

Adult attachment predicts maternal brain and oxytocin response to infant cues

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ABSTRACT

Infant cues, such as smiling faces, are powerful motivators of human maternal behavior, activating dopamine-associated brain reward circuits. Oxytocin, a neurohormone of attachment, promotes maternal care in animals, although its role in human maternal behavior is unclear. We examined whether differences in attachment security of 30 first-time mothers were related to brain reward and peripheral oxytocin response to infant cues. On viewing their own infant's smiling and crying faces during functional MRI scanning, mothers with secure attachment showed greater activation of brain reward regions, including the ventral striatum, and the oxytocin-associated hypothalamus/pituitary region. Peripheral oxytocin response to infant contact was also significantly higher in secure mothers, and was positively correlated with brain activation in both regions. Insecure/dismissing mothers showed insular activation in response to their own infant's sad faces. These results suggest that individual differences in maternal attachment may be linked with development of the dopaminergic and oxytocinergic neuroendocrine systems.

KEY WORDS

Attachment, mother-infant relations, dopamine, oxytocin, reward, functional MRI, maternal.

INTRODUCTION

The attachment relationship between infants and their caregivers is critical for human development, ensuring infant survival and optimal social, emotional and cognitive development (Insel and Young 2001; Sroufe et al. 2005). The relationship between a mother and her infant is particularly salient, with evidence that the biological processes of pregnancy, parturition and lactation may all contribute to the establishment of the mother-infant bond (Strathearn et al. 2009; Kinsley et al. 2008).

In both human and animal research, significant differences in early maternal caregiving have been observed—ranging from sensitive and responsive infant care to maternally perpetrated abuse or neglect (Strathearn et al. 2009; Sroufe et al. 2005), with corresponding differences in infant health and developmental outcomes (Sroufe et al. 2005; Strathearn et al. 2001; Thompson 2008; Francis et al. 1999; Weaver et al. 2004). Understanding the neurobiological processes underlying these differences in maternal behavior may help us to provide more effective treatment and preventative services.

The neurobiology of attachment behavior has been studied extensively in animal models (Insel and Young 2001; Swain et al. 2007), and more recently in humans using functional magnetic resonance imaging (fMRI) (Lorberbaum et al. 2002; Bartels and Zeki 2004; Swain et al. 2007; Strathearn et al. 2008). Although there is likely to be a complex interaction of multiple neuroendocrine systems, two specific systems have been shown to consistently play a role in promoting and maintaining maternal behavior: 1) the dopaminergic reward processing system (Champagne et al. 2004; Strathearn et al. 2008; Ferris et al. 2005) and 2) the oxytocinergic system (Bartels and Zeki 2004; Champagne et al. 2001; Levine et al. 2007) Oxytocin, a neuromodulatory hormone produced in the hypothalamus, has well-described central actions associated with the onset of maternal behavior, as well as peripheral actions in stimulating uterine contraction during labor and milk ejection during lactation. It is rapidly released into the

peripheral circulation following stimuli such as infant suckling, the sight or sound of a nursing mother's infant (Lucas et al. 1980; McNeilly et al. 1983; Johnston and Amico 1986), or even tactile stimulation in rat dams (Uvnas-Moberg et al. 1993). In randomized, placebo-controlled trials, intranasal oxytocin produces a broad range of social effects, including enhanced social memory, eye gaze when viewing faces, increased recognition and memory of facial expressions and identity, and increased manifestations of trust (Domes et al. 2007; Savaskan et al. 2008; Baumgartner et al. 2008; Kosfeld et al. 2005; Guastella et al. 2008b; Guastella et al. 2008a). Oxytocin receptors are located in the ventral striatum, a key dopaminergic brain region, and receptor binding is linked functionally to maternal behavior in the rat (Olazabal and Young 2006a). Thus, oxytocin may link social cues, such as infant facial expressions, with dopamine-associated reinforcement pathways.

The extent to which these biological systems explain differences in the quality of human attachment between mothers and infants, is yet to be explored (Strathearn 2006). In this study, we aimed to measure differences in maternal brain reward activation and peripheral oxytocin release in response to infant cues, based on the mother's adult attachment classification. We hypothesized that mothers with secure patterns of adult attachment would show an increased brain response to their own infant's face in mesocorticolimbic reward regions, including the midbrain ventral tegmental area, the ventral striatum and the medial prefrontal cortex, and that this would be true on viewing both happy and sad infant face cues. We also hypothesized that "secure" mothers would show an enhanced peripheral oxytocin response on interacting with their infants, which would correlate with maternal brain responses.

MATERIALS AND METHODS

Study setting and participants. In this cohort study, we recruited first-time pregnant women during the third trimester of pregnancy from Houston, Texas, and monitored them for 14 months postnatally. Recruitment occurred between August 2004 and April 2006 and was through prenatal clinic visits and advertisements on billboards, in magazines and via the internet. We excluded potential subjects who were on psychotropic medications, using cigarettes during pregnancy, left-handed or had any contraindications to MRI scanning. Research was approved by the Institutional Review Board at Baylor College of Medicine, and all subjects provided written informed consent.

Study design (Fig. 1). *Visit 1: Pregnancy.* During this visit, each enrolled woman participated in a modified version of the Adult Attachment Interview (AAI) (Crittenden 2004; George et al. 1996), a semi-structured 1½-2 hour-long interview involving specified questions and follow-up inquiries relating to childhood relationships with attachment figures, usually parents. The modified version was chosen because of its theoretical links with patterns of information processing in the brain (Strathearn 2006; Crittenden 2008). Each digitally recorded interview was transcribed (with personally identifying details altered to preserve anonymity), and coded blindly to classify each woman's adult attachment pattern, which was not revealed until study completion.

During this visit we also collected sociodemographic data, and screening information for depression (Beck Depression Inventory, BDI) (Beck et al. 1996) and personality disorders (Personality Disorder Questionnaire 4+, PDQ) (see **Supplementary Table 1** online). We repeated the BDI on each post-natal visit, and calculated a mean post-natal score.

Visit 2: Videotaping and oxytocin sampling. Approximately 7 months post-delivery, each mother and infant attended a session at the Human Neuroimaging

Laboratory. We requested that mothers abstain from caffeine and tobacco for 2-3 hours prior to the visit. After separating from their infants, the mothers had an intravenous cannula inserted, and 20 minutes later had blood drawn for baseline measurements of serum oxytocin, free cortisol, epinephrine and norepinephrine. We also measured serum estradiol, progesterone and β human chorionic gonadotropin levels to exclude a current pregnancy and to assess menstrual status. During this separation period, we videotaped each infant to obtain still images for use in the subsequent fMRI visit. Smiling, neutral and crying faces were elicited in a standardized setting, as described elsewhere (Strathearn et al. 2008). The mother and infant were then reunited for a 5-minute "free-play" period in which they physically interacted on the floor, after which another blood sample was drawn. They then participated in a 6-minute modified "still face" procedure (Koos and Gergely 2001), during which mother and infant could hear and see each other (via a mirror) but not interact physically. We then obtained a third blood sample after the mother left the room, followed by a final blood draw after 20 minutes of separation. Before and after the interaction period, each mother rated their current feelings using the Positive and Negative Affect Schedule (PANAS) (Watson et al. 1988), a 5-point rating of 20 affect states, such as "interested", "excited", "irritable" and "nervous".

Each mother also completed a 120 item self-report questionnaire, the Parenting Stress Index (PSI) (Abidin 1995), designed to help identify potentially dysfunctional parent-child relations. We assessed adult and infant temperament using the self-report Adult Temperament Questionnaire—Short form (ATQ) and the Infant Behavior Questionnaire—Revised (IBQ) (Gartstein and Rothbart 2003). The mothers also reported their breastfeeding status, which was repeated at Visit 3.

Visit 3: Scanning. At ~11 months post-delivery, a minimum of 3 months after the videotaping session, each mother underwent fMRI scanning while viewing 60 unique infant face images, 30 of her own baby and 30 of the matched unknown infant face.

Each mother viewed randomly presented baby face images for 2 seconds each within a rapid event-related fMRI design, with a random inter-stimulus interval of 2, 4 or 6 seconds (**Fig. 2**). Visual images were generated using a computer controlled LCD projector, and presented to the mother on an overhead mirror display.

Visit 4: Child follow-up. Finally, at 14 months of age we performed a general assessment of child development using the Screening Test of the Bayley Scales of Infant and Toddler Development (Bayley 2006).

