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ARTICLE

Maternal Oxytocin Response Predicts Mother-to-Infant Gaze

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Abstract

The neuropeptide oxytocin is importantly implicated in the emergence and maintenance of maternal behavior that forms the basis of the mother-infant bond. However, no research has yet examined the specific association between maternal oxytocin and maternal gaze, a key modality through which the mother makes social contact and engages with her infant. Furthermore, prior oxytocin studies have assessed maternal engagement primarily during episodes free of infant distress, while maternal engagement during infant distress is considered to be uniquely relevant to the formation of secure mother-infant attachment. Two patterns of maternal gaze, maternal gaze toward and gaze shifts away from the infant, were micro-coded while 50 mothers interacted with their 7-month-old infants during a modified still-face procedure. Maternal oxytocin response was defined as a change in the mother's plasma oxytocin level following interaction with her infant as compared to baseline. The mother's oxytocin response was positively associated with the duration of time her gaze was directed toward her infant, while negatively associated with the frequency with which her gaze shifted away from her infant. Importantly, mothers who showed low/average oxytocin response demonstrated a significant decrease in their gaze toward their infants during periods of infant distress, while such change was not observed in mothers with high oxytocin response. The findings underscore the

involvement of oxytocin in regulating the mother's responsive engagement with her infant, particularly in times when the infant's need for access to the mother is greatest.

Keywords: oxytocin, mothering, mother-to-infant gaze, distress, engagement

1. Introduction

While the neuropeptide oxytocin has been recognized for its functions in parturition and milk ejection for many decades, there exists now a substantial literature underscoring the role of oxytocin in regulating social behaviors (see reviews, Benarroch, 2013; Feldman, 2012; Meyer-Lindenberg et al., 2011). A sizable number of studies have implicated oxytocin in maternal care (Febo et al., 2005; Pedersen et al., 2006; Strathearn et al., 2009b), pair bonding (Ross et al., 2009; Schneiderman et al., 2012), interpersonal trust (Van IJzendoorn and Bakermans-Kranenburg, 2012), emotion recognition (Lischke et al., 2012; Perry et al., 2013), and empathy (Hurlemann et al., 2010; Rodrigues et al., 2009). Oxytocin has been characterized as a “hormone of affiliation” (Insel, 1992) and the oxytocinergic system has received attention as a key neural substrate of maternal caregiving, involved in the emergence and maintenance of maternal behaviors (Feldman, 2012; Strathearn, 2011). Many important advances in this regard have come from animal models (Francis et al., 2002; Keverne and Kendrick, 1992; Maestriperi et al., 2009; Pedersen et al., 2006; Williams et al., 2001), though they have been extended to human subjects over the past decade, revealing both parallel and divergent findings.

In humans, peripheral oxytocin levels are higher in pregnant and parturient women than non-pregnant women (Feldman et al., 2007; Gordon et al., 2008). Oxytocin levels show high intra-individual stability over the course of pregnancy (Feldman et al., 2007; Levine et al., 2007) and early motherhood (Gordon et al., 2010), suggesting that they may constitute a trait-like characteristic that underpins the expression of maternal behavior. Prospective and cross-sectional studies have demonstrated that maternal oxytocin levels are systematically associated with naturally occurring variations in maternal behavior, with high plasma oxytocin level during pregnancy and postpartum predicting increased maternal behavior in the postpartum months (Atzil et al., 2011; Feldman et al., 2007; Gordon et al., 2010). Interaction with their young in the postpartum period further stimulates oxytocin response in mothers (Feldman et al., 2010a;

Feldman et al., 2010b), though significant inter-individual variations have been found (Strathearn et al., 2012), as with the baseline oxytocin levels. These natural variations in maternal oxytocin response have systematically predicted differences in the quality of maternal care provided by mothers (Feldman et al., 2010a; Feldman et al., 2010b).

