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The Contributions of Maternal Sensitivity and Maternal Depressive Symptoms to Epigenetic Processes and Neuroendocrine Functioning

Elisabeth Conradt
The University of Utah

Katheleen Hawes
The Brown Center for the Study of Children at Risk

Dylan Guerin, David A. Armstrong, and Carmen J. Marsit
Geisel School of Medicine at Dartmouth

Edward Tronick
University of Massachusetts

Barry M. Lester
The Brown Center for the Study of Children at Risk and Warren Alpert Medical School of Brown University

This study tested whether maternal responsiveness may buffer the child to the effects of maternal depressive symptoms on DNA methylation of *NR3C1*, *11β-HSD2*, and neuroendocrine functioning. DNA was derived from buccal epithelial cells and prestress cortisol was obtained from the saliva of 128 infants. Mothers with depressive symptoms who were more responsive and who engaged in more appropriate touch during face-to-face play had infants with less DNA methylation of *NR3C1* and *11β-HSD2* compared to mothers with depressive symptoms who were also insensitive. The combination of exposure to maternal depressive symptoms and maternal sensitivity was related to the highest prestress cortisol levels, whereas exposure to maternal depressive symptoms and maternal insensitivity was related to the lowest prestress cortisol levels.

The negative consequences of child exposure to maternal depressive symptoms have been well documented and range from greater internalizing and externalizing behaviors (Brennan et al., 2000; Essex, Klein, Cho, & Kraemer, 2003; Toth, Rogosch, Sturge-Apple, & Cicchetti, 2009) to dysregulated physiological responses to stress (Laurent, Ablow, & Measelle, 2011). Investigating the biological mechanisms involved in this transmission of risk for depression from mother to child has led to a focus on how the neuroendocrine response to stress in mothers with depression may program the infant hypothalamic–pituitary–adrenal (HPA) axis. At present, however, the processes involved in this "programming" are not fully understood.

In brief, the concept of programming is based on epidemiological studies suggesting that an adverse fetal environment resulting in low birth weight in term infants was associated with the development many decades later of adult cardiovascular and metabolic disorders (Barker, 1998; Barker & Osmond, 1986). This increased risk for disease in adulthood was attributed to fetal adjustments to cues from the intrauterine environment, also known as programming (Gluckman, Hanson, Cooper, & Thornburg, 2008; Godfrey & Barker, 2001). Epigenetic mechanisms have been suggested as one explanation underlying programming and such programming may not be limited to the fetal period. Specifically, research with animal models suggests that program-

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Correspondence concerning this article should be addressed to Elisabeth Conradt, Department of Psychology, The University of Utah, 380 South 1530 East BEHS 602, Salt Lake City, UT 84112. Electronic mail may be sent to elisabeth.conradt@psych.utah.edu.

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ming may occur postnatally as the infant adjusts to the quality of the caretaking environment with concomitant epigenetic effects (Liu et al., 1997; Meaney, 2010; Weaver et al., 2004). For instance, using rodent models, Meaney and colleagues demonstrated that rodent offspring deprived of a particular form of maternal caregiving exhibited reduced expression of the glucocorticoid receptor gene via increased DNA methylation in hippocampal tissue (Liu et al., 1997). Determining whether similar programming processes occur in humans could lead to a greater understanding of the molecular basis for the development of infant HPA axis functioning.

Translating this work to humans requires an understanding of whether the quality of the postnatal environment is related to DNA methylation of genes involved in HPA functioning as well as infant cortisol. DNA methylation is the process by which a methyl group is added to individual cytosines in the context of CpG dinucleotides. When this addition occurs in gene promoters, it is most often associated with transcriptional gene silencing, or the reduction in gene activity (Jones & Takai, 2001). Preliminary human evidence indicates that the experience of depression (Conradt, Lester, Appleton, Armstrong, & Marsit, 2013; Oberlander et al., 2008) while pregnant, and exposure to childhood abuse (Tyrka, Price, Marsit, Walters, & Carpenter, 2012) is related to increased methylation of genes involved in the neuroendocrine response to stress, including the glucocorticoid receptor gene (*NR3C1*) and 11β-hydroxysteroid dehydrogenase Type 2 (*11β-HSD-2*).

The neuroendocrine response to stress is initiated when an individual perceives stress or threat in his or her environment. As a result, limbic brain regions stimulate the release of corticotropin-releasing factor (CRF), which activates the pituitary gland to release adrenocorticotropic hormone, which then stimulates cells in the adrenal cortex to release cortisol into the bloodstream (Gunnar & Vazquez, 2006). A negative feedback is initiated whereby glucocorticoids bind to glucocorticoid receptors in the brain, such as the hippocampus, which then inhibits the synthesis and release of CRF (Zhang & Meaney, 2010), thereby shutting down the HPA axis and the release of more cortisol. Therefore, DNA methylation of *NR3C1* should result in fewer glucocorticoid receptors for binding cortisol resulting in higher levels of cortisol in the blood. Evidence for this hypothesis comes from the work of Oberlander et al. (2008) who found that exposure to prenatal depression was related to greater methylation of *NR3C1*, which in turn was related to greater cortisol reactivity in infancy (Oberlander et al., 2008).

