability to incorporate environmental information in order to function most optimally under local conditions is highly adaptive. Building on this premise, long-term health outcomes would seem to depend on how well the environment during gestation or childhood predicts conditions later in life (7; 8).

One theory regarding the source of individual differences in health outcomes is that a person's phenotype is not simply a product of inherited genes, but rather is conditioned by the environment during sensitive periods of development (9). The hypothesis of *Fetal Programming* states that the prenatal period is a particularly sensitive time in

development during which environmental exposures can have long-term and, through epigenetic mechanisms, heritable effects on physical and mental health trajectories (10; 11). Central to this theory is the idea that adult health is not a genetically preconfigured plan that gradually unfolds over the life course, and that genes and environment are not independent or competing influences on long-term health and disease outcomes. Sources of potentially harmful prenatal exposures include environmental toxins, infectious diseases, diet, and psychosocial stress that can all manifest as physiologic dysregulation in the child (12; 13).

Barker and colleagues provided some of the first evidence of the early environment impacting long-term health trajectories in a maladaptive way. Their studies of cardiovascular and metabolic diseases have shown that fetal undernutrition and altered fetal growth patterns predict higher risk for coronary heart disease and type 2 diabetes in adulthood (14). Building upon these findings, recent investigation has explored other aspects of the prenatal environment – particularly maternal prenatal psychosocial stress. A considerable body of evidence suggests that a fetus whose mother experienced high levels of prenatal stress may be programmed to become more reactive to stressors, to recover less quickly from a stress response, and to be less sensitive to negative feedback from stress response mediators (1; 2). With more than half of all women reporting symptoms of anxiety and depressed mood during pregnancy (15), the potential negative effects on offspring are widespread and deserve the attention of researchers, public health professionals, and communities.

Overall, recent reviews of human and animal research examining elevated prenatal maternal stress and anxiety suggest an association with offspring's poorer mental (1; 16) and physical health outcomes (16) that is independent of medical complications of pregnancy and birth outcomes. Maternal self-report of stress during pregnancy predicts in neonates poorer regulation of state (17; 18), less optimal motor function (17; 19), and prolonged behavioral recovery following a stressful challenge (20). Maternal prenatal stress also predicts neurodevelopmental outcomes in older infants. Maternal report of higher levels of prenatal stress and anxiety (21; 22), as well as increased maternal serum cortisol during pregnancy (22; 23), predict decreased performance on assessments of mental and motor development in infants aged three and eight months. In summary, psychosocial stress experienced by mothers during pregnancy is associated with poorer offspring motor development and self-regulation across the first year of life.

Understanding the mechanisms involved in prenatal programming effects is essential for identifying modifiable prenatal factors that can be targeted by prevention efforts. The HPA axis and the autonomic nervous system are key players in the stress response, and both have been implicated in the transmission of stress biology from mother to fetus (1). As an example, non-human primate and rodent studies have shown that administration of ACTH and glucocorticoids during pregnancy produces a phenotype in infants that is similar to the phenotype of infants whose mothers experienced

psychosocial stress during pregnancy, with delayed motor development, reduced exploration and adaptive behavior, increased emotional and anxious reactions to unfamiliar environments, and impaired attention and learning (24; 25). These parallels to the impaired development, stress response, and cognitive function in human offspring of stressed mothers suggest that the HPA axis plays a mediating role between prenatal stress and infant neurodevelopmental outcomes (24; 26).

Individuals of lower socio-economic status (SES) bear an increased burden of psychosocial stress (27; 28). There is certainly a relation between SES and birth outcomes such as small for gestational age (SGA) or preterm birth (29), though health disparities in offspring may be independent of birth outcomes. There is strong evidence that people of lower SES have higher basal catecholamine and cortisol levels, indicating that they have chronically elevated sympathetic nervous system and HPA axis activity even after controlling for numerous social and biological covariates (30). The HPA axis and the sympathetic nervous system are two systems hypothesized as mediators for maternal prenatal stress programming effects on offspring. If the association between low SES and elevated stress hormones and sympathetic nervous system activity applies to pregnant women as well, then the offspring of lower SES women are at higher risk of prenatal stress programming effects than those of middle or high SES. Thus, not only do women of lower SES experience increased stress (27; 28), they may also have an increased propensity to transmit biologic effects of stress to their offspring. It is therefore imperative that economically diverse samples be included in future research of prenatal stress.

