Aus dem Institut für Medizinische Psychologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

The Role of Functional Oxytocin Receptor Gene Variants in the Intergenerational Transmission of the Effects of Maternal Childhood Maltreatment

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von

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This thesis is dedicated to my mother.

Abstract (English)

Objective: Women exposed to childhood maltreatment (CM) are more likely to exhibit insensitive parenting, which may have consequences for their offspring´s development. Variation in the Oxytocin-receptor gene (*OXTR*) moderates risk of CM-associated long-term sequelae associated with mother-child attachment, although functionality of previously investigated SNPs remained elusive. Here, we investigated the role of *OXTR* rs237895, a brain tissue expression quantitative trait locus (eQTL), as a moderator of the relationship between CM and maternal behavior (MB) and the association between MB and offspring attachment security.

Methods: Of 110 women with information on rs237895 genotype (T-allele=64, CC=46), n=107 have information on CM (CTQ) and n=99 on standardized observer-based ratings of MB at 6 months postpartum (responsivity and detachment), which were used in principal components analysis to obtain a latent factor representing MB. Offspring (n=86) attachment was evaluated at 12 months age. Analyses predicting MB were adjusted for socioeconomic status (SES), age, postpartum depression (PPD), and genotype-based ethnicity. Analyses predicting child attachment were adjusted for infant sex, SES, and PPD.

Results: rs237895 significantly moderates the relationship between CM and MB ($F_{1;66}$=7.99, p<.01), indicating that CM was associated with maternal insensitivity only in high *OXTR*-expressing T-allele carriers but not in low *OXTR*-expressing CC homozygotes. Moreover, maternal insensitivity predicted offspring insecure attachment (B= -.551; p<.05).

Conclusions: Women with a high *OXTR* expressing genotype are more susceptible to CM-related impairments in MB that, in turn, predicts attachment security in their children, supporting the role of the OT-system in the intergenerational transmission of risk associated with maternal CM.

Abstract (Deutsch)

Hintergrund: Frauen, die in ihrer Kindheit Misshandlung erfahren haben, weisen mit größerer Wahrscheinlichkeit wenig feinfühliges mütterliches Verhalten auf, was wiederum Konsequenzen für die Entwicklung des Nachwuchses haben kann. Genetische Variation im Oxytocin-Rezeptor-Gen (*OXTR*) moderiert den Zusammenhang zwischen früher Kindesmisshandlung und Langzeitfolgen, die wichtig für die Mutter-Kind-Bindung sind. Die Funktionalität genetischer Variation blieb bislang jedoch ungeklärt. In der vorliegenden Studie haben wir die Rolle des *OXTR* Einzelnukleotid-Polymorphismus (SNP) rs237895, einen hirnspezifischen *expression quantitative trait locus* (eQTL) als potentiellen Moderator zwischen Kindesmisshandlung und mütterlichem Verhalten als auch den Zusammenhang zwischen mütterlichem Verhalten und Bindungssicherheit des Nachwuchses untersucht.

Methoden: Von N=110 Frauen mit Information zum rs237895 Genotyp (T-Allel=64, CC=46) lagen zusätzlich Informationen von n=107 zu Kindesmisshandlung (Childhood Trauma Questionnaire; CTQ) und von n=99 zu standardisierten Beobachtungs-basierten Einschätzungen mütterlichen Verhaltens (Responsivität und Distanziertheit) vor. Bindungssicherheit der Kinder (n=86) wurde 12 Monate nach der Geburt beurteilt. Analysen, welche mütterliches Verhalten vorhersagen, wurden für sozioökonomischen Status, Alter, postnatale Depressionen und Genotyp-basierter Ethnizität kontrolliert. Analysen zur Vorhersage von Bindungssicherheit wurden kontrolliert für Geschlecht des Kindes, sozioökonomischen Status und postnatale Depression.

Ergebnisse: rs237895 ist ein signifikanter Moderator des Zusammenhangs zwischen Kindesmisshandlung und mütterlichem Verhalten ($F_{1;66}$=7.99, p<.01). Spezifischer konnte gezeigt werden, dass Kindesmisshandlung nur bei Trägerinnen des T-Allels (assoziiert mit stärkerer *OXTR*-Genexpression), nicht jedoch bei CC-homozygoten Frauen (assoziiert mit geringerer *OXTR*-Genexpression), mütterliches Verhalten vorhersagte. Darüber hinaus sagte verringerte mütterliche Feinfühligkeit eine unsichere Bindung des Nachwuchses vorher (B= -.551; p<.05).

Fazit: Frauen mit einem "hoch-exprimierendem" *OXTR*-Genotyp sind anfälliger für die Effekte von Kindesmisshandlung im Hinblick auf ihr späteres mütterliches Verhalten, welches wiederum Bindungssicherheit bei ihren Kindern vorhersagt. Die Ergebnisse der Studie zeigen die Bedeutung genetischer Variation im oxytocinergen System in der intergenerationalen Transmission von Risiko nach Kindesmisshandlung.

Framework for the Dissertation Thesis[a]

[a] The following parts of the theoretical framework are taken from my previously published review article, in which I am the sole first author: Toepfer, P., Heim, C., Entringer, S., Binder, E., Wadhwa, P., & Buss, C. (2017). Oxytocin pathways in the intergenerational transmission of maternal early life stress. *Neuroscience & Biobehavioral Reviews*, *73*, 293-308: 1. Introduction, 2. Oxytocin Signaling in the Central Nervous System – Physiology and its Role in Maternal Behavior (including subsections 2.1, 2.2, 2.3), 3. The Role of Genetic Variations in the Oxytocin Signaling Pathway in the Moderation of Individual Susceptibility to the Long-term Effects of CM Exposure. An additional section of paragraph 3 was taken from the empirical article Toepfer, P., O'Donnell, K. J., Entringer, S., Heim, C. M., Lin, D. T., MacIsaac, J. L., Kobor, M. S., Meaney, M. J., Provencal, N., Binder, E. B., Wadhwa, P. D., & Buss, C. (in press). A role of oxytocin receptor gene brain tissue expression quantitative trait locus rs237895 in the intergenerational transmission of the effects of maternal childhood maltreatment. *Journal of the American Academy of Child & Adolescent Psychiatry*.
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1. Introduction

It is well-established that the exposure to one or multiple forms of childhood maltreatment (CM), like childhood abuse and neglect, constitutes a major risk factor in the etiology of a wide range of somatic and/or psychiatric disorders.[1-3] However, it becomes increasingly apparent that these adverse long-term consequences are not restricted to the exposed individual alone but might be transmitted to the next generation,[4] who also are at increased risk for psychiatric and somatic disorders[5,6] – a phenomenon referred to as *intergenerational transmission.*[7] Several recent studies discuss postnatal transmission pathways, such as non-optimal parenting behavior and psychopathology in CM-exposed parents,[8] as well as offspring victimization.[9]

Within the context of the intergenerational perpetuation of the effects of maternal CM exposure, I emphasize the role of the neuropeptide oxytocin (OT) as a neuroendocrine factor modulating one of the key transmission pathways of maternal CM, i.e., maternal behavior.[10] OT has gained considerable attention in studies of human social behaviors,[11] including parenting[11-14] and attachment formation.[15] Interestingly, there is evidence that CM is associated with lower OT concentrations in the cerebrospinal fluid (CSF) of adult women[16] and non-human primates.[17] This CM-induced reduction in CSF-OT was linked to pronounced deficiencies in social behavior in the study by Winslow et al.[17] This suggests that central availability and functioning of the OT system may be susceptible to environmental factors such as CM, which persist into adulthood and may impact functional integrity of the "maternal brain".

To embed the empirical work of this doctoral thesis into a broader theoretical framework, the following questions need to be answered. First, what is the function of OT in brain circuits that mediate maternal behaviors? Second, what are the potential mechanisms that explain *how* CM exposure may affect OT signaling and thereby influences maternal behavior later in life? Third, what is the role of individual genetic differences in the OT signaling pathway that confer higher risk or resilience to the effects of CM with respect to maternal behavior? Fourth and in order to close the intergenerational cycle of CM exposure effects: What are the consequences of CM-induced impairments in maternal behavior for the subsequent generation?

Recent studies address some of these questions by focusing on common genetic variants in OT-pathway genes (i.e., genes that either code for the peptide and/or its receptor) that may interact with environmental factors (e.g., CM) to account for phenotypic variability, such as risk for emotional dysregulation, insecure attachment, depression, or suboptimal maternal behavior.[18-21] In particular, sequence variations in the coding region of the oxytocin receptor gene (*OXTR*) have emerged as candidates in numerous studies and will thus receive particular attention in the following. One major caveat in these gene-environment interaction studies is the fact that the

biological significance of the investigated *OXTR* genetic variants has not yet been elucidated. The empirical part of the proposed doctoral thesis aims to directly address this critical knowledge gap by investigating the combined effects of maternal CM exposure and functional *OXTR* variants – so called *brain tissue expression quantitative trait loci* (eQTLs) on maternal behavior in an intergenerational transmission framework.

2. Oxytocin Signaling in the Central Nervous System – Physiology and its Role in Maternal Behavior

2.1. Physiological Aspects of Oxytocin Signaling in the Brain

OT is a small nonapeptide which is highly conserved among mammalian species.[22] The OT gene (*OXT*), which first codes for a preprohormone that is then processed to OT and its carrier protein *neurophysin 1*, contains three exonic and two intronic regions and is located on chromosomal region 20p13 in humans.[23] Magnocellular neurons of the hypothalamic *paraventricular nucleus* (PVN) and the *supraoptic nucleus* (SON), the primary sources of OT synthesis, project to the posterior pituitary, where OT is stored in large secretory vesicles, so-called *large dense-core vesicles*. In response to calcium influx as well as intracellular calcium release from the endoplasmic reticulum, OT is released into systemic circulation[12] from axonal terminals within the neurohypophysis.[24,25] The most prominent peripheral OT effects via the classical hypothalamic-neurohypophyseal pathway are the induction of parturition through increased contractibility of the uterine smooth muscles and milk ejection from the mammary gland in response to suckling stimuli in lactating females.[11] In the central nervous system (CNS), however, OT´s communication pathways are more complex[26-28] and still subject to investigation. There are two proposed mechanisms through which OT neurons in the hypothalamus communicate with extrahypothalamic neurons and brain structures. First, it has been suggested that there is a slow, "unwired", and global transmission of OT that is released mainly from neuronal dendrites, but also from axons and soma in the hypothalamus to reach extrahypothalamic brain structures, such as the amygdala or the cingulate cortex.[29] This diffuse mode of communication, referred to as *volume transmission*,[24,27] enables OT to act as a neuromodulator within the brain and implies a slow enzymatic degradation of OT, which in turn permits OT to travel long distances.[27] Second, it has been shown that in the rodent[26] and human brain[29] there is a variety of "hard-wired" oxytocinergic nerve fibers from the hypothalamus to limbic, mesencephalic, and cortical brain regions that allow fine-tuned and fast modulation of target structures.[26,27,30] Effects of OT in the CNS and in the periphery (e.g., heart and cardiovascular system, kidney, reproductive organs) are critically dependent on

presence of OT-receptors (OTR).[31] The OTR is a 389-amino acid polypeptide and belongs to the G-protein coupled receptor superfamily.[31,32] The OTR expressing gene (*OXTR*) is located on chromosomal region 3p25-3p26.2, spans 17 kb, and consists of 3 introns and 4 exons.[31] Immunohistochemical and mRNA expression studies in the human *post mortem* brain demonstrated high OTR abundance in subcortical limbic structures (i.e., amygdala and hippocampus), the striatum (caudate nucleus and putamen), hypothalamic nuclei (medial preoptic area [MPOA], PVN, ventromedial nucleus), and anterior cingulate cortex.[29,33,34]

2.2 OT Brain Circuits Relevant for Maternal Behavior

Given this spatial distribution of OTRs in the brain, OT is able to modulate an array of CNS-dependent processes such as social cognition, motivated behavior, and emotion regulation.[35,36] within an extensive neural network that is involved in human and non-human mammalian caregiving.[35,37-40] This network is comprised of hypothalamic nuclei, importantly the MPOA, the *mesocorticolimbic system*, which encompasses the amygdala, the hippocampus, the *nucleus accumbens* (nAcc), the *ventral tegmental area* (VTA), the *prefrontal cortical regions,* the *ventral pallidum,*[41-43] as well as the *dopaminergic nigrostriatal pathway.*[39] Extensive research in rodents indicates that OTRs are abundantly expressed within this network rendering it sensitive to the effects of OT.[41,44] Already *during* pregnancy, the MPOA, which is thought to constitute the core of this network, is hormonally primed, likely through an estrogen (E)-induced upregulation of *Oxtr* expression.[44-47] This E-primed network is then "triggered" by pup stimuli after parturition to result in immediate onset of maternal behavior, which is then maintained in the postpartum period.[44,48] Infusion of OT into the MPOA and VTA facilitates postpartum onset of maternal behavior and infusion of an OT antagonist into these regions can block pup retrieval and nursing.[49] In rodents, it was demonstrated that dams characterized by high amounts of pup licking and grooming (high LG) as compared to low LG dams showed increased *Oxt* expression in the MPOA and the PVN, as well as stronger oxytocinergic projections from the MPOA and PVN to the VTA.[50] Infusion of OT into the VTA increased dopamine (DA) turnover in the nAcc, and naturally high LG mothers but not low LG mothers showed elevated DA signaling in the nAcc during episodes of interactions with their offspring. Interestingly and similar to the finding by Pedersen et al.,[49] infusion of an OTR-antagonist into the VTA eliminated these differences.