Quality provision of maternal care and formation of secure attachment bonds are of particular importance in the early postpartum months, given their long-term effects on the development of the offspring (Fonagy et al., 2007; Kochanska and Kim, 2013; Sroufe et al., 2005; Weinfield et al., 2004). It is well established that sensitive and responsive maternal behavior has direct bearings on the child's life-long capacity for social adaptation and stress regulation (Kochanska, 2001; Mayes, 2006; Mikulincer and Shaver, 2007; Schore, 2001). One important channel through which maternal sensitive responsiveness is communicated is mother-to-infant gaze. Gaze is a central modality through which mothers signal their availability, establish mutual engagement, and initiate regulation of infant arousal, particularly in times of infant distress (Beebe et al., 2010; Slee, 1984). Infants are highly sensitive to their mothers' gaze (Stern, 1974) and begin to join in mutual gaze with their mothers as early as 3 months, which serves as a basis for the mother-infant synchrony that subsequently emerges in other modalities (e.g., touch, vocalization, facial expression; Colonnese et al., 2012; Feldman, 2007; Lavelli and Fogel, 2013; Tronick et al., 1980).

Despite its significance, the specific association between maternal gaze and maternal oxytocin has not yet been examined. Previous studies have measured maternal gaze, but only as part of a composite of maternal behaviors (also encompassing touch, vocalization, and affect; e.g., Atzil et al., 2011; Feldman et al., 2007; Feldman et al., 2010b; Gordon et al., 2010). Furthermore, while attachment literature has underscored that maternal sensitivity to infant distress uniquely contributes to optimal socioemotional outcomes in the child (Leerkes et al., 2009; McElwain and Booth-LaForce, 2006), prior studies have only examined maternal oxytocin

in relation to indices of maternal synchrony during episodes of positive affect and have not considered episodes of distress (Atzil et al., 2011; Feldman et al., 2010b; Feldman et al., 2011).

In our previous functional magnetic resonance imaging (fMRI) study (Strathearn et al., 2009b), we have demonstrated that mothers' peripheral oxytocin responses predicted their blood oxygenation level-dependent (BOLD) brain responses to their own infants' faces. The greater the mothers' oxytocin responses during interactions with their infants, the greater was their activation in regions known to be rich in oxytocin receptors (i.e., hypothalamic pituitary region) when viewing their infants' faces in the scanner. In the present study, we extended our line of investigation to the mother's actual gaze behavior during real-time interaction with her infant. We sought to examine the relationship between maternal oxytocin response and mother-to-infant gaze during periods of infant non-distress as well as distress.

Mother-infant dyads were observed during 50-minute semi-structured interaction sessions. Maternal oxytocin response was defined as any change in the mother's oxytocin level following interaction with her infant compared to baseline. Two patterns of maternal gaze, duration of maternal gaze toward and frequency of gaze shifts away from the infant, were coded during a well-validated interaction paradigm, a modified still-face procedure (MSFP; Koos and Gergely, 2001). The MSFP is a three-phase procedure, during which the mother interacts freely with the infant in phases 1 and 3, but is instructed to maintain a neutral 'still face' during phase 2, suddenly depriving the infant of maternal contingency and inducing stress in the infant (Koos and Gergely, 2001; Tronick et al., 1978) (Figure 1). The experimental manipulation reliably produces changes in the infant's level of distress: infants display clear signs of distress during phase 2, which have been shown to carry over to phase 3 (Haley and Stansbury, 2003; Mesman et al., 2009). The MSFP thereby offers an opportunity to examine the mother's behavior in the absence and presence of signals of infant distress. We hypothesized that maternal oxytocin response would be positively associated with maternal gaze toward the infant, while negatively associated with maternal gaze shifts away from the infant. We further predicted

that this association would become more pronounced during periods of infant distress than non-distress, in phase 3 than phase 1 of the MSFP.

2. Results

2.1. Participant Characteristics and Preliminary Analyses

Participant characteristics are shown in Table 1. Participants were a non-clinical sample of first-time mothers who were generally of middle to high socioeconomic status, three-quarters of whom held a college or postgraduate degree. All mothers scored below the clinical range for personality disorders, while four mothers scored in the mildly depressed range, one of whom also scored in the clinically significant range for parenting stress. However, these four mothers did not differ from the rest of the sample in oxytocin levels ($p > .65$) or maternal gaze ($p > .20$); neither did the exclusion of the four mothers significantly alter the results reported below. Maternal clinical characteristics are therefore not considered further. Maternal depression was unrelated to baseline oxytocin level ($r_{\text{BDI} \& \text{baseline OT}} = .01, p = .95$), oxytocin response (OTResp; $r_{\text{BDI} \& \text{OTResp}} = .09, p = .57$), maternal gaze variables ($r_{\text{BDI} \& \text{gaze toward}} = .08, p = .62$ and $r_{\text{BDI} \& \text{gaze shift away}} = .05, p = .77$), or infant affect variables ($r_{\text{BDI} \& \text{positive affect}} = -.08, p = .59$ and $r_{\text{BDI} \& \text{negative affect}} = -.04, p = .78$).

