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Dynamic DNA methylation changes in the maternal oxytocin gene locus (*OXT*) during pregnancy predict postpartum maternal intrusiveness

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Abstract

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Conflict of interest

None of the authors declares any conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.01.013.

Maternal behavior (MB) is observable across mammals and represents an important feature of environmental variation during early postnatal development. Oxytocin (OT) plays a crucial role in MB. Even prior to childbirth, pregnancy induces epigenetic and other downstream changes in the maternal OT-system, likely mediated by the actions of steroid hormones. However, little is known about the nature and consequences of epigenetic modifications in the maternal OT-encoding gene (*OXT*) during pregnancy. Our study aims to investigate temporal dynamics of *OXT* promoter DNA methylation (DNAm) throughout pregnancy in predicting MB in humans. In 107 mother-child dyads, maternal *OXT* DNAm was serially analyzed in whole blood in early, mid and late pregnancy. MB was coded based on standardized mother-child interactions at six months postpartum. After controlling for cellular heterogeneity, race/ethnicity, age, and socioeconomic status, *OXT*-promoter DNAm exhibited a dynamic profile during pregnancy ($b = 0.026$, $t = -3.37$, $p < .001$), with decreases in DNAm from early to mid-pregnancy and no further change until late pregnancy. Moreover, dynamic DNAm trajectories of the *OXT*-promoter region predicted MB (intrusiveness) at six months postpartum ($b = 0.006$, $t = 2.0$, $p < 0.05$), with 6% higher *OXT* DNAm in late pregnancy in intrusive compared to non-intrusive mothers. We here demonstrate that *OXT* promoter DNAm changes significantly throughout gestation in peripheral blood and that these changes are associated with variability in MB, providing a novel potential biomarker predicting postnatal MB.

Keywords

Oxytocin; Maternal behavior; DNA methylation; Behavioral epigenetics; Estrogen sensitivity; Pregnancy

1. Introduction

Onset of maternal behavior (MB) immediately after parturition and its maintenance throughout the postpartum period is observable across mammalian species (Rilling and Young, 2014) and its provision is essential for survival and development of the offspring. MB shows naturally occurring variation across and within species (Barrett and Fleming, 2011; Broad et al., 2006) and can, in case of abusive and neglectful parenting, compromise long-term psychosocial adjustment (You and Lim, 2015). It is not only maltreatment, but also suboptimal MB such as intrusiveness that contributes to maladaptive short- and long-term effects on developmental outcomes in the offspring. Intrusive mothers provide excessive stimulation despite the child's gaze aversion, interrupt child-initiated activities, thus undermining the child's autonomy (Ainsworth et al., 1978). Intrusiveness, which is observable in anxious mothers (Kaitz and Maytal, 2005), is associated with insecure-avoidant attachment (Isabella and Belsky, 1991) and anxiety in children (Van Der Bruggen et al., 2008), thus representing a parenting style which may compromise healthy, normative child development. Given the pivotal role of MB as a defining characteristic of the offspring's early environment, it is important to characterize neural and biological underpinnings of variation in MB. Numerous studies highlight a major role of the nonapeptide *oxytocin* (OT) in the modulation of mammalian MB (Rilling and Young, 2014). OT is primarily synthesized in magnocellular neurons of the hypothalamic *paraventricular* (PVN) and *supraoptic* (SON) nuclei, and acts via the G-protein coupled oxytocin receptor

(OTR) as a neuromodulator in neural networks associated with cognitive, affective, and behavioral phenotypes relevant to MB: coding of socially salient cues (Shamay-Tsoory and Abu-Akel, 2015), empathic accuracy (Bartz et al., 2010), anxiolysis (Neumann and Slattery, 2016), child-directed gaze (Kim et al., 2014), and emotion regulation in response to baby cries (Riem et al., 2011). In addition, deficient OT-signaling predicts suboptimal MB such as intrusive parenting (Atzil et al., 2011; Samuel et al., 2015). Importantly, OT-neural circuits of the maternal brain are under influence of surging sex-steroids, especially estrogens and progesterone to facilitate the immediate onset of MB after birth (Brunton and Russell, 2008). Consistent with this notion of a hormonal priming of maternal OT-neural circuits, animal models show that hypothalamic *OXT*-mRNA expression, which gradually increases with progressing pregnancy (Zingg and Lefebvre, 1988), is strongly induced by estradiol (E2) (Sharma et al., 2012) and involves estrogen-receptor β (ERβ) signaling (Patisaul et al., 2003). In humans, ERβ is co-localized with OT-positive magnocellular neurons in the SON and PVN (Hrabovszky et al., 2004) and E2 regulates *OXT* expression (Richard and Zingg, 1990), partly through ERβ-mediated *epigenetic modifications* of the *OXT* gene locus (Sharma et al., 2012).