Despite being less robust than the mechanistic findings in rodents, studies on human parenting also support the notion of an important role of OT in the above described brain circuits. In observational studies, maternal plasma OT correlates with aspects of maternal behavior, such

as “motherese” vocalizations and affectionate touch.[14] Looking at functional changes in the CNS, intranasal OT administration led to an increased activation of the VTA after presentation of reproduction-associated stimuli, e.g., pictures of infant stimuli in healthy women.[51] Furthermore, in response to infant cry sounds, women´s amygdala reactivity was diminished by intranasal OT.[52] Simultaneously, intranasal OT application led to increased activity in the insula and inferior frontal gyrus after hearing infant crying.[52] These findings indicate that OT may selectively attenuate reactivity in limbic structures involved in stress and anxiety (amygdala), while simultaneously enhancing activity of dopaminergic motivational circuits that underlie reward learning and parenting behaviors (VTA). In addition, OT may contribute to enhanced emotion recognition of salient infant social cues through increased activation of specific brain regions (insula and inferior frontal gyrus) that are part of the empathy and salience related brain networks.[53,54] It should be acknowledged, however, that imaging studies using intranasal OT challenge are controversial.[55] For example, although administering supraphysiological doses of OT, only a very small fraction can be detected in the CSF[55] and it is questionable whether this has direct modulating effects on brain functioning and behavior.[56] Furthermore, it is very likely that strong increases of peripheral OT concentrations after intranasal OT application have effects on peripheral organs (e.g., the heart, gastrointestinal tract, reproductive tract) – and might confound the direct effects of OT in the brain.

Despite these methodological drawbacks, central OT seems to play a fundamental role in shaping human maternal behavior, which is substantially supported by animal data. Its effects are in part dependent upon regulation by other hormones (e.g., E), target brain structures (e.g., amygdala vs. VTA), or interactions with other neurotransmitter systems (e.g., DA). Drawing primarily from experimental work in rodents, it must be kept in mind that inferences to humans should be made with necessary precaution due to important cross-species differences in parenting behaviors and its underlying neurobiology. More research in humans is thus warranted to better understand how OT affects brain circuits that are involved in parent-offspring interactions and motivated maternal behavior.

2.3 Effects of CM Exposure on OT Brain Circuits Relevant for Maternal Behavior and Implications for Intergenerational Transmission of Maternal CM

At early stages in the postpartum period, the formation of selective and enduring interpersonal bonds between a mother and her newborn is the basis of healthy, normative child development.[57,58] Critical for the successful establishment of this mother-child bond is the mother´s ability to adequately use a behavioral repertoire, which includes child-directed gaze,

affectionate touch, “motherese” vocalizations, establishment of physical proximity, the expression of positive affect, as well as the regulation of distress and negative emotions. The ability of the mother to react immediately and appropriately to the child´s signals during episodes of dyadic interaction is referred to as maternal sensitivity[59] and is thought to foster secure attachment in children.[60] Importantly, maternal CM exposure may critically impair maternal sensitivity and thus the quality and establishment of the mother-child bond. Consistent with this notion, CM-exposed as opposed to unexposed mothers exhibit less sensitivity,[61,62] more hostility,[4,63] more rejection,[8] and higher intrusiveness[64,65] towards their children. Together, these observational studies strongly suggest altered maternal behavior in women with CM exposure. It is now crucial to understand how the neural circuitry that underlies maternal behavior and involves OT signaling can be influenced by early experiences, such as CM exposure, to perpetuate a vicious circle of dysfunctional attachment formation.

The effects of early life experiences on key regions of the neural circuits that underlie parenting have been mechanistically studied in animal models.[45,66,67] In female offspring of rats, a low level of maternal care (LG) is associated with lower *Oxtr* expression in the MPOA, PVN, the central nucleus of the amygdala, and the lateral septum.[45] Moreover, downstream targets of hypothalamic OT projections in the mesolimbic system, i.e., the VTA and the nAcc, are equally affected by early rearing experiences. Offspring of low LG dams exhibit reduced dopaminergic projections from the VTA, as well as lower expression of dopamine receptors in the nAcc.[67] These experience-induced differences in OT-DA neural circuits that underlie parenting correspond to observable parenting behaviors. Lower levels of *Oxtr* expression are associated with less maternal responsiveness towards pups.[45,66] This intergenerational transmission of maternal behavior in rats is partly mediated by *epigenetic modifications* of the estrogen receptor α gene with downstream effects on *Oxtr* gene expression in the brain. A detailed description of the role of epigenetic modifications in the context of intergenerational transmission of maternal CM and trauma exposure effects is beyond the scope of this dissertation and has been reviewed elsewhere.[68]

An experience-dependent transmission of early life experiences including maternal CM exposure via OT neural pathways is also assumed in humans[38,69-71] although direct empirical evidence is very limited. As indicated above, adult women who report forms of CM, i.e., emotional and physical abuse, exhibit decreased OT concentrations in CSF compared to non-abused women,[16] suggesting that CM has enduring effects on central OT availability. It is highly plausible that these decreased CSF OT concentrations that occur as a function of CM exposure have implications for maternal behavior, but this has not been directly studied to date.

Investigating the neurobiological consequences of CM exposure in the maternal brain, two recent functional MRI studies have demonstrated that mothers with and without CM exposure differ in their brain responses while completing attachment related tasks.[62,72] For example, when asked to imagine a conflictual interaction with their children, CM exposed women show increased reactivity in salience network structures (i.e., amygdala and insula). In contrast, women without CM exposure showed similar activation while imagining pleasant interactions with their children. Another study in primiparous women found that mothers who were classified as insecurely attached as opposed to mothers with a secure attachment style had lower peripheral OT concentrations after interaction with their infants. The maternal OT rise after interaction with their infants correlated positively with maternal brain activation in the *ventral striatum* after presentation of visual stimuli of their own child. Involvement of the ventral striatum, which is part of the reward-related dopaminergic nigrostriatal system, suggests that pictures of one´s own child may have a higher "incentive value" for securely attached mothers and hence facilitate maternal responsiveness and approach.[73] On the other hand, adult women with an insecure attachment representation show increased activation of the amygdala in response to baby cries[74] and heightened amygdala reactivity may partly stem from insufficient OT signaling.[75] One may speculate that mothers with an insecure attachment style, which can be a result of problematic parental care experiences in the mother´s own childhood, are more prone to perceive infant distress as aversive, which can lead to frustration or even abusive behavior towards the child.[76] In addition, hyper-reactivity of the amygdala in response to infant distress is a neural correlate for maternal intrusive parenting,[77] which in turn is a risk factor for higher infant anxious and depressive behaviors.[78] The relevance of these findings to the proposed intergenerational framework is immediately evident: they suggest that early maternal attachment experiences (in her own childhood) or CM may contribute to alterations in CNS OT signaling pathways, which underlie maternal behavior with ensuing consequences for the next generations´ development.

3. The Role of Genetic Variations in The Oxytocin Signaling Pathway in the Moderation of Individual Susceptibility to the Long-term Effects of CM Exposure

It is important to note however that not all women (and their offspring) are equally vulnerable to the long-term consequences of CM exposure. One prominent line of research investigating factors that convey differences in long-term liability after CM exposure has focused on the moderating role of common genetic variants, so-called single nucleotide polymorphisms (SNPs) in genes that play a biologically plausible role in a phenotype of interest, e.g., maternal

behavior.[71] Among several candidate genes, e.g. *DRD2/4* (dopamine receptors 2 and 4) and *5HTT* (serotonin transporter) that have empirical support for either parenting or individual differences in susceptibility after CM exposure, common sequence variations in oxytocin pathway genes (*OXTR* and *OXT*) have attracted considerable attention.[79]

First evidence for a gene-environment interaction including *OXTR* SNPs reported that rs53576 GG homozygous subjects who experienced three or more types of CM were at higher risk for adult emotional dysregulation and a disorganized attachment style than A allele carriers, indicating a higher susceptibility to CM in G allele homozygotes.[18] This finding was partly replicated in a sample of university students.[20] Moreover, it could be shown that rs53576 GG homozygous subjects with CM exposure have a smaller bilateral volume of the ventral striatum, a key structure of the dopaminergic nigrostriatal pathway[80] which is implicated in both depression risk[81] and parenting behavior.[73] A very informative gene-environment interaction in the prediction of maternal parenting found that rs53576 GG mothers exhibit differential maternal sensitivity towards their 2-year old children, varying as a function of inter-parental conflict.[82] Specifically, G allele homozygous women under high marital strain were less sensitive in interaction with their offspring than A allele mothers, whereas under conditions of low marital conflict this pattern reversed – GG allele mothers were more sensitive than their A allele counterparts. Despite the focus on *OXTR* SNPs,[83] there is also evidence for the moderating effects of other SNPs in OT pathway genes that may help to better understand how environmental exposures might affect individuals differently. A sequence variation in the *OXT* gene locus (rs2740210, A/C) has been shown to interact with maternal CM to predict postpartum depression (PPD) and breastfeeding duration in two independent samples.[84] Mothers homozygous for the C allele tended to discontinue breastfeeding much earlier when they were exposed to CM than women who carried the protective A allele. Likewise, CC homozygous mothers were more vulnerable to the long-term effects of CM with regard to development of PPD symptoms at six months postpartum. Moreover, only in CC homozygous individuals, the association of CM and breastfeeding duration was mediated by maternal depression. Another study could indirectly replicate and extend this finding,[21] as maternal CM in interaction with rs2740210, but also with *OXT* rs4813627 (A/G), predicted maternal PPD. CC (rs2740210) homozygous and GG (rs4813627) homozygous mothers exposed to CM reported significantly more depressive symptoms at 6 months postpartum compared to A allele carriers of the respective SNPs. Somewhat counterintuitively, there was also a negative association between maternal CM and maternal instrumental care in mothers homozygous for these genotypes; i.e., homozygous mothers (CC, GG) with CM exposure displayed *more*

instrumental care towards their children compared to women exposed to low CM. In this study, the mother-child interaction had been designed to minimize instrumental care behaviors (e.g., cleaning the child) and elicit spontaneous free play. This may indicate that women with a more susceptible genotype and adverse early experiences may have more difficulties engaging in spontaneous free play and rely on more structured and instrumental caregiving.

As shown above, there is accumulating evidence for common genetic variants in the OT pathway (i.e., *OXTR/OXT) in* moderating the association between CM and long-term risk for a variety of phenotypes that are relevant in the context of mother-child attachment formation, such as adult attachment style, breastfeeding, and maternal behavior. However, it has been unclear until now *why* genetic variants in *OXTR* or *OXT* may have such moderating effects. In other words, nothing is known about the biological functionality of these SNPs. The first study to address functionality of *Oxtr* SNPs demonstrated that in monogamous prairie voles, genetic variation in *Oxtr* strongly predicted *Oxtr* gene expression in the nucleus accumbens, accompanied by significant behavioral effects.[85] Compared to animals carrying the "low *Oxtr*-expression" genotype, homozygous "high *Oxtr*-expression" genotype carriers displayed significantly more attachment towards their partner providing first mechanistic insight into the behavioral consequences of a striatal *Oxtr* expression quantitative trait locus (eQTL).[85] Although not considering the moderating role of environmental variation, this study added a critical piece of evidence to better understand how *Oxtr* genetic variation may contribute to behavioral differences. Collectively, these data suggest that genetic variation in *OXTR* might contribute to individual differences in sensitivity to the (early) social environment, mediated by increased reactivity of neural circuits that underlie response to the social environment as well as MB.

Addressing the critical issue of functional *OXTR* gene variants in the intergenerational transmission of maternal CM was the purpose of the study conducted by Toepfer et al.[86] Detailed information on the methods, results as well as implications for practice and future research will be presented below.

4. The present study: A Role of Oxytocin Receptor Gene Brain Tissue Expression Quantitative Trait Locus rs237895 in the Intergenerational Transmission of the Effects of Maternal Childhood Maltreatment[b]

4.1. Methods

Participants:

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. The cohort is described in greater detail elsewhere.[87] The UCI Institutional Review Board approved all study procedures, and all participants provided written informed consent.

