Adaptive Human Behavior and Physiology (2018) 4:248–263
https://doi.org/10.1007/s40750-018-0090-7

ORIGINAL ARTICLE

Genetic Variation in the Maternal Oxytocin System Affects Cortisol Responsiveness to Breastfeeding in Infants and Mothers

Kathleen M. Krol[1,2] · Mikhail Monakhov[3] · Poh San Lai[4] · Richard P. Ebstein[3] · Markus Heinrichs[5,6] · Tobias Grossmann[1,2]

Received: 22 November 2017 / Revised: 13 March 2018 / Accepted: 14 March 2018 /
Published online: 28 March 2018

Abstract

Objectives The neuropeptide oxytocin regulates milk let-down during breastfeeding and maternal behavior in mammals. Oxytocin has also been shown to reduce stress through inhibitory effects on hypothalamic-pituitary-adrenal (HPA) reactivity. However, it remains unknown whether and how infant cortisol levels are affected by breastfeeding and what role the oxytocin system plays in this process. In the current

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s40750-018-0090-7) contains supplementary material, which is available to authorized users.

✉ Kathleen M. Krol
krol@virginia.edu

✉ Tobias Grossmann
grossmann@virginia.edu

[1] Department of Psychology, University of Virginia, 485 McCormick Rd, Charlottesville, VA 22903, USA

[2] Max Planck Institute for Human Cognitive and Brain Sciences, Stephanstrasse 1a, 04103 Leipzig, Germany

[3] Department of Psychology, National University of Singapore, 9 Arts Link, Singapore 117570, Singapore

[4] Department of Paediatrics, National University of Singapore, 1E Kent Ridge Road, Singapore 119228, Singapore

[5] Department of Psychology, University of Freiburg, Stefan-Meier-Strasse 8, 79104 Freiburg, Germany

[6] Freiburg Brain Imaging Center, Breisacher Strasse 64, 79106 Freiburg, Germany

study, we examined whether genetic variation in the oxytocin system impacts the cortisol response to breastfeeding in 84 mother-infant dyads.
Methods Salivary cortisol was measured before and after a breastfeeding session. Mothers and infants were genotyped for a single-nucleotide polymorphism (SNP) in the *CD38* gene (rs3796863). We compared between CC carriers and CA/AA carriers, as the CC genotype has been associated with reduced release of oxytocin and higher rates of autism in prior studies.
Results Our results show that differences in infant and maternal cortisol responses to breastfeeding were associated with variation in maternal *CD38*. Specifically, CA/AA mothers displayed a significantly greater reduction in cortisol after breastfeeding than mothers with the CC genotype. Moreover, infants of CA/AA mothers showed significantly reduced cortisol levels after breastfeeding, as compared to infants of CC mothers.
Conclusions The current findings demonstrate that maternal cortisol responses to breastfeeding vary as a function of their genetic capacity to release oxytocin, and this may also impact their infant's stress regulation. This suggests a potential mechanism by which breastfeeding contributes to the development of HPA reactivity in infancy.

Keywords *CD38* · Oxytocin · Cortisol · Breastfeeding · Stress · Infancy

Introduction

Stress has been linked to a wide range of mental health outcomes (Gunnar and Vazquez 2001; Lupien et al. 2009). It is crucial to examine what factors may impact the development of the stress response during the earliest stage of postnatal development, as it will foster a better understanding of how stress-related disorders come about and offer new avenues for treatment possibilities. To date, much work has focused on the development of the stress response in infancy and its link to the quality of maternal care (Albers et al. 2008; Francis and Meaney 1999; Haley and Stansbury 2003; Szyf et al. 2005), and also on the role of oxytocin in reducing stress in adults (de Oliveira et al. 2012; Heinrichs et al. 2003; Linnen et al. 2012; Parker et al. 2005). However, little is known as to whether and how maternal factors related to the oxytocin system affect the stress response in infancy. Breastfeeding is a prominent element of maternal care that is dependent on oxytocin (Lincoln and Paisley 1982). Specifically, infant suckling induces a neural reflex in which afferent sensory fibers projecting directly into the hypothalamus initiate the release of oxytocin into the bloodstream; oxytocin then stimulates the contraction of myoepithelial cells to eject milk (Gimpl and Fahrenholz 2001). A significant increase of plasma and salivary oxytocin during suckling occurs in both mothers and offspring (Amico et al. 1994; Dawood et al. 1981; Lupoli et al. 2001). Critically, in line with the notion that oxytocin reduces stress (de Oliveira et al. 2012), breastfeeding results in a strong reduction of hypothalamic-pituitary-adrenal (HPA) axis activity in mothers (for a review, see Heinrichs et al. 2002), as reflected in a marked decrease in plasma adrenocorticotropic hormone (ACTH) and cortisol (Amico et al. 1994; Handlin et al. 2009; Nissen et al. 1996). Furthermore, breastfeeding duration has been linked to a range of maternal outcomes such as lower perceived stress levels, reduced anxiety and depressive symptoms, stronger cardiac vagal tone modulation,

reduced heart rate reactivity, blood pressure, and reduced HPA activity in response to psychosocial stress (Groër 2005; Hahn-Holbrook et al. 2011; Heinrichs et al. 2002; Jansen et al. 2008; Mezzacappa et al. 2005).

Given the existing evidence demonstrating the anxiolytic influence of breastfeeding, the question emerges whether and how these processes seen in mothers may affect the stress response in their infants. While stress responses to breastfeeding have not been examined in human infants, breastfeeding experience has been shown to be associated with differences in infants' stress *reactivity*. Specifically, during the Strange Situation Procedure (Ainsworth et al. 1978), in which the mother leaves her infant for a predefined time and is then reunited, it was shown that infants who were breastfed for longer durations displayed quicker cortisol reductions than infants who were breastfed for shorter durations (Beijers et al. 2013).

There is a body of research indexing bio-behavioral synchrony or co-regulation between mothers and their infants, which is also reflected in synchronized (attuned) cortisol levels between mother and infant (see Feldman, 2012b for a review). This physiological co-regulation (adrenocortical attunement) is thought to be an outcome of extensive and close shared experiences between mother and child (Hibel et al. 2015). It is possible that breastfeeding is one route through which adrenocortical attunement is instantiated. In this context it is important to note that maternal oxytocin and cortisol levels impact the levels of these hormones in the breast milk ingested by the infants (Peaker and Neville 1991; Takeda et al. 1986), thus providing a potential physiological mechanism as to how maternal hormone levels can impact infant stress responses. For example, when rat dams were injected with radioactively labeled oxytocin (^{3}H-oxytocin), stable traces of the same ^{3}H-oxytocin were found in the gastric contents and blood of offspring after suckling (Takeda et al. 1986). Moreover, a wealth of studies in non-human primates have demonstrated that maternal milk cortisol directly impacts behavioral phenotypes of offspring (for a review, see Hinde 2013). Despite these studies demonstrating the transfer of maternal cortisol and oxytocin to offspring through suckling, the immediate impact of breastfeeding on these hormones in human infants is unknown.