11β-HSD2 functions to convert maternal cortisol to inert cortisone. DNA methylation of *11β-HSD2* is thought to reduce activity of this gene, resulting in greater exposure of the fetus to maternal cortisol. Either increased exposure to glucocorticoids or inhibition of *11β-HSD-2* results in decreased birth weight, increases in hyperglycemia and hypertension, increased HPA axis reactivity, and increased anxiety in rodent models (Harris & Seckl, 2011). While this preclinical evidence is promising, no studies that we know of have examined relations between DNA methylation of *11β-HSD2* and HPA functioning in humans.

Exposure to maternal depression may be a risk factor for impaired psychophysiological functioning in infancy as some mothers with mood disorders are less sensitive and responsive when interacting with their infants (Beeghly & Tronick, 2011; Campbell et al., 2004). This insensitivity may be a mechanism by which infants of mothers with mood disorders show alterations in the HPA axis. For instance, infants of insensitive mothers with depression and anxiety had higher baseline cortisol (Brennan et al., 2008) compared to their unexposed counterparts. However, to characterize exposure to maternal depressive symptoms as being a risk factor for all children is shortsighted. Maternal depression is a complicated and heterogeneous disorder, with a great deal of variability in the quality of early parenting (Tronick & Weinberg, 1997). Far less attention has been paid to the specific parenting characteristics that may moderate the effect of exposure to maternal depressive symptoms on child outcomes.

The social buffering hypothesis suggests that maternal sensitivity and responsiveness may buffer the child to the effects of early stress, including maternal depression (DiCorcia & Tronick, 2011; Hostinar, Sullivan, & Gunnar, 2014). An impressive body of research indicates that the HPA response to stress in infants and young children is mitigated in the presence of a sensitive caregiver (Hostinar et al., 2014). In a longitudinal study, women with late, intermittent, or chronic depressive symptoms postnatally *and* who were less sensitive had preschoolers who were more likely to be insecurely attached in comparison to women with depressive symptoms who were more sensitive (Campbell et al., 2004). Therefore, maternal sensitivity or responsiveness may buffer infants from the effects of maternal depressive symptoms.

It is unclear what biologic or molecular mechanism might underlie the effects associated with the buffering of stress by caretaking. The animal litera-

ture suggests that one such process may be epigenetic in nature, though it remains to be seen whether this research translates to human models. The goal of the present study is to investigate whether maternal depressive symptoms and/or maternal sensitive behaviors and responsiveness are related to DNA methylation of genes involved in the neuroendocrine response to stress and to neuroendocrine functioning in infants. Given the comorbidity of maternal depression and anxiety, we also include symptoms of anxiety in our models to determine whether maternal depressive symptoms are related to epigenetic processes above and beyond symptoms of maternal anxiety. We examined maternal sensitive behaviors during the first play phase of the still-face paradigm as we were interested in observing mother–infant interactions during baseline, or more typical conditions. Furthermore, our goal was to understand whether maternal behavior may be related to epigenetic processes. Our first aim was to examine the relations between maternal depressive symptoms and maternal sensitive behaviors and responsiveness and DNA methylation of *NR3C1* and *11β-HSD2*. Our second aim was to examine the main effects of maternal depressive symptoms, maternal sensitive behaviors and responsiveness, and DNA methylation of *NR3C1* and *11β-HSD2* on prestress cortisol and cortisol reactivity. Our third aim was motivated by the social buffering hypothesis. Specifically, we tested interactions between maternal depressive symptoms and maternal sensitive behavior and responsiveness on DNA methylation of *NR3C1* and *11β-HSD2*, prestress cortisol, and cortisol reactivity.

Method

Participants

Mothers and their 4-month-old infants were recruited from an existing cohort of infants born of average weight for gestational age following approval from the Women and Infants Hospital of Rhode Island and Dartmouth College Institutional Review Boards. Only singleton, full-term (≥ 37 weeks gestational age) infants were included in the study. Other exclusion criteria were maternal age < 18 years, a life-threatening medical complication of the mother, and congenital or chromosomal abnormality of the infant. Data collection took place between June 2011 and December 2013. Most of the participants were Caucasian (72.7%), with 12.5% African American, 3.1% Hispanic, 1.6% Asian, 0.8% Native American, and 9.3% identifying themselves as "Other" (see Table 1 for additional sample characteristics). Mother's mean age was 30.5 years (range = 18–40 years). The sample included 128 infants (64 female) with an average age of 19.1 weeks (range = 13–26 weeks). All mothers gave written informed consent.

Measures

Maternal Symptoms of Depression

Maternal symptoms of depression were assessed by the Center for Epidemiologic Studies Depression Scale (CES–D; Radloff, 1977), a 20-item self-report measure designed to assess for symptoms of depression in the past week. It is considered a reliable and a valid indicator of maternal depression in postpartum women (Conradt, Manian, & Bornstein, 2012). The alpha was .99.

