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PAPER

Maternal and paternal plasma, salivary, and urinary oxytocin and parent–infant synchrony: considering stress and affiliation components of human bonding

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Abstract

Studies in mammals have implicated the neuropeptide oxytocin (OT) in processes of bond formation and stress modulation, yet the involvement of OT in human bonding throughout life remains poorly understood. We assessed OT in the plasma, saliva, and urine of 112 mothers and fathers interacting with their 4–6-month-old infants. Parent–infant interactions were micro-coded for parent and child's social behaviors and for the temporal coordination of their socio-affective cues. Parents were interviewed regarding their attachment to the infant and reported on bonding to own parents, romantic attachment, and parenting stress. Results indicated that OT in plasma (pOT) and saliva (sOT) were inter-related and were unrelated to OT in urine (uOT). pOT and sOT in mothers and fathers were associated with parent and child's social engagement, affect synchrony, and positive communicative sequences between parent and child. uOT was related to moments of interactive stress among mothers only, indexed by the co-occurrence of infant negative engagement and mother re-engagement attempts. pOT and sOT were associated with mothers' and fathers' attachment relationships throughout life: to own parents, partner, and infant, whereas uOT correlated with relationship anxiety and parenting stress among mothers only. Similar to other mammals, OT is involved in human attachment and contingent parenting. The dual role of OT in stress and affiliation underscores its complex involvement in processes of social bonding throughout life.

Introduction

The parent–infant bond provides the primary relationship in mammals that supports growth and development and buffers against physiological and social stress (Carter, 2005; Kendrick, Keverne & Baldwin, 1987; Maestripieri, Hoffman, Anderson, Carter & Higley, 2009; Pedersen & Prange, 1979). Research across mammalian species has shown that the neuropeptide oxytocin (OT) plays a key role in processes of bond formation and functions to reduce stress, enhance social competence, initiate maternal behavior, and promote social affiliation throughout life (Francis, Champagne & Meaney, 2000; Gimpl & Fahrenholz, 2001; Keverne & Kendrick, 1992; Pedersen, 2004; Ross, Cole, Smith, Neumann, Landgraf, Murphy & Young, 2009; Waldherr, Nyuyki, Malamby, Bosch & Neumann, 2010; Winslow, Hearn, Ferguson, Young, Matzuk & Insel, 2000). Among the central features of the OT system is its openness to early social experience (Ahern & Young, 2009; Champagne, Bagot, van Hasselt, Ramakers, Meaney, de Kloet, Joels & Krugers, 2008) and the effects of OT on brain organization are shaped early in life through the provision of well-timed maternal behavior (Meaney, 2010). Young mammals who received more maternal grooming and contact exhibited higher OT receptor densities in brain areas central for social affiliation, benefited more from environmental enrichment, were better equipped to handle stress, and provided more optimal parenting to their own infants (Champagne, 2008; Champagne & Meaney, 2007). Yet, in contrast to the abundance of research on OT and bond formation in mammals, the role of OT in human attachment has attracted less research. To date, no study has addressed the relations between OT and contingent parenting or the individual's attachment relationships throughout life. Understanding such links may be important for uncovering the biological basis of human attachment and assessing its consistency with other species.

Oxytocin in plasma, saliva, and urine and attachment-related processes

Research on OT and attachment-related processes in humans has mainly examined plasma OT (*p*OT). For instance, assessing OT repeatedly from early pregnancy to the postpartum, *p*OT levels were individually stable and predicted the amount of maternal postpartum

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behavior, including gaze at infant, 'motherese' vocalizations, positive affect, and affectionate touch, pointing to a priming effect of OT across pregnancy on the development of mothering (Feldman, Weller, Zagoory-Sharon & Levine, 2007). Mothers with secure attachment representations showed *p*OT increases following mother–infant interactions and greater BOLD fMRI response in brain areas rich in OT receptors, such as the medial preoptic area, lateral septum, and paraventricular nucleus of the hypothalamus (Strathearn, Fonagy, Amico & Montague, 2009). *p*OT increased in response to the initiation of breastfeeding (Jonas, Johansson, Nissen, Ejdeback, Ransjo-Arvidson & Uvnas-Moberg, 2009), and newborn suckling and touch stimulated *p*OT release (Matthiesen, Ransjo-Arvidson, Nissen & Uvnas-Moberg, 2001). In addition, *p*OT has been linked with processes of bonding throughout life. Adults with higher *p*OT reported more optimal bonding to their own parents (Gordon, Zagoory-Sharon, Schneiderman, Leckman, Weller & Feldman, 2008) and secure attachment to romantic partners (Tops, van Peer, Korf, Wijers & Tucker, 2007). Similarly, non-verbal displays of love between romantic partners correlated with *p*OT increase (Gonzaga, Turner, Keltner, Campos & Altemus, 2006), underscoring the role of OT in the individual's lifetime attachments: to parents, partners, and infants.