When assessing cortisol responses, it is critical to consider variability in the oxytocin system that may contribute to differences in HPA activity. In particular, there is mounting evidence that there is natural genetic variation impacting the oxytocin system and that such genetic differences are associated with differences in cortisol reactivity during stress (Chen et al. 2011; Kumsta and Heinrichs 2013; Meyer-Lindenberg et al. 2011), and with the sensitivity to exogenous oxytocin administration (Chen et al. 2015). Therefore, another objective of the current study was to examine whether genetic variation related to the oxytocin system is related to differences in infant and maternal cortisol responses during breastfeeding. We focused our investigation on a single-nucleotide polymorphism (SNP) of the *CD38* gene (rs3796863). CD38 is an ectoenzyme that mediates the release of oxytocin through its impact on calcium signaling within oxytocinergic neurons (Jin et al. 2007). Evidence from mouse knock-out models has shown that *CD38* is critical for the display of oxytocin-linked behaviors, such as maternal care in females and social recognition in males. Moreover, knockout mice without *CD38* show significantly reduced levels of oxytocin when measured in blood and brain (Jin et al. 2007). In humans, the common SNP *CD38* rs3796863 has been associated with differences in social functioning. Specifically, the

CC genotype has been associated with lower levels of oxytocin, reduced sensitive parenting, impaired social memory, and reduced responsiveness to gratitude (Algoe and Way 2014; Feldman et al. 2012; Sauer et al. 2013; Sauer et al. 2012). Furthermore, the C allele is more common in autism spectrum disorder (ASD) than controls and is considered a risk allele for atypical social development (Lerer et al. 2010; Munesue et al. 2010). Prior work has also shown that breastfeeding experience interacts with genetic variation at this locus to account for individual differences in infants' attention to emotional cues (Krol et al. 2015a).

Based on the prior work reviewed above, we formulated the following main hypotheses. First, we predicted that maternal cortisol levels would reduce as a result of breastfeeding. Second, similar to what has been shown in breastfeeding mothers, we hypothesized that infants would show a similar reduction in cortisol levels after breastfeeding. Our third and critical hypothesis was that cortisol responses to breastfeeding would be differentially impacted by genetic variation of the *CD38* gene. More specifically, we predicted that genotypes related to higher oxytocin levels (CA and AA genotypes) would be associated with a larger reduction in cortisol in response to breastfeeding. Considering that maternal hormonal levels are likely to impact the breast milk itself, we further predicted that genetic variation within the mothers may in fact have a greater impact on infants' cortisol responses than that of the infants' own oxytocin system.

Material and Methods

Participants The final sample consisted of 84 four-to-six-month-old infants (41 females, 43 males) and their mothers. Infants' ages ranged from 120 to 196 days ($M = 147.90$ days, $SEM = 1.55$), and mothers' ages ranged from 23 to 43 years ($M = 31.57$, $SEM = 0.47$). An additional 17 mother-infant dyads participated in this study: 16 were bottle-feeders, and one infant was fed mashed food before breastfeeding. The current study only included the dyads who breastfed during the feeding session ($N = 84$). This sample size is similar or larger than prior work investigating genetic association effects in infants (Grossmann et al. 2011; Grossmann et al. 2013) and adults (Algoe and Way 2014; Sauer et al. 2013). All infants were typically developing, had a normal birth weight (> 2500 g), were born full-term (37–41 weeks), and were of European descent. Current percentage of breastfed meals ranged from 42.86% to 100% ($M = 92.08\%$, $SEM = 1.50$), with 65.50% of the sample still exclusively breastfeeding (100% breastfed meals). There was no known history of autism spectrum disorder neither in any of the participating mothers nor in any of the older siblings. 67 infants underwent standard vaginal deliveries and 17 were delivered via caesarean section. All mothers were still on maternity leave at the time of testing. Prior to participation in the study, infants' parents provided informed consent. All procedures were approved by the Leipzig University Medical School Ethics Committee, and were performed in accordance with the Declaration of Helsinki. Infants received a toy for participating, and parents were reimbursed for travel.

Procedure Mother-infant dyads were scheduled to arrive in the lab about an hour before a normal feeding time. They were instructed to abstain from eating or drinking anything apart from water thirty minutes prior to their appointment. Upon arrival to the

lab, parents and infants were given time to acquaint themselves with their surroundings and the experimenter. The room was dimly lit with a couch and carpet to make the session as comfortable and relaxing as possible. During this time, the experimenter explained the session protocol in detail, and a consent form was read through and signed by a parent. The first saliva samples were collected immediately after consent was given (about five to ten minutes post-arrival). A timeline of the experimental procedure is depicted in Fig. 1.

After collection of the baseline saliva samples for cortisol analyses (T-60-min pre-breastfeeding), an additional saliva sample was acquired for genetic analyses (see *Genotyping* below). Mothers and infants then had relaxed free time until they were ready to feed. During this time, mothers filled out questionnaires and the experimenter quietly played with the infant. When mothers were ready to feed their infant, the second saliva sample for cortisol was taken (T-1-min pre-breastfeeding). The mothers were instructed to breastfeed as they normally do at home and to take as long as they needed. The experimenter left the room for the feeding session. Breastfeeding lasted an average of twelve minutes (*range*: 5–32 min). After feeding, the third (T + 1-min post-breastfeeding), fourth (T + 8-min post-breastfeeding), and fifth (T + 16-min post-breastfeeding) saliva samples were taken while the mother and infant were relaxed, either filling out questionnaires or quietly playing with the experimenter. Sample time points were selected based off of stress-induction studies in adults, which typically show that salivary cortisol peaks around +10–15 min post-stress (Chen et al. 2011; Klumbies et al. 2014). Based on these studies, we expected peak effects to occur within a similar time frame. The average experimental session lasted a little over an hour (M = 1:06, SEM = 0:01). Although we requested arrival an hour before feeding, the mothers fed their infants as soon as the infants were hungry, which shortened the pre-feeding waiting time for some sessions. Note that variation in experimental session time occurred between baseline and feeding as well as during the feeding itself. Sessions were held most frequently in the late mornings due to typical infant feeding and sleeping schedules (i.e. arrival an hour before lunch and subsequent afternoon nap) (arrival time M = 11:09 AM, SEM = 11 min). Please note that healthy adults display a well-established diurnal rhythm of cortisol levels, characterized by a marked increase in cortisol immediately upon wakening followed by a slow and gradual reduction from about three hours after wakening through the evening (Edwards et al. 2001). While this rhythm is more variable in infants (de Weerth et al. 2003), we included arrival time as a covariate when necessary to account for any diurnal fluctuations (see *Results*).