Briefly, epigenetic mechanisms refer to biochemical modifications of chromatin (e.g., histone modifications or DNAm) or non-coding RNAs, that regulate gene transcription and subsequent gene expression in a cell-lineage and tissue specific manner without altering the underlying DNA sequence itself (Allis and Jenuwein, 2016). DNAm, which refers to the transfer of a methyl group (CH_3) to cytosine is best known for its role in gene silencing, especially when occurring in cis-regulatory elements such as gene promoters that are enriched for CpG dinucleotides. In promoter regions, DNAm represses gene activity through two principal mechanisms: interference with transcription factor binding and recruitment of methyl-CpG binding proteins (e.g., MeCP2) and repressor complexes (see Moore & Fan, 2013 for review). However, associations of increased DNA methylation with both, reduced but also increased gene expression have been reported, in dependence of the genomic location of the methylated sites (Mehta et al., 2013).

Although research on transcriptional control of *OXT* has strongly focused on E2 as the upstream regulatory signal, it is important to note that the presence of a composite hormone responsive element (cHRE) in the proximal 5'-flanking region of the *OXT* transcription start site suggests a more complex transcriptional regulation and involves, in addition to ERβ, other transcription factors such as the thyroid hormone, retinoic acid, as well as several orphan receptors (Burbach et al., 2001; Jurek and Neumann, 2018). Therefore it is likely that, during pregnancy, the coordinated activity of this complex transcriptional machinery is necessary for efficient *OXT* gene expression.

As has been shown before, deficient OT-signaling during pregnancy contributes to impaired MB, (Feldman et al., 2007). We thus speculate that *epigenetic* control of *OXT* gene expression during pregnancy may underlie OT-deficiency with potential consequences for variability in maternal behavior after birth.

Thus far, human studies investigating epigenetic modifications in genes involved in OT-signaling have almost exclusively focused on DNAm of the OTR-coding gene (*OXTR*). For

example, in non-pregnant clinical samples, decreased *OXTR* DNAm predicted social anxiety disorder and increased stress responsiveness (Ziegler et al., 2015). *OXTR* DNAm was also shown to predict postpartum depression in two independent cohorts (Kimmel et al., 2016; Reiner et al., 2015). It is noteworthy, that only one study investigated DNAm of the *OXT*-promoter in non-pregnant adult subjects in saliva and showed that *OXT* DNAm in this tissue is negatively associated with adult secure attachment, the ability to recognize emotional facial expressions, as well as structural and functional differences within brain areas related to social cognition (Haas et al., 2016). These findings suggest a role of *OXT* DNAm in predicting (endo-)phenotypes of direct relevance for MB and, assuming that promoter DNAm is inversely associated with gene transcription, are consistent with the well-established prosocial and attachment facilitating effects of OT (Rilling and Young, 2014). Given the predominance of the *OXTR* locus and the relative lack of the *OXT* locus in the literature on epigenetic modifications in the OT-signaling pathway, we decided to consider both loci in our analyses. Based on the assumption that invariant DNAm cannot be associated with a pheno-type of interest (e.g., MB) and following recent recommendations (Hachiya et al., 2017), we applied a statistically driven CpG-selection strategy to identify variably methylated CpG sites (vCpGs) in these two loci to ultimately increase the likelihood of discovering DNAm-pheno-type associations. *OXT/OXTR* vCpGs will then be carried forward for subsequent analyses intended to answer the following questions: 1) Does DNAm of *OXT/OXTR* vCpGs dynamically change over the course of pregnancy? 2) If dynamic changes occur, do they predict variability in MB? To address these questions, we conducted a prospective longitudinal study in 107 mother-child dyads. *OXT/OXTR* DNAm was repeatedly determined from blood at three time points during early, mid and late pregnancy. At six months postpartum, MB was coded based on a 15-minute long videotaped mother-child interaction during a play situation.