Breastfeeding status also did not correlate with oxytocin response ($r_{\text{breastfeeding} \& \text{OTResp}} = .15, p = .34$), or with maternal gaze variables ($r_{\text{breastfeeding} \& \text{gaze toward}} = .10, p = .54$ and $r_{\text{breastfeeding} \& \text{gaze shift away}} = -.17, p = .28$). Measures of menstrual cycle (i.e., estradiol and progesterone levels) were also not associated with OTResp ($r_{\text{estradiol} \& \text{OTResp}} = .07, p = .65$ and $r_{\text{progesterone} \& \text{OTResp}} = .06, p = .67$), or with maternal gaze variables ($r_{\text{estradiol} \& \text{gaze toward}} = -.01, p = .95$ and $r_{\text{progesterone} \& \text{gaze toward}} = .01, p = .93$; $r_{\text{estradiol} \& \text{gaze shift away}} = .18, p = .27$ and $r_{\text{progesterone} \& \text{gaze shift away}} = .13, p = .38$).

2.2. Infant Affect During Modified Still-Face Procedure

As expected, and consistent with previous research, significant changes were noted in infant affect across the three phrases of the MSFP (positive affect, $F(2, 98) = 33.78, p < .001$;

negative affect, $F(2, 98) = 35.24, p < .001$; Figure 2). Compared to baseline (phase 1), infants displayed increased negative affect during still-face (phase 2) ($M_{\text{phase 1}} = 0.22, SD = 0.26; M_{\text{phase 2}} = 0.51, SD = 0.41; t_{\text{phase 2-1}}(49) = 7.09, p < .001$), as well as decreased positive affect ($M_{\text{phase 1}} = 0.27, SD = 0.24; M_{\text{phase 2}} = 0.04, SD = 0.09; t_{\text{phase 2-1}}(49) = -7.83, p < .001$). While there was a rebound of positive affect during recovery (phase 3) ($M_{\text{phase 3}} = 0.16, SD = 0.23; t_{\text{phase 3-2}}(49) = 4.08, p < .001$), negative affect carried over from phase 2 to phase 3 ($M_{\text{phase 3}} = 0.54, SD = 0.41; t_{\text{phase 3-2}}(49) = 0.86, p < .39$).

2.3. Maternal Gaze During Modified Still-Face Procedure

Means and standard deviations of maternal gaze variables are shown in Table 2 for phases 1 and 3 of the MSFP. Duration of the mother's gaze toward the infant was significantly negatively correlated with the frequency of the mother's gaze shifts away from the infant ($r_{\text{gaze toward \& gaze shift away}} = -.79, p < .001$). Infant affect variables were not significantly associated with either of the maternal gaze variables ($r_{\text{positive affect \& gaze toward}} = -.09, p = .38$ and $r_{\text{negative affect \& gaze toward}} = .09, p = .38; r_{\text{positive affect \& gaze shift away}} = -.01, p = .91$ and $r_{\text{negative affect \& gaze shift away}} = .06, p = .56$), and did not significantly alter any of the observed main or interaction effects reported below when entered into the model.

2.3.1. Maternal gaze toward infant (duration). The optimal model included a subject-level random intercept (LR $\chi^2(1) = 5.86, p = .016$) and a random coefficient for phase (LR $\chi^2(2) = 38.55, p < .0001$), providing a significant fit (Table 2). Consistent with our hypothesis, a significant main effect of OTResp was found, with the duration of mother-to-infant gaze increasing as OTResp increased across participants. The main effect of phase was also significant, indicating that the duration of mother-to-infant gaze decreased in phase 3 as compared to phase 1. OTResp and phase interacted significantly. Decomposition of the interaction revealed that a simple effect of phase was significant at low (i.e., 1 standard deviation below the mean) and average levels of OTResp ($\beta_{\text{phase, at low OT}} = -.010, 95\% \text{ CI} = -.017$ to $-.004, z = -3.06, p = .002$ and $\beta_{\text{phase, at mean OT}} = -.005, 95\% \text{ CI} = -.009$ to $-.0001, z = -2.00, p =$

.045, respectively), but not significant at high (i.e., 1 standard deviation above the mean) levels of OTResp ($\beta_{\text{phase, at high OT}} = .001$, 95% CI = $-.006$ to $.007$, $z = 0.24$, $p = .814$) (Figure 3(a)). This indicated that mothers with high OTResp displayed gaze duration that was similar during phase 1 and phase 3, while maternal gaze duration was significantly reduced for mothers with low/average OTResp during phase 3.