Variables and statistical methods. *Predictor variable – Adult attachment.* We determined each mother’s adult attachment classification using the AAI (George et al. 1996; Fonagy et al. 1991) which categorizes the mother’s capacity to form secure attachment relationships on the basis of a narrative of her own attachment experience. Over the past 25 years, over 200 studies have reported over 10,000 AAIs (van IJzendoorn and Bakermans-Kranenburg 2009). From both cross-sectional and prospective longitudinal studies, adult attachment has been shown to reliably predict maternal behavior patterns, the development of infant attachment (van IJzendoorn 1995), and infant social and emotional development (Sroufe et al. 2005). We chose to measure attachment during pregnancy using a longitudinal design to preclude the possibility that the infant’s temperament or mother-infant interaction patterns might influence the way the mother discusses her own attachment experiences.

The coding is based on the subject’s coherence and consistency in describing attachment-related experiences and their effects on current functioning (Crittenden 2004). The 3 basic styles, which parallel Ainsworth’s original classification of attachment in infancy (Ainsworth and Bell 1970) include Type A “Insecure/Dismissing”, Type B “Secure” and Type C “Insecure/Preoccupied”. Individuals with Type B attachment styles tend to provide balanced descriptions of childhood experiences, using both

temporal/causal order and affect to describe both positive and negative events and feeling states. Individuals with Type A attachment tend to describe events or feelings in more cognitive terms, avoiding or inhibiting displays of negative affect. In contrast, Type C individuals exaggerate affective responses, with omitted or distorted cognitive processing (Crittenden 2008). Fifty percent of the transcripts were double coded to ensure reliability, with an 87% agreement with regard to a 4-group classification ($\kappa=0.78$). Discrepancies were resolved through conferencing between coders.

Potential confounding variables. We measured a variety of socioeconomic and behavioral factors to compare the characteristics of women in the two attachment groups (see **Supplementary Table 1** online). Continuous measures were evaluated using t-tests or the Mann-Whitney U-test for nonparametric data (as determined from histogram analysis). We compared categorical variables using the Chi-Square test, or Fisher Exact test when numbers were insufficient. We used the Kendall's tau-b test for ordinal or ranked nonparametric variables. We compared PANAS ratings of the mothers' affect before and after contact with their infants between groups using a repeated measure analysis of variance (ANOVA).

Serial measurements of cortisol, norepinephrine and epinephrine were also compared between attachment groups using linear mixed modeling. Analyses were performed using SPSS (version 15.0) and $P < 0.05$ (2-tailed) was considered statistically significant.

Outcome Variables. 1) *Oxytocin Response.* We used linear mixed modeling to assess the effects of attachment group, interaction time point, breastfeeding status, and all 2-way interactions, on oxytocin response. Residual plots were used to confirm normality of distribution. Cases with missing data points were excluded (one Type B and two Type A subjects). The difference in mean oxytocin concentration between attachment groups, at each time point, was compared using a z-test (with Bonferroni

correction for multiple comparisons; $\alpha \leq 0.0125$ was considered statistically significant). The mean oxytocin concentration from the two “mother-infant interaction” time points (which were highly correlated: $r_s=0.77$, $P < 0.001$) was recomputed as a percentage change from the first baseline measure, to provide a single index for correlation with fMRI data. To determine the correlation between “% change in oxytocin” and fMRI blood-oxygen-level-dependent (BOLD) activation measured 4 months later (z-transformed beta weights), we calculated a Spearman correlation coefficient. We used a Bonferroni correction to adjust the alpha level for multiple comparisons with differing beta weights for the 6 types of infant face (own-happy, unknown-neutral, etc). An alpha < 0.008 was considered statistically significant.

We measured oxytocin concentrations using a sensitive and specific liquid phase radioimmunoassay, 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 of the assay is 0.5pg ml^{-1} , inter- and intra-assay coefficients of variation are $<10\%$.

2) *Functional MRI Brain Response*. We prepared thirty standardized face images from each infant (10 happy, 10 neutral and 10 sad) for use in the fMRI scanning paradigm, along with 30 images from an “unknown” baby which were matched on age, race and independently-coded degree of affect (**Fig. 2**) (Strathearn et al. 2008). To ensure that the degree of infant facial affect did not vary between attachment groups, all faces were re-coded by three blinded raters using the 9-point Self-Assessment Manikin (Bradley and Lang 1994) (ICC = 0.90). Using a mixed model three-way ANOVA, we saw no main effects for attachment group ($F_{2,28} = 1.9$, NS) or order of presentation (Wilk’s $\Lambda = .502$, $F_{9,20} = 2.0$, NS). Similarly, none of the interactions with attachment security were significant.

Imaging was performed using a 3 Tesla Siemens Allegra head-only MRI scanner. High-resolution T1-weighted structural images (192 slices, in plane resolution 256 x 256; field of view [FOV] 245 mm; slice thickness 1 mm) were first acquired, followed by whole-brain functional runs of around 185 scans (gradient recalled echo planar imaging; 37 slices; repetition time 2000 msec; echo time 25 msec; flip angle, 90°; 64 x 64 matrix [in plane resolution]; FOV 220 mm; slice thickness 3 mm; positioned at 30 degrees in the axial plane to the anterior commissure/posterior commissure line). Imaging data for each subject were preprocessed in BrainVoyager QX, version 1.7.9 and analyzed in version 1.9.10, as previously described (Strathearn et al. 2008).

A BrainVoyager protocol file was created for each functional run, representing the timing of each stimulus event. Each predictor was then convolved using a double-gamma hemodynamic response function. Using the General Linear Model (GLM), effects for the whole group ($n = 30$) and for each attachment subgroup ($n = 15$) were evaluated separately using a random effects analysis between subjects, and fixed effects for individual within factors. After specifying a particular contrast in stimulus types (e.g. own-happy vs. unknown-happy or own-sad vs. unknown-sad), a group t-map was generated onto a template 3-dimensional anatomical image. An activation map threshold was determined using a false discovery rate (FDR) of 5% to control for multiple comparisons, and a cluster threshold of 4 voxels. Smaller cluster thresholds were also examined in the striatum (3 voxels) and brainstem (1 voxel) to reveal activation of smaller nuclei. Anatomical regions were identified using the automated “Talairach Daemon” (Lancaster et al. 2000), and confirmed manually using a human brain atlas (Mai et al. 2004).

Next, we compared activation patterns between attachment groups using an 2-factor random effects ANOVA model, with “infant face category” as a repeated measure within-factor variable and “attachment group” as a between-factor, assessing whole

brain differences in activation using a threshold of $q(\text{FDR}) = 0.05$. Percentage BOLD signal change was calculated between the 2 attachment groups, in *a priori* regions of interest (midbrain, striatum, prefrontal cortex and hypothalamus/pituitary).

RESULTS

Description of subjects

Of 112 women recruited during pregnancy, 61 met eligibility criteria and were enrolled in the study, with 44 participating in fMRI scanning. Ten women were unable to be scanned (9 due to a current pregnancy and one because of a past history of seizures) and 7 had withdrawn from the study or were lost to follow-up. Of the 44 scanned women, 15 were classified as having insecure/dismissing attachment (Type A). A further 16 women demonstrated secure patterns of attachment, without unresolved trauma or loss (Type B). A small group ($n = 4$) were classified as insecure/preoccupied (Type C), with the remaining 9 women having combined or atypical patterns. We specifically compared women from the two predominant attachment groups – Type A and B. To ensure equal numbers in each group, one Type B mother was excluded.

At Visit 1, the 30 women in this study were generally from middle to high socioeconomic backgrounds (based on the *Four-Factor Index of Social Status* [A. B. Hollingshead, PhD, working paper, 1985]: mean score 51.4 ± 9.4). Eighty percent had completed a college or graduate degree and 70% were married at the time of enrollment. The median WTAR-predicted IQ for the group was 112 (range 81-120). Sixty percent identified themselves as non-Hispanic White, one-quarter were Hispanic and one-tenth African American.