Salivary Cortisol Saliva samples were collected from infants and their mothers at five time points across the experimental session (at T-60, T-1, T + 1, T + 8, and T + 16 min

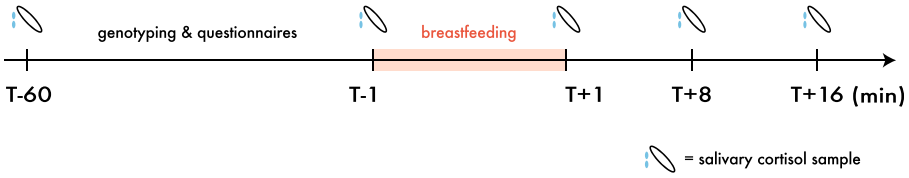

Fig. 1 Procedure timeline. Infant and maternal cortisol levels were measured at five time points across a breastfeeding session

relative to breastfeeding) using commercially available devices (the Salimetrics Infant's Swab (Salimetrics, Suffolk, UK) and the Salivette (Sarstedt, Nümbrecht, Germany), respectively). Samples were stored in a − 80 °C freezer after each experimental session and were later transported at room temperature for analysis. Cortisol levels were analyzed at the Technical University of Dresden, Germany, using luminescence immunoassay kits purchased from IBL International (Hamburg, Germany). The functional sensitivity of the assay was 0.011 (micro)g/dL. Inter- and intra-assay coefficients of variation were < 8%.

Genotyping Sponges were used to collect saliva from infants (CS-2 and OG-250 kits) and collection tubes were used to collect passive drool from mothers (OG-500 kit) from DNA Genotek, Ottawa, Canada. Samples were stored at room temperature prior to DNA extraction and SNP analysis, completed at the National University of Singapore. DNA was extracted using manufacturer's (DNA Genotek) purification protocol. Genotyping of *CD38* rs3796863 was performed with a 5′-nuclease assay. Primers and probes were from Applied Biosystems (TaqMan® SNP Genotyping Assay). PCR was conducted with HotStarTaq Plus DNA polymerase and Q-solution (Qiagen, Venlo, Netherlands) in a Biorad C1000 instrument with CFX96 fluorescence reading module, with the following thermal protocol: denaturation at 95 °C for five minutes; followed by cycling: 95 °C for 15 s, 60 °C for one minute; 45 times.

CD38 rs3796863 genotype frequencies of mothers and infants are displayed in Tables 1 and 2. Frequencies are similar to those observed in other studies (Sauer et al. 2013; Sauer et al. 2012), and no Mendelian inheritance inconsistencies were detected regarding transference of alleles from mothers to offspring. Hardy-Weinberg equilibrium was tested in mothers and infants separately; no deviations were detected (all p-values >0.05). The majority of studies relating the polymorphism rs3796863 (*CD38*) with aspects of social behavior and plasma oxytocin find differences between homozygous risk allele carriers (CC) and A carriers (CA/AA) (Algoe and Way 2014; Feldman et al. 2013; Feldman et al. 2012; Krol et al. 2015a; Sauer et al. 2013; Sauer et al. 2012). In keeping with prior studies, we conducted our analyses by grouping genotypes in the same manner (CC versus CA/AA).

Questionnaires A breastfeeding questionnaire was administered in order to assess feeding behavior of the mother-infant dyad (i.e. the duration of exclusive breastfeeding, percentage of breastfed meals per day), as well as general demographic questions regarding duration of maternity leave, parity, maternal education, and ethnicity. Details of this in-house questionnaire have been published elsewhere (Krol et al. 2014; Krol

Table 1 Maternal *CD38* rs3796863 genotype frequencies

Genotype	Frequency	%
CC	52	61.9
CA	23	27.4
AA	9	10.7

In analyses CA and AA infants were grouped together

Table 2 Infant *CD38* rs3796863 genotype frequencies

Genotype	Frequency	%
CC	37 (16 F, 21 M)	44.0
CA	43 (22 F, 21 M)	51.2
AA	4 (3 F, 1 M)	4.8

F, females, M, males. In analyses CA and AA infants were grouped together

et al. 2015a; Krol et al. 2015b). A short form of the Social Support Questionnaire (SSQ6) was administered in order to gauge the amount of social support the mothers felt they had, as well as how satisfied they were with the support (Sarason et al. 1987). The Parental Sense of Competence (PSOC) questionnaire assessed how knowledgeable and capable each mother felt with parenting (Johnston and Mash 1989). Lastly, the Edinburgh Postnatal Depression Questionnaire (EPDS) was administered to each mother to detect potential signs of postpartum depression (Cox et al. 1987).

Data Analysis In order to identify and rule out potentially confounding factors, analyses (one-way ANOVAs) were performed to uncover any differences between maternal and infant genotype groups with regard to the questionnaire and demographic data. These variables included all questionnaire measures (breastfeeding: current exposure and duration of exclusive breastfeeding, SSQ6, PSOC, and EPDS) as well as the time of arrival, feeding duration during the study, and demographic factors such as maternal age, infant age, years of maternal education, parity, number of caretakers, and current subjective stress level of the mother. As an additional analysis, average maternal and infant cortisol levels were correlated with aforementioned questionnaire and demographic factors to detect potential covariates to be included (and controlled for) in our main analyses. The results of these preliminary analyses are outlined below.