OT measured in saliva and urine has similarly been associated with processes of social affiliation. Carter and colleagues (2007) showed that salivary OT (*s*OT) is a reliable biomarker of peripheral OT, and research has demonstrated the presence of an OT receptor in the human salivary gland (Forsyth & Neville, 2009). *s*OT was found to increase immediately before breastfeeding (White-Traut, Watanabe, Pournajafi-Nazarloo, Schwertz, Bell & Carter, 2009), after massage in adults (Carter, Pournajafi-Nazarloo, Kramer, Ziegler, White-Traut, Bello & Schwertz, 2007), and following touch-related intervention to increase couple communication (Holt-Lunstad, Birmingham & Light, 2008). Following parent–infant interaction, an increase in *s*OT was found in parents who provided more tactile contact to their infants, but not among those who displayed low levels of touch (Feldman, Gordon, Schneiderman, Weisman & Zagoory-Sharon, 2010). Similarly, urinary OT (*u*OT) increased after mother–daughter conversation following a stressful laboratory paradigm (Seltzer, Ziegler & Pollak, 2010). These studies provide initial evidence for the involvement of OT in bonding-related processes as measured in plasma, saliva, and urine; yet no study to date has assessed the correlations between OT measured in these three peripheral systems at the same time.

The dual role of oxytocin in stress and affiliation

The anxiolytic effects of OT and its role in attenuating the stress response are observed in both humans and other mammals (Heinrichs & Gaab, 2007; Neumann, 2008). Authors have pointed to several mechanisms as mediating the effects of OT on stress (Lee, Macbeth, Pagani & Young, 2009). These include the enhancement of neural activity in brain areas central for emotion and cognitive processing while reducing activity in areas controlling autonomic and visceral responses (Febo, Shields, Ferris & King, 2009); modulating the release of serotonin, which reduces anxiety-related behavior (Yoshida, Takayanagi, Inoue, Kimura, Young, Onaka & Nishimori, 2009); and the role of variations in the OT receptor gene in regulating anxiety (Costa, Pini, Martini, Abelli, Gabelloni, Ciampi, Muti, Gesi, Lari, Cardini, Mucci, Bucci, Lacacchini & Cassano, 2009). Yet, human studies on the relations of OT and stress have yielded mixed results. Whereas some found links between OT and higher cortisol, an index of the stress response (Marazziti, Dell'Osso, Baroni, Mungai, Catena, Rucci, Albanese, Giannaccini, Betti, Fabbrini, Italiani, Del Debbio, Lucacchini & Dell'Osso, 2006), others reported correlations between OT and lower levels of stress (Heinrichs & Gaab, 2007).

One reason for the inconsistent findings may relate to the gender-specific effects of OT on stress. Carter and colleagues (2009) suggested that the effects of early rearing experiences on the oxytocinergic system is sexually dimorphic. Both acute and chronic OT manipulations in animals bred for high and low anxiety showed different effects in females and males (Slattery & Neumann, 2010). Similarly, OT was related to relationship distress in women, but not in men (Taylor, Saphire-Bernstein & Seeman, 2010). It has been theorized that since women use the formation of social bonds for the management of stress, the links between OT and stress are tighter in women (Taylor *et al.*, 2000). It is thus possible that among mothers OT would be more closely related to stress, particularly stress within close relationships, due to its role as a signal to the mother to form, maintain, nourish, and repair close bonds as a means for regulating stress.

Oxytocin and parent–infant synchrony

Parent–infant synchrony, which describes the temporal coordination between the parent and infant's affective behavior, is an important component of sensitive parenting that contributes to infant development (Feldman, 2007). Nelson and Panksepp (1998) suggest that hormonal changes in the mother across gestation, particularly in OT and prolactin, prime the initiation of maternal behavior following birth and provide the basis for the development of contingent parenting.