Subjects within the two attachment groups did not differ in age, race, education, socioeconomic status, marital status or predicted IQ (see Methods and **Supplementary Table 1** online). Both groups were also comparable in screening measures of personality disorder risk and parenting stress (at Visit 2) and depression (measured at each study visit). There were no significant differences seen in temperament subscales of either the mother or child, mothers' ratings of emotions before and after mother-infant interaction (based on the PANAS during Visit 2) (Watson et al. 1988) or in scales of

infant development (measured during Visit 4). We also found no significant difference in breastfeeding status at Visits 2 or 3, although Type B mothers tended to breastfeed longer and Type A mothers were significantly more likely to be separated from their child for longer periods of time each week ($P = 0.03$).

Oxytocin response to mother-infant interaction (Visit 2)

During the 7 month postpartum visit, Type B mothers showed a significantly higher peripheral oxytocin response following periods of mother-infant interaction (**Fig. 3a**; time point by attachment group interaction effect adjusted for breastfeeding at this visit, $F = 2.9$, $P = 0.04$). Although there were no differences between attachment groups in the two baseline measurements, after the 5-minute “free-play” interaction Type B mothers had significantly higher oxytocin levels ($P = 0.01$). This difference persisted into an additional mirror-based interaction period, although it was no longer statistically significant ($P = 0.07$). There were no significant differences in serum free cortisol, epinephrine or norepinephrine, or in baseline serum estradiol or progesterone.

Whole group analysis of maternal brain responses (Visit 3)

On the whole brain analysis, when mothers viewed their own infant’s happy faces, compared to unknown happy faces, key dopamine-associated reward processing regions were activated, overlapping previously reported regions (Strathearn et al. 2008) including the substantia nigra, dorsal putamen and thalamic nuclei. In addition, activation was seen in the ventral striatum, caudate nuclei, insular cortex, superior temporal gyrus and pre- and post-central gyri ($P < 0.05$, FDR corrected). As in the prior study, no significant activation was seen on contrasting own vs. unknown sad or neutral infant faces. Combining all affect groups together, contrasting own vs. unknown faces, an activation pattern overlapping our previous study results (Strathearn et al. 2008) was

again seen, including both mesocorticolimbic (ventral tegmental area and ventral striatum) and nigrostriatal pathway (substantia nigra and dorsal striatum) activation, but without activation of the prefrontal or anterior cingulate cortex.

Attachment group comparisons

We next compared own vs. unknown infant face responses between the two attachment groups, to look specifically for hypothesized differences in activation of dopamine-associated brain reward regions (in the midbrain, striatum and forebrain) and the hypothalamus/pituitary region. When comparing happy and sad faces separately, between attachment groups, no significant differences in activation were seen on whole brain analysis (using an alpha of 0.05, FDR corrected). However, when all affect groups were combined, Type B mothers showed significantly more activation in the lateral prefrontal cortex bilaterally and the left medial prefrontal cortex ($P < 0.05$, FDR corrected). In addition, the hypothalamus/pituitary region was strongly activated in Type B mothers (**Table 1; Fig. 3b**), with the activation signal in response to neutral own-infant faces significantly correlated with the mother's peripheral oxytocin response on interaction with her infant (z-transformed beta weights and % change in oxytocin; $r_s=0.60$, $P = 0.001$) (**Fig. 3c**). When attachment groups were compared in this correlation analysis, no differences in line slope ($P = 0.80$) or position ($P = 0.12$) were detected. No correlation was seen between oxytocin response and brain activation when viewing unknown infant faces, or any face type in the medial prefrontal cortex (mPFC).

In post-hoc analyses, we then compared own-infant faces, in each affect state, directly between attachment groups (e.g. own-happy in Type A vs. Type B), without the inclusion of unknown infant face comparisons. From the hypothesized regions of interest, for the happy face contrast, significantly greater activation was seen in the ventral striatum for Type B mothers, as well as the orbitofrontal cortex (OFC) and mPFC

bilaterally (**Table 2**). An equal but opposite BOLD response was seen in Type A mothers in the ventral striatum (**Fig. 4a**). In the mPFC, Type B mothers had a much larger increase in percent signal change compared with Type A mothers. Type A mothers showed significantly more activation in the dorsolateral prefrontal cortex (dlPFC) bilaterally, compared with Type B mothers, in response to both happy and sad own-infant faces.

In response to own infant *sad* faces, the right ventral striatum was also more active in Type B mothers (**Table 2; Fig. 4b**). Type A mothers again showed more activation of the dlPFC in response to own-sad faces, as well as a much stronger activation signal in the anterior insula bilaterally, compared with Type B mothers (**Fig. 4b**). Activation in the right ventral striatum to neutral own-infant faces was also highly correlated with peripheral oxytocin response ($r_s = 0.57$, $P = 0.002$; **Fig. 5**). Unknown-infant faces produced no such correlation. None of these contrasts, for happy or sad faces, showed significant differences in activation of midbrain regions, across attachment groups.

Overall, mothers with Type B attachment tended to show greater left hemisphere activation, whereas Type A had predominantly right hemisphere activation, especially for happy and sad infant faces (Table 1).

DISCUSSION

This study demonstrates group differences in maternal brain and oxytocin responses to infant cues, based on adult attachment patterns measured prior to the birth of the mother's first child. As hypothesized, mothers with secure vs. insecure/dismissing attachment showed increased activation of mesocorticolimbic reward brain regions, on viewing their own infant's smiling face. Furthermore, they showed an increased peripheral oxytocin response while interacting with their infants, which was positively correlated with activation of oxytocinergic and dopamine-associated reward processing regions of the brain (hypothalamus/pituitary and ventral striatum). Finally, striking differences in brain activation were seen in response to their own infant's sad facial affect. Securely attached mothers continued to show greater activation in reward processing regions, while "insecure/dismissing" mothers showed increased activation of the anterior insula, a region associated with feelings of unfairness, pain and disgust (see review, Montague and Lohrenz 2007).

The finding of reduced "reward" activation in mothers with insecure/dismissing attachment is consistent with a recent study of responses to smiling adult faces and positive task feedback (Vrticka et al. 2008), where activation of the ventral striatum was negatively correlated with dismissing attachment scores. In linking attachment security with ventral striatal activation, our findings suggest that for securely attached mothers, infant cues (whether positive or negative in affect) may act as an important signal of "incentive salience" (Berridge 2007), reinforcing and motivating responsive maternal care.

In contrast, mothers with insecure/dismissing attachment styles showed greater activation of dlPFC and anterior insula in response to their own infant's sad face, suggesting cognitive control over a negative affective response (Greene et al. 2004; Sanfey et al. 2003). In line with our current understanding that activation of the anterior

insula may signal “norm violations” (Montague and Lohrenz 2007), insecure/dismissing mothers may cognitively appraise their infant’s sad affect as a violation of an “expected” affect state. This may lead to avoidance or rejection of negative infant cues (Sanfey et al. 2003), rather than the “approach” responses seen in Type B secure mothers. While the ventral striatal activation seen in Type B mothers has been associated with anticipated gain, right anterior insula activation is seen in anticipation of loss (Knutson et al. 2007). These results are consistent with a previously published model of the cortical organization of the attachment system (Strathearn 2006; Crittenden 2008), which postulates that individuals with insecure/dismissing attachment are biased toward cognitive information processing, and tend to inhibit negative affective responses.

Although anterior insula activation has also been linked with empathic responses to a loved one’s feeling of physical pain (Singer et al. 2004), dismissing individuals score much lower on a scale of emotional empathy (Sonny-Borgstrom and Jonsson 2004), making this interpretation less likely.

Oxytocin has long been implicated as an important neuromodulatory hormone involved in maternal behavior (Insel 1992; Insel and Young 2001). Synthesized in the paraventricular nucleus of the hypothalamus, there are oxytocinergic projections to the posterior pituitary gland where it is released into the blood stream. In addition, oxytocin neurons project centrally to regions important in the manifestation of social and maternal behaviors (Numan 2006). There is some evidence to suggest that oxytocin neurons in the hypothalamus may directly project to the ventral striatum, facilitating dopamine release (Liu and Wang 2003) and thus linking social and maternally-related cues to reward processing and behavioral reinforcement (Insel 2003). Rodent studies have demonstrated that oxytocin receptor binding in the nucleus accumbens (a nucleus of the ventral striatum) facilitates the onset of maternal behavior (Olazabal and Young 2006a; Olazabal and Young 2006b).