Maternal *CD38* groups did not significantly differ on any of the aforementioned demographic or questionnaire variables (all p-values >0.35). There was no difference with respect to the delivery method (vaginal birth versus caesarean section) between maternal genotype groups ($\chi^2[1] = 1.20$, $p = 0.16$) or infant *CD38* genotype groups ($\chi^2[1] = 0.54$, $p = 0.46$). However, there were three variables that differed significantly between infant groups: feeding duration during the study ($F[1, 82] = 7.30$, $p = 0.008$), current breastfeeding exposure ($F[1,82] = 6.35$, $p = 0.01$), and maternal postpartum depression score ($F[1,81] = 4.29$, $p = 0.04$). Specifically, infants with the CC genotype fed a few minutes longer than CA/AA infants during the study, had a greater percentage of breastfed meals, and had mothers who scored higher on the Edinburgh Postnatal Depression Scale (see supplementary Table 1). We therefore included these three variables as covariates in our main analyses presented below.

Raw infant and maternal cortisol levels were not normally distributed and thus corrected by using log-transformation (raw maternal cortisol: $Z_{skewness} = 7.39$, $Z_{kurtosis} = 13.59$; raw infant cortisol: $Z_{skewness} = 5.27$, $Z_{kurtosis} = 6.85$). Log-transformed values were used in all analyses. Neither infant nor maternal cortisol levels were significantly correlated with any of the questionnaire or demographic factors (all p-values >0.10). However, as expected by what is already known about diurnal cortisol changes in adults (Edwards et al. 2001), average maternal cortisol levels decreased with later

arrival times ($r[84] = -.37$, $p = 0.001$). There was no such association between infant cortisol levels and arrival time ($r[83] = -.16$, $p = 0.16$). Neither infant nor maternal cortisol levels differed depending on infant gender (p-values >0.83).

Taken together, four potential confounds were controlled for by entering them as covariates in our main analyses. Specifically, in analyses in which: (a) maternal cortisol was a dependent variable, arrival time was included as a covariate, and (b) investigated effects of infant *CD38* genotype, feeding duration, current breastfeeding exposure, and maternal postpartum depression (EPDS) score were included as covariates. In the analyses presented below, Greenhouse-Geisser results are reported when sphericity could not be assumed.

Results

Maternal Cortisol Response is Impacted by Maternal, but not Infant, *CD38* Genotype In order to investigate the impact of maternal genotype on maternal cortisol levels during breastfeeding, a repeated-measures ANCOVA was conducted with sample time point (cortisol T-60 through T + 16) as the within-subjects factor, maternal *CD38* genotype as the between-subjects factor, and arrival time as a covariate. Our analysis revealed a main effect of sample time point such that a general decrease in cortisol levels was observed in response to breastfeeding in all mothers, $F(1.77, 132.34) = 4.23$, $p = 0.021$. Additionally, we found an interaction between sample time point and *CD38* genotype, $F(1.77, 132.34) = 5.03$, $p = 0.01$ (Fig. 2). In order to explore this interaction,

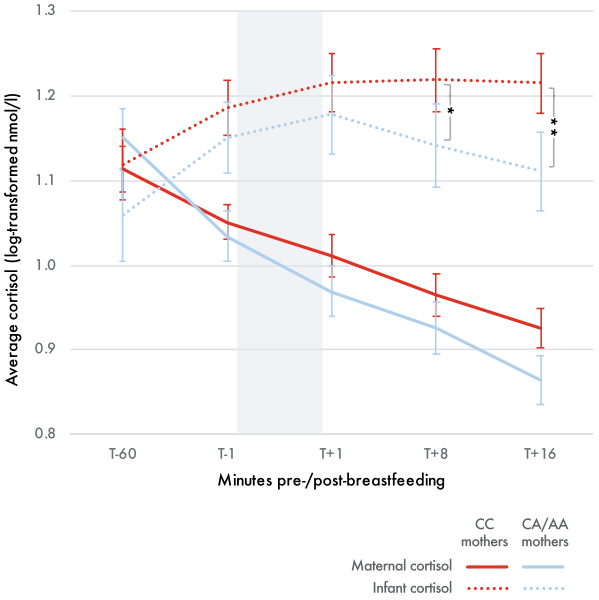

Fig. 2 Infant and maternal cortisol across the breastfeeding session and maternal *CD38* genotype. Graph depicts log-transformed cortisol levels at −60, −1, +1, +8, and + 16 min pre−/post-breastfeeding by maternal *CD38* genotype. Area in gray indicates when breastfeeding took place. Solid lines represent maternal cortisol levels, dashed lines represent infant cortisol levels. Means and standard errors reported are from the original analyses; $*p < 0.05$, $**p < 0.01$

we calculated a difference score to quantify cortisol reactivity over time by taking levels at T + 16 (post-breastfeeding) and subtracting levels at T-60 (baseline). Thus, a difference score that is positive indexes an increase in cortisol across the breastfeeding session, while a difference score that is negative indexes a decrease in cortisol across the session. A univariate ANCOVA with this difference score as the dependent variable and maternal *CD38* genotype as the between-subjects factor revealed that cortisol reduction across the breastfeeding session was significantly greater in CA/AA mothers than in CC mothers, $F(1, 78) = 6.69$, $p = 0.01$ (Fig. 3). This suggests that mothers with genetically higher oxytocin levels had greater reductions of cortisol in response to breastfeeding (CA/AA: $M = -0.28$, $SEM = 0.02$) than those with genetically lower oxytocin (CC: $M = -0.19$, $SEM = 0.02$). Univariate ANCOVAs at each individual sample time point revealed that maternal *CD38* genotype did not significantly impact cortisol levels at specific sample points (all p-values >0.15).

In order to explore the potential impact of infant *CD38* genotype on maternal cortisol levels, a repeated-measures ANCOVA was conducted with sample time (T-60 through T + 16) as the within-subjects factor and infant *CD38* genotype (CC vs. CA/AA) as the between-subjects factor. The interaction between infant *CD38* genotype and sample time point was not significant ($p > 0.077$). There were also no main effects of the covariates feeding duration, current breastfeeding exposure, or postnatal depression score on maternal cortisol levels (all p-values >0.16).