Genotyping, SNP imputation and *OXTR* eQTL selection: DNA extraction was performed on fasting blood samples collected during the first trimester of pregnancy (N=121). Whole-genome SNP genotyping was performed using Illumina HumanOmniExpress BeadChip according to the manufacturer's standard protocols. Quality control (QC) of genotype data was performed using PLINK v1.9.48, where variants with call rates < 98%, minor allele frequency (MAF) < 5%, or Hardy-Weinberg Equilibrium (HWE) test P < 1×10-6 were removed. A total of 599.935 genotypes passed QC and all mothers had genotyping rates > 98% with no gender mismatches. Twelve mothers were identified as relatives (i.e., >25% shared genotype), where one individual of each related pair was removed (n=6), leaving n=115 mothers for the analysis. To complete the dataset and obtain genotype information of variants not covered on the array, we used imputation to predict the unobserved genotypes by linkage disequilibrium (LD) with the

[b] The following sections are taken from my previously published original article, in which I am the sole first author: Toepfer, P., O'Donnell, K. J., Entringer, S., Heim, C. M., Lin, D. T., MacIsaac, J. L., Kobor, M. S., Meaney, M. J., Provencal, N., Binder, E. B., Wadhwa, P. D., & Buss, C. (in press). A role of oxytocin receptor gene brain tissue expression quantitative trait locus rs237895 in the intergenerational transmission of the effects of maternal childhood maltreatment. *Journal of the American Academy of Child & Adolescent Psychiatry*. 4.1 Methods, 4.2 Results, 4.3 Discussion. All referenced tables and figures appear in the original paper.

genotyped SNPs of the array based on an external reference panel of haplotypes. Genotype data were imputed to the 1000 Genomes Phase 3 reference panel using SHAPEIT2[88] and IMPUTE2.[89] After imputation, variants with a MAF < 5% or an INFO metric < 0.8 were removed. The information metric (INFO metric) takes ranges between 0 and 1, where values near 1 indicate that a SNP has been imputed with high certainty. The INFO metric is used to remove poorly imputed SNPs. Although there is no consensus in filtering the imputed datasets based on uncertainty of imputation, we used a conservative threshold of 0.8. To select a functional *OXTR* variant in this subset of n=115 women, we used the GTEx database (gtexportal.org).[90] Using the tissue eQTL visualizer, we identified a haplotype of twelve *OXTR* brain tissue eQTLs (LD cutoff: $r^2 \geq .2$) spanning approximately 8kb (Chr20: 8804371-8812411bp). From this haplotype, we sought to identify an *OXTR* brain tissue eQTL that satisfies two criteria. First, the eQTL should significantly predict gene expression in brain areas known to be involved in MB and social information processing (i.e., ventral striatum, amygdala, anterior cingulate cortex and frontal cortex). To achieve this, GTEx provides a multi-tissue eQTL visualizer indicating the strength, direction and p-value of an eQTL as well as a metric (m-value for posterior probabilities ranging between 0 and 1) that indicates whether a given SNP has meta-analytical evidence for having an eQTL effect (m-value ≥ .9) in a discrete brain region.[91] Second, the eQTL should best represent this *OXTR* eQTL haplotype in our study sample consisting primarily of two ethnicity groups (self-identified non-Hispanic white and self-identified Hispanic). To that end, we conducted a SNP-tagging analyses (https://snpinfo.niehs.nih.gov/snpinfo/snptag.html) for the two ancestry groups separately (based on 1000 genomes CEU and MXL populations) by applying a LD threshold of $r^2 \geq .2$. The resulting SNP that meets these two criteria is rs237895 (T > C). In all brain regions of interest, this SNP has a significant eQTL effect, i.e., predicts gene expression in caudate nucleus (normalized effect size/NES = -0.517, -log10 p-value = 1.2e-10, m-value = 1), putamen (NES = -0.419, p = 1.1e-5, m-value = 1), amygdala (NES = -0.428, p = 6.6e-4, m-value = 1), nucleus accumbens (NES = -0.317, p = 7.6e-4, m-value = 1), ACC (NES = -0.387, p = 2.1e-5, m-value = 1), and frontal cortex (NES = -0.324, p = 7.0e-5, m-value = 1; see supplemental **Table S1**). The negative direction of the effect indicates that the reference allele (T) is associated with higher gene expression compared to the alternative allele (C). As an example, supplemental **Figure S1** depicts *OXTR* gene expression in the caudate nucleus, which is allele-load dependent, i.e. the homozygous T-allele carriers exhibit the highest, T/C heterozygotes intermediate, and the homozygous C-allele carriers the lowest gene expression. Furthermore, also using the xQTL database (http://mostafavilab.stat.ubc.ca/xQTLServe), in an independent

sample of post mortem brain tissue (N=494) rs237895 significantly predicts *OXTR* expression in the dorsolateral prefrontal cortex (r=-.523; p < 8.0e-10).[92] Since rs237895 is not covered on the HumanOmniExpress BeadChip, genotypes of rs237895 from the N=115 genetically unrelated women were extracted from the imputed data for all participants in this study. Rs237895 genotype could be imputed for N=110 women (T/T = 18, T/C = 46, C/C = 46) with sufficient quality (i.e., INFO metric >0.8), who were subsequently included in the statistical analyses. For the analyses, we assigned women to two genotype-groups depending on the presence of the high *OXTR* expressing T-allele (T/T and T/C combined, n=64) or absence of the same, i.e., low expressing CC-homozygotes (n=46).

Maternal CM Exposure: Women provided self-reports about CM exposure using the Childhood Trauma Questionnaire (CTQ),[93] one of the most widely-used, reliable, and valid instruments to retrospectively assess early experiences of abuse and neglect. The CTQ assesses five different types of CM: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect.[93] For each individual CTQ-subscale, we used established cut-off values (emotional abuse ≥ 13; physical abuse ≥ 10; sexual abuse ≥ 8; emotional neglect ≥ 15; and physical neglect ≥ 10) to create a binary variable indicating moderate or severe exposure for any of the five CTQ-subscales. Additionally, an overall binary variable was computed based on the CTQ to indicate moderate/severe childhood maltreatment (CM+) on at least one of the five CTQ-scales vs. no exposure to childhood maltreatment (CM-). We chose to not use the CTQ sum score as a predictor in the statistical analyses because it was not normally distributed as indicated by a Kolmogorov-Smirnov test ($D_{(107)}$= .20; p<.001).

Maternal Postnatal Behavior: At six months postpartum, a home visit was conducted. Research staff was trained to reliably assess maternal emotional and verbal responsivity towards the infant using the Home Observation Measurement of the Environment Infant-Toddler version (HOME-IT).[94] Raters were considered reliable once they had two consecutive observations where 95% of responses matched the responses of a rater with extensive experience in coding the HOME-IT. The responsivity scale of the HOME includes 11 items capturing different aspects of maternal responsivity including maternal vocal reactivity to the infant or display of positive affect towards the infant. The internal consistency was moderate (*Cronbach´s alpha* =.61) and comparable to previous studies reporting psychometric properties of this scale.[94,95] At the same visit and in addition to the HOME assessment, mothers were instructed to engage in a 15-minute standardized play situation as described in Jaeger.[96] The play situation was

video-recorded and subsequently coded by two trained and reliable independent observers (intra-class correlation coefficient [ICC] >.9) using the coding manual of the NICHD Early Child Care Research Network.[96] We here focused on non-optimal MB and coded maternal detachment (1 = "not at all characteristic" – 5 = "highly characteristic"). Highly detached mothers appear emotionally uninvolved and disengaged during dyadic play, do not react to the child´s signals in a contingent manner and can thus be considered unresponsive. In a next step, we conducted a principal component analysis (PCA) using the two maternal behavioral phenotypes responsivity and detachment. Results of the PCA indicated a one-factor solution (eigenvalue of factor 1 = 1.19; eigenvalue of factor 2 =.81). Detachment (.78) loaded positively, while maternal responsivity (-.78) loaded negatively on this extracted latent factor. Our one-factor solution explained 60% of the total variance. Higher scores on this latent variable indicate less optimal MB (i.e., higher detachment and lower responsivity) and we thus termed it "maternal insensitivity".

Infant Attachment at 12 Months Age: Infant attachment security was assessed at twelve months during the Strange Situation Procedure (SSP).[60] The SSP, a standardized laboratory observation consists of eight episodes, each three minutes long. These episodes include short periods of interaction between the mother and child, interaction between the child and an unfamiliar female stranger, and separation of the child from the mother followed by a reunion episode during which infant attachment is coded. Infants were categorized into three different types of attachment: securely attached (B), insecure-avoidant (A), and insecure-ambivalent (C). The relative frequencies of attachment categories was as follows: 62.2% were classified as securely attached (B), 30.6% as insecure-avoidant (A), and 7.1% as insecure-ambivalent (C). We used the dichotomous secure–insecure grouping (i.e., B vs. A and C) for data analysis because of the relatively small group size of type C attachment. Classification of attachment was completed by one rater with extensive experience in the assessment of attachment.

Covariates: Analyses testing the predictive value of maternal CM and rs237895 genotype interactions for MB were adjusted for the potential confounding effects of variables that have been shown to be associated with CM exposure, the observed phenotype (MB), or both (see supplemental **Table S2**). For this set of analyses, the covariates included maternal age, socio-economic status (SES), maternal depressive symptoms and racial/ethnic differences in genetic background. Information on annual household income and education (highest degree obtained) were aggregated to a composite measure indicative of socio-economic status (SES) as

previously described in our study sample.[87] Women provided self-reports on postpartum depressive symptoms (PPD symptoms) using the Edinburgh Postnatal Depression Scale (EPDS),[97] a widely-used valid screening tool for PPD symptoms on three occasions during the first postnatal year (i.e., at one, six and twelve months). In order to account for the different time points in assessing the maternal phenotype (at six months) and infant attachment (at twelve months), we averaged EPDS-scores (all highly correlated across postnatal visits: r= .606 - .767; all correlation p-values <.001) depending on the predicted outcome. More specifically, we used an average of EPDS scores including the one and six month's assessments for analyses predicting maternal behavior at six months and an average including all three EPDS scores (one, six, twelve months) in the model predicting infant attachment at twelve months. Racial/ethnic 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 (see supplemental **Figure S2**). We also took parity status into account, which has been shown to predict differences in MB. All analyses testing the association between MB and offspring attachment were adjusted for SES, infant sex, and PPD symptoms.

Statistical Analyses: All statistical analyses were performed using IBM SPSS version 22©. Prior to testing the gene-environment interaction in the prediction of MB, we evaluated the main effect of CM exposure on maternal behavior using a linear regression model, while controlling for all potential confounding variables (i.e., maternal age, SES, PC1-3, PPD symptoms). Maternal G-E analyses, were conducted using the SPSS PROCESS macro.[98] In the simple moderation analyses (Model 1), the binary maternal CM exposure variable (CM+/CM-) was entered as the main predictor, the latent MB factor as the outcome, maternal dichotomous *OXTR* rs237895 genotype (T-allele vs. CC-homozygotes) as the moderator and maternal age, SES, genotype-based ethnicity, and maternal PPD symptoms as the covariates. For the prediction of attachment security (secure vs. insecure), a logistic regression analysis was performed using MB as the predictor and infant sex, SES, and PPD symptoms as covariates.

4.2 Results

Sample characteristics: Information on socio-demographic characteristics, CM-experience, MB, PPD symptoms, and infant attachment are shown in **Table 1** for the total sample (N=110) and stratified by rs237895 genotype as well as CM-exposure status. Importantly, neither CM-

exposure (p >.93), nor maternal insensitivity (p >.82), nor infant attachment (p >.5) were significantly different between the genotype groups. Compared to women in the CM – group, CM+ subjects have a lower SES (p<.01) and report more PPD symptoms throughout the first year postpartum (all p-values <.05). CM groups did not differ in MB or infant attachment (**see Table 1**). Inter-correlations of all study variables are displayed in **s**upplemental **Table S2**.

Maternal CM exposure and rs237895 genotype effects on MB: The linear regression model that tested the association between CM exposure and MB revealed no significant main effect of CM exposure (*b* = -.31, p=0.2). The main moderation model accounted for 38.36% of the variance in MB (F(9,66) = 4.56, p < 0.001; see **Table 2**). The maternal CM x rs237895 interaction was significantly associated with MB (F(1,66) = 7.99, p < 0.01). Confirming our hypothesis, post-hoc analyses revealed that only women carrying the high *OXTR* expressing T-allele showed significant differences in maternal insensitivity depending on CM exposure (t = -.26; p = 0.015; Cohen´s d = .92), with CM-exposed women showing greater maternal insensitivity than non-CM exposed women. (**Figure 1**, p. 62). In low-expressing CC-homozygous women, CM was not associated with MB (t=.83; p =0.4; Cohen´s d = -.26).

MB and Infant Attachment at 12 Months: MB significantly predicted offspring attachment security at 12 months (B= -.551; p<.05; Cohen´s d = -.51; **Figure 2**) after controlling for SES, infant sex, and PPD symptoms. Infant attachment was higher in children of women with less maternal insensitivity. In other words, securely attached infants are overrepresented in the group of women who, based on median split, exhibited higher sensitivity (77.8% securely attached infants versus 22.2% insecurely attached). In women with greater insensitivity (> median), the prevalence of insecurely attached children increased 2-fold (45.0 % insecure attachment) compared to the group exhibiting low insensitivity.

4.3 Discussion

To the best of our knowledge, the findings described here provide first evidence of the moderating role of a functional *OXTR* variant in the process of intergenerational transmission of the effects of maternal CM exposure. Only women carrying the high *OXTR* expressing T-allele exhibited significant differences in MB in conjunction with CM experience, with CM-exposed women experiencing greater insensitivity than non-CM-exposed women do. MB in C-allele homozygous women appears to be less impacted by CM exposure, indicating reduced behavioral adaptations after CM exposure in these individuals. Unlike previous studies that

have either demonstrated a main effect of CM exposure[61] or *OXTR* genotype[13] in predicting MB, our findings highlight the importance of gene-environment interactions to predict MB. It appears that these observations are in accordance with the Differential Susceptibility Theory (DST).[99] However, the mere absence of early adversity (i.e., CM-) (conditions under which T-allele carrying mothers show the least amount of insensitivity) does not, *per se*, implicate the presence of a supportive and enriched early environment, which is an important premise of the DST framework that cannot be addressed in the current study. The CTQ, our environmental exposure, is not designed to capture positive aspects of the early environment. Nevertheless, our results support *OXTR* rs237895 functioning as a genetic moderator, and they are in line with prior research. It has been previously shown that genetic variation in *OXTR* predicts limbic reactivity to social cues[100] and MB[13] and moderates the association between CM exposure and depression as well as disorganized adult attachment.[18,20] By adopting a biologically informed SNP-selection strategy,[90,92] the present study corroborates, extends, and strengthens this line of research. In accordance with recent theoretical frameworks that postulate a role for oxytocin in modulating the salience of social cues,[54] we propose that genetic variation in *OXTR* eQTLs (e.g., rs237895) may operate through increased genotype-dependent *OXTR* expression in socially sensitive neural networks as an important neurobiological mechanism conferring heightened social-environmental susceptibility.

