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Research Article

Evidence for a Neuroendocrinological Foundation of Human Affiliation

Plasma Oxytocin Levels Across Pregnancy and the Postpartum Period Predict Mother-Infant Bonding

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ABSTRACT—*Although research on the neurobiological foundation of social affiliation has implicated the neuropeptide oxytocin in processes of maternal bonding in mammals, there is little evidence to support such links in humans. Plasma oxytocin and cortisol of 62 pregnant women were sampled during the first trimester, last trimester, and first postpartum month. Oxytocin was assayed using enzyme immunoassay, and free cortisol was calculated. After the infants were born, their interactions with their mothers were observed, and the mothers were interviewed regarding their infant-related thoughts and behaviors. Oxytocin was stable across time, and oxytocin levels at early pregnancy and the postpartum period were related to a clearly defined set of maternal bonding behaviors, including gaze, vocalizations, positive affect, and affectionate touch; to attachment-related thoughts; and to frequent checking of the infant. Across pregnancy and the postpartum period, oxytocin may play a role in the emergence of behaviors and mental representations typical of bonding in the human mother.*

As social animals, humans are biologically prepared to form selective and enduring bonds to other individuals. These bonds provide protection and caregiving, ensure survival, and offer soothing during times of distress (Carter & Keverne, 2002). Bond formation is essential for survival and involves a set of

genetic and epigenetic, neurological, behavioral, and cognitive processes that serve as the basis for the capacity to form meaningful relationships throughout life (Leckman et al., 2004). The mother-infant bond, the primary bond across mammalian species, is expressed in a clearly defined set of maternal postpartum behaviors that emerge or intensify during the bonding stage. These behaviors include proximity seeking, touch, and contact. Additional maternal bonding behaviors in humans are gaze at the infant, “motherese” vocalizations, positive expression, and adaptation to cues expressed by the infant. Pedersen (1999) suggested that maternal behavior revolutionized child care not only by sustaining protection and nurturing, but also by permitting a longer period of brain development, a prerequisite for higher intelligence. Animal research supporting this idea has demonstrated the profound effects of maternal postpartum behavior on brain morphology and physiology, lifelong propensity for stress reactivity, and the pup’s ultimate capacity for social affiliation (Meaney, 2001). Studies in humans similarly indicate that maternal postpartum behavior has long-term effects on infants’ cognitive, neurobehavioral, and social-emotional growth (Feldman & Eidelman, 2003, 2007; Feldman, Eidelman, & Rotenberg, 2004).

Although bonding in humans integrates physiological and behavioral processes shared by all mammals, human bonding is organized by cognitive and metacognitive processes that coordinate responses at the biological, perceptual, emotional, and behavioral levels (Hinde, 1989). Thus, the fear component in human bonding includes not only bonding-related physiological and behavioral processes that activate the fear system—for example, heightened arousal, vigilance, and increased anxiety, which are mediated by brain stem and limbic structures—but

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also preoccupations with the well-being and safety of the infant. Similarly, the reward component, which addresses the motivational and incentive value placed on the loved person and is mediated by midbrain, thalamic, and hypothalamic structures, is expressed not only in selective contact, hormonal release, and affiliative touch, but also in attachment representations of the infant and the shared future (Panksepp, 1998). A longitudinal assessment from late pregnancy to 3 months postpartum found a typical mental constellation of maternal bonding, including both anxious preoccupations and pleasurable attachment representations (Leckman et al., 1999). These bonding-related representations intensified at the first postpartum month and then decreased, and they were highly sensitive to risk conditions, such as maternal postpartum depression and premature birth (Feldman, Weller, Leckman, Kvint, & Eidelman, 1999).