Previously published studies of maternal prenatal stress and infant development have demonstrated prenatal programming effects, though they have largely been conducted in white, middle or high SES samples (18; 20; 21), thus limiting the generalizability of their findings. Given the disproportionate levels of psychosocial stress experienced by women of low SES, their exclusion from prenatal stress research constitutes a critical gap in the literature. The prospective study presented here begins to fill that gap by investigating the relation between maternal prenatal perceived stress and infant neurodevelopment in fifty ethnically and socioeconomically diverse mother-infant dyads. Further, this study utilizes observation of infants early in the neonatal period (at approximately four weeks), which presents a unique opportunity to assess neurobehavioral outcomes with minimal influence of maternal bias and other aspects of the postnatal environment. We hypothesized that the offspring of women who reported higher levels of stress during pregnancy would demonstrate less optimal performance on a behavioral measure of neurodevelopment than the infants of women who reported lower levels of prenatal stress.

III. METHODS

Participants

The sample included fifty mother-infant dyads who were recruited from prenatal clinics and community centers throughout the San Francisco Bay Area. All mothers were enrolled to participate in a larger parent study – a controlled trial of an intervention to reduce prenatal stress and excess weight gain. At the time of recruitment, women were required to be 18-45 years of age and 8-23 weeks pregnant with a singleton gestation. Additional inclusion criteria included a pre-pregnancy BMI of 25-40 and income less than 500% of federal poverty level. Due to the metabolic outcomes of the parent project, women with substance abuse, mental health, and medical conditions (e.g. polycystic ovarian syndrome or diabetes) that may interfere with participation in group interventions or with baseline body composition were excluded from the study. Shortly after their estimated delivery dates, women were contacted regarding participation in the follow-up study of infants. All women who enrolled in the parent project and their infants were invited to participate. Data for this sub-study was collected at two visits: the post-intervention visit at approximately 28 weeks of pregnancy and the post-natal visit at approximately four weeks following the birth.

Assessments

Demographics. Maternal demographics were collected at the prenatal visit. Mothers reported age, parity, race/ethnicity, annual household income, and education. At the postnatal visit, mothers were asked about infant race/ethnicity and birth characteristics (i.e. length of gestation, weight, height, mode of delivery).

Maternal Prenatal Stress. Prenatal stress data was collected at approximately 28 weeks of pregnancy. Maternal perception of stress was measured using Cohen's Perceived Stress Scale (PSS) (31), a widely used and well-validated assessment of generalized stress and coping over the previous month. The version utilized in this study consisted of ten items (e.g. "In the last month, how often have you felt that you were unable to control the important things in your life?") on a 5-point Likert scale ranging from 0 to 4 (never, rarely, sometimes, often, or very often). Questionnaires were administered verbally and in-person. To analyze the PSS data, ratings of positively-worded items were reverse coded, and a mean score was created.

Infant Neurobehavioral Development. Infants were assessed as soon following birth as was feasible, with a target of four weeks of age. Participants were given the option of completing the infant assessments at the UCSF pediatric research ward or in their homes. The measure used to assess neurodevelopment was the Newborn Behavioral Observation (NBO) (32). The NBO is a clinical observation tool that is based on the more extensive Neonate Behavioral Assessment Scale (NBAS; a.k.a. "The Brazelton") (33). The published NBO consists of twenty observations rated on a three-point scale. However this study piloted a new version of the NBO that uses the same twenty observations rated on a five-point scale (unpublished work by J. Kevin Nugent and Beth McManus). The twenty NBO items are subcategorized into four domains: Autonomic, Motor, Organization of State, and Responsivity. The Autonomic subscale includes three

items: tremors, startles, and skin color changes. Motor includes rooting, sucking, hand grasp, and crawling reflexes, as well as muscle tone of the neck, shoulders, and extremities, and a rating of optimality of the neonates overall activity during the session. The third subscale, Organization of State, consists of five items: habituation to light and sound, crying, soothability, and overall state regulation throughout the session. Finally, the five-item Responsivity subscale includes: the ability to track a face, a face plus a voice, and an inanimate object (i.e. red ball), as well as the ability to locate a voice and a rattle. The NBO assessments were conducted by one of two examiners who received extensive training and coaching from the original NBO author, J. Kevin Nugent, PhD, and ongoing supervision by child psychologist and study PI, Nicole Bush, PhD.