While there has been some controversy surrounding the relationship between peripheral and central oxytocin production (McGregor et al. 2008), these results are consistent with the idea that differences in peripheral oxytocin response may reflect central oxytocin production and contribute to individual differences in maternal caregiving behavior. Other studies have shown reduced peripheral oxytocin responses in cocaine addicted mothers (Light et al. 2004) and in pregnant women with lower maternal-fetal attachment scores (Levine et al. 2007). Furthermore, reduced peripheral oxytocin levels have been seen in orphanage-adopted children with histories of early neglect, who display severe impairments in social reciprocity (Fries et al. 2005). The observation that oxytocin levels are higher in securely attached mothers following interaction with their infants suggests the importance of this neuropeptide in mediating attachment and social behaviors, as seen in human randomized placebo-controlled trials of intranasal oxytocin (Baumgartner et al. 2008; Guastella et al. 2008b), as well as in rodent studies (Insel and Young 2001; Champagne et al. 2001; Insel 1992; Liu and Wang 2003). In our study, the correlation of interaction-elicited peripheral oxytocin with the activation of reward regions in the brain suggests that oxytocin may be one mechanism by which socially-relevant cues activate dopaminergic pathways and thus reinforce behavior. Mothers with secure attachment patterns when interacting with their infants may produce more oxytocin which increases the experience of reward, and in turn may contribute to the mother's ability to provide consistent, nurturant care. One limitation of these findings is that oxytocin measurements during real-time interaction were collected 4 months prior to fMRI scanning, providing no opportunity to examine simultaneous correlations. However, the correlation between oxytocin and hemodynamic response across time suggests that the oxytocin response may reflect an enduring trait difference associated with attachment security.

Numerous previous investigations have shown that mothers with insecure attachment patterns are less likely to establish secure relationships with their children, and that their children tend to have greater difficulties regulating affect, forming peer relationships and establishing secure attachment relationships themselves (Sroufe et al. 2005; van IJzendoorn 1995). While the transgenerational transmission of attachment has been frequently observed, its mechanism is still poorly understood (van IJzendoorn 1995). This study may help shed light on this question, with evidence that secure attachment is associated with more intense maternal reward activation to infant facial expressions, while insecure/dismissing mothers show greater insula response to negative infant cues. Additional research is needed to confirm these findings in larger cohorts of mothers, including mothers with insecure/preoccupied attachment. A randomized controlled trial of intranasal oxytocin may also help to clarify any causal relationship between oxytocin response and maternal brain activation.

In conclusion, this study is the first to examine the neuroendocrine basis of human mother-infant attachment. As such, it may help us to better understand the transmission of attachment patterns across generations and how secure maternal attachment may confer developmental advantages on infant development (Sroufe et al. 2005; van IJzendoorn 1995).

DISCLOSURE / CONFLICT OF INTEREST

The authors have no disclosures or conflicts of interest.

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Table 1 Areas of significant activation within the prefrontal cortex, striatum and midbrain, when comparing Type A and Type B attachment groups. All regions-of-interest $P \leq 0.0001$; voxel threshold=4, except as noted. Talairach coordinates (x, y, z) represent centre-of-gravity mean values for each region-of-interest.

Region-of-Interest / Cluster (Brodmann Area, BA)	Right Hemisphere		Left Hemisphere	
	x, y, z	Mean t-score	x, y, z	Mean t-score
A. Own > Unknown (all affect groups combined):				
Secure > Insecure/Dismissing				
Prefrontal cortex				
Middle frontal gyrus (BA 10)	44, 46, 18	3.42	-	-
Medial frontal gyrus (BA 10)	-	-	-7, 58, 10	3.45
Superior frontal gyrus (BA 10)	-	-	-32, 51, 25	3.62
Insula / Frontal operculum (BA 13)	-	-	-40, 17, 11	3.71
Hypothalamus / pituitary region	-	-	-3, 2 -16	4.04
Insecure/Dismissing > Secure				
Dorsolateral prefrontal cortex				
Precentral gyrus (BA 6)	44, -16, 25	3.67	-24, -16, 50	3.35
Precentral gyrus (BA 9)	-	-	-31, 5, 34	3.41
Superior frontal gyrus (BA 9)	39, 34, 30	4.23	-	-
Inferior frontal gyrus (BA 9)	36, 10 22	3.54	-	-
Middle frontal gyrus (BA 8/6)	24, 21, 35	3.58	-24, 6, 44	3.67
Middle frontal gyrus (BA 46)	-	-	-41, 29, 21	3.75
Medial prefrontal cortex				
Superior frontal gyrus (BA 8)	13, 36, 44	3.42	-	-

Anterior Insula (BA 13)	-	-	-31, -3, 21	3.57
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B. Own-Happy Faces:

Secure > Insecure/Dismissing

Medial prefrontal cortex

Medial frontal gyrus (BA 10)	7, 64, 8	3.97*	-6, 60, 9	3.54
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Orbitofrontal cortex

Inferior frontal gyrus (BA 46/45)	48, 40, 5	3.66	-54, 17, 8	3.65*
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Superior frontal gyrus (BA 10)	-	-	-20, 55, 5	3.73
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Medial frontal gyrus (BA 9)	-	-	-22, 36, 24	3.52
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Striatum

Ventral striatum / nucleus	-	-	-2, 10, -4	3.39*
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accumbens

Insecure/Dismissing > Secure

Dorsolateral prefrontal cortex

Middle frontal gyrus (BA 46)	44, 30, 17	3.78	-41, 37, 14	3.86
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Middle frontal gyrus (BA 9)	28, 24, 34	3.82	-40, 21, 27	3.57*
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Middle frontal gyrus (BA 9)	36, 32, 31	3.98	-	-
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Middle frontal gyrus (BA 6)	21, 18, 50	3.54	-	-
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Subcallosal gyrus	1, 13, -15	3.64	-	-
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C. Own-Sad Faces:

Secure > Insecure/Dismissing

Lateral prefrontal cortex

Inferior frontal gyrus	-	-	-38, 41, -2	3.54
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Middle frontal gyrus (BA 9)	-	-	-26, 40, 32	3.74
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Striatum

Ventral striatum / Nucleus accumbens	12, 10, -3	3.47	-	-
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Insecure/Dismissing > Secure

Dorsolateral prefrontal cortex

Inferior frontal gyrus (BA 44)	-	-	-53, 4, 13	3.65
Middle frontal gyrus (BA 9)	35, 31, 34	3.77	-	-
Middle frontal gyrus (BA 8)	23, 20, 42	3.49	-	-
Medial frontal gyrus (BA 6)	18, 6, 49	3.79	-	-
Medial frontal gyrus (BA 9)	10, 47, 30	3.48	-	-
Precentral gyrus (BA 44)	47, 12, 11	3.59	-	-

Anterior insula

Anterior Insula (BA 13)	37, 19, 18	3.57	-34, 27, 16	3.54
Anterior Insula (BA 13)	38, 17, -1	3.66	-	-
Anterior Insula (BA 13)	27, 18, -7	3.59	-	-

Medial frontal lobe

Medial frontal gyrus – posterior (BA 6)	14, -13, 55	3.40	-	-
Uncus (BA 28)	15, -9, -24	3.62	-	-
Anterior cingulate cortex (BA 32)	14, 30, 7	3.68	-	-
Medial frontal gyrus / Gyrus rectus (BA 25)	3, 10, -15	3.61	-	-

* Only seen at a threshold of 3 voxels.

Supplementary Table 1 Comparison of Cohort Groups: Type A and Type B mothers.