Infant Cortisol Response is Impacted by Maternal, but not Infant, *CD38* Genotype Consistent with the previous analyses of maternal cortisol responses, a repeated-measures ANCOVA was conducted with sample time point (cortisol T-60 through T + 16) as the within-subjects factor and maternal *CD38* genotype as the between-subjects factor. A main effect of sample time point was present $F(2.18, 126.54) = 3.60$, $p = 0.027$, indexing a general increase from baseline. While no interaction between maternal *CD38* genotype and sample time point was observed ($p > 0.68$), we decided to statistically explore potential differences between maternal genotype groups among infants in a follow-up analysis, because visual inspection of the cortisol response data suggested elevated levels of cortisol in infants of CC mothers (who showed elevated cortisol responses themselves as seen in the analysis of the maternal data detailed above). Using univariate ANCOVAs, this analysis revealed that

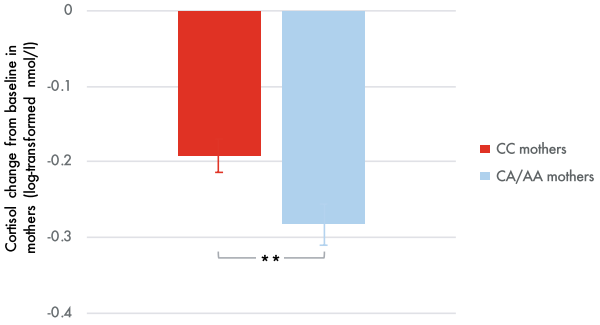

Fig. 3 Maternal cortisol reduction across the breastfeeding session and maternal *CD38* genotype. Graph depicts the change (reduction) in maternal cortisol levels across the breastfeeding session by maternal *CD38* genotype. Means and standard errors reported are from the original analysis; $^{**}p < 0.01$

maternal *CD38* genotype significantly impacted infant cortisol levels *post* breastfeeding at T + 8 and T + 16, $F(1, 78) = 4.17$, $p = 0.04$ and $F(1, 74) = 5.71$, $p = 0.02$, respectively. Specifically, infants of CC genotype mothers had significantly higher cortisol levels after breastfeeding than infants with CA/AA mothers (Fig. 2). The addition of arrival time as a covariate in these analyses did not change our findings, and in fact strengthened them (T + 8: $F(1, 77) = 5.05$, $p = .027$; T + 16: $F(1, 73) = 7.911$, $p = .006$). Note that these significant comparisons would not survive conservative Bonferroni correction.

In order to explore the potential impact of infant *CD38* genotype on infant cortisol levels, the same repeated-measures ANCOVA was conducted with sample time point (T-60 through T + 16) as the within-subjects factor and infant *CD38* genotype (CC vs. CA/AA) as the between-subjects factor, including feeding duration, current breastfeeding exposure, and maternal postnatal depression as covariates. No significant main effects or interactions were observed for these variables or any of the covariates, all *p*-values >0.06. Univariate analyses revealed no group differences with regard to any specific sample time point, all *p*-values >0.15.

Maternal and Infant Cortisol In order to investigate whether there was an association between maternal and infant cortisol levels, *Pearson's* correlations were performed. Average cortisol levels across the session did not significantly correlate between mothers and infants, $r(83) = 0.14$, $p = 0.20$. In addition, we examined whether there is a correlation between maternal cortisol levels and infant cortisol levels specifically after breastfeeding (sample time points: T + 1, T + 8, and T + 16), and whether any such correlation differs between maternal *CD38* genotype groups. This was done because cortisol and oxytocin transfer from the mother to her infant through breast milk (Hinde 2013; Takeda et al. 1986), so maternal levels may predict infant levels after milk ingestion. As a whole, maternal and infant cortisol levels did not correlate after breastfeeding. Examining maternal *CD38* groups separately suggests a marginal association between average cortisol levels within CC mothers and their infants, $r(51) = 0.25$, $p = 0.08$ but not for CA/AA mothers and their infants $r(32) = -0.03$, $p = 0.43$. Relevant data files have been deposited in the Center for Open Science digital repository (https://osf.io/g59tb/).

Discussion

This is the first study to examine how both infant and maternal cortisol levels are affected by breastfeeding and what role variation in *CD38* plays in this process. In mothers, our results suggest that breastfeeding is linked to a general reduction in cortisol. It is important to note that time of day was included in this analysis, suggesting that the reduction in cortisol was present after taking diurnal changes into account. This reduction of cortisol levels in mothers confirms previous work that has also found a reduction in HPA activity during breastfeeding (Amico et al. 1994), and may help inform the finding that breastfeeding mothers generally feel less stressed than bottle-feeding mothers (Groër 2005), and that increased duration of breastfeeding is associated with lower HPA reactivity when faced with a stressful situation (Heinrichs et al. 2002). With regard to infant cortisol levels, our hypothesis regarding a general

reduction of cortisol was not confirmed. Instead, we observed a general increase in cortisol levels from baseline. In this context, it should be noted that in adults food intake has been shown to activate the HPA axis (Gibson et al. 1999). It is thus possible that, in infants, breastfeeding is associated with a general increase in cortisol levels.

The current data further revealed that both maternal and infant cortisol levels were critically modulated by genetic variation in the maternal oxytocin system (*CD38* rs3796863). Our results show that the degree to which cortisol levels decrease in response to breastfeeding is impacted by variation at the *CD38* rs3796863 locus. Specifically, mothers with CA/AA genotypes exhibited a stronger decline of cortisol than mothers with the CC genotype. This suggests that mothers with lower endogenous oxytocin levels (CC genotype) may have more elevated cortisol levels after breastfeeding because they have less oxytocin available to reduce HPA activity. This is important because it indicates that mothers with genetically (endogenously) higher levels of oxytocin might be most sensitive to the reported anxiolytic effects of breastfeeding. It is also possible that mothers with genetically lower levels of oxytocin (CC genotype) might be more susceptible to stress. However, there are two limitations to this conjecture: first, it is not clear whether this effect generalizes to situations other than breastfeeding and second, we do not have direct evidence for reduced oxytocin levels in mothers with the CC genotype, because we did not directly measure oxytocin in the current study.

Our results further demonstrated that infants' cortisol responses were modulated by maternal *CD38* genotype. In particular, we found that infants of mothers with the CC genotype exhibited higher levels of cortisol than infants of mothers with CA/AA genotypes eight minutes and sixteen minutes after breastfeeding. This indicates that variability within *CD38* does not only impact the mothers' own but also her infants' cortisol response. It is thus possible that, through breastfeeding, genetic variation in the maternal oxytocin system is associated with an increased susceptibility to stress in both mothers and their infants.

It is important to mention that infant *CD38* genotype was not associated with infant or maternal cortisol responses; the effects seen in response to breastfeeding were specifically related to the maternal *CD38* genotype. Why infant *CD38* genotype did not have an impact on infants' cortisol response remains unclear. However, it should be mentioned that in prior work with older infants (7 months of age), infant *CD38* genotype has been shown to impact differences in infants' attention to emotional eyes as a function of duration of exclusive breastfeeding (Krol et al. 2015a). This indicates that in a different context (emotion perception and exclusive breastfeeding duration) and at an older age (7 instead of 4–6 months of age) infant *CD38* genotype has an effect on socio-emotional processes. Regardless, the current data show that it is genetic variation within the maternal oxytocin system that affects infants' stress physiology at this young age. This supports the general notion that maternal variables play an important role in regulating emotional (stress) responses in infants (Feldman 2012a).