Maternal Symptoms of Anxiety

Maternal symptoms of anxiety were assessed with the Beck Anxiety Inventory (Beck & Steer, 1987), a 21-item self-report inventory. The alpha was .99.

Maternal Sensitivity and Responsiveness

Maternal sensitivity and responsiveness were assessed using a coding scheme adapted from Gun-

Table 1
Participant Characteristics

Demographic variable	*M* (range) or %
Household income	
Maternal employment status: full-time work	49.2
Maternal employment: part-time work	18.8
Household income: $0–24,999	20.0
Household income: $25,000–49,999	22.6
Household income: > $50,000	57.4
Caucasian	72.7
African American	12.5
Hispanic	3.1
Asian	1.6
Native American	0.8
Other	9.3
Maternal age	30.5 years (18–40 years)
Infant sex: female	50
Infant age	19.1 weeks (13–26 weeks)

ning, Fiori-Cowley, and Murray (1999) and included four scales. Maternal *acceptance* included the willingness and ability of the mother to follow her infant's lead, *demandingness* (reverse scored) was defined as the degree to which the mother required her infant to behave in a certain way, *responsiveness* was operationalized as both the mother's awareness of her infant's signals and her response to them (regardless of the appropriateness of the response), and appropriate *touch* was defined as the mother's ability to touch her infant in a gentle and affectionate manner as opposed to a more intrusive style.

Maternal sensitivity and responsiveness were coded every 30 s during a 2-min face-to-face play episode by coders trained to reliability against a set of 10 training tapes coded by three experts in the field of maternal sensitivity. The first play episode was part of the face-to-face still-face paradigm (Tronick, Als, Adamson, Wise, & Brazelton, 1978), which includes three episodes: a 2-min play episode, a 2-min still-face episode in which mothers are asked to be unresponsive to their infant, and a 2-min reunion episode. The modification by Haley and Stansbury (2003) was conducted, which includes an additional second still-face and reunion episodes. Only the first 2-min play episode was used in this study due to our interest in measuring maternal sensitivity to nondistress. Coders then coded an additional 20% of tapes for reliability. The intraclass correlations were .78 for accepting, .90 for demandingness, .95 for responsiveness, and .83 for touch. Each score within each maternal sensitivity and responsiveness domain was significantly and positively correlated (*r*s ranged from .40 to .60 for accepting, .26 to .43 for demandingness, .42 to .67 for responsiveness, and .46 to .68 for touch). The values were therefore averaged to create a single score. We then ran a principal component analysis to reduce the number of variables tested in the analyses. Two factors emerged that accounted for 80.5% of the variance, and all sensitivity and responsiveness variables had factor loadings > .64. The first factor was the responsiveness/appropriate touch factor and the second factor was the accepting/nondemanding factor. These two factors were employed in our analyses.

Cortisol

Prestress cortisol samples were taken from infants upon entry into the laboratory and two poststress cortisol samples were taken following the still-face paradigm (Tronick et al., 1978). Following Haley and Stansbury (2003), the first poststress saliva sample was taken 30 min after the end of the first still-face episode and the second poststress saliva sample was taken 40 min after the end of the first still-face episode. Salivary cortisol was collected from the infant using a small sponge that was swabbed in the infant's mouth until it became saturated with saliva. The swab was then placed into a storage vial and frozen until analyzed. If infants ate or drank 30 min prior to sample collection, their mouths were first swabbed with a wet paper towel. Samples were analyzed by Salimetrics (Arizona) for analysis.

Buccal Sample Collection, DNA, and Bisulfite Modification

Buccal-derived DNA was collected from saliva samples following the still-face paradigm using the Oragene-DNA saliva collection system. Buccal cells were taken from the infants' cheeks using a small swab. The swabs were then placed in a collection tube and sealed, releasing a stabilizing solution into the collected sample to allow for processing of the sample at a later period. Batches of sample collection tubes were sent to Dartmouth College for DNA isolation. DNA was isolated from the collection tubes following the Oragene methods. Purified DNA was quantified using a ND-1000 spectrophotometer (Nanodrop, Wilmington, DE), and DNA samples (500 ng) were bisulfite modified using the EZ DNA Methylation Kit (Zymo Research, Irvine, CA) and stored at −20°C.

Bisulfite Pyrosequencing DNA Methylation Analysis

NR3C1. Of the 13 CpG sites in the *NR3C1* exon 1_F promoter region, our primary interest was in Sites 1–3, which have previously shown variability in DNA methylation associated with maternal depression and cortisol response in infant cord blood. Pyrosequencing, which allows for quantitative assessment of DNA methylation in short sequence regions, was performed on PCR product amplified from bisulfite-modified DNA as described previously (Conradt et al., 2013).