Synchronous interactions are organized in repetitive-rhythmic sequences that consist of matched affective behaviors in the gaze, vocal, facial expression, and touch modalities. Such moments of affect synchrony are important for the maturation of the infant's physiological systems and shape the individual's attachment relationships throughout life (Feldman, 2010). Synchronous exchanges may come in three forms according

to the timed relationships between the parent and child's social behavior. Concurrent synchronous relations indicate that the parent and child coordinate their affective behavior, and positive affective expressions co-occur in the two partners. Sequential synchronous relations imply that positive affective behaviors in one partner are followed by similar positive behaviors in the other, creating sequences of positive engagement. Finally, communications systems are dynamic in nature and are underlaid by patterned cross-correlations between the partners' behaviors that include a time-lag of responsivity, for instance, when one partner becomes more positive and the other follows by increasing positive engagement within a time-lag. Inherent in such moments of synchrony are repeated episodes of mismatch and repair, for instance, when the infant displays negative affect and the parent attempts to re-establish social contact (Tronick, 1989). Such mismatched moments are likely to increase the parent's stress, particularly among mothers who are more attentive to mismatch within the relationship (Feldman, 2003) and use social relationships for the management of stress (Taylor, Klein, Lewis, Gruenewald, Gurung & Updegraff, 2000).

The current study

In light of the above, the current study had three goals. First, we examined similarities and differences between maternal and paternal OT as expressed in plasma, saliva, and urine. Although much less research examined OT and fathering, OT in biparental fathers has been associated with paternal behavior and pup exposure (Ziegler, 2000), and similar neuroendocrine pathways are theorized to mediate the initiation of fathering and mothering in mammals (Wynne-Edwards & Timonin, 2007). Thus, similar levels of *p*OT, *s*OT, and *u*OT were expected in mothers and fathers.

The second goal aimed to assess the relations between OT and synchronous parent–infant interactions. Both moments of concurrent affect synchrony and sequences of positive engagement were expected to correlate with OT in both mothers and fathers. The degree of stress inherent in synchronous interactions, indexed by the co-occurrence of infant negative emotions and the parent's re-engagement attempts, was expected to correlate with higher maternal, but not paternal, OT.

The final goal was to test the relations between OT in the three fractions and the parent's attachment relationships throughout life, including bonding to own parents, romantic attachment, and attachment to infant. Human bonds include components of both stress and affiliation: romantic attachment involves anxieties for the exclusivity of the relationship and parental attachment involves biologically based preoccupations and worries with regard to infant safety (Leckman, Feldman, Swain, Eicher, Thompson & Mayes, 2004). Based on the dual role of OT, we expected that indices of both stress and affiliation would correlate with OT but that the links between OT and stress would be more notable among women.

Method

Participants

Participants were 112 parents and their infants, including 71 mothers and 41 fathers (not couples) and their 4–6-month-old infants (M = 166.3 days, SD = 12.6). Parents were of middle-class SES, healthy, and with at least 12 years of education. Mothers' age was, M = 28.7, SD = 5.29 years, and education, M = 15.17, SD = 2.47 years, and 81.3% were breastfeeding. Fathers' age was, M = 29.1, SD = 4.28 years and education, M = 15.50, SD = 2.73 years. Infants were born at term (birthweight: M = 3319.4 gr, SD = 452.1), mainly (96.3%) by vaginal delivery, received an Apgar score of 9.40 (SD = 1.56), and 55% were firstborns. Infants were healthy since birth, and parents were screened for depression and anxiety. Fathers reported at least medium-level participation in childcare on a scale of 1–5, which assessed how much parents shared childcare responsibilities in terms of both time spent with child and types of activities performed (e.g. bathing, feeding, diapering, doctor's visits). The study was approved by the Institutional Review Board and all parents signed informed consent.

Procedure

Parents and infants arrived at the lab during the early afternoon (1–4pm) and following a 10-minute acquainting period when no touch or parent–child interaction occurred (parent completed questionnaires and RA took care of the infant), provided baseline plasma, saliva, and urine samples in consecutive order. Assessments were arranged to take place at least half an hour after breastfeeding and to end at least half an hour before breastfeeding, in light of White-Traut *et al.* (2009), who showed that following a peak in OT during the period surrounding breastfeeding, OT in breastfeeding mothers returns to baseline levels. Then parent and child entered an observation room with an infant-seat mounted on a table and were filmed from an adjoining room by two cameras that were integrated into a single frame using a split-screen generator. Parents were asked to engage in a 15-minute interaction that would include any type of touch they typically use. Fifteen minutes after play, post-interaction saliva and urine samples were collected. Blood was drawn only once at baseline as our pilot study indicated that multiple draws created high levels of stress. Parents were then interviewed and completed self-report measures.