With respect to a potential link between maternal and infant cortisol responsiveness, we found no general association between maternal and infant cortisol levels. However, our data suggest a mild association between infant and maternal cortisol levels between infants and mothers with the CC genotype. These findings provide some evidence for adrenocortical attunement between mother and child, and may raise the possibility that, through breastfeeding, cortisol may transfer from the mother to the infant. This is a

plausible scenario because previous work has shown that maternal hormones such as cortisol and oxytocin are: (1) stable in breast milk and (2) can be transferred to the gastric contents and blood plasma of the offspring upon suckling (Peaker and Neville 1991; Takeda et al. 1986). Indeed, a recent study compared breastfed infants and formula-fed infants and found a significant correlation in night cortisol levels between mothers and infants only for the breastfeeding dyads (Neelon et al. 2015). Future work is needed in which cortisol and oxytocin levels are directly examined in breast milk and saliva to test this possibility.

Previous research has related breastfeeding with positive health, cognitive, and social outcomes in infants (Deoni et al. 2013; Isaacs et al. 2010; Krol et al. 2015b). The current study provides evidence for one possible mechanism accounting for some of these effects, which is the regulation of HPA activity in infants through endogenous maternal oxytocin. In fact, our findings point towards a form of oxytocin-mediated co-regulation of HPA activity (stress) between mothers and infants (Feldman 2012b). Our results suggest that mothers with more endogenously available oxytocin (CA and AA genotypes) more effectively regulate (reduce) their own HPA activity and that of their infants in response to breastfeeding. Conversely, mothers with less endogenously available oxytocin (CC genotypes) less effectively regulate (reduce) their own HPA activity and that of their infants. It is possible that in the long-term with repeated breastfeeding over the course of infancy these genetic differences in the maternal oxytocin system might result in chronic differences in stress regulation patterns among mothers and their infants. Investigating long-term outcomes in stress regulation outside the breastfeeding context will also be of crucial importance to understand the clinical relevance of these genetic differences.

Breastfeeding is a highly complex process manifested in hormonal, tactile, and behavioral changes in both mother and infant, shaped by mammalian evolution. In the current study, as in most of the existing breastfeeding research with humans, this complexity makes it difficult to mechanistically identify what specific aspect of the breastfeeding process accounts for observed effects. Not only does breastfeeding depend upon the oxytocin system, but it also involves touch, warmth, and eye contact; all of which may contribute to the reported differences in cortisol levels. The current study is only a first step and future research is needed that extends these findings and identifies potential underlying mechanisms. Specifically, future work would benefit from comparing cortisol responses in infants and mothers during breastfeeding, bottle feeding, no feeding, and a condition with no mother-infant interaction. Moreover, it is possible that oral contraceptive use in mothers may have contributed to our findings as this has been shown to impact HPA activity (Kirschbaum et al. 1999). Another limitation to improve upon in future research is that participants, depending on the time needed to complete consent forms, had only about five to ten minutes to acclimate to the laboratory environment. This may have resulted in increased stress levels at baseline. While this cannot account for the effects observed in the current study, future studies may benefit from a longer warm-up period in the laboratory, and perhaps the use of additional measurement points before and after breastfeeding to better map the cortisol responses.

In summary, the current study presents novel insights into how HPA reactivity to breastfeeding in infants and mothers is influenced by the maternal oxytocin system. The current findings provide support for the notion that the maternal oxytocin system

may play a critical role in infant development and highlight the importance of extending this line of research with infants to the study of stress. The observed patterns of differential cortisol reactivity to breastfeeding depending on maternal *CD38* genotype in infants provides: (a) foundational insights into the early development of the stress response and (b) will likely serve as a vital basis for inspiring future work looking at the long-term consequences of these individual differences.

Acknowledgments Open access funding provided by Max Planck Society. We would like to thank Caterina Boettcher and Jenny Tippmann for their assistance with data collection, Dr. Clemens Kirschbaum and his colleagues for salivary cortisol analysis, and all families who participated in this study. This work was supported by funds awarded by the Max Planck Society.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest.

References

Ainsworth, M. D. S., Blehar, M. C., Waters, E., & Wall, S. (1978). *Patterns of attachment: A psychological study of the strange situation*. Hillsdale, NJ: Lawrence Erlbaum Associates Publishers.

Albers, E. M., Riksen-Walraven, J. M., Sweep, F. C. G. J., & de Weerth, C. (2008). Maternal behavior predicts infant cortisol recovery from a mild everyday stressor. *Journal of Child Psychology and Psychiatry, 49*(1), 97–103. https://doi.org/10.1111/j.1469-7610.2007.01818.x.

Algoe, S. B., & Way, B. M. (2014). Evidence for a role of the oxytocin system, indexed by genetic variation in CD38, in the social bonding effects of expressed gratitude. *Social Cognitive and Affective Neuroscience, 9*(12), 1855–1861.

Amico, J. A., Johnston, J. M., & Vagnucci, A. H. (1994). Suckling-induced attenuation of plasma cortisol concentrations in postpartum lactating women. *Endocrine Research, 20*(1), 79–87.

Beijers, R., Riksen-Walraven, J. M., & de Weerth, C. (2013). Cortisol regulation in 12-month-old human infants: Associations with the infants' early history of breastfeeding and co-sleeping. *Stress, 16*(3), 267–277. https://doi.org/10.3109/10253890.2012.742057.

Chen, F. S., Kumsta, R., von Dawans, B., Monakhov, M., Ebstein, R. P., & Heinrichs, M. (2011). Common oxytocin receptor gene (OXTR) polymorphism and social support interact to reduce stress in humans. *Proceedings of the National Academy of Sciences of the United States of America, 108*(50), 19937–19942.

Chen, F. S., Kumsta, R., Dvorak, F., Domes, G., Yim, O. S., Ebstein, R. P., & Heinrichs, M. (2015). Genetic modulation of oxytocin sensitivity: A pharmacogenetic approach. *Translational Psychiatry, 5*, e664. https://doi.org/10.1038/tp.2015.163.

Cox, J. L., Holden, J. M., & Sagovsky, R. (1987). Detection of postnatal depression. Development of the 10-item Edinburgh postnatal depression scale. *The British Journal of Psychiatry, 150*(6), 782–786.