2.3.2. Maternal gaze shift away from infant (frequency). The optimal model was obtained with a random effects structure that included a subject-level random intercept (LR $\chi^2(1) = 26.75$, $p < .0001$) and a random coefficient for phase (LR $\chi^2(2) = 8.62$, $p = .013$; Table 2). As hypothesized, a significant main effect was found for OTResp, with mothers displaying less frequent gaze shifts away from their infants as OTResp increased (Figure 3(b)). The main effect of phase was also significant, indicating that mothers' gaze shifts from infants increased in phase 3 compared to phase 1. OTResp and phase did not interact significantly.

3. Discussion

The present results are the first, to our knowledge, to document that measures of maternal peripheral oxytocin are systematically associated with individual variations in mother-to-infant gaze. As hypothesized, maternal peripheral oxytocin response was positively associated with the duration of mother-to-infant gaze, while negatively associated with the frequency with which maternal gaze was directed away from infants. Also consistent with our expectation, these associations were more pronounced under conditions of infant distress than non-distress. It is worth noting that mothers with low/average peripheral oxytocin responses demonstrated a significant decrease in their mother-to-infant gaze during periods of infant distress, while such change was not observed in mothers with high peripheral oxytocin responses.

The association documented here between maternal oxytocin and mother-to-infant gaze is consistent with and extends previous studies that have reported on the links between

maternal oxytocin and other forms of synchronous maternal behavior (e.g., affectionate touch; Atzil et al., 2011; Feldman et al., 2010a; Feldman et al., 2010b; Feldman et al., 2011). In keeping with previous data from animal (Snowdon et al., 2010) and human research (Dawood et al., 1979; de Geest et al., 1985; Feldman et al., 2007; Levine et al., 2007), we found significant individual variations in mothers' peripheral oxytocin responses, spanning from those that demonstrate a decrease from baseline oxytocin level following interactions with infants to those demonstrating an increase. This variation, likely reflecting naturally occurring differences in mothers' oxytocinergic system functioning, was associated with two patterns of mother-to-infant gaze observed at a micro-behavioral level. Our present finding, in conjunction with the existing literature (Feldman et al., 2010a; Feldman et al., 2011; Strathearn et al., 2009b; Strathearn et al., 2012), points to the role of oxytocin in regulating the mother's responsive engagement with the infant. It is of note that the strength of the observed association between oxytocin and maternal gaze increased during periods of infant distress. From the inception of attachment theory (Bowlby, 1969/1982), the biological function of mother-infant attachment has been thought to be one of ensuring the infant's access to the mother in times of distress (Goldberg et al., 1999; Mikulincer and Shaver, 2003). Our data underscore the involvement of oxytocin in preparing mothers for such a function.

The significant difference reported here between mothers with high versus low/average oxytocin response is worthy of attention. A corollary to this can be seen in a series of reports on rodents demonstrating that rat mothers who exhibit low licking-and-grooming and arched-back nursing (LG-ABN), a rodent equivalent of neglectful mothering, showed a reduced density of oxytocin receptors in brain regions critical for the emergence of maternal behavior (i.e., the medial preoptic area, the lateral septum, the paraventricular nucleus of the hypothalamus; Champagne et al., 2001; Francis et al., 2000). These mothers' low LG-ABN behavior, in turn, was associated with decreased oxytocin receptor expression in similar brain regions in the offspring (Champagne et al., 2001; Champagne et al., 2003b; Champagne et al., 2006) who,