2. Materials and methods

2.1. Participants and study design

Mothers and children were part of an ongoing, longitudinal study, conducted at the University of California, Irvine (UCI), for which mothers were recruited during the first trimester of pregnancy. All women had singleton, intrauterine pregnancies. Women were not eligible for study participation if they met the following criteria: use of psychopharmacological treatment, corticosteroids, or illicit drugs during pregnancy (verified by urinary cotinine and drug toxicology). Exclusion criteria for the newborn were preterm birth (i.e., less than 37 weeks of gestational age at birth), as well as any congenital, genetic, or neurologic disorders at birth. Of all participating mother-child dyads (N = 146), maternal DNAm data was available for 121 women. Six women were excluded from analyses because they shared more than 25% of genotype (i.e., sisters or 1st degree cousins). Of the remaining 115 women, 107 women provided sufficient information for the analyses, i.e., data on *OXT* DNAm during pregnancy and participation in the play situation with their child at 6 months postpartum. For a sub-sample of these women (N = 54), DNAm was also available at 3 months postpartum. The UCI institutional review board approved all study procedures and all participants provided written informed consent. Study visits occurred on three occasions

during early (T1: 12.8 ± 1.8, mean ± SD, gestational weeks), mid (T2: 20.5 ± 1.5 weeks), and late (T3: 30.4 ± 1.4 weeks) pregnancy and six months (5.8 ± 0.6 months) postpartum.

2.2. OXT DNA methylation and CpG selection strategy

Genomic DNA was bisulfite converted using the EZ DNA methylation kit (Zymo Research, Irvine, CA, USA). DNAm analysis using the Infinium Illumina MethylationEPIC BeadChip (Illumina, Inc., San Diego, CA) was performed according to the manufacturer's guidelines in whole blood collected during pregnancy. Among the N = 107 women included in the current analyses, N = 99 had data at T1, N = 103 had data at T2 and N = 95 had data at T3, with 7.5% (n = 8) having provided 1 sample, 7.5% (n = 8) 2 samples, and 85% (n = 91) having provided samples across the three pregnancy visits. DNAs for all time points from individual mothers were hybridized on the same BeadChip and randomized across arrays. Raw probe intensities were loaded into R. In order to reduce technical variability between samples, functional normalization (Fortin et al., 2014) was applied using three principal components of control probes on the EPIC array, which are designed to measure technical (but not biological) variability with the *R*-package *Minfi* (Aryee et al., 2014).

From all available blood samples (in N = 121 women), one was removed due to a low call rate (< 98%). Probes with low detection p-values, probes containing single nucleotide polymorphisms (SNPs) and cross-hybridizing probes were removed according to Zhou et al (2017). Principal component analysis on normalized data identified two additional technical batches (the plate in which samples were processed and BeadChip row of the array) that were removed using *ComBat* (Johnson et al., 2007). In total, the EPIC-array covers 19 CpG sites within the *OXT* locus and 22 CpG sites within *OXTR* locus. Beta values from these 41 CpG sites were extracted from the normalized and quality controlled EPIC array data and used in our analysis. Subsequently, we sought to identify representative *OXT/OXTR* promoter CpGs that show two characteristics: First, a significant degree of inter-individual variability in DNAm across all three time-points and second, high inter-correlation with the other *OXT/OXTR* promoter CpGs. To achieve the first goal and in order to identify vCpGs, the R-package DMRcate (Peters et al., 2015) was used. We provided all 41 CpGs (*OXT:* N = 19; *OXTR:* N = 22) to this tool. After correcting for multiple comparisons, we identified one CpG in the *OXT* promoter region (cg16887334; location: Chr20:3052151, hg19) as the significant, most variable CpG of all interrogated CpG sites across the two loci (average inter-quartile range [IQR] for beta-value = .124; FDR p-value = 0.01). We further demarcated a variably methylated region (VMR) consisting of N = 9 CpGs around that vCpG. Cg16887334 was consistently flagged as the significant, most variable CpG across all three time points (see Figure S1). For *OXTR,* we also identified one vCpG site, located in intron 1 outside the promoter region (cg03987506; Chr3:8810549, hg19) with significant albeit much lower variability than the *OXT* cg16887334 (average IQR = .035; FDR p-value = 0.01). Next, we calculated Pearson inter-correlations between CpGs for the two loci separately. As can be seen in Figure S2 (Supplement), across all time points, cg16887334 is located within a cluster of N = 10 highly inter-correlated *OXT* CpGs spanning the promoter region. *OXTR* cg03987506 only has very limited inter-correlation with other *OXTR* CpGs (see Supplement Figure S3). Interestingly, cg16887334 also appeared to exhibit relatively high positive correlation between blood and brain as determined using BECon (https://

redgar598.shinyapps.io/BECon/; Edgar et al., 2017; see Supplement Table 1). According to our two selection criteria (significant variability and inter-correlation), we carried cg16887334 (Fig. 1) but not cg03987506 forward as the representative CpG for the identified *OXT* VMR in our DNAm-phenotype association analyses.