Hormone collection and analysis

Plasma

Blood was drawn from antecubital veins into 9 mL chilled vacutainer tubes containing lithium heparin that was supplemented with 400 KIU of Trasylol (Trasylol–Bayer, Germany) per 1 mL blood. Blood samples were kept ice-chilled for up to 2 hours before being centrifuged at 4°C at 1000 × g for 15 minutes. Supernatants were collected and stored at −80°C until assayed.

Saliva

*s*OT was collected by Sallivatte (Sarstedt, Rommelsdorft, Germany). Parents were asked to chew a roll of cotton for 40 seconds. Salivettes were kept ice-chilled for up to 1 hour before being centrifuged at 4°C at 1500 × g for 15 minutes. The liquid samples were stored at −80°C. To concentrate the samples by 3 or 4 times, the liquid samples were lyophilized overnight and kept at −20°C until assayed. The dry samples were reconstructed in the assay buffer immediately before analysis by Oxytocin EIA commercial kit, consistent with previous research (Carter *et al*., 2007).

Urine

The method for OT extraction was taken from the kit insert, with the following modifications to adjust the low concentration of *u*OT. Two ml of urine were loaded on HBL extraction cartridge 3 cc/60 mg (Waters Oasis, MA, USA). The cartridges were washed twice with 0.1% TFA and 10% acetonitrile solution. Samples were eluted by 0.1% TFA and 80% acetonitrile solution, dried by speed-vac, and kept at −20°C until assayed. For each urine collection the process was repeated twice. The dry samples were reconstructed in the assay buffer immediately before analysis by OT EIA commercial Kit.

Determination of oxytocin

OT was determined using a commercial OT ELISA kit (Assay Design, MI, USA) consistent with previous research (Carter *et al*., 2007; Feldman *et al*., 2007). Measurements were performed in duplicate and the concentrations of samples were calculated by using MatLab-7 according to relevant standard curves. The observed intra-assay and inter-assay coefficients were < 12.4% and 14.5%, respectively.

Coding of parent–infant interaction

Interactions were micro-coded on a computerized system (Noldus, The Vaggenigen, Netherlands), consistent with previous research on parent–infant synchrony (Feldman & Eidelman, 2004, 2007). Four non-verbal categories of parental behavior and four non-verbal categories of infant behavior were coded and each category included a set of mutually exclusive codes (an 'uncodable' code was added to each category in instances during which codes could not be determined). Categories and codes were as follows:

Parent

Parent Gaze: This category assessed the direction of parent gaze and included the following codes: gaze to infant's face, gaze to infant's body; gaze to object or environment; and gaze aversion, indicating that parent gazes away from the infant but gaze is not focused on other objects or the environment. *Parent Affect*: Parent's expressed affect was coded on the basis of facial expressions, body tone, movements, and other non-verbal signals and included positive, neutral, and negative affective expression. *Parent Vocalizations*: The parent's vocal output was coded along four codes: 'motherese' vocalizations, which are infant-directed speech that is high pitched and typically includes sing-song vocalizations; 'typical' adult speech to the infant in a normal range and regular rhythm; adult speech to other adult; and no speech. *Parent Touch* included six codes: affectionate touch – loving touch such as hugging, kissing, stroking, or light pokes; touch of infant extremities – touch of infant's hands or feet often with another object; functional touch – touch that has a functional goal such as wiping the infant's mouth; proprioceptive touch – includes touch that changes the infant's position in space, for instance, pulling the infant to a sitting position; stimulatory touch – indicating touch that intends to stimulate and increase arousal; and no touch.

Infant

Infant Gaze was coded similar to parent's gaze along the following codes: gaze to parent, gaze to object or environment, and gaze aversion. *Infant Affect* was similarly coded as positive, neutral, or negative. *Infant Vocalizations*: included positive vocalizations, such as positive babbling, cooing, or giggles, negative vocalizations, including fussing and crying. *Infant Touch* included intentional, accidental, and no touch. Inter-rater reliability was computed for 15 interactions and reliability kappas averaged .84 (range = .76–.93)

The following composites were computed as the sum proportions of several codes: *Parent Positive Engagement* included parent gaze to infant, positive affect, and 'motherese' vocalizations. *Infant Positive Engagement* included infant gaze to parent, positive affect, and positive vocalizations. *Infant Negative Engagement* included fuss-cry vocalizations and gaze aversion.