The primers for amplification were forward: 5′-TTT TTT TTT TGA AGT TTT TTT A-3′ and reverse: 5′-Biotin-CCC CCA ACT CCC CAA AAA-3′. The first sequencing primer was designed to sequence the first 5 CpG sites (5′-GAG TGG GTT TGG AGT-3′), and the second sequencing primer was designed to sequence the following 8 CpG sites (5′-AGA AAA GAA TTG GAG AAA TT-3′) for a total of 13 sites sequenced.

11β-HSD-2. Pyrosequencing was performed on PCR product amplified from bisulfite-modified DNA as described previously (in citation blinded for review) based on the region sequenced and displaying differential methylation in human placenta from Alikhani-Koopaei, Fouladkou, Frey, and Frey (2004). Amplification primers were HSD11B2-F, 5′-GGA AGTGGGGTTGTGYGTTTTTAGGTTTAAGTT-3′ and HSD11B2-R, 5′-biotin-ATACCCTTTACTAATCRCA CCACC-3′ (IDT Inc., Coralville, IA), and the sequencing primer designed to interrogate four CpG sites HSD11B2-seq, 5′-GGGGTAGAGATTTTAAGAA-3′.

For both *NR3C1* and *HSD11B2*, the percent methylation at each CpG site was quantified using the Pyro Q-CpG Software, version 1.0.11 (Qiagen, Germantown, MD). For both assays, bisulfite conversion controls were included on each sequencing read. In order for the sample's methylation extent to be called, the bisulfite conversion rate must be > 93%, and for all samples examined the conversion rate was > 95%. All assays were performed in triplicate on the same bisulfite converted DNA template on all samples, and if any of the repeats differed by > 10% those assays on that sample were repeated. To prevent batch effects from bisulfite treatments interfering with the analysis, samples were randomized across batches.

Missing Data

There were 128 infants with complete *11β-HSD2* methylation and maternal sensitivity and responsiveness data. Of these, 9 children had missing *NR3C1* methylation data due to insufficient saliva volume needed for testing and 6 had missing cortisol data because the quantity of saliva was insufficient ($n = 5$) or because their cortisol values were extreme outliers ($n = 1$). One participant had missing CES–D data.

There were no significant differences in maternal sensitive behaviors or responsiveness between infants with and without missing *NR3C1* methylation data (ps > .21) or maternal depression among infants with and without missing cortisol data, $t(126) = -.48$, $p = .63$. Infants with missing *NR3C1* methylation values had mothers with significantly greater symptoms of depression, $t(126) = -2.26$, $p = .03$. Tests for birth and demographic differences between infants with and without missing data revealed that there were no differences in birth weight, gestational age, ethnicity, education level, or maternal age among infants with and without missing *NR3C1* methylation data (all ps > .15) or missing cortisol data (all ps > .10).

We controlled for false discovery among the 10 tests of interaction using the Benjamini and Hochberg (1995) procedure. This method was used to determine the percentage of findings that could be a false discovery. Instead of a corrected p value, a q value is obtained, which represents the proportion of tests below which are false positives. As is standard in the epigenetic literature, we chose a q value of .10. In the results we present both the p and q values.

Results

Descriptive Statistics

Data were examined for outliers and violations of normality. In addition to examining outliers among individual variables, we checked the assumption that the error term residuals should be normally distributed by looking at normal p-p plots of regression standardized residuals and found that residuals were normally distributed. The raw cortisol values (μg/dl) and *11β-HSD2* methylation scores were positively skewed and normalized using a log transformation. Outliers above or below 3 *SD* in all three samples and the difference scores were winsorized by replacing the value with the value at 3 *SD* (< 1% of values were affected).

Table 2 includes the means, standard deviations, and correlations among our variables of interest. There were no significant associations between maternal depressive symptoms or DNA methylation of either gene. Greater levels of maternal accepting and nondemanding behavior were related to greater methylation of *11β-HSD2* CpG 1. Greater levels of maternal sensitive behaviors (both factors) were related to lower levels of cortisol at the first poststress sample, but not cortisol reactivity (difference score of $\text{cortisol}_{\text{poststress 1 or 2}}$–$\text{cortisol}_{\text{prestress}}$). Greater levels of DNA methylation of *NR3C1* CpG 1 were related to lower levels of cortisol at the first and second poststress sample, but not cortisol reactivity.

Covariates

Because of the diurnal rhythm of cortisol, all assessments took place in the morning between 8:00 and 11:30 a.m. (range = 8:11–11:20 a.m.). We examined whether the time of each of the three assessments was associated with each measure of cortisol (e.g., whether time of the prestress measurement was correlated with the prestress cortisol value). Time of measurement was not significantly related to the time-specific measurement of cortisol