Recently, models proposing a neuroendocrinological foundation for social affiliation in mammals have received attention and support. Specifically, research in animals has implicated the neuropeptide oxytocin (OT) in processes of parental, pair, and filial bonding. OT is a uniquely mammalian hormone consisting of nine amino acids. It is observed in brain regions implicated in attachment and functions to integrate autonomic states with social behavior. OT is secreted in association with uterine contraction and milk ejection and plays a role in the initiation of maternal behavior (Insel & Young, 2001). A study of female voles demonstrated that hypothalamic oxytocin gene expression and receptor binding increased in the postpartum (Wang, Liu, Young, & Insel, 2000), and deficits in maternal behavior, including lower pup retrieval and less licking and self-grooming, were found in oxytocin knockout mice (Pedersen, Vadlamudi, Boccia, & Amico, 2006). Cross-fostering studies indicated that individual differences in OT receptors in female pups were related to variations in early maternal behavior that these pups received as infants, as well as to the amounts of maternal postpartum behaviors, including licking, grooming, and arched-back nursing, that they provided later to their own young (Meaney, 2001); these results point to the involvement of OT in the cross-generation transmission of attachment.

Little research has addressed the role of OT in human bonding. Although animal studies have focused on brain OT, human research necessarily has tested peripheral levels. Still, peripheral OT has been associated with bonding-related factors, such as empathy, closeness, and trust (Grewen, Girdler, Amico, & Light, 2005), and early parental neglect was found to alter peripheral OT (Fries, Ziegler, Kurian, Jcoris, & Pollak, 2005). Other studies have shown that following birth, mother-infant touch and contact stimulate OT release (Matthiesen, Ransjo-Arvidson, Nissen, & Uvnas-Moberg, 2001), and intranasal administration of OT increases trusting behavior (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005). Thus, OT is thought to play a special role in the initiation of bonding, possibly by decreasing stress, increasing trust, and integrating psychological

and physiological states that enable calmness and approach (Uvnas-Moberg, 1998).

In light of these findings, we examined the relations between OT and maternal bonding in humans. OT was measured in the first trimester of pregnancy, the last trimester, and the first postpartum month, and bonding was assessed through both maternal behaviors and bonding-related representations. Given the inverse associations between OT and cortisol (Heinrichs, Baumgarten, Kirshbaum, & Ehlert, 2003), cortisol was also assessed at each time point. The relations between OT and human bonding are of theoretical importance at several levels. First, such links may suggest that similar mechanisms are at work across species and that human bonding draws on its mammalian heritage. Second, associations between OT in early pregnancy and maternal postpartum behavior may point to potential priming effects, indicating that OT across pregnancy sensitizes mothers to the initiation of bonding. Finally, it is of interest whether OT is related to the behavioral aspect of bonding, shared by all mammals, or whether it also correlates with the exclusively human component of bonding, expressed in cognitive representations.

METHOD

Subjects

Subjects were 62 pregnant women in their first trimester (mean age = 27.8 years, $SEM = 0.70$, range = 18.4–43.2), and 52% of the sample were primiparous. Of these 62 women, 50% had completed high school, 26% had some college education, and 24% had completed college; 37% were unemployed, 10% worked part-time, and 53% worked full-time. Fifty-six percent of the mothers breast-fed full-time, 27% breast-fed part-time, and 17% did not breast-feed. All the mothers were married, were in good health, and used no medication. Nurses in nationwide well-baby clinics invited all mothers fitting the inclusion criteria to participate. The study was approved by the institutional review board of the Israel Ministry of Health, and the women signed informed consent.

Procedure

The initial assessment (T1) was during the first trimester ($M = 10$ weeks, $SEM = 0.32$, range = 6–16). At this time, the mothers completed questionnaires assessing demographics, anxiety, and depression, and blood samples were drawn by nurses trained to handle blood assays for this study. The second assessment (T2) was early in the third trimester ($M = 27.35$ weeks, $SEM = 0.27$, range = 22.43–32.72). Mood questionnaires were administered and blood samples were drawn as at T1. The final assessment (T3) was during the first postpartum month ($M = 1.84$ weeks, $SEM = 0.11$, range = 0.57–4.14). The mothers completed the same questionnaires as earlier, and blood samples were drawn again. Additionally, 15 min of mother-infant interaction were

videotaped in a private room, and the mothers were interviewed for 30 to 45 min. Data pertaining to maternal anxiety and depression are not presented in this report.