Statistical Analysis

Analyses were performed using Stata/SE version 10.1. Visualization of PSS data revealed a bimodal distribution. Women were therefore classified as "low stress" (n=26, mean=1.03, SD=0.27, range=0.3-1.4) or "high stress" (n=24, mean=1.96, SD=0.41, range=1.5-3.6) based on a median split.

A mean score was created for each of the four NBO subscales as long as data was present for at least two items within the subscale. This resulted in an n of 49 for the Autonomic subscale (3 items), an n of 50 for the Motor (7 items) and Organization of State subscales (5 items), and an n of 47 for the Responsivity subscale (5 items). Missing data for the Responsivity subscale was expected since infants must be in an "available state" (i.e. not fussing or crying) in order to assess their ability to track or locate animate and inanimate visual and auditory stimuli (32). Additionally, there was considerable missing data was on two items within the Organization of State subscale: Habituation to Light and Habituation to Sound. Assessment of these two items requires that the infant be asleep at some point during the visit. These data were therefore missing for the majority of infants (n=33), and were thus excluded from all subjects' mean Organization of State scores, resulting in a 3-item scale.

Bivariate correlations were assessed to identify sociodemographic (race/ethnicity, income, education) or biologic (infant and maternal age, length of gestation, mode of delivery, birth weight, sex) variables that might influence maternal stress or infant neurobehavioral outcomes. Due to the small sample size, variables with trend level association ($p<0.10$) were included in the multiple regression model as covariates (see table 3).

IV. RESULTS

Sample Description

Demographic characteristics of the full sample, low- and high-stress mothers, and infants are shown in Table 1. The mean age of the full sample of mothers was 27.8

years (SD=5.64, range=19–41). The sample was ethnically diverse, with greater than 80% of women identifying as minority or multiethnic. Median household income was $15,000, with a range from $0 to $100,000. Education level varied widely from less than high school (14%) to graduation from college (19%). The mean infant gestational age at birth was 39.5 weeks (SD=1.8, range=30.9-42.7 weeks). At the time of assessment, the corrected gestational age ranged from 39.7 to 47.7 weeks, with a mean of 44.2 weeks (SD=1.9). This corresponded to 2.7 to 9.1 postnatal weeks (mean=4.6, SD=1.6). The sample of infants consisted of 25 boys and 25 girls. Of the women invited to participate, 96% enrolled in and completed the study. There were no significant demographic differences between the low- and high-stress groups (see Table 1).

Table 1. Maternal and Infant Demographic Characteristics; Comparisons among High- and Low-stress Subsamples