Table 2
Means, Standard Deviations, and Correlations of Variables of Interest

Variable	*M*	*SD*	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Maternal symptoms of depression	9.01	8.63	—												
2. Maternal symptoms of anxiety	6.25	8.06	.76***	—											
3. Maternal responsiveness and appropriate touch factor	0.00	1.00	.07	.07	—										
4. Maternal accepting and nondemanding factor	0.00	1.00	−.17	−.004	.13	—									
5. *NR3C1* CpG 1	1.09	1.35	.01	−.02	−.09	.04	—								
6. *NR3C1* CpG 2	1.15	1.46	.05	−.03	−.07	−.05	.51***	—							
7. *NR3C1* CpG 3	1.98	1.95	.09	.16	−.02	.08	.04	−.10	—						
8. *11β-HSD2* CpG 1	0.66	.76	−.09	.08	.03	.21*	−.03	−.05	.22**	—					
9. *11β-HSD2* CpG 2	1.30	1.37	.04	.06	−.05	−.05	−.03	−.07	−.05	.29***	—				
10. *11β-HSD2* CpG 3	0.81	1.04	.01	−.04	−.05	−.10	−.13	−.10	−.01	.16	.65***	—			
11. *11β-HSD2* CpG 4	3.26	0.82	.11	.05	−.08	.04	−.06	.16	.09	.45***	.41***	.51***	—		
12. Prestress cortisol (μg/dL)	0.22	0.19	.14	.05	−.13	−.14	−.05	−.11	−.05	−.12	−.09	−.13	−.10	—	
13. Poststress cortisol Sample 1 (μg/dL)	0.24	0.26	.08	.004	−.15	−.43***	−.16	−.15	−.06	.02	−.03	−.04	.04	.55***	—
14. Poststress cortisol Sample 2 (μg/dL)	0.22	0.18	−.07	−.08	−.11	−.33***	−.19*	−.10	−.05	−.01	.02	.03	.11	.14	.73***

Note. *11β-HSD2* = 11β-hydroxysteroid dehydrogenase Type 2; *NR3C1* = glucocorticoid receptor gene.
*$p < .05$. **$p < .01$. ***$p < .001$.

(all *p*s > .35). We also examined whether either infant or maternal prescription and/or nonprescription steroid medication, or maternal use of caffeine impacted cortisol concentrations. Steroid use within the last 12 hr by either mother or infant was not significantly associated with the cortisol values (all *p*s > .40), and neither was maternal consumption of caffeine that morning (*p*s > .11). If infants had eaten < 30 min prior to cortisol sampling their mouths were rinsed with water. As nap times may also affect cortisol values we examined whether time of nap and/or time of awakening affected cortisol. Neither was related to our cortisol values (*p*s > .18).

We also examined covariates that may be related to DNA methylation of *11β-HSD2*, *NR3C1*, or cortisol. These covariates include birth weight, gestational age, ethnicity, and sex. None of these covariates were significant predictors of DNA methylation of *11β-HSD2*, *NR3C1*, or cortisol (all *p*s > .08).

Aim 1: Main effects of maternal sensitive behaviors and responsiveness and depressive symptoms on DNA methylation of 11β-HSD2 and NR3C1

We tested the main effects of maternal sensitive behaviors (responsiveness/appropriate touch factor and accepting/nondemanding factor, entered separately), and maternal depressive symptoms on DNA methylation of *11β-HSD2* CpG Sites 1–4 and *NR3C1* CpG Sites 1–3 in infants. Of the 14 regressions tested, we found one main effect. Greater levels of the accepting/nondemanding factor were related to greater methylation of *11β-HSD2* CpG 1, $b = 0.23$, $p = .02$, $q = .007$.

Aim 2: Main effects of maternal sensitive behaviors and responsiveness and depressive symptoms on prestress cortisol and cortisol reactivity

We again tested the main effects of maternal sensitive behaviors (responsiveness/appropriate touch factor and accepting/nondemanding factor, entered separately), and maternal depressive symptoms on prestress cortisol and cortisol reactivity in infants (outcomes tested separately). Of the 21 regressions tested, no main effects emerged.

Aim 3: Test of maternal sensitive behavior as a moderator of the effect of maternal depressive symptoms on DNA methylation of 11β-HSD2 and NR3C1 and cortisol

We next tested the hypothesis that the effect of maternal depressive symptoms on DNA methylation of *11β-HSD2* and *NR3C1* may depend on maternal sensitive behaviors. In other words, we examined whether these sensitive behaviors buffered, or were moderators of, the effect of maternal depressive symptoms on DNA methylation of *11β-HSD2* and *NR3C1*. Our regression models included maternal depressive symptoms, the maternal responsiveness/appropriate touch factor and the maternal accepting/nondemanding factor entered as main effects in Step 1, the interaction between maternal responsiveness/appropriate touch and maternal depressive symptoms, and the maternal accepting/nondemanding factor entered in Step 2 of all models. Ten outcomes were tested separately: four CpG sites for *11β-HSD2*, three for *NR3C1*, and our three cortisol outcomes (prestress and the two reactivity measures). These results are reported in Table 3.