like their mothers, subsequently displayed reduced levels of LG-ABN behavior with their offspring (Champagne et al., 2003a; Francis et al., 1999; Lovic et al., 2001). While we have not examined infant outcomes in the present study, Beebe et al. (2010)'s micro-analysis of the interaction between mothers and their 4-month-old infants provides particularly noteworthy information in this regard. Beebe et al. (2010) reported on a group of mothers whose behaviors strikingly parallel the behaviors of mothers who demonstrated reduced oxytocin responses in the present study. Mothers of infants who went on to develop profoundly insecure attachment at 12 months were characterized not by their global failure of attunement, but rather by a remarkably specific failure to attend to their infants' distress. Maternal disengagement from infant distress is seen as a critical precursor to the formation of disrupted attachment (Allen, 2013). Similarly, reduced gaze directed to infant distress reported here in a subsample of our mothers, and those mothers' decreased oxytocin response as a possible biological marker for their impoverished gaze and mirroring response, may subsequently be linked to less-than-optimal outcomes in their infants, particularly in terms of affect dysregulation (Fonagy et al., 2011). Future research should examine the longitudinal links between maternal oxytocin, maternal gaze, and infant developmental outcomes.

Several limitations of the study should be recognized. First, we relied on peripheral measures of oxytocin, using a radioimmunoassay technique. While a degree of concordance has been reported between central and peripheral measures of oxytocin (Carter et al., 2007), peripheral oxytocin levels may not accurately reflect central oxytocin activity (Amico et al., 1990). This represents a limitation yet to be fully overcome in human oxytocin research, although we have previously demonstrated a correlation between peripheral oxytocin response and brain activation in the hypothalamic/pituitary region, where oxytocinergic neurons are concentrated (Strathearn et al., 2009b). Second, we did not track changes in maternal oxytocin or gaze direction as a function of changes in infant affect. We were therefore unable to examine how maternal oxytocin moderated the temporal process by which infant distress modifies and is, in

turn, modified by maternal gaze. This would be a fruitful area for further investigation. Third, we considered oxytocin concentration during the initial mother-infant separation as ‘baseline,’ although we acknowledge that this baseline measurement may have been confounded by the stress experienced during the separation procedure. Finally, our sample consisted largely of mothers of middle to high socioeconomic status, which may limit the generalizability of our findings.

The present study provides the first evidence for the unique relationship between maternal oxytocin response and mother-to-infant gaze. We have found results consistent with our understanding that maternal oxytocin may be substantially implicated in the mother’s responsive engagement with her infant, particularly in times when the infant’s need for the mother is greatest. The data presented here may have important implications for intervention in conditions that challenge optimal mothering. Helping mothers maintain their engagement during moments of infant distress may be an important focus of intervention for mothers whose oxytocin response may be compromised, who suffer from postpartum depression, or who have struggled with maternal substance abuse or trauma. Breastfeeding, though it may not lead to long-term changes in baseline oxytocin levels, induces a short-term release of oxytocin in mothers, and may hence be beneficial for these mothers (Strathearn et al., 2009a). Finding pharmacological or therapeutic ways to enhance maternal oxytocin release may be another important focus of future intervention.

4. Experimental Procedure

4.1. Participants

Participants were 50 first-time mothers aged 19 to 41 ($M = 28.0 \pm 0.7$) years, recruited through prenatal clinics and community advertisements as part of a larger study. Of 116 participants initially recruited during the third trimester of pregnancy, 61 met eligibility criteria, and 50 completed oxytocin sampling and MSFP at 7 months postpartum. Exclusion criterion

included mothers with past or present alcohol or substance abuse, mothers who used cigarettes during pregnancy, and mothers who were on psychotropic medication at the time of the study. The institutional review board at Baylor College of Medicine approved the research protocol, and all participants provided written informed consent.

4.2. Measures and Procedure

4.2.1. Oxytocin sampling. Mothers attended the study session with their infants 7 months postpartum. Mothers were instructed to abstain from caffeine and tobacco 2 to 3 hours prior to the scheduled visit. During the visit, mother-infant dyads participated in a semi-structured mother-infant interaction procedure, which consisted of two periods of separation and an intervening period of mother-infant interaction. Four serial measurements of plasma oxytocin were obtained from the mothers during the procedure (Figure 4).

4.2.1.1. Baseline. Upon an initial separation of mother and infant, an intravenous cannula was inserted into the mother's forearm and a blood sample was taken for estradiol and progesterone levels. Blood was drawn again 20 minutes later to determine the first baseline measurement of plasma oxytocin (OT 1).