MATERNAL DEMOGRAPHICS	**Full Sample** (n=50)		**Low Stress** (n=26)	**High Stress** (n=24)	**Test Statistic**	***p***
	Mean (SD) or #(%)	*Range*	*Mean or #(%)*	*Mean or #(%)*		
Maternal age (yrs)	27.80 (5.64)	19-41	27.44	28.20	t=-0.48	0.63
Parity					χ2=1.05	0.31
Primiparous	25 (50%)		14 (56%)	11 (44%)		
Multiparous	25 (50%)		12 (48%)	13 (52%)		
Marital Status[1]					χ2=0.32	0.57
Married or partnered	25 (66%)		14 (56%)	11 (44%)		
Single	13 (34%)		5 (38%)	8 (62%)		
Maternal education					χ2=1.35	0.72
Less than high school	9 (18%)		5 (56%)	4 (44%)		
High school grad	13 (26%)		5 (38%)	8 (62%)		
Some college/vocational	18 (36%)		10 (56%)	8 (44%)		
College degree or higher	10 (20%)		6 (60%)	4 (40%)		
Household income ($1,000/yr)	$23.8 ($23.3)	$0-100.0	$23.6	$24.0	t=-0.06	0.95
5th percentile	$0					
25th percentile	$6,192					
50th percentile	$15,000					
75th percentile	$33,600					
95th percentile	$70,000					
INFANT DEMOGRAPHICS						
Birth weight (kg)	3.25 (0.44)	1.90–4.40	3.29	3.21	t=0.60	0.55
Gest. age at birth (wks)	39.3 (1.9)	30.9–42.4	39.6	39.0	t=1.17	0.25
Gest. age at assessment (wks)	44.1 (2.0)	39.7–47.7	44.3	43.9	t=0.55	0.60
Postnatal age (wks)	4.6 (1.6)	2.7–9.1	4.4	4.9	t=-1.14	0.26
Mode of Delivery					χ2=0.06	0.81
Vaginal	41 (82%)		21 (51%)	20 (49%)		
Cesarean	19 (18%)		5 (56%)	4 (44%)		
Sex					χ2=0.00	0.99
Male	25 (50%)		13 (52%)	12 (48%)		
Female	25 (50%)		13 (52%)	12 (48%)		
Infant race/ethnicity[2]						
African American	29 (58%)		14 (48%)	15 (52%)	χ2=0.38	0.54
Asian	6 (12%)		3 (50%)	3 (50%)	χ2=0.01	0.92
Latino	20 (40%)		10 (50%)	10 (50%)	χ2=0.05	0.82
Native American	2 (4%)		2 (100%)	0	--	
White	10 (20%)		6 (60%)	4 (40%)	χ2=0.32	0.57
Multi-ethnic	8 (16%)		4 (50%)	4 (50%)	χ2=0.02	0.90

1=Marital status data was only available for 38 of 50 participants; 2=Participants were given the option to report more than one race/ethnicity

Maternal PSS scores indicated low-to-moderate stress in this sample, with mean scores of 1.5 out of a maximum of 4 (SD 0.6, range 0.3-3.6). Infant scores on the NBO were moderate-to-high for the Motor, Organization of State, and Responsivity subscales. On the Autonomic subscale, there was very little variability and a high mean of 4.7 out of a possible 5 (SD=0.4, range=3.3-5) and was thus excluded from further analyses. Cronbach's alpha for Motor, Organization of State, and Responsivity subscales was 0.39, 0.89, and 0.77, respectively. Descriptive statistics of the PSS and NBO are shown in Table 2.

Table 2. Intercorrelations and Bivariate Correlations between Maternal Perceived Stress Scale and Newborn Behavioral Observation

				Motor		**Org. of State**		**Responsivity**	
	Mean	*SD*	*Range*	*r*	*p*	*r*	*p*	*r*	*p*
Motor (n=50)	3.298	0.457	2.1 – 4.3[1]	*(0.39)*		--		--	
Org. of State (n=50)	3.560	1.122	1.0 – 5.0[1]	0.047	0.359	*(0.89)*		--	
Responsivity (n=47)	3.355	0.930	1.5 – 5.0[1]	**0.124**	**0.072 †**	**0.466**	**0.001** ***	*(0.77)*	
PSS (n=50)	1.480	0.576	0.3 – 3.6[2]	0.003	0.982	**-0.730**	**0.020***	0.089	0.747

*† p< 0.10, * p< .05, ** p<.01, ***p<.001; Reliability coefficients (Cronbach's α) are shown in parentheses.*
1=Possible range 1-5; 2=Possible range 0-4

Covariates

Correlations between maternal PSS, infant NBO scores, and various biologic and sociodemographic characteristics were examined (see Table 3). Due to the small sample size, only variables significantly associated with the predictor or outcome variables were included in the final regression model. Hispanic/Latino race was significantly associated with lower infant motor function (B=-0.292, p=0.040). There was a trend toward a significant association between African American race and better infant responsivity (B=0.613, p=0.053). The final model included minority race as a dichotomous variable since the study was insufficiently powered to include in the model all race variables separately, and because minority status is associated with increased stress (34) and birth outcomes (35). Infant biologic variables such as gestational or postnatal age, sex, birth weight, and mode of delivery were unrelated to NBO scores in our sample (all ps>0.3) and were therefore excluded from multiple regression models.