Variable	Insecure/ Dismissing Attachment (Type A) (N=15)	Secure Attachment (Type B) (N=15)
Age of mother, Visit 1, y ± SD	29.6±3.6	28.0±4.3
Timing of visits, mo ± SD		
Visit 2 (months after birth)	6.2±1.7	7.5±2.2
Visit 3 (months after Visit 2)	4.0±2.0	4.5±1.5
Visit 4 (months after Visit 3)	3.5±1.9	2.7±2.8
Maternal race, N (%)		
White	8 (53%)	10 (67%)
Non-white	7 (47%)	5 (33%)
Maternal education, N (%)		
Graduate professional training	7 (47%)	4 (27%)
Some college experience	8 (53%)	11 (73%)
Married, Visit 1, N (%)	10 (67%)	11 (73%)
Hollingshead SES (joint with partner), Visit 1, score ± SD	51.3±11.6	45.9±13.1
Maternal IQ (WTAR-predicted WAIS-III) ± SD	108.3±9.5	110.8±7.7
Personality disorder screener (PDQ- 4+), Visit 1, N		
No positive screens	4	5

1 or 2 positive screens	5	4
3 or more positive screens	6	6
Depression (BDI), N		
Prenatal depression, Visit 1		
Minimal	12	13
Mild	1	2
Moderate	2	0
Postnatal depression (mean score, Visits 2-4)		
Minimal	14	14
Mild	1	1
Parenting Stress Index (PSI), Visit 2, raw score \pm SD	(N=13)	(N=14)
Child Domain	95.9 \pm 12.7	93.6 \pm 15.8
Parent Domain	116.3 \pm 18.4	113.0 \pm 25.6
Total Stress	212.2 \pm 23.1	206.6 \pm 35.9
Temperament of Mothers (ATQ), raw score \pm SD		
Negative Affect	4.0 \pm 0.9	4.2 \pm 0.6
Extraversion/Surgency	4.7 \pm 0.9	4.7 \pm 0.6
Effortful Control	4.7 \pm 0.6	4.3 \pm 0.6
Orienting Sensitivity	4.9 \pm 0.6	5.3 \pm 0.7
Infant Temperament (IBQ), raw score \pm SD		
Approach	5.5 \pm 0.9	5.9 \pm 0.6

Vocal reactivity	5.4±1.1	5.3±0.7
High intensity pleasure	6.1±0.6	6.0±0.5
Activity level	5.0±0.7	5.0±0.7
Perceptual sensitivity	4.4±0.9	5.2±0.8
Distress to limitations	4.0±0.6	4.1±0.7
Fear	2.8±1.1	2.7±0.7
Low intensity pleasure	5.1±0.9	4.6±1.5
Cuddliness/affiliation	5.7±0.7	5.5±0.7
Duration of orienting	4.0±1.1	4.4±1.1
Mother-infant separation, >20 hrs per week, Visit 3, N (%) ^a	10 (67%)	4 (27%)
Still breastfeeding, N (%)		
Visit 2	8 (53%)	11 (73%)
Visit 3	8 (53%)	9 (60%)
Child development, Visit 4, Bayley	(N=13)	(N=15)
mean raw score ± SD		
Cognitive	17.2±1.9	17.4±1.6
Receptive communication	13.8±2.2	14.5±2.4
Expressive communication	13.3±1.6	13.5±1.8
Fine motor	14.8±1.8	15.5±1.7
Gross motor	17.8±2.0	18.1±1.3

^a P=0.03. No significant differences seen between groups in all other comparisons.

FIGURE LEGENDS

Figure 1 Study timeline and data collected at each of 4 study visits. Abbreviations: AAI, Adult Attachment Interview; PDQ, Personality Disorder Questionnaire 4+; BDI, Beck Depression Inventory; PANAS, Positive and Negative Affect Schedule; ATQ, Adult Temperament Questionnaire—Short form; IBQ, Infant Behavior Questionnaire—Revised; PSI, Parenting Stress Index; WTAR, Wechsler Test of Adult Reading.

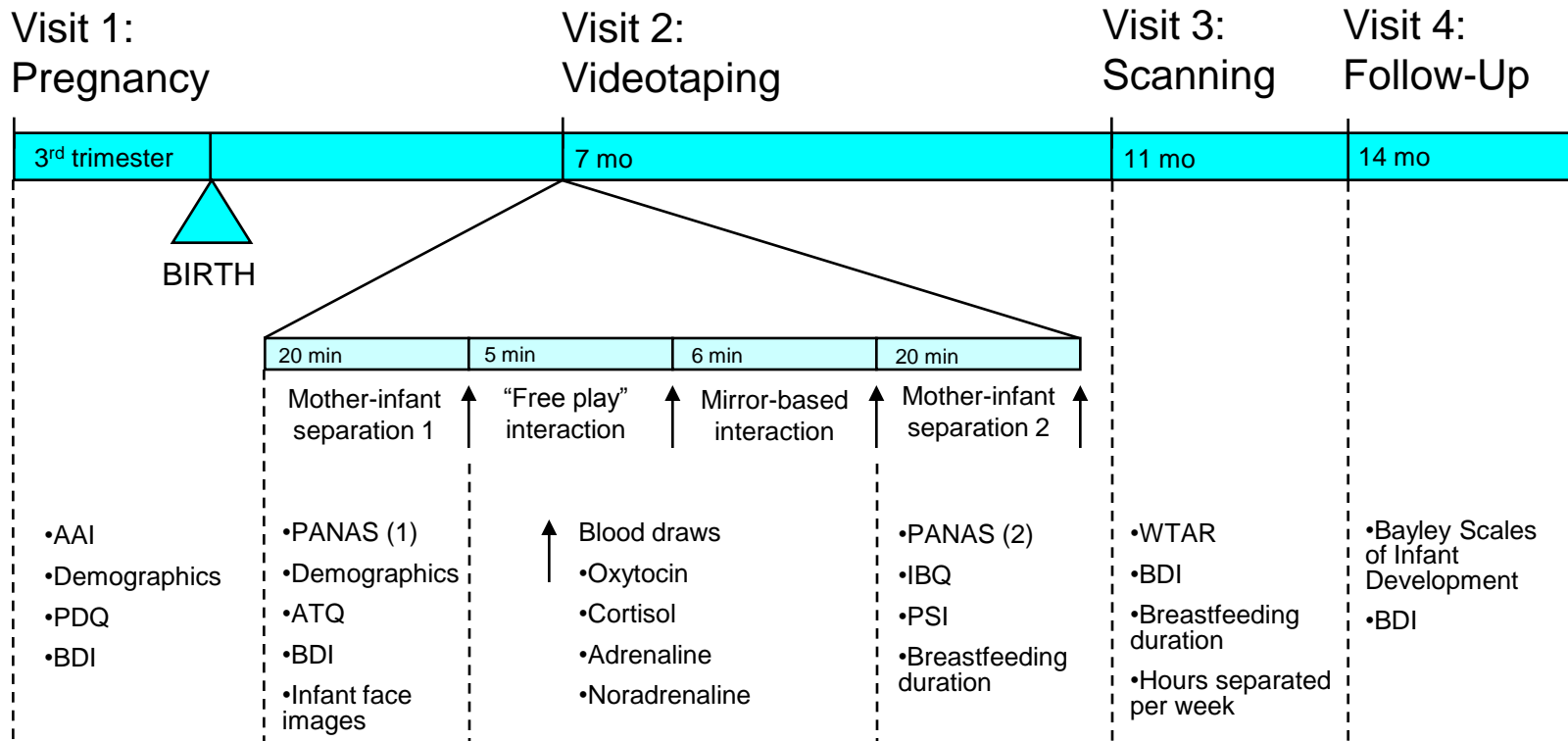
Figure 2 Baby face presentation paradigm in functional MRI experiment. Infant face images were presented for 2 seconds, followed by a variable 2-6 second period of a plain black screen. Six stimulus types were presented in random order: own-happy (OH), own-neutral, own-sad, unknown-happy, unknown-neutral, unknown-sad. Reproduced with permission from *Pediatrics*, Vol. 122, Pages 40-51, Copyright © 2008 by the AAP.

Figure 3 Peripheral oxytocin and related brain activation in response to infant cues. **(a)** Mothers with Type B (secure) attachment patterns show a greater peripheral oxytocin response during an episode of physical interaction with their infant (mean \pm sem; Bonferroni corrected comparison at free play time point, $P = 0.01$). The first baseline sample was collected 20 minutes after mother-infant separation; the second immediately after a 5-minute “free-play” involving direct physical contact between the mother and infant. The third sample was after a modified still-face procedure, in which the mother was in direct visual and auditory contact with her infant (via a mirror) but was physically separated by a screen divider. The final sample was collected after a further 20-minute period of complete mother-infant separation. **(b)** Type B mothers show greater activation of the hypothalamus/pituitary region in response to own vs. unknown infant face images (all affect groups combined) (mean \pm sem, $t=4.2$, $P < 0.001$). Structural brain image

created from average of all subjects. Inset of magnified hypothalamic/pituitary region (single subject image to improve anatomical clarity). **(c)** Peripheral oxytocin response correlates with activation of hypothalamus/pituitary region in response to neutral own infant face cues ($r_s = 0.60$, $P = 0.001$). A single outlying value was omitted from the graph, but not the statistical calculations.