Synchrony

Two variables were created as measures of synchrony: (1) *Affect Synchrony* – conditional probability indicated the proportion of time parent and child coordinated their

positive engagement. Using the median split, parents were divided into High and Low Affect Synchrony groups (median = .25). (2) *Positive Communicative Sequences* were computed using lag sequential analysis and indicated the number of times the infant's positive affective engagement was followed by the parent's positive engagement.

Interactive Stress

Interactive Stress was the conditional probability of the proportion of time the infant was in negative engagement while the parent attempted to re-engage the child by positive affect or 'motherese' vocalizations.

Measures of stress and affiliation in the parent's attachment relationships

Parental attachment to infant and parenting preoccupations and worries

The adapted Yale Inventory of Parent Thought and Action (YIPTA; Feldman, Weller, Leckman, Kuint & Eidelman, 1999) is an instrument that assesses parent–infant bonding and consists of an interview and self-report measure. Both the interview and the self-report consider nine topics pertaining to the parent's thoughts, worries, feelings, attachment behaviors, and attachment-related thoughts in the postpartum months, and the instrument has been validated in several studies of healthy and high-risk infants (Feldman *et al*., 2007; Feldman *et al*., 1999; Leckman *et al*., 1999). A clinician interviewed the parent for 30–45 minutes and first probed the parent's free narrative on each topic. Narratives were audiotaped, transcribed, and coded by two coders on a scale from 1 (little) to 5 (a lot) for each topic, with an inter-rater reliability performed on 10 interviews averaging 95% (intraclass r = .93). Next, parents completed questionnaires organized by the same topics. Two composites were included. *Parental Preoccupations* was the average of the six items on the self-report questionnaire, and the coded preoccupation score (alpha = .87). *Attachment Representations* was the average of four items on the self-report questionnaire and the parallel narrative attachment score (alpha = .86).

Romantic attachment

The Adult Attachment Style (Brennan & Shaver, 1998) defines two dimensions of adult romantic attachment: anxiety and avoidance, each measured with a reliable and valid 18-item scale. Low anxiety and avoidance index attachment security. High attachment anxiety indicates stress within romantic relationships.

Bonding to parents

The Parental Bonding Instrument (PBI; Parker, Tupling & Brown, 1979) includes 25 questions for mother and father. The average of mother and father care scores indexed bonding to own parents and was used here.

Parenting stress

The Parenting Stress Index (PSI; Abidin, 1983) is a 36-item questionnaire measuring the magnitude of stress in the parent–child system with good reliability and validity.

Results

Mean levels of OT and interactive behaviors in mothers and fathers

Prior to data analysis, Pearson correlations were computed to test potential relationships between OT and background variables. No correlations were found between OT in plasma, saliva, and urine and demographic variables, including parent age, height, weight, body mass index, smoking, use of medications, and time of last meal. Maternal OT was unrelated to menstrual cycle phase, contraceptive intake, mode of delivery (vaginal vs. c-section), feeding style (breastfeeding vs. bottle-feeding), postpartum interval (weeks from birth to date of assessment), or the interval from prior breastfeeding. No differences in maternal OT level in either fraction were observed between breastfeeding and non-breastfeeding women, consistent with previous research which found that when OT is not sampled during breastfeeding, no differences are found between breastfeeding and non-breastfeeding women (Feldman *et al*., 2010; Gordon, Zagoory, Leckman & Feldman, 2010).

Descriptive statistics for baseline OT in the three fractions and interactive behavior are presented in Table 1 and show no differences between mothers and fathers in OT levels, parent and child's Positive and Negative Engagement, and Affect Synchrony. Mothers showed more Interactive Stress and more Positive Communicative Sequences during play. All following correlation and regression analyses were conducted with baseline OT measures.

Significant correlations emerged between *p*OT and *s*OT, r = .41, $p < .001$, indicating some degree of concordance between the two measures, and the correlations were of similar magnitudes in mothers and fathers. *u*OT was unrelated to *p*OT, $r = -.06$, *ns*, or *s*OT, $r = -.03$, *ns*, in the combined parents groups and were of similar magnitudes in mothers and fathers.