Regarding maternal characteristics, there was a trend toward a significant association between maternal age and infant organization of state (B=0.048, p=0.085). However, maternal age was strongly related to education (B=0.452, p=0.000) and to household income (B=0.379, p=0.005) and is possibly indicative of socioeconomic status rather than a biologic process related to maternal aging. In our study sample, the predictor variable, prenatal maternal perceived stress, was not significantly associated with any

biologic or sociodemographic characteristics of mothers or infants (all *p*s>0.2). Although we did not find a link between prenatal stress and sociodemographics, the variability in income and education in our sample was low, giving us little power to detect such associations. In summary, the final regression model adjusted only for covariates significantly associated with variables of interest: ethnic minority status and maternal age.

Table 3. Bivariate Correlations between Possible Covariates, Newborn Behavioral Observation, and Perceived Stress Scale

	Motor		**Org. of State**		**Responsivity**		**PSS**	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Maternal Age	0.00	0.90	**0.05**	**0.09 †**	0.02	0.37	0.01	0.64
Primiparity	0.13	0.37	-0.13	0.36	-0.09	0.53	-0.08	0.58
Education	-0.01	0.75	0.10	0.38	-0.01	0.88	-0.03	0.52
Household Income	0.00	0.77	0.00	0.31	0.00	0.67	0.00	0.95
Race/Ethnicity								
African American[1]	-0.07	0.62	-0.24	0.56	**0.61**	**0.05 †**	0.18	0.34
Latino[1]	**-0.29**	**0.04 ***	0.08	0.83	0.12	0.69	0.15	0.44
Asian[1]	0.12	0.51	0.27	0.60	-0.08	0.88	0.11	0.65
Minority[1]	0.09	0.52	-0.27	0.06	0.11	0.48	0.08	0.58
Birthweight	0.05	0.68	0.23	0.50	-0.20	0.45	-0.10	0.55
Gest. Age at Birth	0.00	0.52	-0.01	0.32	0.01	0.46	-0.01	0.25
Gest. Age at Assessment	0.00	0.67	0.01	0.50	0.00	0.99	0.00	0.55
Infant Postnatal Age	0.00	0.99	0.00	0.89	0.01	0.41	0.01	0.26
Vaginal Mode of Delivery	-0.11	0.45	0.27	0.47	-0.36	0.28	0.04	0.82
Infant Sex[2]	-0.04	0.77	0.18	0.56	0.19	0.46	0.00	0.99

*† p< 0.10, * p< 0.05, 1=in comparison to white race/ethnicity, 2=Females scored as 1*

Maternal Perceived Stress and Infant Neurobehavioral Outcomes

Before adjusting for covariates, regression analyses revealed that maternal perceived stress predicted lower infant Organization of State scores (β=-0.33, p=0.02), as shown in Table 2. Figures 1 and 2 show this unadjusted association, with PSS presented as a continuous and dichotomous variable, respectively. After adjusting for minority status

and maternal age, the relation between maternal stress and infant Organization of State remained significant (β=-0.33, p=0.02). Maternal perceived stress was not significantly associated with Motor and Responsivity scores (ps>0.7). Multiple regression results are shown in Table 4.

Figure 1. Scatterplot of Perceived Stress Scale (as a continuous variable) and Newborn Behavioral Observation (Organization of State subscale)

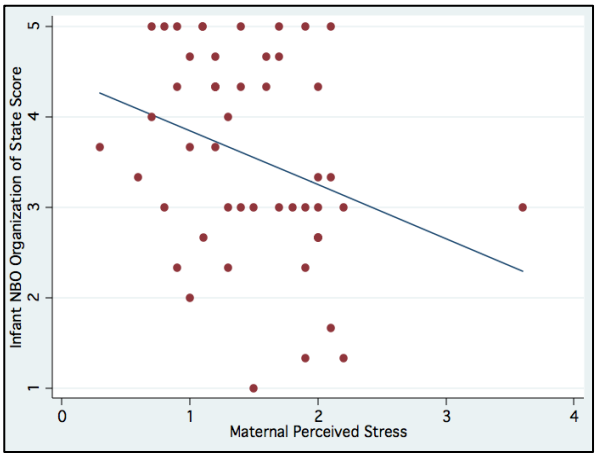

Figure 2. Boxplot of Maternal Perceived Stress Scale (as a dichotomous variable) and Newborn Behavioral Observation (Organization of State subscale)