Figure 4 Brain responses to happy and sad own-infant faces, contrasting mothers with Type A (insecure/dismissing) and B (secure) attachment classifications (% signal change \pm sem) **(a)** Type B mothers show greater activation of the ventral striatum (VS; $t=3.1$, $P<0.005$) and medial prefrontal cortex (mPFC; $t=3.0$, $P<0.01$) in response to happy own-infant faces. **(b)** Type B mothers show greater activation of the right ventral striatum ($t=3.0$, $P<0.01$) in response to sad own-infant faces. Type A mothers show greater activation of the right anterior insula ($t=-3.9$, $P<0.0005$).

Figure 5 Peripheral oxytocin response after episodes of mother-infant interaction correlates with activation in the right ventral striatum (area shown in Figure 4b) in response to neutral own infant face cues ($r_s=0.57$, $P=0.002$). Percent oxytocin change calculated from the first baseline measurement and a mean of the second and third samples, which were taken during episodes of mother-infant interaction.



2 sec



2 sec



2 sec



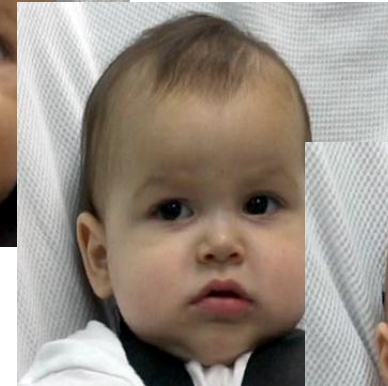
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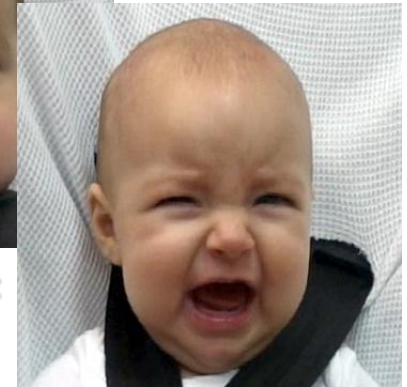
2-6 sec random inter-stimulus interval



2 sec



2 sec



Own:
Happy
(OH)

Unknown:
Happy
(UH)

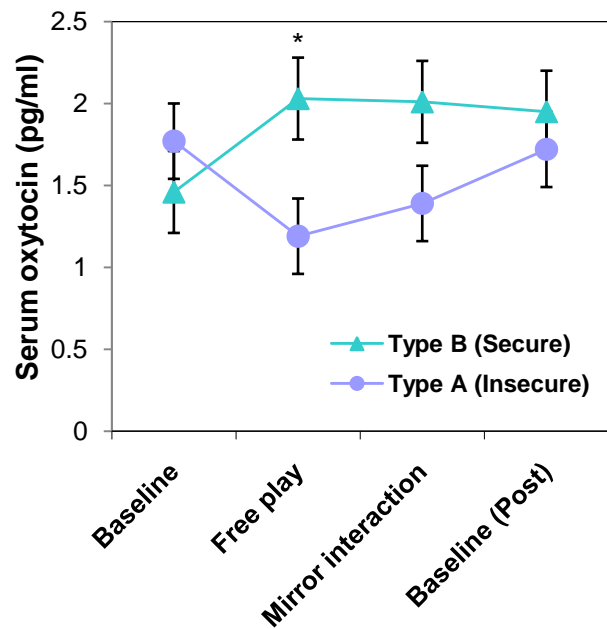
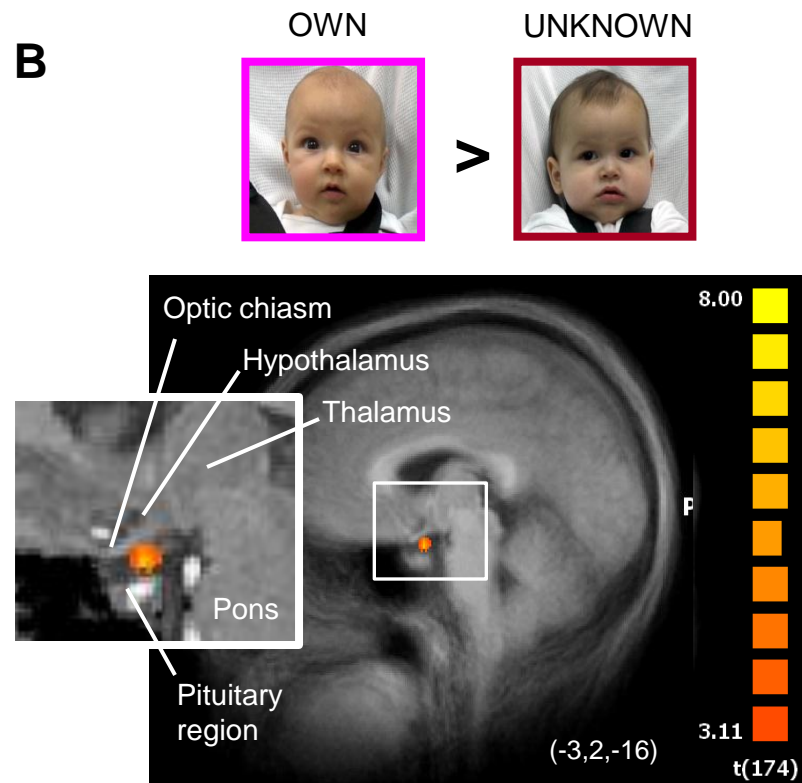
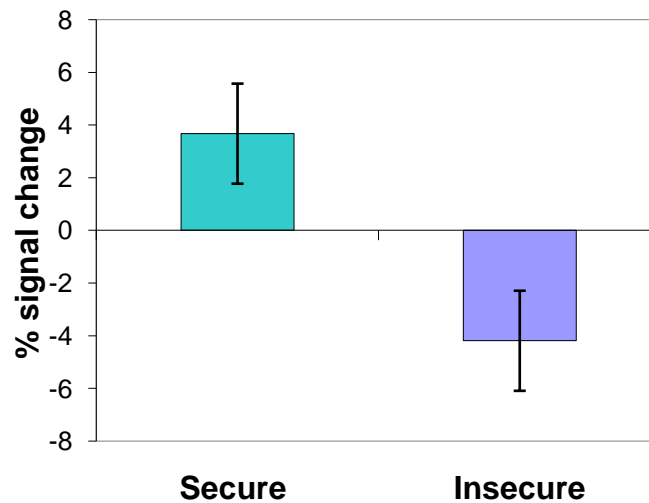
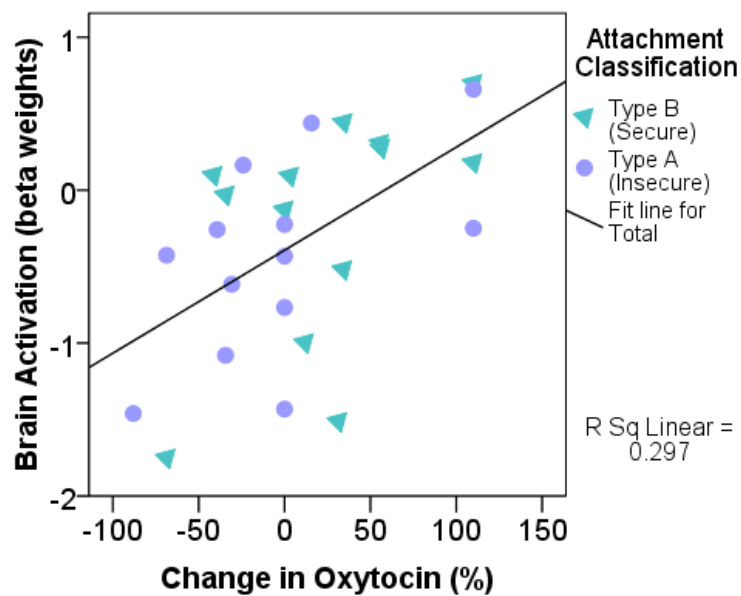
Unknown:
Sad
(US)