Measures

Maternal Behavior

Coders blind to the mothers' hormonal status coded the videotapes using the *Coding Interactive Behavior Manual–Newborn Version* (Feldman, 1998), a validated system for coding mother–newborn interactions (Feldman & Eidelman, 2003, 2007). In this system, interactions are coded for mutually exclusive behaviors in each of four categories of maternal behavior: gaze (gaze at infant's face, gaze at surrounding, gaze aversion), affect (positive, neutral, angry, withdrawn), touch (affectionate touch, functional touch, rocking, stimulating touch, no touch), and vocalization (motherese, adult speech, no talk). For each 10-s epoch, one behavior from each category is selected, and the proportion of time the mother spends enacting that behavior is computed. Interrater reliability, computed for 13 interactions (21%), averaged 92%, $\kappa = .85$. As in previous research, we calculated a *maternal-behavior* composite, which was the sum of the proportions of the following behaviors from the four categories: mother's gaze at infant's face, positive affect, affectionate touch, and motherese vocalizations.

Maternal Representations

We assessed the mothers' cognitive representations with the adapted Yale Inventory of Parent Thought and Action (Feldman et al., 1999). This instrument includes a maternal interview and a self-report measure, each subdivided into nine topics pertaining to bonding-related thoughts, feelings, and behaviors. First, a clinician conducted the interview, eliciting a free narrative on each topic. These narratives were audiotaped and transcribed, and two blind coders scored the degree to which each narrative focused on the assigned topic, using a scale from 1 (*low*) to 5 (*high*). Interrater reliability, calculated for 10 interviews, averaged 94% (intraclass $r = .92$). Next, the mothers completed the self-report questionnaire, which included a series of questions on each of the topics covered in the interview.

We calculated three composite scores: *maternal preoccupation*, the average of responses to six questionnaire items (e.g., preoccupations with the infant's health, safety, and future) and the coded preoccupation score from the interview ($\alpha = .88$); *attachment representations*, the average of responses to four questionnaire items (e.g., imagining the infant when not with him or her) and the coded attachment score from the interview ($\alpha = .85$); and *checking behavior*, the average of two self-report items and the coded interview score for checking the infant during the day and night ($\alpha = .84$).

Hormones

Blood was sampled between 8:00 and 10:00 a.m. OT is not pulsatile when mothers are not nursing (Russell, Leng, & Douglas, 2003). A single draw of 5 ml of blood was taken into Li Heparin vacutainer tubes supplemented with 2,000 kallikrein inhibitor units of aprotinin (Bayer, Wuppertal, Germany) for OT determination, and 5 ml of blood was drawn into EDTA (ethylenediaminetetraacetic acid) vacutainer tubes for cortisol and cortisol binding globulin (CBG) determination.

The OT samples were kept ice-chilled up to 4 hr before being centrifuged at 4 °C at 1,800 G for 15 min. Supernatants were collected and stored at –70 °C until assayed. Determination of OT was performed using a 96-plate commercial OT enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN). This ELISA is an immunoassay for the quantitative determination of OT and is considered sensitive and reliable (Kramer, Cushing, Carter, Wu, & Ottinger, 2004). The kit recognizes exclusively OT, not other nanopeptides (vasopressin, somatostatin). Measurements were performed in duplicate. All samples for each subject were assayed in the same batch to avoid differences due to interassay variance. Samples were diluted 1:5 in the assay buffer and treated according to the instructions of the kit. The concentrations of samples were calculated using MatLab 6 from the relevant standard curves (range: 3.9 pg/ml–1,000 pg/ml of OT). For each plate, a separate standard curve was constructed. The intra-assay and interassay coefficients of variability were 10.2 to 12% and 5.5 to 20.6%, respectively.