But why would mothers differ in the degree to which they adapt their reproductive (i.e., MB) strategies after CM exposure? Environmental variation, especially early social experiences (e.g., the mother´s CM exposure) may operate via MB to shape offspring development, thereby ultimately promoting reproductive fitness in the next generation.[101] Strong support for this "maternal mediation hypothesis" comes from rodent studies showing how natural variations in MB (licking and grooming [LG]) may induce persistent behavioral and neurobiological changes in offspring.[101] As examples, offspring of low LG dams exhibit heightened stress-reactivity[102] and increased fearfulness,[103] phenotypes that promote survival in a dangerous environment. Furthermore, female offspring of low LG dams show alterations in MB consistent with their own rearing experience.[66] Directly translating this line of research to humans, we would predict that women exposed to CM should adapt their MB (i.e., lower responsivity, higher detachment) accordingly to transmit information about their own past aversive environment to their offspring. However, our data suggest otherwise, since the association between maternal CM exposure and MB appears to be dependent on maternal *OXTR* genotype. A possible explanation for this observation is the concept of bet-hedging.[104] Since the future is inherently unpredictable and early experiences (e.g., CM exposure) may not always accurately predict the future

environment (e.g., dangerous/adverse environment for offspring), natural selection has maintained genes for both environmentally susceptible (e.g., high *OXTR* expression) as well as less susceptible (e.g., low *OXTR* expression) developmental strategies, to ultimately increase fitness payoffs regardless of environmental continuity.[99]

These possibly adaptive reproductive strategies may, however, come at a cost from the lens of a developmental psychopathology perspective rather than an evolutionary one. We show that less responsive and more detached MB is associated with insecure attachment in her child at 12 months age, which is in accordance with prior research.[60,105] Insecure attachment itself predicts anxiety,[106] internalizing and externalizing behavior[107] among other phenotypes, closing the cycle of intergenerational transmission of early life experiences.

Previous research in humans and animals has shown that MB is hormonally primed, and that this process starts as early as during pregnancy itself,[108] partly mediated via estrogen-induced up-regulation of oxytocin receptors.[109] An open question now is whether *OXTR* eQTLs exert their effects on brain gene expression through variable accessibility of transcription factors to chromatin. Given the fundamental role of sex-steroids in regulating *OXTR* gene expression and the fact that sex-steroids dramatically increase during pregnancy, this period represents a time window of critical importance to better understand the contribution of *OXTR* genetic variation in the association between CM and MB. Moreover, it is possible that additional prenatal factors, such as alterations in CM-associated maternal-placental-fetal stress physiology operate as mechanisms in the intergenerational transmission of risk associated with maternal CM exposure.[110] It remains to be elucidated whether these transmission pathways differ systematically between women carrying high or low susceptibility variants of rs237895. Moreover, in addition to maternal interactive behavior, future studies in the context of intergenerational transmission during the postnatal period should consider other postnatal variables such as breastfeeding status and breast milk composition, which may be different based on maternal CM experience. This is a relevant avenue of research aimed at understanding the mechanisms underlying intergenerational transmission of maternal CM given the common underlying neurobiology for breastfeeding and MB that crucially involve efficient OT-signaling.[111]

MB is a complex phenotype emerging from extensive inter-connected neural circuitry underlying a wide array of executive, cognitive, motivational and self-regulatory functions,[40] and can be modulated by early childhood experiences,[10,66,112] OT-signaling,[35] and interactions of OT with other neurotransmitters such as dopamine among many others.[40] It would be informative for future studies to employ neuroimaging assessments to characterize neural

functional and/or structural differences after CM exposure in genetically susceptible women. This will then provide further insights into the neural underpinnings of the associations between CM exposure and variation in MB. The SNP under investigation here, rs237895, predicts *OXTR* expression across multiple brain regions that are critical for MB, cognition and motivation (e.g., amygdala, ventral striatum [VS], ACC, PFC), raising the possibility that alterations in some or even most of the above-mentioned OT-associated functions might be critically altered in T-allele carriers after CM. Intriguingly, a previous study by Loth and colleagues has shown that another intronic *OXTR* SNP (rs237893, A>G), which tags the same *OXTR* eQTL haplotype as rs237895, predicts activity in the VS in response to social cues in an allele-load dependent manner.[100] VS reactivity was highest in high *OXTR*-expressing AA carriers and lowest in low expressing GG carriers.[100] Bearing in mind the well-documented role of OT-signaling in the VS for MB (e.g., affecting salience and reward of infant stimuli as well as infant-directed behavior),[77] the findings by Loth et al, by supporting the notion of higher social sensitivity in individuals carrying a high *OXTR*-expression genotype, provide important insights into intermediate phenotypes at the intersection of gene-behavior associations that may theoretically vary depending on the early environment.

There are several limitations of the current study including the relatively small sample size and the lack of an independent replication sample. From a methodological point of view, a moderated mediation analyses would have been more suitable to test the entire intergenerational pathway from maternal CM-exposure to infant attachment in the next generation. However, the resulting sample size in the full model with no missing data for both mothers and children would have been relatively small (n=69). Consequently, the full model predicting attachment security, while including all covariates would have been vulnerable to overfitting in such a small sample, which is why we decided to test the paths in 2 separate models. In addition, we had to group the T-allele carrying women together for practical reasons because the homozygous T-allele group only included n=18 individuals. Given the allele-load dependent eQTL effect of rs237895, it would be interesting, in future larger samples to test the CM-MB association for all three groups of genotype separately. Also, rs237895 is not covered on the array used for genotyping. Thus, we performed an LD-based imputation and applied a conservative threshold (INFO metric >0.8) to acquire maternal genotype data with sufficient, albeit not perfect certainty. Moreover, only healthy pregnant women and their children participated in the study, limiting the number of women with severe CM exposure. Nevertheless, the prevalence estimate of CM exposure in the study sample is comparable with recent epidemiological data on CM exposure in the general population.[113] A retrospective self-report measure (CTQ) was used to

assess maternal CM. While there were no differences in reported severity of CM between genotype groups and analyses adjusted for current mood, other potential variables that may influence self-reported childhood experiences (e.g., forgetting, recollection bias, or non-disclosure) cannot be ruled out entirely. Following recent recommendations,[114] we utilized objective observation-based ratings of MB to quantify our outcome, and raters were blind to maternal genotype and CM exposure, thereby strengthening confidence in the current findings. Also, we did not investigate offspring rs237895 genotype as a potential moderator in the association between MB and attachment security at 12 months. To do so, we would have needed to statistically control for maternal genotype (with whom children share 50% of genetic variation), thereby greatly reducing our ability to detect moderation effects that are exclusively attributable to offspring genotype in this small sample. Lastly, it is noteworthy that no infant was classified as being disorganized during the Strange Situation Procedure. This finding indicates that our study sample may not be entirely representative with respect to this characteristic, given prevalence estimates of disorganized attachment of ca. 15% in low-risk populations.[115]

With these caveats in mind, we conclude that OT-associated bio-behavioral mechanisms may be implicated in the postnatal transmission of the effects of maternal CM exposure to her offspring. From a translational point of view, two issues warrant particular attention. First, the SNP-selection strategy used here critically advances interpretability of gene-environment interactions involving *OXTR* gene variants in conferring differential susceptibility to the environment. Investigating the role of genetic variants with known effects on gene expression in the brain could help identify susceptible individuals at increased risk for possible maladaptive developmental trajectories after CM exposure. Second, once identified, women at risk and their children could benefit from early interventions that have proven effective in promoting maternal sensitivity and secure attachment. As we have argued earlier,[112] it is likely that individuals with a genetic predisposition for increased social sensitivity may not only show greater impairments after adverse early experience, but also may be the ones who disproportionately profit from psychosocial interventions.

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Affidavit

I, Philipp Töpfer certify under penalty of perjury by my own signature that I have submitted the thesis on the topic: “The Role of Functional Oxytocin Receptor Gene Variants in the Intergenerational Transmission of the Effects of Maternal Childhood Maltreatment”. I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The section on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) corresponds to the URM (s.o) and are answered by me. My contribution in the selected publication for this dissertation corresponds to those that are specified in the following joint declaration with the responsible person and supervisor.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

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Date Signature

Detailed Declaration of Contribution

Philipp Töpfer had the following share in the following publication:

Publication 1: Toepfer, P., O´Donnell, K.J., Entringer, S., Heim, C. M., Lin, D. T., MacIsaac, J. L., Kobor, M. S., Meaney, M.J., Provencal, N., Binder, E.B., Wadhwa, P.D., & Buss, C. (2019). A Role of Oxytocin Receptor Gene Brain Tissue Expression Quantitative Trait Locus rs237895 in the Intergenerational Transmission of the Effects of Maternal Childhood Maltreatment. *Journal of the American Academy of Child & Adolescent Psychiatry (in press)*.

Contribution in detail:

As single first author, Mr. Töpfer contributed substantially to the preparation of the present publication. More specifically, Mr. Töpfer established the theoretical rationale for the selection of *OXTR* expression QTL data and coded the quality of the dyadic mother-child interactions. Moreover, he conducted the statistical analyses and wrote the manuscript. He also made the tables, figures and supplemental material associated with the manuscript.

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Signature, date and stamp of the supervising University teacher

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Signature of the doctoral candidate

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6	DEVELOPMENT AND PSYCHOPATHOLOGY	10,349	4.357	0.013270
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8	DEVELOPMENTAL SCIENCE	6,769	4.078	0.012110
9	AUTISM	3,348	3.906	0.007030
10	CHILD DEVELOPMENT	29,042	3.779	0.023270
11	Autism Research	2,439	3.768	0.006980
12	Journal of Attention Disorders	3,100	3.668	0.006190
13	EUROPEAN CHILD & ADOLESCENT PSYCHIATRY	4,492	3.553	0.007980
14	JOURNAL OF AUTISM AND DEVELOPMENTAL DISORDERS	17,270	3.476	0.024100
15	JOURNAL OF ABNORMAL CHILD PSYCHOLOGY	8,572	3.287	0.012620
16	JOURNAL OF YOUTH AND ADOLESCENCE	8,000	3.247	0.012800
17	Child Development Perspectives	2,196	3.207	0.007560
18	DEVELOPMENTAL PSYCHOLOGY	20,748	2.934	0.023350
19	Journal of School Violence	624	2.721	0.001170
20	JOURNAL OF EXPERIMENTAL CHILD PSYCHOLOGY	6,790	2.424	0.012200

Original Publication

NEW RESEARCH

A Role of Oxytocin Receptor Gene Brain Tissue Expression Quantitative Trait Locus rs237895 in the Intergenerational Transmission of the Effects of Maternal Childhood Maltreatment

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Objective: Women exposed to childhood maltreatment (CM) are more likely to exhibit insensitive parenting, which may have consequences for their offspring's development. Variation in the oxytocin-receptor gene (*OXTR*) moderates risk of CM-associated long-term sequelae associated with mother–child attachment, although functionality of previously investigated single nucleotide polymorphisms (SNPs) remained elusive. Here, we investigated the role of *OXTR* rs237895, a brain tissue expression quantitative trait locus (eQTL), as a moderator of the relationship between CM and maternal behavior (MB) and the association between MB and offspring attachment security.

Method: Of 110 women with information on rs237895 genotype (T-allele = 64, CC = 46), 107 had information on CM (CTQ) and 99 on standardized observer-based ratings of MB at 6 months postpartum (responsivity and detachment), which were used in principal component analysis to obtain a latent factor representing MB. Offspring (n = 86) attachment was evaluated at 12 months of age. Analyses predicting MB were adjusted for socioeconomic status, age, postpartum depression, and genotype-based ethnicity. Analyses predicting child attachment were adjusted for infant sex, socioeconomic status, and postpartum depression.

Results: rs237895 significantly moderated the relationship between CM and MB ($F_{1;66} = 7.99$, $p < .01$), indicating that CM was associated with maternal insensitivity only in high–*OXTR*-expressing T-allele carriers but not in low–*OXTR*-expressing CC homozygotes. Moreover, maternal insensitivity predicted offspring insecure attachment (B = –0.551; $p < .05$).

Conclusion: Women with a high *OXTR* expressing genotype are more susceptible to CM-related impairments in MB that, in turn, predict attachment security in their children, supporting the role of the OT system in the intergenerational transmission of risk associated with maternal CM.