4.2.1.2. Post free-play. Following an initial 20-minute separation, mother and infant were reunited for a 5-minute 'free-play' period, which included direct physical interaction between mother and infant on the floor with age-appropriate toys. The second blood sample was then drawn through the previously inserted cannula (OT 2).

4.2.1.3. Post modified still-face procedure. A 6-minute MSFP was then conducted. The MSFP is a structured experimental paradigm whereby the mother interacts with her infant through three successive phases, including a still-face phase (see section 4.2.2) (Figure 1). Mother and infant were able to see each other via a mirror and hear one another during the procedure, but were separated by a dividing screen and thus could not physically interact. A third blood sample was obtained following the MSFP (OT 3).

4.2.1.4. Baseline (Post). A second period of 20-minute separation occurred between mother and infant, after which the final oxytocin blood sample (OT 4) was obtained.

4.2.2. Modified still-face procedure (MSFP). The MSFP (Koos and Gergely, 2001) adhered to the standard still-face procedure (Tronick et al., 1978) with one exception, the mother and infant were seated beside one another (the mother in a chair and the infant in a high chair), separated by a divider and facing a one-way mirror (Figure 1(a)). The purpose of the divider and one-way mirror was to prevent physical interaction and touch, but allow mother and infant to see each other reflected in the mirror.¹ On the opposing side of the one-way mirror were two cameras, generating a split-screen recording of the mother and infant. Interactions between mother and infant were videotaped during each of the three 2-minute phases (Figure 1(b)): (1) the baseline normal interaction phase (phase 1), (2) the still-face phase, during which the mother was asked to assume a neutral face (phase 2), and (3) the recovery phase, in which the mother resumed free interaction with the infant (phase 3). An intercom was used to communicate the start of each phase to the mother. Trained raters, who had had no prior contact with study participants and were blind to the study hypotheses, coded the videotaped interactions. Coding of each behavior category was performed in 1-second time intervals during multiple viewings of videotapes (independently for each behavior). Tapes were viewed at normal speed during coding, although they were frequently stopped or run in slow motion to confirm codes and to accurately determine the beginning and ending of episodes of maternal and infant behavior. Onset and offset times of behaviors were entered into custom-built software, which generated frequency and duration data for each variable of interest. The median inter-rater reliability was $r_s = 0.86$ for 18 double-coded interactions (36%).

4.2.3. Additional mother and infant characteristics. Several characteristics of interest for mothers and infants were also examined. The Beck Depression Inventory-II (BDI-II; Beck et al., 1996), the Personality Disorder Questionnaire 4+ (PDQ-4+; Hyler et al., 1992), and the Parenting Stress Index (PSI; Abidin, 1995) were administered to assess symptoms of

depression, personality disorders, and parenting stress in mothers. We also collected information regarding infant's breastfeeding and daycare status (i.e., if the mother was still breastfeeding or not, and the number of hours per week the infant was cared for by someone other than the mother). Details on the psychometric properties of all administered measures can be found in Shah et al. (2010).

4.3. Blood Collection and Assay

Blood samples were collected and processed by registered nurses from the General Clinical Research Center. Estradiol and oxytocin samples were placed in chilled heparinized tubes and kept on ice. These tubes were centrifuged to separate plasma within 2 hours after collection, and the plasma was rapidly frozen and maintained at -80 degrees Centigrade. Before centrifuging, blood samples for progesterone were placed in a serum separator tube and allowed to clot at room temperature. A commercial laboratory determined plasma estradiol and serum progesterone concentrations using quantitative chemiluminescent immunoassay. Dr Janet Amico's laboratory at the University of Pittsburgh received batches of oxytocin samples that were sent on dry ice by overnight courier. A sensitive and specific liquid phase radioimmunoassay (RIA) of oxytocin in plasma was performed on acetone-ether extracted material, using previously published and validated methods in which oxytocin antiserum does not cross-react with arginine vasopressin or other oxytocin-like peptides (Amico et al., 1985). The lower limit for detectability for this assay is 0.5 pg/ml and inter- and intra-assay coefficients of variation are each < 10%. Although more labor-intensive than widely used alternate methods such as the enzyme immunoassay, RIA on extracted plasma has consistently produced valid and reliable results (McCullough et al., 2013).