Total cortisol was assessed using an immunofluorescent polarization enzyme-linked immunoassay (IFPEIA; Abbott Laboratories, Abbott Park, IL). Interassay and intra-assay coefficients of variability were 5 to 7% and 7.5 to 12%, respectively. Next, CBG was determined by radioimmunoassay (CBG-RIA-100, BioSource, Nivelles, Belgium). Samples were diluted 1:25 in the assay buffer and treated according to instructions. All CBG samples were run simultaneously using several kits; for each kit, a separate standard curve was constructed. The range of the curves was from 0.46 to 8.7 $\mu\text{g/ml}$ of CBG. Calculated free cortisol was determined from total cortisol and CBG, by using Coolen's equation (for details, see Levine, Zagoory-Sharon, Feldman, Lewis, & Weller, 2007).

RESULTS

No correlations were found between either OT or cortisol and any demographic factors, and none of the study variables showed differences between breast-feeding and non-breast-feeding women. With regard to OT and breast-feeding, previous studies (e.g., van der Post et al., 1997) have similarly reported no differences in OT between breast-feeding and non-breast-feeding mothers when OT is not sampled during breast-feeding. Descriptive statistics appear in Table 1.

TABLE 1
Descriptive Statistics for the Study Variables

Variable	Mean	SEM	Range
Oxytocin (pM): T1	328.73	86.32	11.64–3,648.70
Oxytocin (pM): T2	267.25	60.93	59.58–3,300.00
Oxytocin (pM): T3	267.87	49.63	55.40–2,775.00
Calculated free cortisol (nM): T1	26.38	3.55	2.37–114.62
Calculated free cortisol (nM): T2	61.44	8.18	7.62–265.30
Calculated free cortisol (nM): T3	43.00	5.98	2.69–206.64
Maternal behavior ^a	.53	.12	.06–.88
Maternal preoccupation ^b	2.85	0.54	0.67–4.75
Attachment representations ^b	3.39	0.12	2.2–4.56
Checking behavior ^b	2.56	0.33	1.00–5.00

Note. T1 = first assessment (first trimester); T2 = second assessment (third trimester); T3 = third assessment (first postpartum month).

^aThis composite score was obtained from the coded videotapes of mother-infant interactions. The score was calculated as the sum of the proportions of time spent enacting the following behaviors: mother’s gaze at infant, motherese vocalization, positive affect, and affectionate touch. ^bThese scores were derived from the interview and questionnaire of the Yale Inventory of Parent Thought and Action (Feldman, Weller, Leckman, Kvint, & Eidelman, 1999); narratives and responses were coded on a scale from 1 to 5.

Although OT levels ranged widely at each assessment, a repeated measures analysis of variance showed no significant change in the mean level of OT across the three assessments, sphericity-assumed $F(2) = 2.24, p = .12, \eta^2 = .04$ (see Fig. 1, top panel). No linear, cubic, or quadratic trends were found, indicating that peripheral levels of OT remained constant from early pregnancy to the postpartum period. OT also showed high individual stability: $r = .93, p < .001$, between T1 and T2; $r = .96, p < .001$, between T2 and T3; and $r = .92, p < .001$, between T1 and T3.

A repeated measures analysis of variance revealed that cortisol levels changed significantly across the study period, sphericity-assumed $F(2) = 7.01, p = .002, \eta^2 = .22$. The linear trend was significant, $F(1) = 4.91, p_{rep} = .912, \eta^2 = .09$, as was the quadratic trend, $F(1) = 6.23, p_{rep} = .941, \eta^2 = .11$. Cortisol increased substantially across pregnancy and then decreased, without reaching initial levels. A moderate degree of individual stability was found: $r = .29, p < .05$, between T1 and T2; $r = .31, p < .05$, between T2 and T3; and $r = .37, p < .01$, between T1 and T3 (see Fig. 1, bottom panel).