Key words: gene-environment interaction, childhood maltreatment, intergenerational transmission, cxytocin receptor gene, parenting

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The potentially deleterious long-term consequences of exposure to childhood maltreatment (CM) on mental and physical health are well established.[1] Furthermore, the detrimental effects of CM exposure do not seem to be restricted to the exposed individual alone, but might also have an impact on the next generation.[2] There is empirical evidence that offspring of CM-exposed mothers are at increased risk for depressive symptoms and insecure attachment.[3,4] In the context of maternal CM exposure, this intergenerational transmission of CM-associated sequelae is hypothesized to occur during the pre- and postnatal periods of development via multiple, partly overlapping pathways,[5,6] including altered gestational maternal–placental–fetal stress physiology in CM-exposed women,[7] increased risk for maternal depression,[8] and non-optimal maternal behavior (MB).[9] To date, most research has focused on behavioral aspects of the postnatal mother-to-child transmission of maternal CM exposure. It is, however, evident that not all women (and their offspring) are equally vulnerable to the long-term consequences of CM exposure. Addressing this issue of interindividual differences in susceptibility, we have proposed a conceptual framework[6] that highlights the crucial role of the oxytocin (OT) neuropeptide system in the intergenerational transmission of

maternal CM exposure for the following reasons: first, substantial evidence highlights the importance of OT in MB,[10] which is considered a primary postnatal transmission pathway of maternal CM exposure to her offspring.[11] Second, women with exposure to CM exhibit decreased concentrations of OT in plasma and cerebrospinal fluid.[12,13] Lower OT concentrations, in turn, have been shown to be associated with non-optimal MB,[14] which is a significant predictor for offspring attachment problems.[15] Third, growing evidence suggests an important role of genetic variation in oxytocin pathway genes (ie, oxytocin receptor gene [*OXTR*] and the oxytocin gene [*OXT*]) for MB[16] in moderating the association between CM exposure and subsequent risk for psychopathology,[17] and sub-optimal MB among others.[18] At the neural level, genetic variation in *OXTR* predicts differential activation within the social salience network (SSN) during perception of social stimuli.[19,20] Within the SSN that comprises highly interconnected meso-cortico-limbic structures (eg, ventral striatum [VS], anterior cingulate cortex [ACC], amygdala), oxytocin, via its receptor, synchronizes neural activity between SSN nodes,[19] providing a potential mechanism to confer greater sensitivity to the social environment. Most studies of gene−environment interactions investigating OT-pathway genes have focused on specific single nucleotide polymorphisms (SNPs) in *OXTR*, namely, rs53576 and rs2254298, without clarifying their functionality.[6] The first study to address functionality of *Oxtr* SNPs demonstrated that in monogamous prairie voles, genetic variation in *Oxtr* strongly predicted *Oxtr* gene expression in the nucleus accumbens, accompanied by significant behavioral effects.[21] Compared to animals carrying the "low−*Oxtr*-expression" genotype, homozygous "high−*Oxtr*-expression" genotype carriers displayed significantly more attachment toward their partner, providing the first mechanistic insight into the behavioral consequences of a striatal *Oxtr* expression quantitative trait locus (eQTL).[21] Although it did not consider the moderating role of environmental variation, this study added a critical piece of evidence to better understand how *Oxtr* genetic variation may contribute to behavioral differences. Collectively, these data suggest that genetic variation in *OXTR* might contribute to individual differences in sensitivity to the (early) social environment, mediated by increased reactivity of neural circuits that underlie response to the social environment as well as MB. Based on this evidence, we hypothesize that a genetic predisposition for increased social sensitivity (due to increased *OXTR* expression) may be associated with higher risk for suboptimal MB after CM exposure, with ensuing consequences for offspring development. We took advantage of publicly available resources providing information on genetic variation and genotype-specific gene expression in discrete post mortem brain tissues, so-called brain tissue eQTLs.[22,23] Here, we tested the hypothesis that women carrying a "high−*OXTR*-expressing" genotype are more sensitive to their (early) environment, and we expected them to exhibit greater CM-associated long-term adaptations in their MB compared to "low−*OXTR*-expressing" genotype carriers. Moreover, we expected variation in MB to predict attachment security in the next generation, as has been shown previously,[15] thus providing evidence for a pathway transmitting the effects of maternal CM exposure to the next generation. To test these hypotheses, we conducted a prospective longitudinal study in a total of 121 mother−child dyads. Mothers were genotyped and provided information on their own CM exposure. During a home visit at 6 months postpartum, MB was assessed by video recording behavior of mother−child dyads in standardized situations, which was later coded by trained observers. At 1 year of age, infant attachment security was characterized during the Strange Situation Paradigm (SSP).

METHOD

Participants

The study was conducted in the Development, Health and Disease Research Program at the University of California, Irvine, in a sample of 121 pregnant women with singleton pregnancies and their children. The cohort is described in greater detail elsewhere.[24] The University of California, Irvine, Institutional Review Board approved all study procedures, and all participants provided written informed consent.

Genotyping, SNP Imputation, and *OXTR* eQTL Selection

DNA extraction was performed on fasting blood samples collected during the first trimester of pregnancy (N = 121). Whole-genome SNP genotyping was performed using Illumina HumanOmniExpress BeadChip according to the manufacturer's standard protocols. Quality control of genotype data was performed using PLINK v1.9.48, where variants with call rates <98%, minor allele frequency <5%, or Hardy−Weinberg equilibrium test $p < 1\times10^{-6}$ were removed. A total of 599.935 genotypes passed quality control, and all mothers had genotyping rates >98% with no sex mismatches. Twelve mothers were identified as relatives (ie, >25% shared genotype), of whom one individual of each related pair was removed (n = 6), leaving 115 mothers for the analysis. To complete the dataset and to obtain genotype information of variants not covered on the array, we used imputation to predict the unobserved

genotypes by linkage disequilibrium (LD) with the genotyped SNPs of the array based on an external reference panel of haplotypes. Genotype data were imputed to the 1000 Genomes Phase 3 reference panel using SHAPEIT2[25] and IMPUTE2.[26] After imputation, variants with a minor allele frequency of <5% or an INFO metric <0.8 were removed. The information metric (INFO metric) takes ranges between 0 and 1, where values near 1 indicate that a SNP has been imputed with high certainty. The INFO metric is used to remove poorly imputed SNPs. Although there is no consensus in filtering the imputed datasets based on uncertainty of imputation, we used a conservative threshold of 0.8. To select a functional *OXTR* variant in this subset of 115 women, we used the GTEx database (gtexportal.org).[22] Using the tissue eQTL visualizer, we identified a haplotype of 12 *OXTR* brain tissue eQTLs (LD cutoff: $r^2 \geq 0.2$) spanning approximately 8 kb (Chr20: 8804371−8812411bp). From this haplotype, we sought to identify an *OXTR* brain tissue eQTL that would satisfy two criteria. First, the eQTL should significantly predict gene expression in brain areas known to be involved in MB and social information processing (ie, ventral striatum, amygdala, anterior cingulate cortex, and frontal cortex). To achieve this, GTEx provides a multi-tissue eQTL visualizer indicating the strength, direction, and p value of an eQTL as well as a metric (m-value for posterior probabilities ranging between 0 and 1) that indicates whether a given SNP has meta-analytical evidence for having an eQTL effect (m-value $\geq$ 0.9) in a discrete brain region.[27] Second, the eQTL should best represent this *OXTR* eQTL haplotype in our study sample consisting primarily of two ethnicity groups (self-identified non-Hispanic white and self-identified Hispanic). To that end, we conducted SNP-tagging analyses (https://snpinfo.niehs.nih.gov/snpinfo/snptag.html) for the 2 ancestry groups separately (based on 1,000 genomes CEU and MXL populations) by applying an LD threshold of $r^2 \geq 0.2$. The resulting SNP that meets these two criteria is rs237895 (T > C). In all brain regions of interest, this SNP has a significant eQTL effect, that is, it predicts gene expression in the caudate nucleus (normalized effect size/NES = −0.517, −log10 p value = 1.2e-10, m-value = 1), putamen (NES = −0.419, p = 1.1e-5, m= 1), amygdala (NES = −0.428, p = 6.6e-4, m = 1), nucleus accumbens (NES = −0.317, p = 7.6e-4, m = 1), ACC (NES = −0.387, p = 2.1e-5, m = 1), and frontal cortex (NES = −0.324, p = 7.0e-5, m = 1) (see Table S1, available online). The negative direction of the effect indicates that the reference allele (T) is associated with higher gene expression compared to the alternative allele (C). As an example, Figure S1 (available online) depicts *OXTR* gene expression in the caudate nucleus, which is allele-load dependent, that is, the homozygous T-allele carriers exhibit the highest, T/C heterozygotes intermediate, and the homozygous C-allele carriers the lowest gene expression. Furthermore, also using the xQTL database (http://mostafavilab.stat.ubc.ca/xQTLServe), in an independent sample of post mortem brain tissue (N = 494), rs237895 significantly predicts *OXTR* expression in the dorsolateral prefrontal cortex (r = –0.523; p < 8.0e-10).[23] Because rs237895 is not covered on the HumanOmniExpress BeadChip, genotypes of rs237895 from the 115 genetically unrelated women were extracted from the imputed data for all participants in this study. The rs237895 genotype could be imputed for 110 women (T/T = 18, T/C = 46, C/C = 46) with sufficient quality (ie, INFO metric >0.8), who were subsequently included in the statistical analyses. For the analyses, we assigned women to 2 genotype groups depending on the presence of the high−*OXTR*-expressing T-allele (T/T and T/C combined, n = 64) or absence of the same, that is, low−*OXTR*-expressing CC-homozygotes (n = 46).

Maternal CM Exposure

Women provided self-reports about CM exposure using the Childhood Trauma Questionnaire (CTQ),[28] one of the most widely used, reliable, and valid instruments to retrospectively assess early experiences of abuse and neglect. The CTQ assesses five different types of CM: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect.[28] For each individual CTQ subscale, we used established cut-off values (emotional abuse, ≥13; physical abuse, ≥10; sexual abuse, ≥8; emotional neglect, ≥15; and physical neglect, ≥10) to create a binary variable indicating moderate or severe exposure for any of the five CTQ-subscales. In addition, an overall binary variable was computed based on the CTQ to indicate moderate/severe childhood maltreatment (CM+) on at least one of the five CTQ scales versus no exposure to childhood maltreatment (CM−). We chose not to use the CTQ sum score as a predictor in the statistical analyses because it was not normally distributed as indicated by a Kolmogorov−Smirnov test ($D_{(107)}$= 0.20; p < .001).

Maternal Postnatal Behavior

At 6 months postpartum, a home visit was conducted. Research staff was trained to reliably assess maternal emotional and verbal responsivity toward the infant using the Home Observation Measurement of the Environment Infant−Toddler version (HOME-IT).[29] Raters were considered reliable once they had 2 consecutive observations in which 95% of responses matched the responses of

a rater with extensive experience in coding the HOME-IT. The responsivity scale of the HOME includes 11 items capturing different aspects of maternal responsivity, including maternal vocal reactivity to the infant or display of positive affect toward the infant. The internal consistency was moderate (Cronbach α =0.61) and comparable to that in previous studies reporting psychometric properties of this scale.[29,30] At the same visit and in addition to the HOME assessment, mothers were instructed to engage in a 15-minute standardized play situation as described elsewhere.[31] The play situation was video-recorded and subsequently coded by two trained and reliable independent observers (intraclass correlation coefficient [ICC] >0.9) using the coding manual of the NICHD Early Child Care Research Network.[31] We here focused on nonoptimal MB and coded maternal detachment (1 = "not at all characteristic" to 5 = "highly characteristic"). Highly detached mothers appear to be emotionally uninvolved and disengaged during dyadic play, do not react to the child's signals in a contingent manner, and can thus be considered unresponsive. In a next step, we conducted a principal component analysis (PCA) using the two maternal behavioral phenotypes, namely, responsivity and detachment. Results of the PCA indicated a one-factor solution (eigenvalue of factor 1 = 1.19; eigenvalue of factor 2 = 0.81). Detachment (0.78) loaded positively, whereas maternal responsivity (–0.78) loaded negatively on this extracted latent factor. Our one-factor solution explained 60% of the total variance. Higher scores on this latent variable indicate less optimal MB (ie, higher detachment and lower responsivity), and we thus termed it "maternal insensitivity."

Infant Attachment at 12 Months of Age

Infant attachment security was assessed at 12 months during the Strange Situation Procedure (SSP).[32] The SSP, a standardized laboratory observation, consists of 8 episodes, each 3 minutes long. These episodes include short periods of interaction between the mother and child, interaction between the child and an unfamiliar female stranger, and separation of the child from the mother followed by a reunion episode during which infant attachment is coded. Infants were categorized into three different types of attachment: securely attached (B), insecure−avoidant (A), and insecure−ambivalent (C). The relative frequencies of attachment categories was as follows: 62.2% were classified as securely attached (B), 30.6% as insecure−avoidant (A), and 7.1% as insecure−ambivalent (C). We used the dichotomous secure–insecure grouping (ie, B versus A and C) for data analysis because of the relatively small group size of type C attachment. Classification of attachment was completed by one rater with extensive experience in the assessment of attachment.

Covariates

Analyses testing the predictive value of maternal CM and rs237895 genotype interactions for MB were adjusted for the potential confounding effects of variables that have been shown to be associated with CM exposure, the observed phenotype (MB), or both (see Table S2, available online). For this set of analyses, the covariates included maternal age, socio-economic status (SES), maternal depressive symptoms, and racial/ethnic differences in genetic background. Information on annual household income and education (highest degree obtained) were aggregated to a composite measure indicative of socio-economic status (SES) as previously described in our study sample.[24] Women provided self-reports on postpartum depressive symptoms (PPD symptoms) using the Edinburgh Postnatal Depression Scale (EPDS),[33] a widely used valid screening tool for PPD symptoms on 3 occasions during the first postnatal year (ie, at 1, 6, and 12 months). To account for the different time points in assessing the maternal phenotype (at 6 months) and infant attachment (at 12 months), we averaged EPDS scores (all highly correlated across postnatal visits: r = 0.606−0.767; all correlation p values <.001) depending on the predicted outcome. More specifically, we used an average of EPDS scores including the 1- and 6-month assessments for analyses predicting maternal behavior at 6 months and an average including all 3 EPDS scores (1, 6, and 12 months) in the model predicting infant attachment at 12 months. Racial/ethnic 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 (see Figure S2, available online). We also took parity status into account, which has been shown to predict differences in MB. All analyses testing the association between MB and offspring attachment were adjusted for SES, infant sex, and PPD symptoms.

Statistical Analyses

All statistical analyses were performed using IBM SPSS version 22. Prior to testing the gene−environment interaction in the prediction of MB, we evaluated the main effect of CM exposure on maternal behavior using a linear regression model, while controlling for all potential confounding variables (ie, maternal age, SES, PC1-3, PPD

symptoms). Maternal G-E analyses were conducted using the SPSS PROCESS macro.[34] In the simple moderation analyses (model 1), the binary maternal CM exposure variable (CM+/CM−) was entered as the main predictor, the latent MB factor as the outcome, maternal dichotomous *OXTR* rs237895 genotype (T-allele versus CC-homozygotes) as the moderator and maternal age, SES, genotype-based ethnicity, and maternal PPD symptoms as the covariates. For the prediction of attachment security (secure versus insecure), a logistic regression analysis was performed using MB as the predictor and infant sex, SES, and PPD symptoms as covariates.