Assessing differences in baseline OT between the high- and low-Affect Synchrony groups, differences between the high- and low-Affect Synchrony groups were found for *p*OT, $F(1, 111) = 6.24$, $p < .01$, *Effect Size* (*ES*) = .11, and *s*OT, $F(1, 111) = 4.76$, $p < .05$, *ES* = .06 (Figure 1).

Table 1 *Plasma, salivary, and urinary OT and interactive behavior among mothers and fathers*

	Mothers		Fathers		
	M	SEM	*M*	SEM	*F*(1, 111)
Plasma OT – *p*OT (pg/ml)	365.59	16.56	405.10	23.72	1.86
Salivary OT – *s*OT (pg/ml)	6.17	.37	7.09	.37	1.73
Urinary OT – *u*OT (pg/ml)	10.34	1.25	9.81	2.03	.05
Parent Positive Engagement	.74	.09	.71	.11	1.59
Infant Positive Engagement	.45	.05	.52	.08	.45
Infant Negative Engagement	.34	.05	.37	.07	.50
Affect Synchrony	.23	.06	.27	.08	1.84
Interactive Stress	.13	.03	.07	.02	3.92*
Positive Communicative Sequences	3.4	.04	1.9	.01	4.13*

*$p < .05$
Note: Numbers represent baseline OT levels.

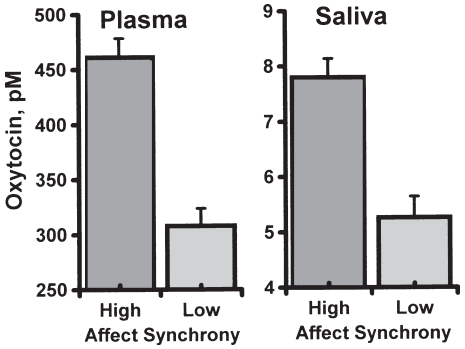

Figure 1 *Baseline plasma and salivary oxytocin in mothers and fathers high and low on affect synchrony.*

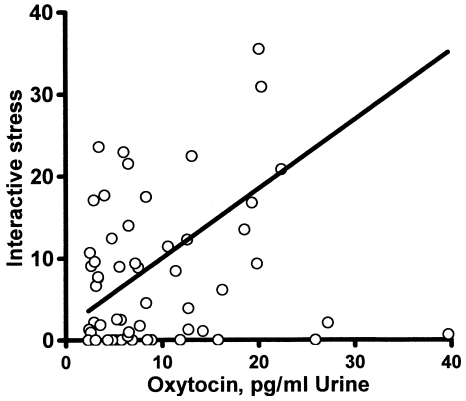

Figure 2 *Correlation between urinary oxytocin and mothers' interactive stress.*

Relations between OT and parent–infant interactive behavior

Pearson correlations between *p*OT, *s*OT, and *u*OT and parent–infant interactive behavior appear in Table 2. As seen, *p*OT and *s*OT correlated with parent Positive Engagement, Affect Synchrony, and Positive Communicative Sequences between parent and child. Infant Positive Engagement was related to parent *p*OT and marginally to *s*OT. These correlations were significant for both mothers and fathers. However, correlations between *u*OT and any interactive variable were significant among mothers only. *u*OT correlated with the infant's Negative Engagement with mother and with the mother's Interactive Stress (Figure 2).

Table 2 *Correlations between oxytocin in parents' baseline plasma, saliva, and urine and parent–infant interaction*

	*p*OT	*s*OT	*u*OT[a]
Parent Positive Engagement	.31*	.26*	.08
Infant Positive Engagement	.22*	.16+	.04
Infant Negative Engagement	−.06	−.08	.38**
Affect Synchrony	.34**	.27*	−.05
Interactive Stress	−.10	.02	.45**
Positive Communicative Sequences	.28*	.17	−.07

+$p < .10$; *$p < .05$; **$p < .01$.
Note: Correlations with *u*OT are reported for mothers only.

Relations between OT and indices of stress and affiliation

Parent–infant attachment

Oxytocin was associated with the parent's attachment representations of the infant. *p*OT and *s*OT correlated with the parent's Attachment Representations, $r = .30$, $p < .01$, $r = .26$, $p < .05$, respectively. *s*OT also correlated with Parental Preoccupations, $r = .29$, $p < .01$.

Bonding to parents

Bonding to own parents correlated with *p*OT, $r = .30$, $p < .01$.

Romantic attachment

*s*OT correlated with attachment security as indexed by low attachment anxiety, $r = -.28$, $p < .05$, and low avoidance, $r = -.32$, $p < .01$.