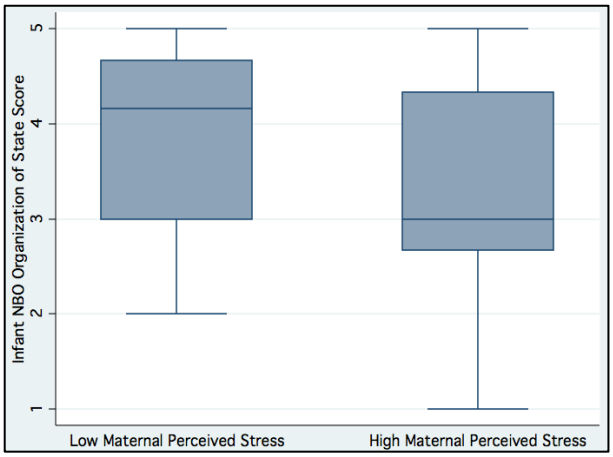

Table 4. Multiple regression analysis of maternal Perceived Stress Scale and Newborn Behavioral Observation, adjusted for minority status and maternal age

	Motor				**Organization of State**				**Responsivity**			
	B	β	Std. Error	p	B	β	Std. Error	p	B	β	Std. Error	p
Minority Status	0.12	0.10	0.18	0.52	-0.47	-0.17	0.40	0.24	0.38	0.17	0.36	0.30
Maternal Age	0.00	0.03	0.01	0.87	0.04	0.20	0.03	0.16	0.03	0.18	0.03	0.27
Maternal PSS	-0.01	-0.01	0.13	0.96	**-0.73**	**-0.33**	**0.30**	**0.02 ***	0.03	0.01	0.28	0.92
	Total R^2=0.01, p=0.93				**Total R^2=0.20, p=0.01***				Total R^2=0.014, p=0.608			

*$p< .05$

V. DISCUSSION

In line with our hypothesis, study findings showed that neonates whose mothers reported high prenatal stress during their second trimester demonstrated poorer state organization than neonates whose mothers perceived low levels of prenatal stress. More specifically, an independent rater observed that offspring born to mothers reporting lower stress during pregnancy cried less frequently, were more easily soothed,

and generally showed better regulation of state when presented with a variety of visual and auditory stimuli. Sustained attention to such stimulation presents a considerable challenge to a four-week-old neonate. Neonates with more optimal self-regulation have been shown to exhibit less behavioral problems (36; 37), better mental (38) and psychomotor (36; 38) development, increased school readiness (38), intelligence (38; 39), and communication skills (40) in early childhood. This evidence suggests that the neonatal period may be a clinically useful time point for the assessment of neurodevelopmental characteristics that have relevance to later childhood functioning. Further, the study finding that high prenatal stress predicts poorer neonatal state regulation suggests that stress reduction interventions early in pregnancy could improve infant outcomes.

The proportion of variance in infant Organization of State scores explained by maternal perception of prenatal stress was sizeable (R^2=0.20), particularly given the innumerable factors influencing infant behavior and self-regulation. The clinical utility of this finding is less clear, though limited evidence suggests that neonatal state regulation seems to predict early childhood development and behavior (3; 36-38; 40; 41). It is also interesting to note that the variance could not be explained by biologic and sociodemographic characteristics, lending confidence to our finding that maternal prenatal stress predicts infant self-regulation. Additionally, despite the racial and socioeconomic diversity of this sample, most women reported low-to-moderate levels of perceived stress, as shown in Figure 1. One possible explanation for lower than anticipated maternal reports of stress is that perceived stress was measured relatively late in pregnancy (at 28 weeks), and psychosocial stress has been shown to decrease over the course of pregnancy (42). Assuming that the association between prenatal stress and offspring state organization is linear, it is likely that women who experience very high stress during pregnancy might have infants with even poorer observed emotion regulation than the high-maternal stress group of infants in our sample – a possible future direction for this research.