RESULTS

Sample Characteristics

Information on socio-demographic characteristics, CM experience, MB, PPD symptoms, and infant attachment are shown in Table 1 for the total sample (N = 110) and stratified by rs237895 genotype as well as CM exposure status. Importantly, neither CM exposure ($p > .93$) nor maternal insensitivity ($p > .82$) nor infant attachment ($p > .5$) was significantly different between the genotype groups. Compared to women in the CM– group, CM+ subjects had a lower SES ($p < .01$) and reported more PPD symptoms throughout the first year postpartum

TABLE 1 Characteristics of the Total Sample and Stratified by Maternal *OXTR* rs237895 Genotype and Childhood Maltreatment (CM) Exposure

		***OXTR* rs237895 genotype**			**CM exposure[a]**		
Characteristics	**Total sample (N = 110)**	**T-Allele carriers (n = 64)**	**CC Carriers (n = 46)**	***p***	**CM+ (n = 38)**	**CM− (n = 69)**	***p***
Maternal age, y, mean (SD) at study entry	27.80 (5.07)	27.41 (5.14)	28.44 (4.71)	NS	26.81 (5.79)	28.37 (4.39)	NS
Maternal SES[b]	3.25 (0.97)	3.21 (0.96)	3.27 (0.98)	NS	2.87 (0.91)	3.44 (0.94)	<.01
Maternal race/ethnicity, self-report, n (%)[c,f]							
Non-Hispanic white	44 (43.1%)	21 (35%)	23 (54.8%)	NS	9 (25.7%)	33 (51.6%)	NS
Hispanic white	38 (37.3%)	25 (41.7%)	13 (31%)	NS	18 (51.4%)	20 (31.3%)	NS
Asian	7 (6.9%)	5 (8.3%)	2 (4.8%)	NS	4 (11.5%)	3 (4.7%)	NS
Other	13 (12.8%)	9 (15%)	4 (9.6%)	NS	4 (11.4%)	8 (12.4%)	NS
Maternal behavior[d]							
Responsivity, HOME, range 0−11	8.28 (1.81)	8.17 (2.12)	8.42 (1.38)	NS	7.87 (2.43)	8.47 (1.46)	NS
Detachment, play situation, range 1−5	1.49 (0.83)	1.41 (0.76)	1.61 (0.92)	NS	1.61 (0.90)	1.45 (0.81)	NS
PPD symptoms, EPDS score, 1 mo (SD)	6.00 (4.66)	5.63 (4.25)	6.56 (5.24)	NS	7.54 (4.91)	5.16 (4.41)	<.05
PPD symptoms, 6 mo	4.96 (4.66)	4.63 (4.02)	5.43 (5.50)	NS	6.30 (5.88)	4.16 (3.70)	<.05
PPD symptoms, 12 mo	5.51 (4.37)	5.62 (4.17)	5.34 (4.72)	NS	7.92 (4.35)	4.29 (3.87)	<.01
CTQ Total Score, range 25−125[a]	37.39 (14.97)	38.13 (16.46)	36.89 (12.80)	NS	51.37 (17.55)	30.04 (4.46)	<.001
CM+, n (%)	38 (35.5%)	21 (33.3%)	17 (38.6%)				
CM−, n (%)	69 (64.5%)	42 (66.7%)	27 (61.4%)				
Infant attachment at 12 months[e,f]							
Securely attached, n (%)	56 (65.1%)	29 (61.7%)	27 (69.2%)	NS	18 (66.7%)	37 (64.9%)	NS
Insecurely attached, n (%)	30 (34.9%)	18 (38.3%)	12 (30.8%)	NS	9 (33.3%)	20 (35.1%)	NS

Note: *CTQ = Childhood Trauma Questionnaire; EPDS = Edinburgh Postnatal Depression Scale; NS = not significant; OXTR = Oxytocin Receptor Gene; PPD = postpartum depression; SES = socioeconomic status.*

[a]*Missing values in CM exposure: n = 3.*

[b]*SES is a composite measure of maternal education and annual household income, coded from 1 (low SES) to 5 (high SES).*

[c]*Missing values in race/ethnicity self-report: n = 8 (n = 4 in each genotype group).*

[d]*Missing values for maternal behavior: n = 11*

[e]*Missing values for attachment security: n = 24.*

[f]*For categorical variables (race/ethnicity and infant attachment), χ^2 tests were calculated.*

(all p values <.05). CM groups did not differ in MB or infant attachment (Table 1). Intercorrelations of all study variables are displayed in Table S2, available online.

Maternal CM Exposure and rs237895 Genotype Effects on MB

The linear regression model that tested the association between CM exposure and MB revealed no significant main effect of CM exposure ($b = –0.31$, $p = .2$). The main moderation model accounted for 38.36% of the variance in MB ($F_{9,66} = 4.56$, $p < .001$) (Table 2). The maternal CM × rs237895 interaction was significantly associated with MB ($F_{1,66} = 7.99$, $p < .01$). Confirming our hypothesis, post hoc analyses revealed that only women carrying the high−*OXTR*-expressing T-allele showed significant differences in maternal insensitivity depending on CM exposure ($t = –0.26$; $p = .015$; Cohen d = 0.92), with CM-exposed women showing greater maternal insensitivity than non−CM-exposed women (Figure 1). In low-expressing CC-homozygous women, CM was not associated with MB ($t = 0.83$; $p = .4$; Cohen d = –0.26).

TABLE 2 Regression Table for Maternal Childhood Maltreatment (CM) x rs237895 Predicting Maternal Insensitivity[a]

Predictor	Coefficient	SE	t Statistic	p	95% CI
Constant	2.00	0.62	3.21	<.01	0.76 to 3.25
Maternal Age	−0.08	0.02	-3.18	<.01	−0.13 to −0.03
SES[b]	−0.05	0.13	−0.39	.70	−0.32 to 0.21
PC1[c]	−3.72	1.35	-2.75	<.01	−6.41 to −1.02
PC2	1.41	1.14	1.24	.22	−0.87 to 3.7
PC3	−0.54	1.47	−0.34	.71	−3.47 to 2.40
PPD symptoms[d]	0.47	0.23	2.02	<.05	0.01 to 0.93
OXTR rs237895[e]	0.23	0.21	1.05	.30	−0.21 to 0.66
Maternal CM[f]	−0.26	0.25	−1.05	.30	−0.76 to 0.23
CM x rs237895	−1.18	0.42	−2.83	<.01	−2.02 to −0.35

Note: *OXTR = Oxytocin Receptor Gene; PC = principal component; SES = socioeconomic status; SE = standard error.*

[a]*Maternal insensitivity: latent factor derived from principal components analyses (see Methods for details).*

[b]*Maternal SES comprised of annual household income and highest degree of education obtained (see Methods for details).*

[c]*PC representing maternal genotype-based ethnicity (see Methods).*

[d]*Postpartum depressive symptoms: mean 6-month postpartum symptoms assessed at 1 month and 6 months (Edinburgh Postnatal Depression Scale);*

[e]*Maternal OXTR rs237895 genotype dichotomized (T-allele vs CC-homozygous);*

[f]*Maternal CM exposure (dichotomous groups: CM– vs CM+; see Methods for details).*

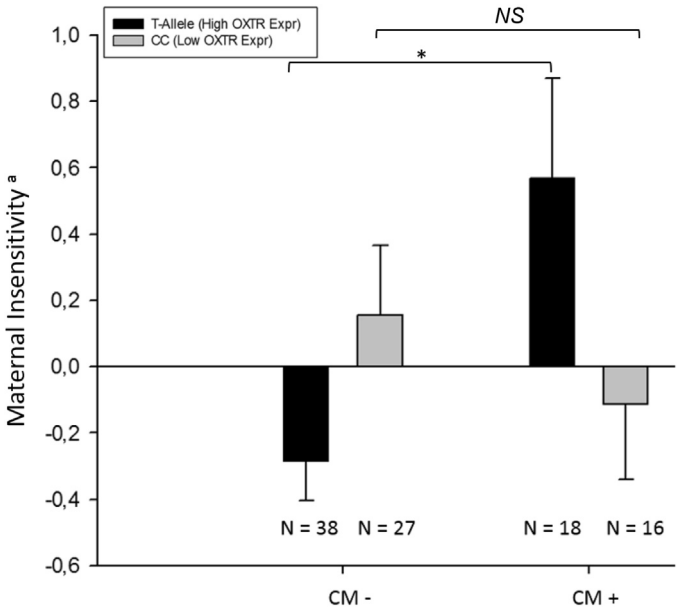
MB and Infant Attachment at 12 Months

MB significantly predicted offspring attachment security at 12 months (B = –0.551; $p < .05$; Cohen d = –0.51) (Figure 2) after controlling for SES, infant sex, and PPD symptoms. Infant attachment was higher in children of women with less maternal insensitivity. In other words, securely attached infants were overrepresented in the group of women who, based on median split, exhibited higher sensitivity (77.8% securely attached infants versus 22.2% insecurely attached). In women with greater insensitivity (> median), the prevalence of insecurely attached children increased 2-fold (45.0 % insecure attachment) compared to that in the group exhibiting low insensitivity.

DISCUSSION

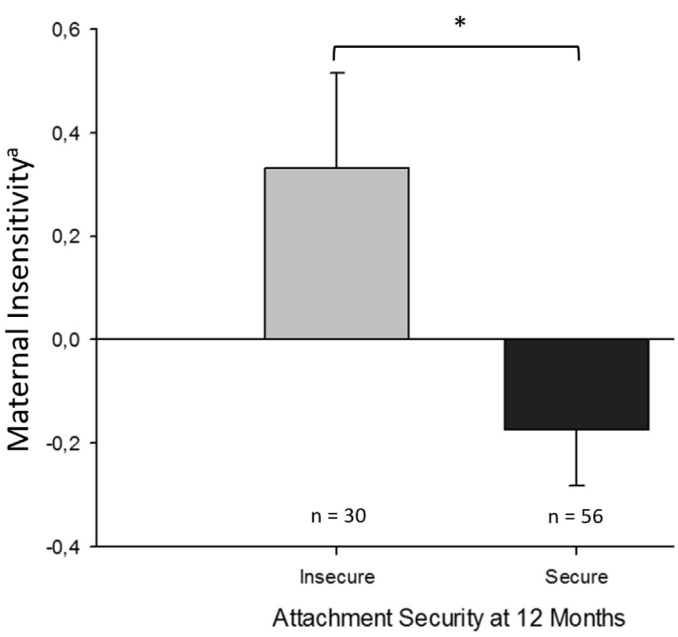
To the best of our knowledge, the findings described here provide the first evidence of the moderating role of a functional *OXTR* variant in the process of intergenerational transmission of the effects of maternal CM exposure. Only women carrying the high−*OXTR*-expressing T-allele exhibited significant differences in MB in conjunction with CM experience, with CM-exposed women experiencing greater insensitivity than non−CM-exposed women. MB in C-allele homozygous women appeared to be less affected by CM exposure, indicating reduced behavioral adaptations after CM exposure in these individuals. Unlike previous studies that have demonstrated either a main effect of CM exposure[9] or *OXTR* genotype[16] in predicting MB, our findings highlight the importance of gene−environment interactions to predict MB. It appears that these observations are in accordance with the Differential Susceptibility Theory (DST).[35] However, the mere absence of early adversity (ie, CM−) (conditions under which T-allele−carrying mothers show the least amount of insensitivity) does not, per se, implicate the presence of a supportive and enriched early environment, which is an important premise of the DST framework that cannot be addressed in the current study. The CTQ, our environmental exposure, is not designed to capture positive aspects of the early environment. Nevertheless, our results support *OXTR* rs237895 functioning as a genetic moderator, and they are in line with prior research. It has been previously shown that genetic variation in *OXTR* predicts limbic reactivity to social cues[20] and MB[16] and moderates the association between CM exposure and depression as well as disorganized adult attachment.[17,36] By adopting a biologically informed SNP-selection strategy,[22,23] the present study corroborates, extends, and strengthens this line of research. In accordance with recent theoretical frameworks that postulate a role for oxytocin in modulating the salience of social cues,[37] we propose that

FIGURE 1 Maternal Insensitivity Stratified by Maternal Childhood Maltreatment (CM) Exposure and *OXTR* rs237895 Genotype



Note: *CM– indicates no Childhood Trauma Questionnaire (CTQ). Q category above moderate cut-off. CM+ indicates one or more CTQ categories above moderate cut-off. OXTR = Oxytocin Receptor Gene.* [a]*Latent factor representing maternal insensitivity includes measures of maternal responsivity (HOME) and maternal detachment during play (see Method for details). Higher scores indicate greater insensitivity. NS = not significant.*
*p < .05.

FIGURE 2 Maternal Insensitivity Stratified by Offspring Attachment Security



Note: [a]*Latent factor representing maternal insensitivity includes measures of maternal responsivity (HOME) and maternal detachment during play (see Method for details). Higher scores indicate greater maternal insensitivity.*
*p < .05.

genetic variation in *OXTR* eQTLs (eg, rs237895) may operate through increased genotype-dependent *OXTR* expression in socially sensitive neural networks as an important neurobiological mechanism conferring heightened social–environmental susceptibility.