The correlations reported for *p*OT and *s*OT were for the entire sample of parents but were significant when testing mothers and fathers separately, as expected.

Relationship stress

As hypothesized, *u*OT was related to relationship-related stress among mothers only: to romantic attachment anxiety, $r = .29$, $p < .05$, and parenting stress, $r = .34$, $p < .01$.

Predicting Affect Synchrony and Interactive Stress

Two regression equations were computed predicting Affect Synchrony and Interactive Stress from measures of stress and affiliation. In light of the gender-specific correlations reported above, the model predicting Interactive Stress included mothers only, whereas the model predicting Affect Synchrony included both mothers and fathers. Each model contained six predictor variables. The first was the parent's OT (*p*OT was entered for Affect Synchrony and *u*OT for Interactive Stress). The next two predictors included Parental Preoccupations and Attachment Representations of the parent–child relationship, followed by bonding to own parents, attachment anxiety, and parenting stress. Results appear in Table 3. As seen, *p*OT, Attachment Representations, and bonding to own parents each explained unique variance in Affect Synchrony. Interactive Stress was uniquely predicted by *u*OT, maternal preoccupations, and parenting stress, with marginal beta for attachment anxiety among mothers. Overall, these physiological and psychological variables explained 22% of the variance in Affect Synchrony and Interactive Stress.

Discussion

OT is the most abundant brain neuropeptide that provides the foundation for the capacity to form close relationships (Gimpl & Fahrenholz, 2001). The present study utilized a broad perspective on the relations of OT and human bonding by analyzing OT in three peripheral systems, using diverse methodologies such as micro-analysis of non-verbal social cues and in-depth interviews, focusing on the parent's multiple attachments throughout life, and assessing indices of both stress and affiliation within each relationship. Results demonstrated the involvement of OT in micro-level processes of parent–infant synchrony; in the parent's attachments to his or her own parents, partner, and infant; and in mothers', but not fathers' relationship distress within the spousal relationship, the parenting role, and the parent–infant interaction. The study is also the first to compare OT levels in plasma, saliva, and urine. Overall, the findings point to substantial consistencies between human and mammalian parenting and bonding-related processes, and suggest that OT supports bond formation in humans similar to its role in other mammals. The comparable levels of *p*OT, *s*OT, and *u*OT in mothers and fathers similarly accord with animal research on the role of OT in the development of paternal behavior in biparental species (Ziegler, 2000). Notwithstanding cross-species consistency, the relations between OT and micro-level synchrony of visuo-affective facial signals and the parent's mental representations of attachment relationships highlight the unique features of attachment in humans and their specific links with the OT system.

Synchrony is a concept coined by the first researchers on parenting in social animals that describes the coordination of hormonal, behavioral, and physiological stimuli between parent and infant during social contact, providing critical inputs for growth and development of the young. Through such bio-behavioral synchrony, mammalian mothers adapt their physiological systems to those of the infants and the process facilitates physiological maturation and social adaptation (Fleming, O'Day & Kraemer, 1999). In humans, parent–infant synchrony appears in a species-typical form that involves the coordination of visual, vocal, and affective signals, and this experience similarly organizes the infant's physiology and socialization and supports later development (Feldman, 2007). The present findings point to similarities between humans and other mammals and suggest that the species-typical form of well-adapted parenting in humans is similarly supported by the OT system and is likely to carry a similar organizing impact on infant growth (Champagne, 2008).

Attachment theory is built on the assumption that representational models of attachment and their

Table 3 *Predicting Affect Synchrony and Interactive Stress*

	Affect Synchrony			Interactive Stress			
Criterion:	Beta	R^2Change	*F*Change	Beta	R^2Change	*F*change	*DF*
Oxytocin	.29*	.05	4.16*	.28*	.04	3.85*	1, 118
Parental Preoccupation	.17	.02	1.94	.37**	.06	5.44**	2, 117
Attachment Representations	.31*	.05	4.22*	.12	.01	.93	
Attachment Anxiety	−.23	.03	3.26	.28+	.04	4.02*	5, 114
Bonding to Parents	.28*	.05	4.96*	−.11	.02	1.27	6, 113
Parenting Stress	−.15	.02	2.53	.34*	.05	4.36*	7, 112