Our hypotheses that maternal stress would predict offspring motor functioning and responsivity were not supported. This finding may indicate that infant motor function is not as sensitive to maternal stress as infant state organization. Alternatively, neurodevelopmental differences within these two domains may be better detected later in infancy when new skills related to motor function and attention emerge. Another possible explanation is that the lack of association may be due to insufficient power, given the small sample size, or to low reliability of our measure. We will reexamine these associations once the full sample of 180 completes the study.

Study Limitations

As mentioned above, this study's small sample may have limited its power to detect neurobehavioral differences in the infants of low- and high-stress mothers. Examinations of the associations presented here will therefore be repeated using data

from the full sample. Additionally, the subjectivity of observational data constitutes a potential source of bias in the data. To minimize the impact on inter-rater reliability, the two NBO administrators were trained extensively by the original NBO author J. Kevin Nugent and supervised by child psychologist and study PI, Nicole Bush, PhD. In instances in which behavioral coding was unclear, both NBO administrators, as well as Dr. Bush, reviewed the infant's NBO video and discussed coding. Also of note, the study was conducted in a sample of overweight and obese women, which may limit the generalizability of findings to women who are under- or normal weight.

As noted in table 2, the motor subscale of the NBO showed low internal consistency (Cronbach's alpha=0.39), which is perhaps concerning for analysis. Though no prior data on internal consistency for the NBO is available, studies using the more extensive NBAS, on which the NBO is based, have often reported low levels of internal consistency on the motor subscale (43). For this reason, we did not make any alternations to the motor subscale in our analyses. Motor development results were interpreted in light of this limitation.

Future Directions

A question unanswered by the present study relates to the influence of the timing of stress during the prenatal period. Previous studies of the timing of prenatal psychosocial stress and stress hormone exposure have found that that exposure early in gestation has the greatest negative impact on offspring developmental outcomes (44). Though our findings suggest a link, the ability of late pregnancy stress to predict infant neurodevelopmental outcomes is less clear (23; 44). Additionally, different types of stress (e.g. generalized versus pregnancy-specific) have distinct influences on infant outcomes (44). As noted, these prior studies were conducted in predominately white, educated samples. Women of low SES are disproportionately exposed to psychosocial stress (34), and since they may be experiencing unique stressors during pregnancy in comparison to women of higher SES (45). While the current study assessed an ethnically and socioeconomically diverse sample of women, an important future direction is to investigate a variety of sources of prenatal stress at multiple time points in pregnancy in such a population. Such a study design would offer important insights that could be used to maximize the benefit of prenatal interventions by targeting the most sensitive periods of pregnancy and the narrowest type of stressors.

An additional future direction of this research relates to the prediction of emotion regulation problems later in childhood. Previous studies have investigated childhood developmental outcomes predicted by neonate neurobehavioral assessments in substance exposed (38), preterm, and low birth weight (37; 40; 41) samples. To our knowledge, only one study has examined the predictive value of neonatal neurobehavioral assessment in healthy, term infants (36). While these data are limited, they consistently show that the neonatal period constitutes an important early opportunity for the screening and identification of children who may have poor

developmental trajectories. Neurobehavioral assessment of the neonate is therefore justified, though further study of the specific long-term developmental outcomes predicted by neonate neurodevelopment is needed. Such investigation is crucial for the most effective and efficient allocation of early intervention resources (4).

VI. CONCLUSION

The finding that maternal prenatal stress predicts poorer infant self-regulation corroborates the work of others (18; 21; 23), yet in a sample with broader ethnic and socioeconomic variation than samples previously studied and using systematic observation of offspring neurodevelopment. Given the predictive value of early life behavioral regulation for later life mental and physical health, interventions to reduce maternal prenatal stress may provide an opportunity to improve health trajectories for their offspring during childhood and possibly beyond. This may be especially true for low-SES women who are disproportionately burdened by psychosocial stressors. Our findings add to the growing understanding that policies designed to promote health across the life course should take into account the influence of the prenatal environment.

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