However, the question remains as to why mothers would differ in the degree to which they adapt their reproductive (ie, MB) strategies after CM exposure. Environmental variation, especially early social experiences (eg, the mother's CM exposure) may operate via MB to shape offspring development, thereby ultimately promoting reproductive fitness in the next generation.[38] Strong support for this "maternal mediation hypothesis" comes from rodent studies showing how natural variations in MB (eg, licking and grooming) may induce persistent behavioral and neurobiological changes in offspring.[38] As examples, offspring of dams low in licking and grooming exhibit heightened stress reactivity[39] and increased fearfulness,[40] phenotypes that promote survival in a dangerous environment. Furthermore, female offspring of dams low in licking and grooming show alterations in MB consistent with their own rearing experience.[41] Directly translating this line of research to humans, we would predict that women exposed to CM should adapt their MB (ie, lower responsivity, higher detachment) accordingly to transmit information about their own past aversive environment to their offspring. However, our data suggest otherwise, as the association between maternal CM exposure and MB appears to be dependent on maternal *OXTR* genotype. A possible explanation for this observation is the concept of bet-hedging.[42] Since the future is inherently unpredictable and early experiences (eg, CM exposure) may not always accurately predict the future environment (eg, dangerous/adverse environment for offspring), natural selection has maintained genes for both environmentally susceptible (eg, high *OXTR* expression) as well as less susceptible (eg, low *OXTR* expression) developmental strategies, to ultimately increase fitness payoffs regardless of environmental continuity.[35]

These possibly adaptive reproductive strategies, however, may come at a cost from the lens of a developmental psychopathology perspective rather than an evolutionary one. We show that less responsive and more detached MB is associated with insecure attachment in her child at 12 months age, which is in accordance with prior research.[15,32] Insecure attachment itself predicts anxiety[43] and

internalizing and externalizing behavior,[44] among other phenotypes, closing the cycle of intergenerational transmission of early life experiences.

Previous research in humans and animals has shown that MB is hormonally primed, and that this process starts as early as during pregnancy itself,[45] partly mediated via estrogen-induced up-regulation of oxytocin receptors.[46] An open question now is whether *OXTR* eQTLs exert their effects on brain gene expression through variable accessibility of transcription factors to chromatin. Given the fundamental role of sex steroids in regulating *OXTR* gene expression and the fact that sex steroids dramatically increase during pregnancy, this period represents a time window of critical importance to better understand the contribution of *OXTR* genetic variation in the association between CM and MB. Moreover, it is possible that additional prenatal factors, such as alterations in CM-associated maternal−placental−fetal stress physiology operate as mechanisms in the intergenerational transmission of risk associated with maternal CM exposure.[5] It remains to be elucidated whether these transmission pathways differ systematically between women carrying high- or low-susceptibility variants of rs237895. Moreover, in addition to maternal interactive behavior, future studies in the context of intergenerational transmission during the postnatal period should consider other postnatal variables such as breastfeeding status and breast milk composition, which may be different based on maternal CM experience. This is a relevant avenue of research aimed at understanding the mechanisms underlying intergenerational transmission of maternal CM, given the common underlying neurobiology for breastfeeding and MB that crucially involve efficient OT signaling.[47]

Maternal behavior is a complex phenotype emerging from extensive interconnected neural circuitry underlying a wide array of executive, cognitive, motivational, and self-regulatory functions,[48] and can be modulated by early childhood experiences,[6,11,41] OT signaling,[49] and interactions of OT with other neurotransmitters such as dopamine among many others.[48] It would be informative for future studies to use neuroimaging assessments to characterize neural functional and/or structural differences after CM exposure in genetically susceptible women. This will then provide further insights into the neural underpinnings of the associations between CM exposure and variation in MB. The SNP under investigation here, rs237895, predicts *OXTR* expression across multiple brain regions that are critical for MB, cognition, and motivation (eg, amygdala, ventral striatum [VS], ACC, PFC), raising the possibility that alterations in some or even most of the above-mentioned OT-associated functions might be critically altered in T-allele carriers after CM. Intriguingly, a previous study by Loth *et al.* has shown that another intronic *OXTR* SNP (rs237893, A>G), which tags the same *OXTR* eQTL haplotype as rs237895, predicts activity in the VS in response to social cues in an allele-load−dependent manner.[20] VS reactivity was highest in high−*OXTR*-expressing AA carriers and lowest in low-expressing GG carriers.[20] Bearing in mind the well-documented role of OT-signaling in the VS for MB (eg, affecting salience and reward of infant stimuli as well as infant-directed behavior),[50] the findings by Loth *et al.*, by supporting the notion of higher social sensitivity in individuals carrying a high *OXTR*-expression genotype, provide important insights into intermediate phenotypes at the intersection of gene−behavior associations that may theoretically vary depending on the early environment.

There are several limitations of the current study, including the relatively small sample size and the lack of an independent replication sample. From a methodological point of view, a moderated mediation analysis would have been more suitable to test the entire intergenerational pathway from maternal CM exposure to infant attachment in the next generation. However, the resulting sample size in the full model with no missing data for both mothers and children would have been relatively small (n = 69). Consequently, the full model predicting attachment security, while including all covariates, would have been vulnerable to overfitting in such a small sample, which is why we decided to test the paths in two separate models. In addition, we had to group the T-allele−carrying women together for practical reasons because the homozygous T-allele group included only 18 individuals. Given the allele-load−dependent eQTL effect of rs237895, it would be interesting, in future larger samples, to test the CM−MB association for all three groups of genotype separately. Also, rs237895 is not covered on the array used for genotyping. Thus, we performed an LD-based imputation and applied a conservative threshold (INFO metric >0.8) to acquire maternal genotype data with sufficient, albeit not perfect, certainty. Moreover, only healthy pregnant women and their children participated in the study, limiting the number of women with severe CM exposure. Nevertheless, the prevalence estimate of CM exposure in the study sample is comparable with recent epidemiological data on CM exposure in the general population.[51] A retrospective self-report measure (CTQ) was used to assess maternal CM. Although there were no differences in reported severity of CM between genotype groups and analyses adjusted for current mood, other potential variables that may influence self-reported childhood experiences (eg, forgetting, recollection bias, or nondisclosure) cannot be ruled out entirely.

Following recent recommendations,[52] we used objective observation-based ratings of MB to quantify our outcome, and raters were blinded to maternal genotype and CM exposure, thereby strengthening confidence in the current findings. Also, we did not investigate offspring rs237895 genotype as a potential moderator in the association between MB and attachment security at 12 months. To do so, we would have needed to statistically control for maternal genotype (with whom children share 50% of genetic variation), thereby greatly reducing our ability to detect moderation effects that are exclusively attributable to offspring genotype in this small sample. Finally, it is noteworthy that no infant was classified as being disorganized during the Strange Situation Procedure. This finding indicates that our study sample may not be entirely representative with respect to this characteristic, given the prevalence estimates of disorganized attachment of approximately 15% in low-risk populations.[53]

With these caveats in mind, we conclude that OT-associated bio-behavioral mechanisms may be implicated in the postnatal transmission of the effects of maternal CM exposure to her offspring. From a translational point of view, two issues warrant particular attention. First, the SNP-selection strategy used here critically advances interpretability of gene−environment interactions involving *OXTR* gene variants in conferring differential susceptibility to the environment. Investigating the role of genetic variants with known effects on gene expression in the brain could help to identify susceptible individuals at increased risk for possible maladaptive developmental trajectories after CM exposure. Second, once identified, women at risk and their children could benefit from early interventions that have proved effective in promoting maternal sensitivity and secure attachment. As we have argued earlier,[6] it is likely that individuals with a genetic predisposition for increased social sensitivity may not only show greater impairments after adverse early experience, but also may be the ones who disproportionately profit from psychosocial interventions.

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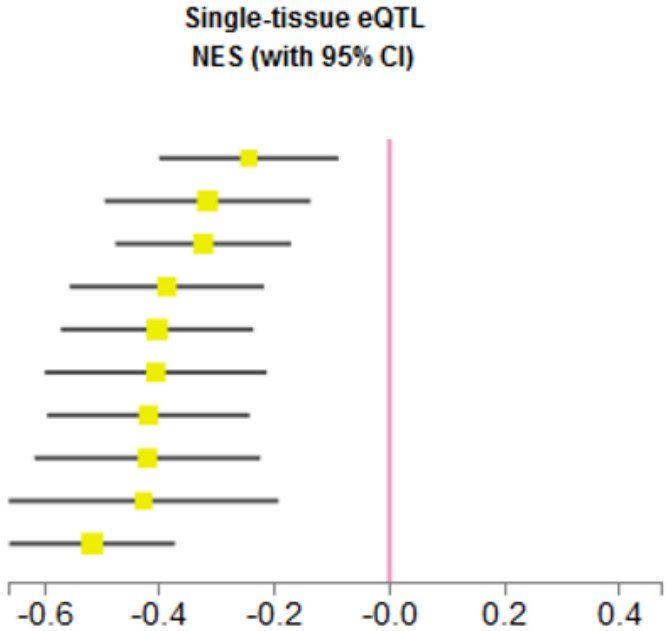
Supplemental Material

Table S1: eQTL Effect of *OXTR* rs237895 Across Brain Tissues (Table Generated from gtexportal.org)

Tissue	Samples	NES	p-value	m-value
Brain - Substantia nigra	80	-0.245	3.2e-3	0.984
Brain - Nucleus accumbens (basal ganglia)	130	-0.317	7.6e-4	1.00
Brain - Frontal Cortex (BA9)	118	-0.324	7.0e-5	1.00
Brain - Anterior cingulate cortex (BA24)	109	-0.387	2.1e-5	1.00
Brain - Cortex	136	-0.404	6.5e-6	1.00
Brain - Hippocampus	111	-0.407	8.3e-5	1.00
Brain - Putamen (basal ganglia)	111	-0.419	1.1e-5	1.00
Brain - Hypothalamus	108	-0.421	6.4e-5	1.00
Brain - Amygdala	88	-0.428	6.6e-4	1.00
Brain - Caudate (basal ganglia)	144	-0.517	1.2e-10	1.00



Note: sample sizes, normalized effect sizes, p-values and m-values (m-value ≥ .9 indicates significant eQTL effect of a SNP) are shown for brain regions separately. Negative directions of NES indicate that the reference allele (T) is associated with higher gene expression. NES = normalized effect size

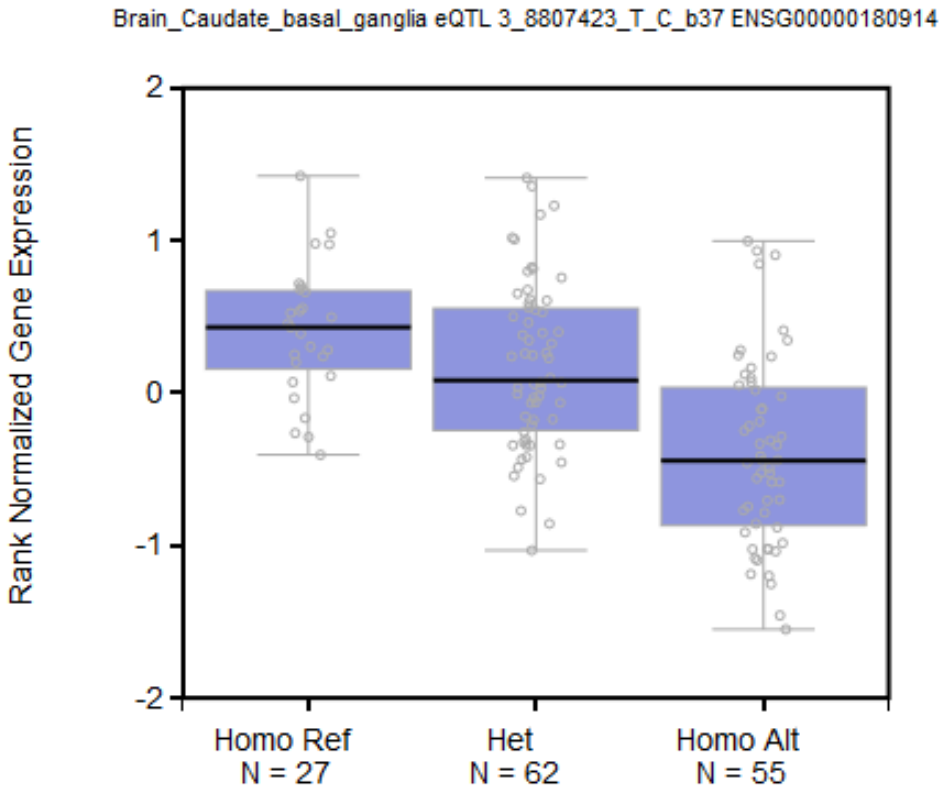


Figure S1: eQTL Effect of *OXTR* rs237895 in Caudate Nucleus Stratified by Genotype (Figure Generated from gtexportal.org).