Dawood, M. Y., Khandawood, F. S., Wahi, R. S., & Fuchs, F. (1981). Oxytocin release and plasma anterior-pituitary and gonadal-hormones in women during lactation. *The Journal of Clinical Endocrinology & Metabolism, 52*(4), 678–683.

de Oliveira, D. C., Zuardi, A. W., Graeff, F. G., Queiroz, R. H., & Crippa, J. A. (2012). Anxiolytic-like effect of oxytocin in the simulated public speaking test. *Journal of Psychopharmacology, 26*(4), 497–504.

de Weerth, C., Zijl, R. H., & Buitelaar, J. K. (2003). Development of cortisol circadian rhythm in infancy. *Early Human Development, 73*(1–2), 39–52. https://doi.org/10.1016/S0378-3782(03)00074-4.

Deoni, S. C., Dean, D. C., 3rd, Piryatinksy, I., O'Muircheartaigh, J., Waskiewicz, N., Lehman, K., … Dirks, H. (2013). Breastfeeding and early white matter development: A cross-sectional study. *NeuroImage, 82*(100), 77–86.

Edwards, S., Evans, P., Hucklebridge, F., & Clow, A. (2001). Association between time of awakening and diurnal cortisol secretory activity. *Psychoneuroendocrinology, 26*(6), 613–622. https://doi.org/10.1016/S0306-4530(01)00015-4.

Feldman, R. (2012a). Bio-behavioral synchrony: A model for integrating biological and microsocial behavioral processes in the study of parenting. *Parenting-Science and Practice, 12*(2–3), 154–164. https://doi.org/10.1080/15295192.2012.683342.

Feldman, R. (2012b). Parent-infant synchrony: A biobehavioral model of mutual influences in the formation of affiliative bonds. *Monographs of the Society for Research in Child Development, 77*(2), 42–51.

Feldman, R., Zagoory-Sharon, O., Weisman, O., Schneiderman, I., Gordon, I., Maoz, R., … Ebstein, R. P. (2012). Sensitive parenting is associated with plasma oxytocin and polymorphisms in the OXTR and CD38 genes. *Biological Psychiatry, 72*(3), 175–181.

Feldman, R., Gordon, I., Influs, M., Gutbir, T., & Ebstein, R. P. (2013). Parental oxytocin and early caregiving jointly shape children's oxytocin response and social reciprocity. *Neuropsychopharmacology, 38*(7), 1154–1162.

Francis, D. D., & Meaney, M. J. (1999). Maternal care and the development of stress responses. *Current Opinion in Neurobiology, 9*(1), 128–134. https://doi.org/10.1016/S0959-4388(99)80016-6.

Gibson, E. L., Checkley, S., Papadopoulos, A., Poon, L., Daley, S., & Wardle, J. (1999). Increased salivary cortisol reliably induced by a protein-rich midday meal. *Psychosomatic Medicine, 61*(2), 214–224.

Gimpl, G., & Fahrenholz, F. (2001). The oxytocin receptor system: Structure, function, and regulation. *Physiological Reviews, 81*(2), 629–683.

Groër, M. W. (2005). Differences between exclusive breastfeeders, formula-feeders, and controls: A study of stress, mood, and endocrine variables. *Biological Research for Nursing, 7*(2), 106–117.

Grossmann, T., Johnson, M. H., Vaish, A., Hughes, D. A., Quinque, D., Stoneking, M., & Friederici, A. D. (2011). Genetic and neural dissociation of individual responses to emotional expressions in human infants. *Developmental Cognitive Neuroscience, 1*(1), 57–66. https://doi.org/10.1016/j.dcn.2010.07.001.

Grossmann, T., Vaish, A., Franz, J., Schroeder, R., Stoneking, M., & Friederici, A. D. (2013). Emotional voice processing: Investigating the role of genetic variation in the serotonin transporter across development. *PLoS One, 8*(7), e68377. https://doi.org/10.1371/journal.pone.0068377.

Gunnar, M. R., & Vazquez, D. M. (2001). Low cortisol and a flattening of expected daytime rhythm: Potential indices of risk in human development. *Development and Psychopathology, 13*(3), 515–538. https://doi.org/10.1017/S0954579401003066.

Hahn-Holbrook, J., Holt-Lunstad, J., Holbrook, C., Coyne, S. M., & Lawson, E. T. (2011). Maternal defense: Breast feeding increases aggression by reducing stress. *Psychological Science, 22*(10), 1288–1295.

Haley, D. W., & Stansbury, K. (2003). Infant stress and parent responsiveness: Regulation of physiology and behavior during still-face and Reunion. *Child Development, 74*(5), 1534–1546. https://doi.org/10.1111/1467-8624.00621.

Handlin, L., Jonas, W., Petersson, M., Ejdeback, M., Ransjo-Arvidson, A. B., Nissen, E., & Uvnas-Moberg, K. (2009). Effects of sucking and skin-to-skin contact on maternal ACTH and cortisol levels during the second day postpartum-influence of epidural analgesia and oxytocin in the perinatal period. *Breastfeeding Medicine, 4*(4), 207–220. https://doi.org/10.1089/bfm.2009.0001.

Heinrichs, M., Neumann, I. D., & Ehlert, U. (2002). Lactation and stress: Protective effects of breast-feeding in humans. *Stress, 5*(3), 165–203.

Heinrichs, M., Baumgartner, T., Kirschbaum, C., & Ehlert, U. (2003). Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biological Psychiatry, 54*(12), 1389–1398.

Hibel, L., Granger, D., Blair, C., Finegood, E., & Key, F. L. P. (2015). Maternal-child adrenocortical attunement in early childhood: Continuity and change. *Developmental Psychobiology, 57*(1), 83–95. https://doi.org/10.1002/dev.21266.

Hinde, K. (2013). Lactational programming of infant behavioral phenotype. In K. B. H. Clancy, K. Hinde, & J. N. Rutherford (Eds.), *Primate developmental trajectories in proximate and ultimate perspectives* (pp. 187–220). New York: Springer.

Isaacs, E. B., Fischl, B. R., Quinn, B. T., Chong, W. K., Gadian, D. G., & Lucas, A. (2010). Impact of breast milk on intelligence quotient, brain size, and white matter development. *Pediatric Research, 67*(4), 357–362.