In the first model, only the interaction between maternal depressive symptoms and the maternal responsiveness/appropriate touch factor was a significant predictor of *11β-HSD2* CpG 3, $p = .04$, $q = .04$. We used the online computational tools provided by Preacher, Curran, and Bauer (2006; http://www.quantpsy.org/interact/mlr2.htm) to clarify the nature of this interaction. The simple slopes of maternal responsiveness and maternal depressive symptoms were computed at 1 *SD* above and below their respective means. As seen in Figure 1A, there were no differences in DNA methylation of *11β-HSD2* CpG 3 among infants whose mothers scored high on responsiveness/appropriate touch, regardless of the number of depressive symptoms the mother endorsed ($b = -1.55$, $p = .12$). The highest levels of DNA methylation of *11β-HSD2* CpG 3, however, were found among infants of mothers who were less responsive and with high depressive symptoms ($b = 2.09$, $p = .04$).

In the second model, we examined DNA methylation of *11β-HSD2* CpG 4 and *NR3C1* CpG 2 (tested separately). There was a main effect of maternal depressive symptoms and the responsiveness/appropriate touch factors on DNA methylation of *11β-HSD2* CpG 4, $p = .03$, $q = .03$, and *NR3C1* CpG 2, $p = .01$, $q = .02$ (Table 3). This main effect, however, was qualified by a significant interaction between maternal responsiveness/appropriate touch and maternal depressive symptoms. Again, a test of simple slopes revealed no differences in DNA methylation of *11β-HSD2* CpG 4 ($b = -1.02$, $p = .31$; Figure 1B) or *NR3C1* CpG 2 ($b = -.96$, $p = .34$; Figure 1C) among infants whose mothers were more responsive, regardless of

Table 3
Hierarchical Regression Predicting DNA Methylation and Prestress Cortisol

Predictors	β Step 1	β Step 2	R^2	F
Outcome: DNA methylation of *11β-HSD2* CpG 3				
Responsiveness/appropriate touch factor	−.05	−.22		
Accepting/nondemanding factor	.04	.09		
Maternal depressive symptoms	.02	.06		
Maternal anxious symptoms	−.05	−.20		
Maternal Depressive Symptoms × Accepting/Nondemanding Factor	—	−.13		
Maternal Depressive Symptoms × Responsiveness/Appropriate Touch Factor	—	−.26*		
		ΔR^2 = .06	.07	3.09*
Outcome: DNA methylation of *11β-HSD2* CpG 4				
Responsiveness/appropriate touch factor	−.11	−.32**		
Accepting/nondemanding factor	.09	.16		
Maternal depressive symptoms	.14	.19		
Maternal anxious symptoms	−.01	.02		
Maternal Depressive Symptoms × Accepting/Nondemanding Factor	—	−.19		
Maternal Depressive Symptoms × Responsiveness/Appropriate Touch Factor	—	−.30**		
		ΔR^2 = .08	.10	4.73**
Outcome: *NR3C1* CpG 2				
Responsiveness/appropriate touch factor	−.09	−.33*		
Accepting/nondemanding factor	.02	−.01		
Maternal depressive symptoms	.30*	.39**		
Maternal anxious symptoms	−.21	−.20		
Maternal Depressive Symptoms × Accepting/Nondemanding Factor	—	.03		
Maternal Depressive Symptoms × Responsiveness/Appropriate Touch Factor	—	−.34*		
		ΔR^2 = .05	.11	2.57
Outcome: prestress cortisol				
Responsiveness/appropriate touch factor	−.09	.13		
Accepting/nondemanding factor	−.14	−.10		
Maternal depressive symptoms	.02	−.07		
Maternal anxious symptoms	.05	.06		
Maternal Depressive Symptoms × Accepting/Nondemanding Factor	—	−.08		
Maternal Depressive Symptoms × Responsiveness/Appropriate Touch Factor	—	.37**		
		ΔR^2 = .09	.12	4.89*

Note. *11β-HSD2* = 11β-hydroxysteroid dehydrogenase Type 2; *NR3C1* = glucocorticoid receptor gene.
*p < .05. **p < .01.

depressive symptom severity. Infants with the highest levels of DNA methylation of *11β-HSD2* CpG 4 (b = 3.27, p = .001) or *NR3C1* CpG 2 (b = 2.83, p = .01), however, had mothers who were both less responsive and who reported greater depressive symptoms.

In our final model, the same predictors were used to test prestress cortisol as our outcome (Table 3). While there were no significant main effects, there was a significant interaction of maternal depressive symptoms and the responsiveness/appropriate touch factor, p = .003, q = .01. Simple slopes testing revealed infants of mothers who had lower levels of responsiveness/appropriate touch and higher levels of depressive symptoms had the lowest prestress cortisol levels (b = −2.43, p = .02; Figure 1D). Infants of mothers who had higher levels of responsiveness/appropriate touch and higher levels of maternal depression had the highest prestress cortisol levels (b = 2.70, p = .01).