R^2 Total = .22, $F(6, 103) = 2.94$, $p < .01$; .23, $F(6, 63) = 3.11$, $p < .01$.
$+p > .10$; $*p < .05$; $**p < .01$.
Note: Model for Affect Synchrony included both mothers and fathers. Model for Interactive Stress was conducted for mothers only.
Plasma oxytocin was entered in the first step predicting Affect Synchrony, whereas urinary oxytocin was entered in the prediction of Interactive Stress.

behavioral and physiological underpinnings are formed early in life through the experience of sensitive parenting, continue with the individual's romantic attachment, and culminate in the capacity to provide adequate parenting to the next generation (Bowlby, 1969). Cross-fostering animal studies show that the cross-generation transmission of maternal behavior rides on mechanisms of early experience and shapes the organization of brain OT (Meaney, 2010). The present findings indicate that OT is related to the entire constellation of attachment in humans, including the parent's experience of being cared for as a child, attachment to romantic partner, and the ability to provide optimal parenting to the next generation, as expressed in developmentally adequate preoccupations and worries regarding infant well-being, clear and coherent representations of the parent–infant attachment, and the ability to engage in positive, well-timed synchronous interactions with the child.

Unlike the cross-gender associations of OT and synchrony, the correlations between OT and markers of relationship distress were specific to women, consistent with the findings for mammals (Neumann, 2008). The data indicate that maternal *u*OT was related to moments of interactive stress, maternal attachment anxiety, and stress in the maternal role, in line with theories suggesting that women are more sensitive to relationship distress and utilize close relationships as a strategy for stress regulation (Taylor *et al*., 2000). Possibly, relationship distress functions to increase OT production through its impact on HPA functioning (Pedersen, 2004), consistent with recent findings on the effects of stress on the expression of magnocellular neurons and their secreted neurohypophysial peptides in the hypothalamic paraventricular nucleus (Herman, Flak & Jankord, 2008). OT increase during moments of stress may activate a feedback loop that up-regulates the woman's employment of affiliative processes and social behavior in the service of well-being, calmness, and physical health (Uvnas-Moberg, 1998).

Interestingly, the associations between the stress components of bonding and OT were expressed in urine, whereas the links with the affiliation components emerged in plasma and saliva. OT plays a complex role in urination. Like vasopressin, OT functions as an anti-diuretic hormone via the kidney vasopressin type-2 receptor (Chou, DiGiovanni, Luther, Lolait & Knepper, 1995). Moreover, micturition is partially regulated by neurological pathways sending inputs to the limbic system, including preoptic regions, the central nucleus of the amygdala, bed nucleus of the stria terminalis, and hypothalamic nuclei, particularly the PVN where OT is produced (Holstege, 2005). Future research is required to assess the role of OT in psychophysiological processes leading to micturition. The differential relations of *p*OT and *u*OT with markers of affiliation and stress may also accord with the bi-phasic theory of OT (Lancel, Kromer & Neumann, 2003), which suggests that when physiological systems are in a calm state, OT functions as a soporific agent that reduces central activity and induces calmness, while under conditions of stress OT acts as a stimulant and increases social vigilance and active social behaviors.

It must be emphasized, however, that there are currently no comprehensive explanations for the specific associations between OT in urine and indices of maternal stress. Similarly, the lack of correlations between OT in saliva and plasma and OT in urine are not fully understood. One possibility may relate to the longer time it takes OT to be expressed in urine as compared to the other peripheral systems. This is the first study to assess OT in plasma, saliva, and urine simultaneously and assess their relationships with observed behavior and indices of stress and affiliation across several attachment systems. Much further research is required to examine the conditions under which OT levels measured in these three systems converge or diverge and the differential associations between the various markers of OT with parenting behavior and attachment experiences.

A central limitation of the present study is the peripheral measure of OT, which is unavoidable in humans. Although the relations between central and peripheral OT are not fully understood, studies in animals (Carter *et al*., 2007) and humans (Strathearn *et al*., 2009) as well as theoretical accounts of the functioning of the oxytocinergic system (Ross & Young, 2009) suggest that central and peripheral levels are coordinated. The consistency between the present findings – which show associations between bonding-related processes and peripheral OT as measured in various fractions – and those observed centrally and peripherally in other mammals provides further evidence for the utility of assessing peripheral OT. However, much further research is required to uncover the involvement of OT in processes of human attachment and understand the behavioral indicators, physiological correlates, genetic expressions, brain structures, and meta-representational models that contribute to the human capacity to form selective and enduring attachments throughout life.

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