2.3. Maternal behavior

A home visit occurred at six months postpartum. The mothers were instructed to engage in a 15-minute standardized play situation as described by the NICHD Early Child Care Research Network (1999). The play situation was video-recorded and subsequently coded by two well-trained and reliable independent observers, who were blind to all maternal biological data (i.e., DNAm). To evaluate the quality of the dyadic mother-child interaction, we used the coding manual of the NICHD Early Child Care Research Network (1999), which allows to quantify affective and behavioral phenotypes in both mothers and children as well as their dyadic reciprocity on 5-point Likert-scales. As described above, maternal intrusive parenting, is non-contingent with the child's social signals, excessive and over-controlling (Kaitz and Maytal, 2005). Also, given the well-documented role of OT in both anxiolysis (Neumann and Slattery, 2016) and parenting (Rilling and Young, 2014), we speculated that intrusiveness represents a parenting phenotype where these well-established OT-associated phenotypes would converge and thus be best observable. After an extensive rater training, very high inter-rater reliability was achieved for maternal intrusiveness (ICC > .9), which was coded from 1 (= not characteristic) to 5 (= highly characteristic).

2.4. Covariates

All analyses were adjusted for the potential confounding effects of variables that have been shown to have effects on either the observed phenotype (MB), DNAm, or both. The covariates included maternal age, annual household income, and education (highest degree obtained), the latter two being aggregated to a composite measure indicative of socio-economic status (SES). Differences in genetic background were accounted for by population stratification using principal component analysis on genotype data obtained using the Illumina OmniExpress array (Illumina, Inc., San Diego, CA). Genotype data for 593.229 SNPs survived quality control and SNP filtering (minor allele frequency ≥ 5%). The first three principal components were added to account for differences in genetic background (Supplement Figure S4). To correct for cellular heterogeneity across samples, prediction of blood cell proportions using the DNAm data with a previously published algorithm (Houseman et al., 2012) was performed. Variability in DNAm of cg16887334 that was explained by the estimated proportions of blood cells (i.e., B-cells, CD4 T-cells, CD8 T-cells, monocytes, granulocytes and natural killer cells) was regressed out in linear regression models at each trimester, and residualized (cell-type corrected) DNAm of this CpG site was used in statistical analyses. Since maternal reproductive experience (i.e., parity) has been shown to predict differences in maternal behavior (Maupin et al., 2016), we also considered parity as a potentially confounding variable.

2.5. Statistical analyses

To model temporal variability in *OXT* promoter DNAm across gestation for all women and to further investigate the moderating role of maternal behavior (intrusiveness) on the

association between time during pregnancy and *OXT* promoter DNAm, we performed a linear mixed effects model on the data using the R-package *lmerTest* (Kuznetsova et al., 2015). More specifically, time variable *OXT* promoter DNAm (cg16887334, corrected for cell-type heterogeneity) throughout pregnancy was entered as the dependent variable and predicted by a combination of level 2 (MB [intrusiveness], maternal age, SES, three genotype PCs representing genetic background) and level 1 predictors (time in pregnancy) as well as a cross-level interaction term (time x intrusiveness). To evaluate the direction of the effects of our predictors of interest, i.e., time during pregnancy on DNAm in the entire sample, the gestational trajectories within and between intrusiveness groups (based on median split: high vs. low intrusiveness) in *OXT DNAm,* we conducted post-hoc analyses with the appropriate *t*-tests for dependent and independent samples using SPSS version 22.

3. Results

3.1. Sample characteristics

Socio-demographic characteristics are shown in Table 1 for the entire sample and stratified by maternal intrusiveness (median split). In addition, inter-correlations between the main predictors, outcomes and co-variates are depicted in supplemental Table S2. None of these co-variates were associated with DNAm at any time point but maternal intrusiveness was significantly associated with age, SES, and genotype-based ethnicity (PC1; all correlations $p < .05$) but not with parity status. We therefore included age, SES and the 3 PCs but not parity status as covariates in our statistical models.