4.4. Variables

4.4.1. Maternal oxytocin response (OTResp). OTResp (Strathearn et al., 2012) was calculated by computing the change in oxytocin concentration between measurement at baseline (OT 1) and measurement following mother-infant interactions (mean of OT 2 and OT 3;

see Figure 4). OT 2 and OT 3 were highly correlated with each other ($r_{s_{OT2 \& OT3}} = .61, p < .001$). OT 4 was omitted from further analyses as a carry-over effect was seen from the mother-infant interaction phases; OT 4 was associated with both OT 3 and OT 2 ($r_{s_{OT3 \& OT4}} = .41, p = .004$ and $r_{s_{OT2 \& OT4}} = .43, p = .002$), but not with baseline ($r_{s_{OT1 \& OT4}} = .15, p = .30$). Positive OTResp values indicated a relative increase in oxytocin during mother-infant interactions, while negative OTResp values indicated a relative decrease. For four mothers, a single missing oxytocin value was imputed using linear interpolation.

4.4.2. Maternal gaze variables. We coded maternal eye gaze toward and gaze shifts away from the infant during the two interactive phases (i.e., phase 1 and phase 3) of the MSFP. Maternal gaze toward infant was quantified by the total duration of time, in seconds, that the mother looked at her infant. Maternal gaze shifts away from infant was assessed by the total frequency with which the mother's gaze first fixated on the infant (i.e., remained stationary on the infant for a minimum of 1 second), then shifted away from the infant. MSFP phases were recorded for 2 minutes each with slight variations in timing due to parent compliance with procedure instructions and infant behavior. Thus, gaze duration and frequency values were adjusted for the total length of time in each phase of the MSFP.

4.4.3. Infant affect variables. Infant facial expressions and vocalizations were coded for positive and negative affect at 1-second intervals throughout the MSFP. Total duration of positive and negative affect was calculated for the three phases of the MSFP, adjusting for the total length of time in each respective phase.

4.5. Statistical Analysis

All variables were inspected for normality via quantile-quantile plots of residuals against fitted values. Logarithmic transformations were performed on maternal gaze variables to optimize the approximation to normal distribution. Changes in infant positive and negative affect were examined in repeated-measures ANOVAs with MSFP phase as a within-subject factor, which were followed by post hoc mean comparisons. Maternal gaze variables were centered

prior to being submitted to mixed-effects linear regression analysis. Model building was carried out as follows. (a) The initial model included the fixed main effects of OTResp and MSFP phase (phase 1 vs. phase 3). (b) Subject-level random intercept and slope were added to model systematic inter-individual variability. (c) Interaction terms were added sequentially and retained in the model if they improved model fit. (d) Infant affect variables were added as covariates to examine whether variability in infant affect altered the significance of the model fit or parameter estimates. The model was fitted by maximum likelihood estimation, and likelihood-ratio chi-square tests were used to assess the relative fit of nested models. All analyses were conducted using STATA/SE version 12.1 and SPSS version 21.

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Figure Captions

Figure 1. The modified still-face procedure (MSFP): (a) diagram of the experimental setting and (b) example still frames from all three phases.

Figure 2. Duration of positive and negative affect displayed by infants across modified still-face procedure (MSFP) phases. The duration values on y-axis were adjusted for the total length of the each respective phase of the MSFP and represent mean proportion values. Positive affect dropped and negative affect increased in phase 2, while a rebound in positive affect and a carry-over of negative affect were seen in phase 3.

*** $p < .001$

Figure 3. (a) Duration of maternal gaze toward infant and (b) frequency of maternal gaze shift away from infant as a function of maternal oxytocin response (OTResp; mean \pm SD) and phase of the modified still-face procedure. The y-axes indicate log-transformed values. The statistical significance of the gaze differences between phases 1 and 3 is noted at low (i.e., -1 SD) and average (i.e., mean) levels of maternal oxytocin response.

* $p < .05$, ** $p < .01$

Figure 4. Mother-infant interaction procedure and oxytocin response (OTResp) calculation. Measurements of plasma oxytocin were obtained from mothers at four time points: (a) following the first period of mother-infant separation (OT1), (b) following two periods of mother-infant interaction (OT 2 and OT 3), and (c) following the final period of mother-infant separation (OT 4)

Footnote

¹ The purpose of the modification of the still-face procedure was to test separate hypotheses regarding infant gaze preference (self vs. mother), which were not explored in this paper focusing on mother-to-infant gaze.