Jansen, J., de Weerth, C., & Riksen-Walraven, J. M. (2008). Breastfeeding and the mother-infant relationship-a review. Developmental Review, 28(4), 503–521. doi:https://doi.org/10.1016/j.dr.2008.07.001.

Jin, D., Liu, H. X., Hirai, H., Torashima, T., Nagai, T., Lopatina, O. … Higashida, H. (2007). CD38 is critical for social behaviour by regulating oxytocin secretion. *Nature, 446*(7131), 41–45.

Johnston, C., & Mash, E. J. (1989). A measure of parenting satisfaction and efficacy. *Journal of Clinical Child Psychology, 18*(2), 167–175.

Kirschbaum, C., Kudielka, B., Gaab, J., Schommer, N., & Hellhammer, D. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosomatic Medicine, 61*(2), 154–162. https://doi.org/10.1097/00006842-199903000-00006.

Klumbies, E., Braeuer, D., Hoyer, J., & Kirschbaum, C. (2014). The reaction to social stress in social phobia: Discordance between physiological and subjective parameters. *PLoS One, 9*(8), e105670. https://doi.org/10.1371/journal.pone.0105670.

Krol, K. M., Kamboj, S. K., Curran, H. V., & Grossmann, T. (2014). Breastfeeding experience differentially impacts recognition of happiness and anger in mothers. *Scientific Reports, 4*, 7006.

Krol, K. M., Monakhov, M., Lai, P. S., Ebstein, R. P., & Grossmann, T. (2015a). Genetic variation in CD38 and breastfeeding experience interact to impact infants' attention to social eye cues. *Proceedings of the National Academy of Sciences of the United States of America, 112*(35), E5434–E5442. https://doi.org/10.1073/pnas.1506352112.

Krol, K. M., Rajhans, P., Missana, M., & Grossmann, T. (2015b). Duration of exclusive breastfeeding is associated with differences in infants' brain responses to emotional body expressions. *Frontiers in Behavioral Neuroscience, 8*, 459.

Kumsta, R., & Heinrichs, M. (2013). Oxytocin, stress and social behavior: Neurogenetics of the human oxytocin system. *Current Opinion in Neurobiology, 23*(1), 11–16.

Lerer, E., Levi, S., Israel, S., Yaari, M., Nemanov, L., Mankuta, D., … Ebstein, R. P. (2010). Low CD38 expression in lymphoblastoid cells and haplotypes are both associated with autism in a family-based study. *Autism Research, 3*(6), 293–302.

Lincoln, D. W., & Paisley, A. C. (1982). Neuroendocrine control of milk ejection. *Journal of Reproduction and Fertility, 65*, 571–586.

Linnen, A. M., Ellenbogen, M. A., Cardoso, C., & Joober, R. (2012). Intranasal oxytocin and salivary cortisol concentrations during social rejection in university students. *Stress-the International Journal on the Biology of Stress, 15*(4), 393–402. https://doi.org/10.3109/10253890.2011.631154.

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience, 10*(6), 434–445. https://doi.org/10.1038/nrn2639.

Lupoli, B., Johansson, B., Uvnäs-Moberg, K., & Svennersten-Sjaunja, K. (2001). Effect of suckling on the release of oxytocin, prolactin, cortisol, gastrin, cholecystokinin, somatostatin and insulin in dairy cows and their calves. *Journal of Dairy Research, 68*(2), 175–187.

Meyer-Lindenberg, A., Domes, G., Kirsch, P., & Heinrichs, M. (2011). Oxytocin and vasopressin in the human brain: Social neuropeptides for translational medicine. *Nature Reviews Neurosience, 12*(9), 524–538.

Mezzacappa, E. S., Kelsey, R. M., & Katkin, E. S. (2005). Breast feeding, bottle feeding, and maternal autonomic responses to stress. *Journal of Psychosomatic Research, 58*(4), 351–365.

Munesue, T., Yokoyama, S., Nakamura, K., Anitha, A., Yamada, K., Hayashi, K. … Higashida, H. (2010). Two genetic variants of CD38 in subjects with autism spectrum disorder and controls. *Neuroscience Research, 67*(2), 181–191.

Neelon, S. E. B., Stroo, M., Mayhew, M., Maselko, J., & Hoyo, C. (2015). Correlation between maternal and infant cortisol varies by breastfeeding status. *Infant Behavior & Development, 40*, 252–258. https://doi.org/10.1016/j.infbeh.2015.06.005.

Nissen, E., Uvnas-Moberg, K., Svensson, K., Stock, A. M., Widstrom, A. M., & Winberg, J. (1996). Different patterns of oxytocin, prolactin but not cortisol release during breastfeeding in women delivered by caesarean section or by the vaginal route. *Early Human Development, 45*(1–2), 103–118. https://doi.org/10.1016/0378-3782(96)01725-2.

Parker, K. J., Buckmaster, C. L., Schatzberg, A. F., & Lyons, D. M. (2005). Intranasal oxytocin administration attenuates the ACTH stress response in monkeys. *Psychoneuroendocrinology, 30*(9), 924–929. https://doi.org/10.1016/j.psyneuen.2005.04.002.

Peaker, M., & Neville, M. C. (1991). Hormones in milk - chemical signals to the offspring. *Journal of Endocrinology, 131*(1), 1–3. https://doi.org/10.1677/Joe.0.1310001.

Sarason, I. G., Sarason, B. R., Shearin, E. N., Pierce, E. N., & Pierce, G. R. (1987). A brief measure of social support: Practical and theoretical implications. *Journal of Social and Personal Relationships, 4*(4), 497–510.

Sauer, C., Montag, C., Wörner, C., Kirsch, P., & Reuter, M. (2012). Effects of a common variant in the CD38 gene on social processing in an oxytocin challenge study: Possible links to autism. *Neuropsychopharmacology, 37*(6), 1474–1482.

Sauer, C., Montag, C., Reuter, M., & Kirsch, P. (2013). Imaging oxytocin × dopamine interactions: An epistasis effect of CD38 and COMT gene variants influences the impact of oxytocin on amygdala activation to social stimuli. *Frontiers in Neuroscience, 7*, 45.

Szyf, M., Weaver, I. C. G., Champagne, F. A., Diorio, J., & Meaney, M. J. (2005). Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. *Frontiers in Neuroendocrinology, 26*(3–4), 139–162. https://doi.org/10.1016/j.yfrne.2005.10.002.

Takeda, S., Kuwabara, Y., & Mizuno, M. (1986). Concentrations and origin of oxytocin in breast milk. *Endocrinologia Japonica, 33*(6), 821–826.