3.2. OXT DNAm throughout pregnancy and maternal intrusiveness

Results of the linear mixed model revealed a significant time during pregnancy effect on DNAm ($b = -0.026$, $t = -3.372$, $p < .001$). As shown in Fig. 2a and confirmed with post-hoc analyses (*t*-test for dependent measures), *OXT* promoter DNAm significantly decreased from early to mid-pregnancy ($\Delta_{T1\text{-}T2} = 2.5\%$, $t = 4.53$, df = 94, $p < .001$) with no further change occurring from mid to late pregnancy ($\Delta_{T2\text{-}T3} = 0.04\%$, $t = -0.187$, df = 93, $p = 0.85$). A time during pregnancy x intrusiveness interaction on *OXT* promoter DNAm ($b = 0.006$, $t = 2.0$, $p < 0.05$) was also significant. Post-hoc analyses using a dichotomous intrusiveness variable (based on median split) indicated that intrusive mothers showed a decrease in DNAm from T1 to T2 ($t = 4.53$, df = 57, $p < .001$) and a trend for an increase from T2 to T3 ($t = -1.77$, df = 55, $p = .082$). Non-intrusive mothers, on the other hand, exhibited a trend for a decrease from T1 to T2 ($t = 1.72$, df = 36, $p = .095$) and further significant decrease from T2 to T3 ($t = 2.04$, df = 37, $p < .05$), indicating a continuous decrease of DNAm over pregnancy (Fig. 2b). These different patterns of change in DNAm between intrusive and non-intrusive mothers lead to a significant difference in DNAm in late pregnancy, with intrusive mothers showing approximately 6% higher DNAm compared to non-intrusive mothers ($t = -2.41$, df = 93, $p = 0.018$; *Cohen's d* = 0.52). Since none of the covariates included in the main model were significantly associated with *OXT* DNAm, we did not account for them in the post hoc analyses.

To address the hypothesis that it is the prenatal rather than post-natal changes in *OXT* DNAm that predict MB, we tested our main model in a subgroup of women (N = 54) for

whom data on *OXT* DNAm were available at three months postpartum. The results indicated that in this subgroup, like in the complete sample and despite reduced power, the time during pregnancy x intrusive interaction also significantly predicted DNAm ($b = 0.008$, $t = 2.24$, $p = 0.028$) after adjusting for the same co-variates as in the analyses that were run for the complete sample. We then tested whether DNAm at three months postpartum was different between intrusive and non-intrusive mothers and conducted multiple linear regression models to determine whether 3rd trimester DNAm, when adjusting for DNAm at three months postpartum, remained significantly associated with maternal intrusiveness. The results indicate that 1) at 3 months postpartum, there is no association between DNAm and intrusiveness (b = .197, t = 1.47, p > .05) and 2) that after controlling for 3 months postpartum DNAm, DNAm in late gestation still significantly predicts maternal intrusiveness (b = .489, t = 2.51, p = 0.016).

4. Discussion

To the best of our knowledge, our results provide first insights into the *dynamic* epigenetic adaptations of the maternal oxytocinergic pathway during human pregnancy that may support transition to motherhood. We demonstrated that non-intrusive mothers show a trend for a decrease of *OXT* promoter DNAm from the first to the second trimester and a further significant decrease from the second to the third trimester. Intrusive mothers on the other hand only exhibit a decrease of DNAm from the first to the second trimester and a trend towards an increase from the second to the third trimester. These different patterns of change in *OXT* promoter DNAm during pregnancy between intrusive and non-intrusive mothers result in group differences of about 6% (effect size $d = 0.52$) in DNAm in the third trimester of pregnancy, with intrusive mothers showing higher *OXT* promoter DNAm compared to non-intrusive mothers. These differential trajectories of *OXT* DNAm may serve as a potential prenatal biomarker in the prediction of variation in postnatal MB although it is unclear at this point, whether the observed differences in late pregnancy do actually reflect differences in *OXT* transcription. Moreover, it is important to note that prenatal rather than postnatal changes in *OXT* DNAm predict maternal behavior, supporting prior work on hormonal priming of the maternal brain. Regarding the upstream mechanisms that may contribute to variability in dynamic *OXT* DNAm throughout pregnancy and postnatal MB, future research should investigate at the molecular level, whether *OXT* promoter DNAm is dynamically changing in response to increasing levels of bioactive hormones (e.g. thyroid and steroid hormones) and whether the efficiency of these interactions predict variability in postnatal MB. Pregnancy represents a time of unparalleled increases of sex steroids, especially estrogens and progesterone, which readily enter the brain to induce and maintain profound, long-lasting structural and functional brain reorganizational processes in cortical (e.g., anterior cingulate cortex) and sub-cortical (e.g., *medial preoptic area*) regions associated with social cognition and MB, to ensure successful transition to motherhood (Brunton and Russell, 2008; Hoekzema et al., 2017). As an example, steroid hormones, via their nuclear receptors, have the capacity to alter accessibility of transcription factors to DNA (via histone modifications and DNAm), and thus alter gene-expression within brain areas that are sensitive to OT and are involved in MB (Stolzenberg and Champagne, 2015). Consistent with this notion, DNAm within promoter regions of steroid responsive genes is

extraordinarily dynamic in nature, which is especially well established for estrogen-responsive loci (Kangaspeska et al., 2008; Métivier et al., 2008). Together, these data suggest that the efficiency of these molecular steroid-peptide interactions may partly contribute to variability in pregnancy-related reorganization processes of the maternal brain and the subsequent quality in postnatal MB.