Discussion

Decades of research with animals have demonstrated that the quality of maternal care may be protective in the face of environmental challenge. What biologic mechanism underlies this process is unknown though animal studies suggest that epigenetic mechanisms may be at play. This study was an attempt to determine if similar effects could be observed in humans. These initial findings provide some support for the hypothesis that maternal responsiveness may buffer infants from the effects

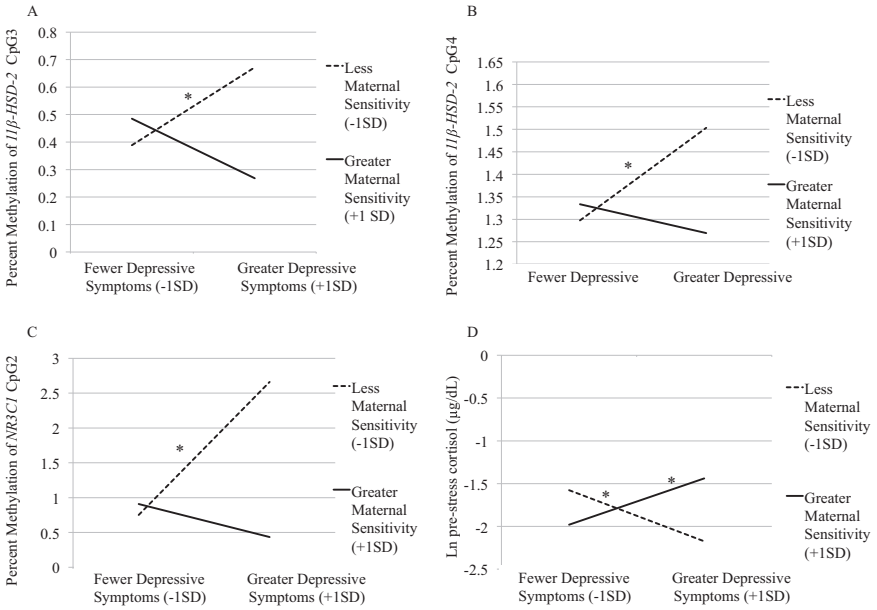

Figure 1. Interactions between maternal depressive symptoms and maternal sensitivity on 11β-hydroxysteroid dehydrogenase Type 2 (*11β-HSD2*) CpG 3 (A), *11β-HSD2* CpG 4 (B), *NR3C1* CpG 2 (C), and prestress cortisol (D). Simple slopes were tested at ± 1 *SD* from the mean.
*$p < .05$. **$p < .01$. ***$p < .001$.

of maternal depressive symptoms. This could suggest that epigenetic processes are sensitive to environmental input. These findings are similar to those of Meaney and colleagues and could have translational implications by suggesting that particular forms of maternal caregiving is related to less methylation of genes involved in HPA axis functioning to humans (Meaney, 2010).

There was a significant positive correlation between DNA methylation of *11β-HSD2* CpG 1 and the accepting/nondemanding factor, which was not expected. There are no known transcription factor (proteins that regulate the transcription, or the first step in gene expression, of genes) binding sites on *11β-HSD2* CpG 1, and thus it is difficult to interpret why maternal behavior would be associated, in the opposite direction, with methylation at this site. For instance, CpG Site 4 is the binding site for transcription factor GATA1 (Armstrong, Lesseur, Conradt, Lester, & Marsit, 2014). GATA1 is involved in the regulation of the immune response (Hirasawa et al., 1995) and may be a more important site for regulation of the neuroendocrine response to stress than is CpG 1. Methylation at Site 4 could decrease GATA1 binding and subsequent transcription, which may ultimately interfere with HPA axis regulation. This process could explain why we found relations between maternal responsiveness and maternal depressive symptoms in this site implicated in GATA1 binding. In previous work examining *11β-HSD2* from placenta samples, we also found relations between maternal prenatal depression exposure and methylation at CpG Site 4, but not CpG 1 (Conradt et al., 2013). Therefore, it may be that some CpG sites play a stronger role in HPA axis regulation, and subsequent neuroendocrine/behavior relations than others, because of their proximity to transcription factor binding sites.

It was only by examining maternal depressive symptoms that the effect of maternal sensitive behavior on DNA methylation and HPA axis functioning became clear. Mothers with depressive symptoms who were more responsive and engaged in more appropriate touch during face-to-face play had infants with less DNA methylation compared to mothers with depressive symptoms who were also less sensitive. This interaction emerged for three of the seven CpG sites tested and thus appears to be a robust effect. Furthermore, the combination of exposure to maternal depressive symptoms and maternal responsiveness was related to the highest prestress cortisol levels, whereas exposure to maternal depressive symptoms and maternal unresponsiveness was related to the lowest prestress cortisol levels. The false discovery rates were low, indicating that our results likely represent true discoveries. However, like all findings

from initial studies, our results should be replicated in an independent sample.

These results could be interpreted in favor of the social buffering hypothesis as maternal sensitive behavior may buffer the effects that exposure to maternal depression has on genes that regulate the infant HPA axis and on the HPA axis itself. Even in the face of maternal depressive symptoms, having a mother who is responsive and engages in appropriate touch during play may dampen HPA axis activity via decreased methylation of genes involved in the neuroendocrine response to stress. Furthermore, DNA methylation outcomes were similar between infants whose mothers were more responsive, regardless of the mother's report of her own depressive symptoms. While these data are preliminary, they could suggest that having a responsive caregiver may buffer infants to the exposure of maternal depressive symptoms. Put another way, infants do not know the diagnosis or symptom levels of their mother, they only know what they experience.