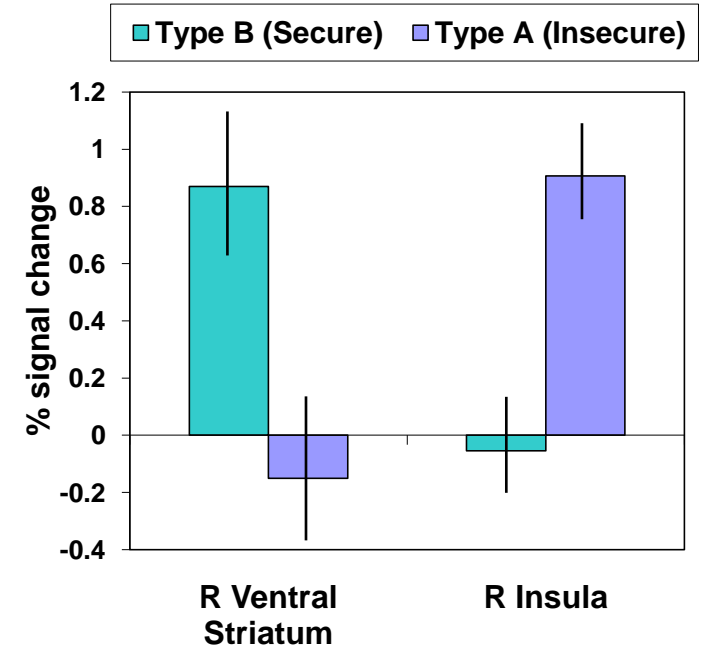
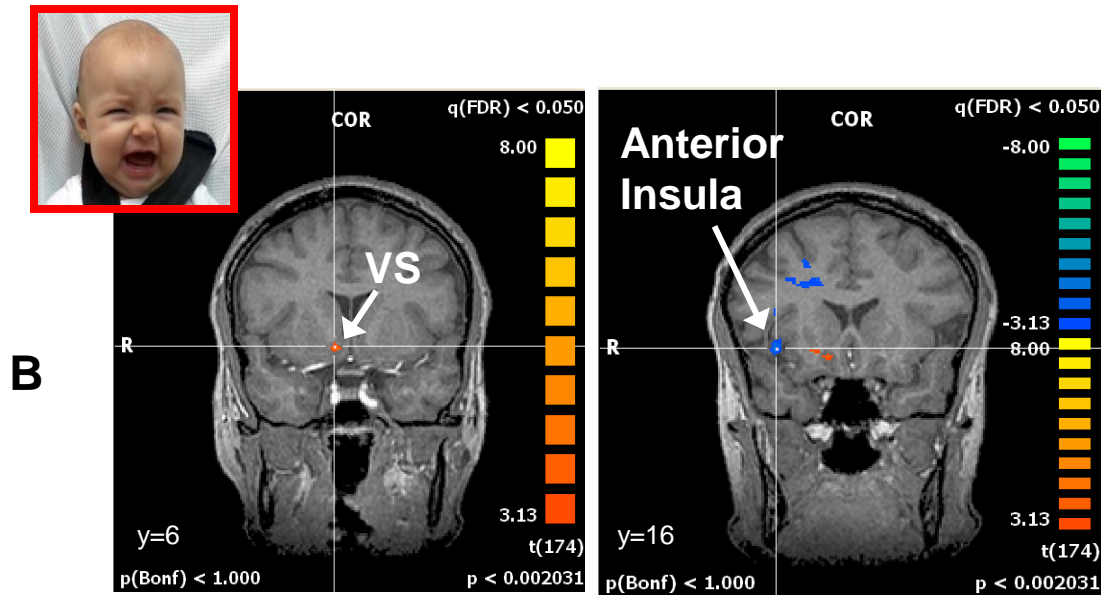
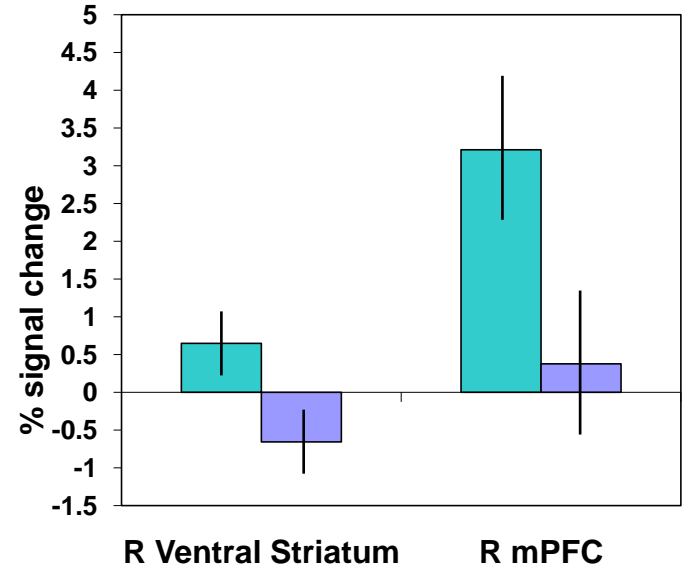
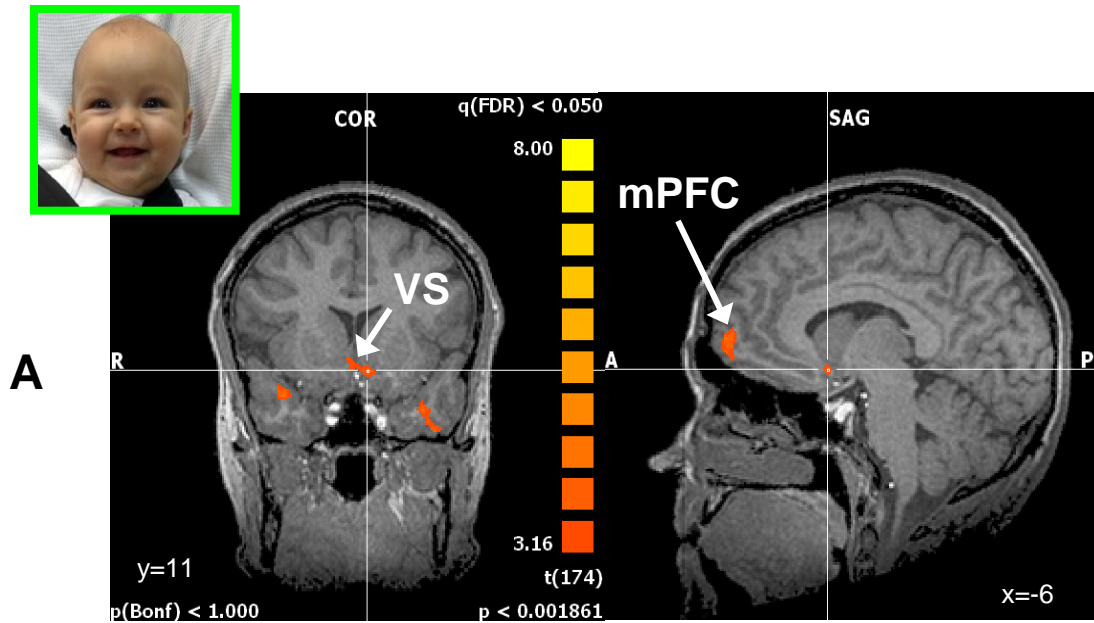
Own:
Neutral
(ON)