Moreover, our data are consistent with previous work in animals (Pedersen, 1997) and humans (Feldman et al., 2007; Levine et al., 2007) that have shown prenatal OT concentrations in association with postnatal MB. However, neither Feldman et al. (2007) nor Levine et al. (2007) did show a dynamic change in plasma levels of OT during pregnancy, which does not contradict our findings. It is well established that complex allopregnanolone, GABAergic, and opioid mechanisms restrain neurohypophyseal release of OT from magnocellular PVN and SON neurons during pregnancy (Brunton and Russell, 2008), which allows accumulation of OT in vesicles of the posterior pituitary, while preventing premature onset of uterus contractions and thus premature birth. Consequently, while no increases of plasma OT are observable until parturition, pregnancy induces upregulation of *OXT* mRNA in the hypothalamus (Zingg and Lefebvre, 1988), and we suggest that epigenetic modifications (i.e., DNAm) of the *OXT* promoter may partly underlie this upregulation of *OXT* mRNA. It may thus be more informative to focus on peripheral OT markers that show a dynamic gestational profile (like DNAm) as opposed to relatively stable ones (plasma OT) when interested in characterizing the association between oxytocinergic adaptations during pregnancy and MB. Our findings are furthermore in concordance with previous work by Haas et al. (2016), who in non-pregnant subjects of both sexes have shown that lower DNAm of the *OXT* promoter is associated with less anxious attachment, improved emotion recognition, as well as structural and functional brain endo-phenotypes associated with social cognition. Importantly, *OXT* promoter DNAm in the study by Haas et al was measured in saliva, while in the current study it was measured in blood, which indicates that across tissues lower *OXT* DNAm, probably indicative of higher gene transcription, may confer a more prosocial phenotype in humans.

Based on our observation that these dynamic changes in DNAm predict MB, future studies should investigate what factors may underlie inter-individual differences in *OXT* DNAm changes during pregnancy of that locus. Among these factors, early environmental conditions, e.g. exposure to childhood trauma (Heim et al., 2009), is a candidate that should be considered.

While our study is the first to show dynamic gestational epigenetic regulation of the *OXT* gene promoter in association with MB, several limitations and considerations need to be acknowledged. First, DNAm is highly tissue and cell-type specific. This issue is the critical limitation in the interpretation of our findings and relates to the question of the biological validity of peripheral epigenetic markers in the prediction of behavioral and thus centrally mediated phenotypes. However, a recent cross-species study, combining rodent neural tissue and peripheral blood from humans have provided promising results to address this challenge (Guintivano et al., 2014). In this study, E2 treatment lead to differential DNAm in the mouse hippocampus and some of these E-responsive loci in the mouse brain overlapped with regions showing differential DNAm in peripheral blood cells in high-risk individuals with

and without postpartum depression (Guintivano et al., 2014). Given the fact that the *OXT* locus is E-responsive, these data support the notion that sex-steroids can induce changes in DNAm in the brain that are mirrored by changes in peripheral blood, an assumption that is further supported by the relatively high blood-brain correlation of our representative CpG (cg16887334; Supplemental Table 1). Thus, despite the fact that DNAm was measured in the periphery, concordance between blood and brain DNAm of steroid-responsive loci has been shown before corroborating the notion that peripheral DNAm may be a biologically valid marker in the prediction of behavioral phenotypes. In addition, we would like to emphasize that the exploratory nature of the study and the relatively small sample size warrant a cautious interpretation of the results. We therefore encourage others to replicate our findings in independent samples in order to validate these data.

Finally and despite referring to maternal intrusiveness as 'non-optimal' maternal behavior, for example with regard to their children's development, a more controlling and intrusive strategy may prove adaptive under certain circumstances. Others have noted (Belsky et al., 1991) that parenting behavior conveys information about the predictability and safeness of the environment the offspring grows into. Thus, women should adapt their parenting strategies according to their environment, thereby *enhancing* reproductive fitness in the next generation. Another aspect that should be discussed is the observation that women, who self-identified as Hispanic are more likely to be characterized as intrusive and insensitive. This finding suggests cultural differences in parenting practices and/or a cultural bias in the coding process: coders were White/non-Hispanic and the coding instrument (NICHD, E.C.C.R.N., 1999) was standardized in a predominantly White/non-Hispanic sample and thus not designed to capture cultural differences in parenting. Research on parenting strategies that is informed by ethnology has reliably found that cultural norms (focus on autonomy vs. interdependence) shape parenting behavior (Keller et al., 2006). Therefore, the observed differences in race/ethnicity with respect to MB are not unexpected but rather represent "normal" cross-cultural differences in MB. Importantly, the association between DNAm and MB remains significant after controlling for race/ethnicity.