Exposure to maternal depressive symptoms at 4 months could be a proxy for exposure to prenatal maternal depression, which may program the infant HPA axis in utero. It is possible that exposure to prenatal maternal depression is related to increased glucocorticoid exposure, as some adults with depression hypersecrete and exhibit prolonged elevations in cortisol (Parker, Schatzberg, & Lyons, 2003), and their offspring tend to have higher cortisol levels (Field et al., 2004), though other work finds null results (Huot, Brennan, Stowe, Plotsky, & Walker, 2004). Indeed, in previous work we have found that exposure to prenatal maternal depression is related to more DNA methylation of *NR3C1* and *11β-HSD2* (Conradt et al., 2013). Therefore, at birth, these infants may exhibit greater cortisol levels compared to infants who are not exposed to maternal depression, and may be more reactive to stress. By contrast, more responsive caregiving and greater infant capacity for self-buffering may lead to demethylation of genes involved in HPA axis functioning. Indeed, Meaney and colleagues (Liu et al., 1997) found that at postnatal Day 1, all of the rats exhibited hypermethylation of specific CpG sites on exon 1_F of *NR3C1* and it was the experience of receiving high levels of licking and grooming that led to demethylation; perhaps a similar process is occurring in humans.

We found interaction effects for the factor that included maternal appropriate touch, but not for the accepting factor. This research was informed by animal models suggesting that maternal licking and grooming is related to the expression of genes regulating the HPA axis, and we expected that maternal sensitivity is a good proxy for this licking and grooming behavior in rats. As others have argued, licking, grooming, and maternal sensitivity reflect species-specific parenting practices, both of which are involved in the offspring response to stress, and buffer HPA axis reactivity in infancy (Loman & Gunnar, 2010). In humans, for instance, studies have found relations between maternal caregiving and infant stress reactivity, over and above the effects of infant negative temperament (Conradt & Ablow, 2010; Hane & Fox, 2006). Gusella, Muir, and Tronick (1988) found that maternal holding of the infant during the still face, even when the infant was in an infant seat, reduced negative affect compared to infants not touched. Our research suggests that appropriate touch in human mothers may be a better proxy for rat licking and grooming than global measures of maternal sensitivity per se. There is also a large literature suggesting that touch dampens the stress response and reduces cortisol levels (Field et al., 2004), negative affect (Feldman, Weller, Sirota, & Eidelman, 2003), and stress (Hernandez-Reif, Diego, & Field, 2007) in preterm infants. Future research may even include measures of appropriate touch during a feeding interaction to determine whether touch further dampens the HPA response to stress via DNA methylation.

Although we find support for the social buffering hypothesis, alternative explanations are still warranted. For instance, maternal depression could moderate the effect of maternal sensitivity on DNA methylation and neuroendocrine functioning. These findings could then be interpreted in light of a "dual-risk" framework by which the combination of exposure to maternal depression and maternal insensitivity is related to the poorest outcomes. It may also be that epigenetic factors could be related to increased behavioral reactivity, which in turn could affect maternal responsiveness. On the other hand, it is critical to consider the infant's capacity to cope or buffer him- or herself from the stress. In addition, we need to keep in mind that the effects of depression, maternal sensitivity, reactivity and methylation along with other processes are ongoing dynamic processes which may change or maintain initial effects.

This research has several limitations that should be noted. Given the small sample size, these are initial findings and need to be replicated in an independent sample. Second, all measures assessed were concurrent and thus we cannot imply direction of effect with these data. While animal models using cross-fostering paradigms to determine direc-

tion of effect found that maternal licking and grooming do indeed drive DNA methylation effects, this type of design cannot be conducted in humans due to obvious ethical implications (but see Zeanah et al., 2000, for their intervention with children reared in orphanages). Relatedly, there was a lack of independence between our measures of maternal sensitive behaviors and cortisol reactivity. It is important that replication studies assess cortisol reactivity in settings different from assessments of maternal behavior. In addition, the percent methylation values for our outcomes were low but are consistent with our prior work and work from independent laboratories (e.g., Oberlander et al., 2008) where effects of maternal prenatal depression exposure were found on *NR3C1* CpG 2. While this information gives us more confidence that we are identifying a meaningful relation between depression, responsiveness, and DNA methylation, the presences of low methylation may also represent cellular heterogeneity. Future work should therefore consider how to account for this heterogeneity. Our sample size was also restricted due to missing *NR3C1* data for women with depressive symptoms. Given that we found significant effects of maternal depressive symptoms on *NR3C1* CpG 2 on this "milder" (e.g., less depressed) portion of the sample highlights the more robust nature of the findings. In addition, increasing variability in socioeconomic status and/or risk of clinical depression will be important in future work to determine whether maternal sensitivity may buffer infants against exposure to clinical levels of depression and describe the epigenetic pathways involved.

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