Pearson correlations between hormones at T3 and bonding variables showed that OT was related to maternal behavior, $r = .28, p < .05$; to attachment representations, $r = .35, p < .01$; and to checking behavior, $r = .42, p < .001$. OT at Time 1 was also correlated with maternal behavior, $r = .25, p = .04$; attachment representations, $r = .28, p < .05$; and checking behavior, $r = .34, p < .01$. Cortisol at T3 was negatively related to maternal behavior, $r = -.33, p < .01$. OT and cortisol were unrelated at each time point.

Finally, hierarchical multiple regressions were computed to examine the unique effects of hormones, cognitive representa-

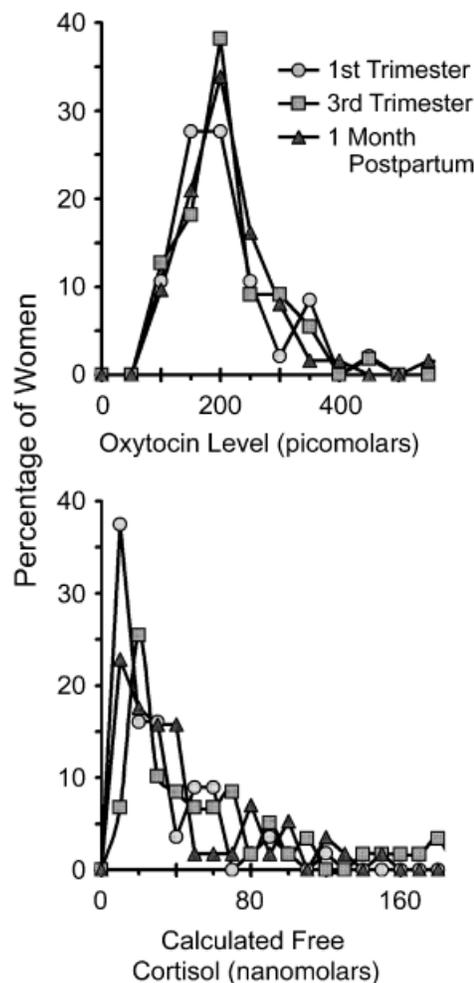


Fig. 1. Distribution curves for levels of oxytocin (top panel) and calculated free cortisol (bottom panel) at the first trimester, third trimester, and first postpartum month. For oxytocin, percentages are presented for every 50-pM interval; each percentage is plotted against the highest level of oxytocin in the corresponding interval. Four women with values between 551 and 3,648 pM are not included in this graph. For calculated free cortisol, percentages are presented for every 10-nM interval; each percentage is plotted against the highest level of free cortisol in the corresponding interval.

tions, and checking on maternal relational behavior. In the first two steps, OT and cortisol from T3 were entered. Maternal preoccupation and attachment representations were entered in the next two steps, and checking behavior was entered in the final step. All variables were normally distributed.

As Table 2 shows, higher OT and lower cortisol levels were uniquely predictive of maternal behavior. Maternal preoccupations with infant safety explained unique variance, and the contribution of attachment representations was marginally significant. In combination, the predictor variables explained 27.5% of the variance in maternal behavior.

Similar regressions were computed with OT at T1 and OT at T2 entered in the first step and cortisol at T1 and cortisol at T2 entered in the second step. The following three steps were identical to those in the regression reported in Table 2. OT at

TABLE 2
Results of the Hierarchical Multiple Regression Predicting Maternal Behavior

Predictor	β	R	ΔR^2	ΔF	df
Oxytocin: T3	.24*	.27	.07	4.25*	1, 60
Calculated free cortisol: T3	-.26*	.39	.09	4.77*	2, 59
Maternal preoccupation	.33*	.47	.07	4.27*	3, 58
Attachment representations	.30*	.52	.05	3.61†	4, 57
Checking behavior	.03	.53	.00	0.13	5, 56

Note. The model accounted for 28% of the variance, $F(5, 56) = 3.06, p = .013$. T3 = third assessment (first postpartum month).