Despite these limitations, the findings of the current study contribute to a better understanding of the molecular mechanisms that occur already during pregnancy and contribute to phenotypic variation in human MB. Future studies should systematically address the outstanding questions and limitations by using a combination of animal and human studies going beyond DNAm of the *OXTR* gene locus to achieve a more nuanced and comprehensive picture of epigenetic modifications in OT pathways and their association with behavioral phenotypes. This said, our study may stimulate future replication efforts in independent samples and extension by integrating genetic variation, environmental exposures (e.g., childhood trauma), and ideally rodent hypothalamic OT-expressing neuronal tissue. As noted above, MB is the primary source of environmental variability for the offspring and therefore plays a crucial role in offspring development (Isabella and Belsky, 1991; Wagner et al., 2015). Thus, there is a compelling need to better understand the biological mechanisms that prepare pregnant women for their postpartum behavior and, if better understood, these mechanisms could be targets of early, prenatal interventions holding the promise to improve offspring health and development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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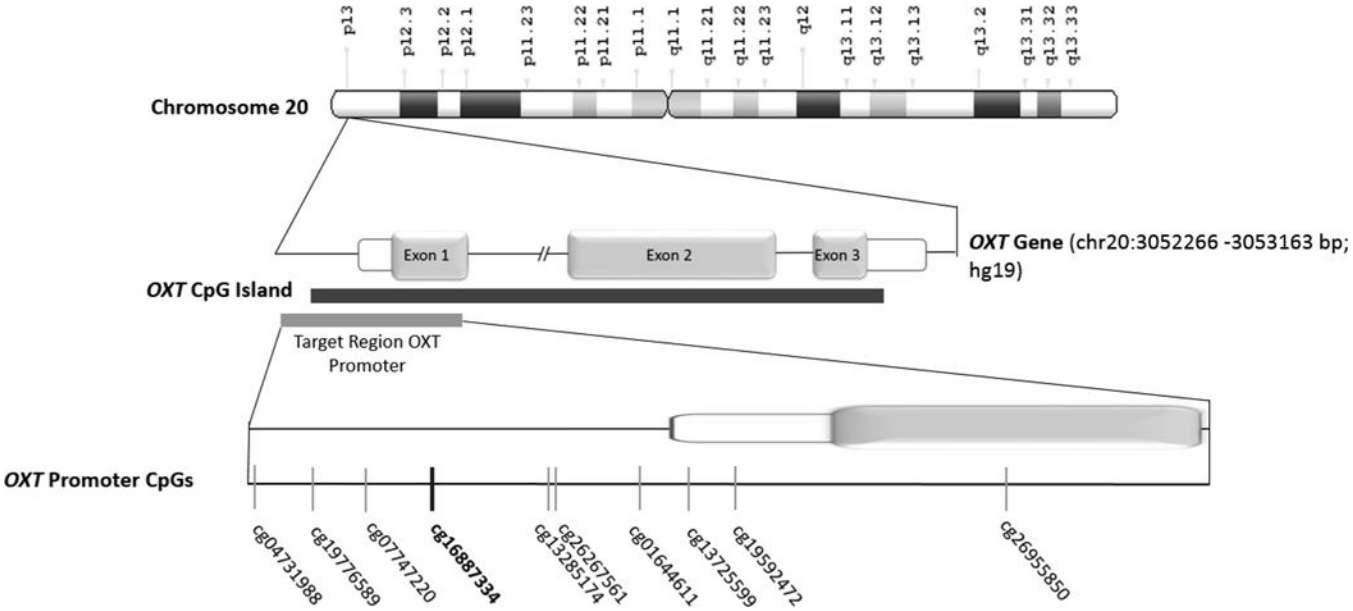

Fig. 1.

Localization and schematic representation of the structure of the oxytocin gene locus *(OXT)* on chromosome 20.p13, *OXT* promoter CpG sites, and the representative *OXT* promoter CpG (cg16887334; Chr20:3052151, hg19) highlighted in bold.