Note: Homo Ref = homozygous reference allele (T/T); Het = heterozygous (T/C); Homo Alt = homozygous alternative allele (C/C)

Table S2: Inter-correlations between Study Variables

	SES	Maternal Age	Parity	PC1	PC2	PC3	CTQ Score	PPD symptoms (average 1 & 6 months)	PPD symptoms (average 1, 6, 12 months)	Maternal insensitivity	Sex of Infant	Attachment security
SES	-											
Maternal Age	.410***	-										
Parity	-.093	.332**	-									
PC1	.412***	.036	-.038	-								
PC2	-.280**	-.188	.147	.036	-							
PC3	-.125	-.006	-.047	-.020	.066	-						
CTQ score	-.215**	-.135	.079	-.221*	.104	-.034	-					
PPD symptoms (average 1 & 6 months)	-.123	-.114	.082	-.059	.075	-.016	.404***	-				
PPD symptoms (average 1, 6, 12 months)	-.144	-.100	.084	-.035	.072	-.028	.433***	.968***	-			
Maternal insensitivity	-.315***	-.391***	.052	-.340**	.210	.072	.177	.261**	.217*	-		
Sex of Infant[a]	-.030	-.011	-.021	-.014	.019	-.014	.061	.090	.035	.164	-	
Attachment Security[b]	.164	.145	.122	.209	.073	.022	-.006	-.001	-.011	-.251*	.020	-

Note: All correlations shown are Pearson correlation coefficients, except for categorical variables (i.e., infant sex and attachment security), for which Spearman rank correlations are displayed. CTQ = Childhood Trauma Questionnaire; PC = Principal component for population stratification; PPD = Postpartum depression (assessed via Edinburgh Postnatal Depression Scale); SES = Socioeconomic status (composite measure of maternal education and annual household income).
[a]Infant sex coded as 1 = male, 2 = female;

[b]Attachment security coded as 0 = insecure, 1 = secure.

* p<.05; ** p<.01; *** p<.001

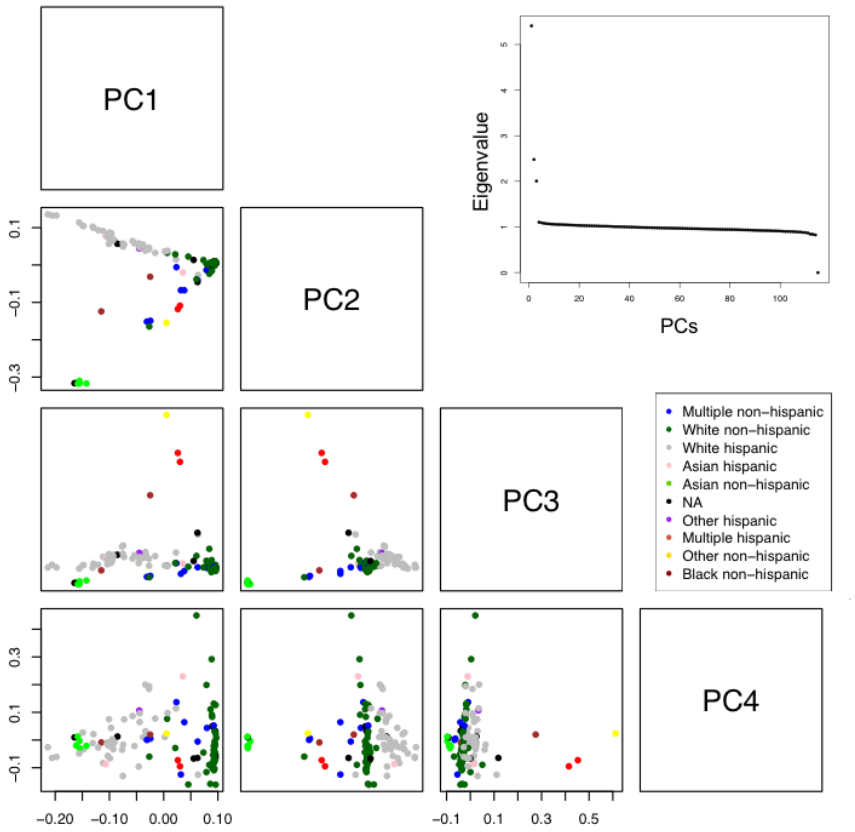


Figure S2: Scatter Plot Showing Population Stratification of the First Four Principal Components from the Principal Component Analysis (PCA) Using Genotyping Data on 593229 Variants. **Note:** Colors indicate reported race and ethnicity by the mothers. Top right panel shows the eigenvalues for each PC where the first 3 PCs explain most of the variance associated with genotype. As can be seen in the figure, our sample primarily consists of two major self-identified race/ethnicity groups: White/non-Hispanic and White Hispanic. In reference to self-reported race/ethnicity groups presented in **Table** 1, "Asian" (n=7) refers to women, who self-identified as Asian/Hispanic (n=2) or Asian/Non-Hispanic (n=5). Lastly, "other" (n=13) includes "Black/non-Hispanic (n=1), "Other/Non-Hispanic" (n=1), "Multiple Races/non-Hispanic" (n=8), "Other/Hispanic" (n=1), and "Multiple races/Hispanic" (n=2). Women who self-identified as white-non Hispanic show consistently higher factor loadings on PC1, while self-identified white Hispanic women exhibit higher factor loadings on PC2. PC = principal component.

Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

List of publications

ORIGINAL AND REVIEW ARTICLES

1. Toepfer, P., Heim, C. M., Entringer, S., Binder, E. B., Wadhwa, P. D., & Buss, C. (2017). Oxytocin pathways in the intergenerational transmission of maternal early life stress. *Neuroscience and biobehavioral reviews, 73,* 293 - 308. **Impact Factor (2017): 8,037.**

2. Buss, C., Entringer, S., Moog, N. K., Toepfer, P., Fair, D. A., Simhan, H. N., Heim, C.M. & Wadhwa, P. D. (2017). Intergenerational Transmission of Maternal Childhood Maltreatment Exposure: Implications for Fetal Brain Development. *Journal of the American Academy of Child & Adolescent Psychiatry, 56,* 373 – 382. **Impact Factor (2017): 6,250.**

3. Toepfer, P., O'Donnell, K. J., Entringer, S., Garg, E., Heim, C. M., Lin, D. T., Kobor, M. S., Meaney, M.J., Provencal, N., Binder, E. B., Wadhwa, P.D., & Buss, C. (2019). Dynamic DNA methylation changes in the maternal oxytocin gene locus (OXT) during pregnancy predict postpartum maternal intrusiveness. *Psychoneuroendocrinology, 103,* 156-162. **Impact Factor (2017): 4,731.**

4. Toepfer, P., O´Donnell, K.J., Entringer, S., Heim, C. M., Lin, D. T., MacIsaac, J. L., Kobor, M. S., Meaney, M.J., Provencal, N., Binder, E.B., Wadhwa, P.D., & Buss, C. (2019). A Role of Oxytocin Receptor Gene Brain Tissue Expression Quantitative Trait Locus rs237895 in the Intergenerational Transmission of the Effects of Maternal Childhood Maltreatment. *Journal of the American Academy of Child & Adolescent Psychiatry (in press).* **Impact Factor (2017): 6,250.**

PUBLISHED ABSTRACTS

1. Toepfer, P., Heim, C., Entringer, S., Wadhwa, P. D., Provencal, N., Binder, E. B., Buss, C. (2016). A Variation in the Oxytocin Receptor Gene Moderates the Relationship Between Early Maternal Care and Interleukin-6 (IL-6) Concentrations During Pregnancy. *Psychoneuroendocrinology, 71,* 15.

2. Toepfer, P., O´Donnell, K. J., Heim, C. M., Lin, D. T., MacIsaac, J. L., Kobor, M. S., Meaney, M.J., Provencal, N., Binder, E.B., Entringer, S., Wadhwa, P.D., Buss, C. (2017). Dynamic DNA methylation changes in the oxytocin locus (OXT) during pregnancy are associated with maternal parenting behavior. *Psychoneuroendocrinology, 83,* 25.

3. Toepfer, P., O´Donnell, K.J., Entringer, S., Heim, C. M., Lin, D. T., MacIsaac, J. L., Kobor, M. S., Meaney, M.J., Provencal, N., Binder, E.B., Wadhwa, P.D., & Buss, C. (2019). (2019). OXTR brain tissue expression quantitative locus rs237895 moderates the association between maternal childhood maltreatment and non-optimal maternal behavior with implications for offspring socio-emotional development and attachment security. *Psychoneuroendocrinology, 100,* S48.

ORAL CONFERENCE PRESENTATIONS

1. Toepfer, P., O´Donnell, K.J., Entringer, S., Heim, C. M., Lin, D. T., MacIsaac, J. L., Kobor, M. S., Meaney, M.J., Provencal, N., Binder, E.B., Wadhwa, P.D., & Buss, C. (2019). (2019). OXTR brain tissue expression quantitative locus rs237895 moderates the association between maternal childhood maltreatment and non-optimal maternal behavior with implications for offspring socio-emotional development and attachment security. *48th Annual Meeting of the International Society of Psychoneuroendocrinology*. Irvine, California, USA, September 2018.

2. Toepfer, P., Heim, C., Entringer, S., Provencal, N., Binder, E. B., Wadhwa, P. D., Buss, C. A variation in the oxytocin receptor gene moderates the relationship between early maternal care in childhood and interleukin 6 (IL-6) concentrations during pregnancy. *15th Conference of European Society for Traumatic Stress Studies*. Odense, Dänemark, Juni 2017.

3. Toepfer, P., Heim, C., Entringer, S., Provencal, N., Binder, E. B., Wadhwa, P. D., Buss, C. Eine Variation im Oxytocin-Rezeptor-Gen moderiert den Zusammenhang zwischen frühkindlicher mütterlicher Fürsorge und Interleukin 6 Konzentrationen während der Schwangerschaft. *Jahreskongress der Deutschen Gesellschaft für Biologische Psychiatrie.* Würzburg, September 2016.

4. Toepfer, P., Heim, C., Entringer, S., Provencal, N., Binder, E. B., Wadhwa, P. D., Buss, C. Eine Variation im Oxytocin-Rezeptor-Gen moderiert den Zusammenhang zwischen frühkindlicher mütterlicher Fürsorge und Interleukin 6 Konzentrationen während der Schwangerschaft. *Gemeinsame Jahrestagung der Deutschen Gesellschaft für Medizinische Soziologie (DGMS) und zur der Deutschen Gesellschaft für Medizinische Psychologie (DGMP). Berlin, September 2016.*

POSTER CONFERENCE PRESENTATIONS

1. Toepfer, P., O´Donnell, K.J., Entringer, S., Heim, C. M., Kobor, M. S., Meaney, M.J., Provencal, N., Binder, E.B., Thomas, E., Schifsky, E., Fair, D.A., Graham, A. M., Wadhwa, P.D., & Buss, C. Maternal Intrusive Parenting and Infant Regulation of Negative Affect - The Moderating Role of a Functional Oxytocin Receptor Gene (*OXTR*) Variant. *74th Annual Meeting of the Society of Biological Psychiatry*, Chicago, IL, USA, May 2019.

2. Toepfer, P., Pokhvisneva, I., Garg, E., Entringer, S., Heim, C.M., Kobor, M. S., Provencal, N., Binder, E. B., Wadhwa, P. D., Meaney, M. J., Buss, C., O´Donnell, K.J. Maternal Prenatal Depression and Infant DNA Methylome Maturation: Developmental Regulation of DNA Methylation and Relevance for Infant Behavior. *74th Annual Meeting of the Society of Biological Psychiatry*, Chicago, IL, USA, May 2019.

3. Toepfer, P., O´Donnell, K.J., Entringer, S., Heim, C. M., Lin, D. T., MacIsaac, J. L., Kobor, M. S., Meaney, M.J., Provencal, N., Binder, E.B., Wadhwa, P.D., & Buss, C. OXTR brain tissue expression quantitative locus rs237895 moderates the association between maternal childhood maltreatment and non-optimal maternal behavior with implications for offspring socio-emotional development and attachment security. *73rd Annual Meeting of the Society of Biological Psychiatry*, New York, N.Y, Mai 2018.

4. Toepfer, P., O´Donnell, K., Heim, C.M., Lin, D. T., MacIsaac, J. L., Kobor, M. S., Meaney, M.J., Provencal, N., Binder, E.B., Entringer, S., Wadhwa, P.D., Buss, C. Dynamic DNA methylation changes in the oxytocin gene locus (*OXT*) during pregnancy are associated with maternal parenting behavior. *1st Annual UK Maternal Mental Health Alliance Conference*, London, UK, September 2017.

5. Toepfer, P., O´Donnell, K., Heim, C.M., Lin, D. T., MacIsaac, J. L., Kobor, M. S., Meaney, M.J., Provencal, N., Binder, E.B., Entringer, S., Wadhwa, P.D., Buss, C. Dynamic DNA methylation changes in the oxytocin gene locus (*OXT*) during pregnancy are associated with maternal parenting behavior. *47th Annual Meeting of the International Society of Psychoneuroendocrinology*. Zürich, Schweiz, September 2017.

6. Toepfer, P., Heim, C., Entringer, S., Provencal, N., Binder, E. B., Wadhwa, P. D., Buss, C. A variation in the oxytocin receptor gene moderates the relationship between early maternal care in childhood and interleukin 6 (IL-6) concentrations during pregnancy. *43. Gemeinsame Jahrestagung Deutschen Gesellschaft für Psychophysiologie und ihre Anwendung (DGPA) e.V und der Fachgruppe Biologische Psychologie und Neuropsychologie in der Deutschen Gesellschaft für Psychologie (DGPs) "Psychologie und Gehirn"*. Trier, Juni 2017.

7. Toepfer, P., Heim, C., Entringer, S., Provencal, N., Binder, E. B., Wadhwa, P. D., Buss, C. A variation in the oxytocin receptor gene moderates the relationship between early maternal care in childhood and interleukin 6 (IL-6) concentrations during pregnancy. *46th Annual Meeting of the International Society of Psychoneuroendocrinology*. Miami, FL, USA, September 2016.

8. Toepfer, P., Heim, C., Entringer, S., Binder, E. B., Wadhwa, P. D., Buss, C. Influence of Early Life Stress Exposure on Oxytocinergic Adaptations Over the Course of Pregnancy – Implications for Parenting Behavior. *ERA Net Neuron, mid-term symposium*. Helsinki, Finnland, September 2015.

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