† $p < .10$. * $p < .05$.

T1 was a meaningful predictor of maternal behavior, $\beta = .23$, $\Delta R^2 = .07$, $\Delta F(1, 60) = 4.03$, $\eta^2 = .06$, $p_{\text{rep}} = .886$, as was OT at T2, $\beta = .25$, $\Delta R^2 = .08$, $\Delta F(1, 60) = 4.45$, $p < .05$, $\eta^2 = .07$, $p_{\text{rep}} = .893$.

DISCUSSION

This study is the first to demonstrate that maternal OT across pregnancy and the postpartum period is linked to the set of behaviors and the mental constellation typical of bonding in the human mother. The results suggest that the neuroendocrine system associated with bond formation in mammals may play a similar role in humans. OT was found to be related to a well-defined cluster of maternal behaviors, attachment representations, and a specific maternal behavior that appears across mammalian species—repeated checking. The findings, therefore, shed light on the three theoretical issues raised in our introduction: OT is related to human bonding; OT levels are consistent across pregnancy, and initial levels predict postpartum bonding behaviors; and OT is related to the mental, as well as the behavioral, aspect of bonding. These findings lend support to ethological and evolutionary perspectives on human bonding. It is important to note, however, that causal relations between hormones and behavior cannot be inferred from this study and that both hormone levels and behavior may be related to a third underlying mechanism (e.g., anxiety).

The relations between OT and the representational aspect of bonding suggest that OT supports the cognitive processes that organize bonding in humans, much as OT plays a role in initiating maternal proximity, the organizer of bonding in mammals. These findings indicate that there is cross-species continuity in the mechanisms that underlie the species-specific expressions of bonding, that is, the specific postpartum behaviors observed in each species and the representational component in humans. Note that OT was associated with the pleasurable aspect of bonding, attachment representations, but not with the anxiety-provoking preoccupation component. Such findings are consistent with perspectives suggesting that OT functions primarily

by reducing anxiety, increasing calmness, and intensifying the incentive value of the attachment target (Uvnas-Moberg, 1998).

There have been few longitudinal studies on OT across pregnancy, and even fewer that have used the new, more sensitive type of assay—enzyme immunoassay (EIA). Our OT values are consistent with those reported by De Geest, Thiery, Piron-Possuyt, and Vandenriessche (1985), who used radioimmunoassay methods. The fact that our EIA assessment showed a consistent, predominantly flat pattern over time and high individual stability supports the validity of this assessment.

The high individual stability in OT and the association of OT in the first trimester with postpartum behaviors and representations may suggest that OT functions to prime species-specific postpartum behaviors, expressed in vocalizations, gaze, and touch, and that OT similarly primes the mental processes required for affiliative bonds, including exclusivity of focus, repeated checking, and pleasurable mental states. Such a priming function, which has been found in other mammals (Pederson, 1999), may suggest that the central role of OT in humans relates to the initiation of affiliative bonds.

Whereas OT was related to higher levels of maternal behavior, cortisol was associated with reduced levels of maternal behavior. Research has shown that the relation between cortisol and maternal behavior is a complex one, modified by the mother's age, parity, and childhood experiences (Krpan, Coombs, Zinga, Steiner, & Fleming, 2005). Future research is required to fully elucidate the relations between cortisol and mother-infant attachment.

The study of OT has generated new insights into affiliation and bonding in mammals; however, the role of this hormone in human bonding is poorly understood. Future research may assess the role of OT in fathers or in mothers at risk for poor bonding, such as those with postpartum depression or whose infants are born prematurely. Better understanding of the way neuroendocrine systems interact with behavioral tendencies and representational models may shed further light on the biological foundation of early human attachment under both healthy and at-risk conditions.

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