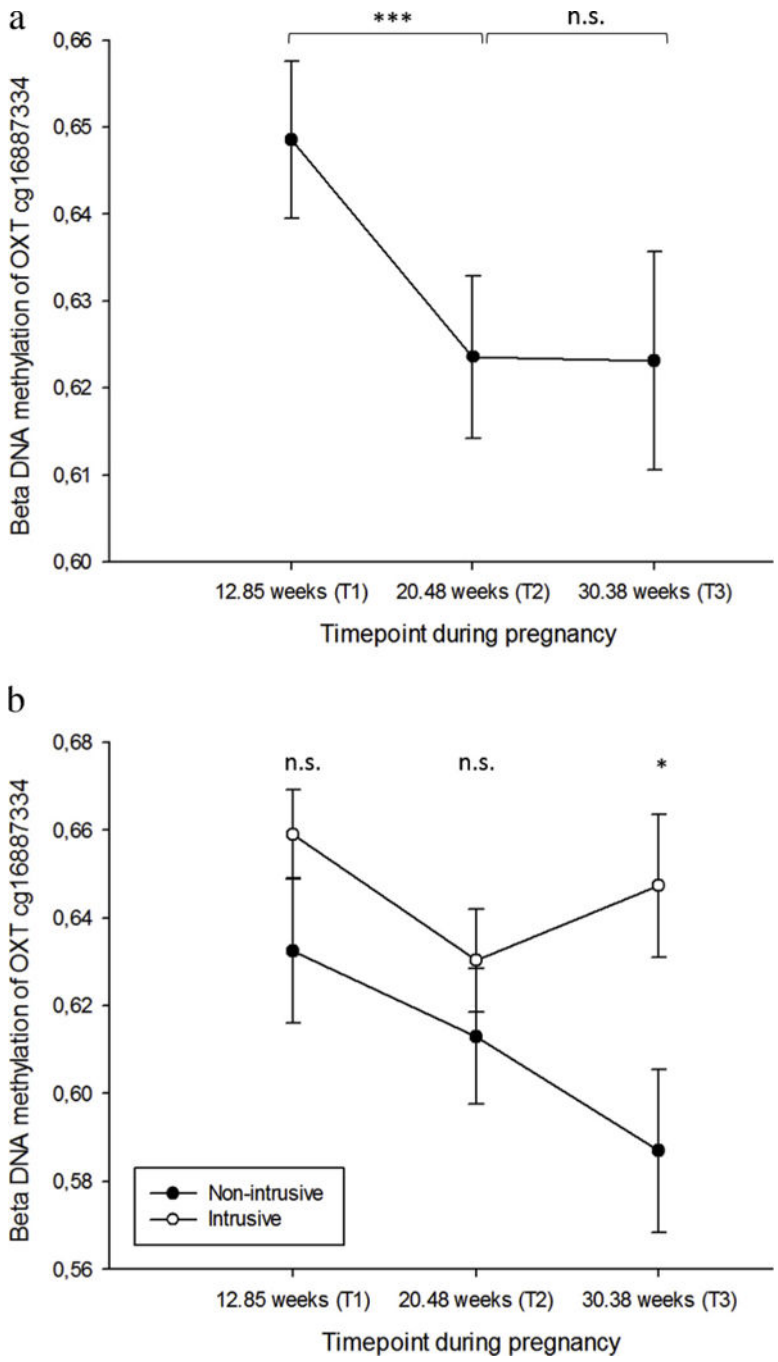

Fig. 2.

a DNA methylation of cg16887334 throughout gestation. Note: DNAm data are shown as mean ± SEM. Fig. 2b DNA methylation of cg16887334 throughout gestation by maternal intrusiveness (median split). Note: DNAm data are shown as mean ± SEM. Group sizes are N = 42 (39.25% of total sample) for the non-intrusive group and N = 65 (60.75% of total sample) for the intrusive group, respectively.

*p < .05.

Table 1

Sociodemographic characteristics of the sample by maternal intrusiveness[1].

Characteristics	Non-intrusive (N = 42; 39.25% of total sample)	Intrusive (N = 65; 60.75% of total sample)	Total sample (N = 107)
Maternal age (years; ± SD) at study entry	29.59 (3.28)	26.51 (5.70)[4]	27.75 (5.09)
Parity status (number of children ± SD) at study entry	1.12 (0.99)	1.03 (1.21)	1.07 (1.12)
Mother's Race/ Ethnicity (self-report; n and percentages)[2]			
Non-Hispanic White	24 (60.0)	20 (33.9)	44 (41.1)
White Hispanic	9 (22.5)	27 (45.8)	36 (33.6)
Black	–	1 (1.7)	1 (0.9)
Asian	2 (5.0)	5 (8.5)	7 (6.5)
Other	5 (12.5)	6 (10.2)	11 (9.0)
Maternal socioeconomic status (SES)[3]	3.65 (0.84)	3.01 (0.97)[4]	3.26 (0.97)

Note:

[1]Maternal intrusiveness and sensitivity groups are based on median split;

[2]missing values in race/ethnicity self-report: n = 8 (7.5%);

[3]SES is a composite measure of maternal education and annual household income, coded from 1 [low SES] to 5 [high SES]);

[4]significantly different from non-intrusive ($p < .01$).