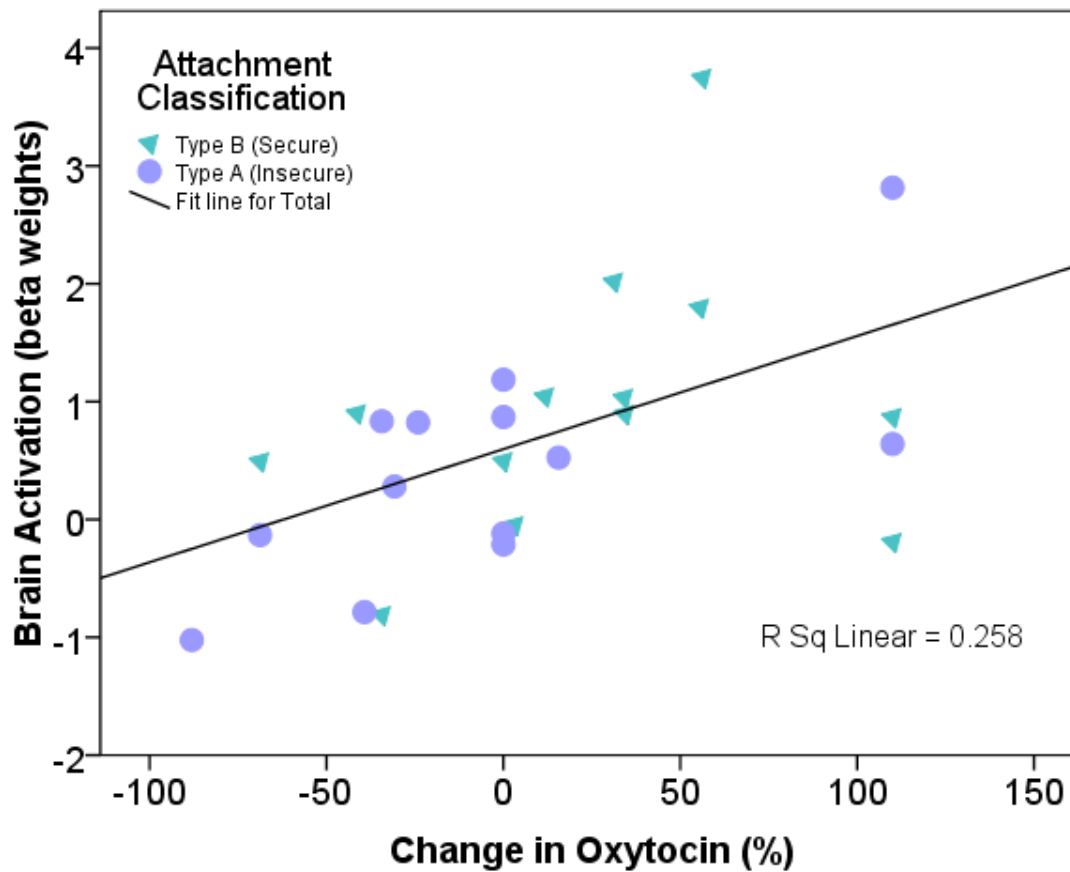
Unknown:
Neutral
(UN)

Own:
Sad
(OS)

STIMULUS TYPES		IDENTITY	
		Own Infant	Unknown Infant
A F F E C T	Happy	OH	UH
	Neutral	ON	UN
	Sad	OS	US

A**B****C**





Response to Reviewers

We appreciate the reviewer's thoughtful comments and their positive overall assessment of "a well written article with extremely interesting findings", "rare and admirable... [in] attempt[ing] to relate brain activity... to real-life maternal behavior and physiology" and "an elegant and demanding study design" to "address an issue at the very core of biological psychiatry."

We have addressed each of the reviewer's suggestions as outlined below:

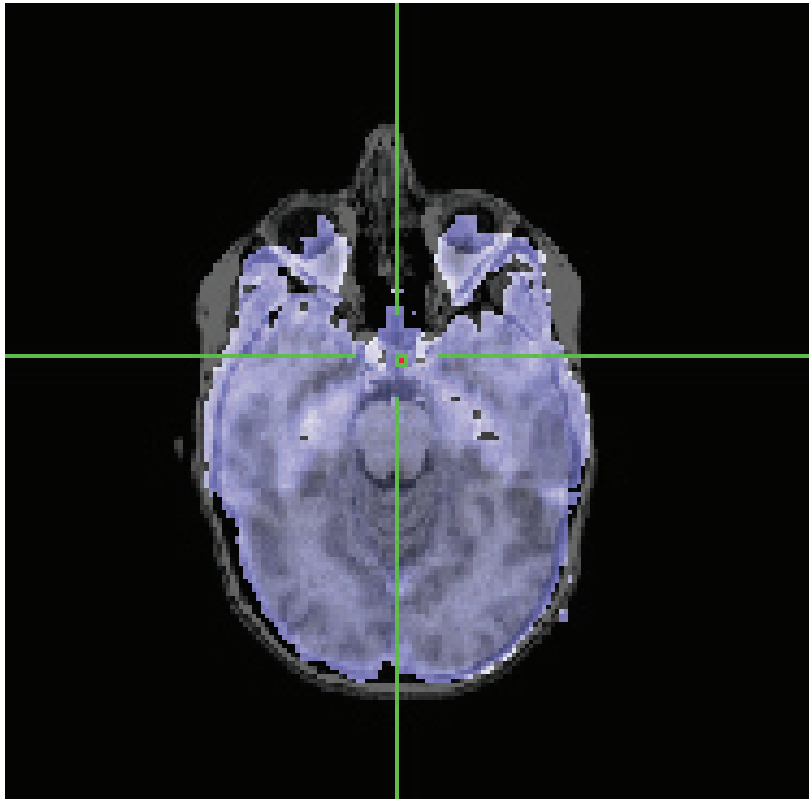
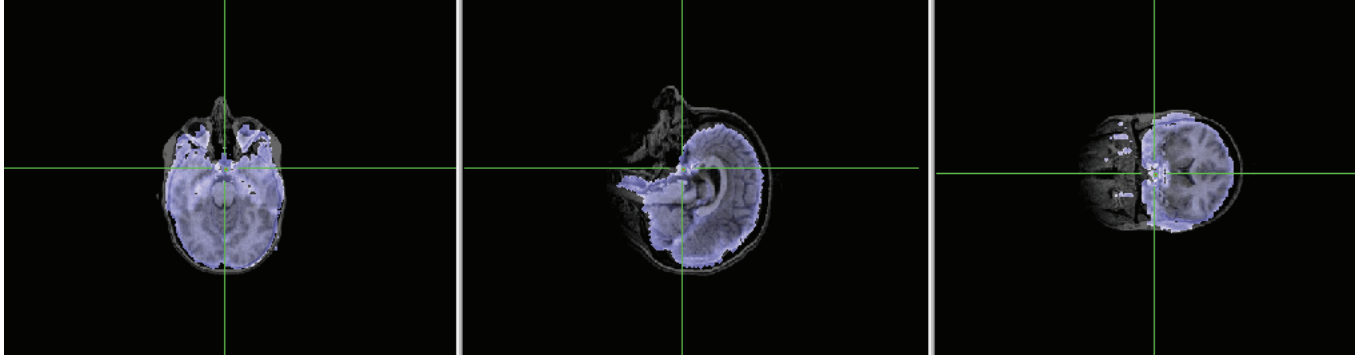
Reviewer #1:

1. "Figure 3b shows a stronger hypothalamic response in type B compared with type A mothers for the contrast own - unknown. Given this, it would seem that the data points for type B mothers in figure 3c should lie mostly above $y=0$, but this is not the case. How can this be? Also, the figure legend indicates that the contrast between own and unknown is being plotted in figure 3c, but the y axis is labeled beta weight. Is this the beta weight for own or for the contrast value for own-unknown? This needs to be clarified. The only way I can reconcile these two findings is if 3c is plotting the beta value for own and 3b is plotting the contrast value. But in this case, the difference between maternal types would be driven by a differential response to unknown faces and it is hard to see why this would be correlated with the OT response to interacting with one's own infant (Figure 3c)."
 - We appreciate the insightful feedback from this reviewer. Figure 3c was incorrectly labeled, giving rise to these inconsistencies. As correctly suggested by the reviewer, Figure 3c plots the beta values of own (neutral) faces, whereas Figure 3b is the contrast between own and unknown infant faces (happy/neutral/sad affect combined). Thus, the hypothalamic activation in response to own vs. unknown infant faces is greater in mothers with secure attachment, and the activation in response to neutral own-infant faces is correlated significantly with peripheral oxytocin response when the mothers interact with their child. This is now clarified in the Figure 3 legend and text (page 14).
2. "The ventral striatum is activated for own vs. unknown happy faces for the combined sample of type A and type B mothers (page 13), but figure 4a shows it deactivating for own happy faces in the type A mothers? How can these two findings be reconciled?"
 - A number of factors may contribute to this apparent inconsistency. Firstly, the two analyses differ in the contrast used. The first is a contrast between face types (own vs. unknown happy faces), while the second is a contrast between attachment groups (Type B vs. Type A). Each contrast uses different analysis methods and has different numbers of contrasted groups (i.e. a random effects analysis of 30 individual subjects in the first analysis, vs. a fixed effects analysis of 2 attachment groups in the second). There were also differences in the specific location of activation within the ventral striatum. The most likely explanation is that mothers in general show greater activation of the ventral striatum when viewing their own vs. an unknown infant's smiling face, but the subgroup of "insecure/dismissing" mothers show more deactivation in this region, compared with "secure" mothers. These two results link the findings of our previous paper (Strathearn, 2008) with the present study.

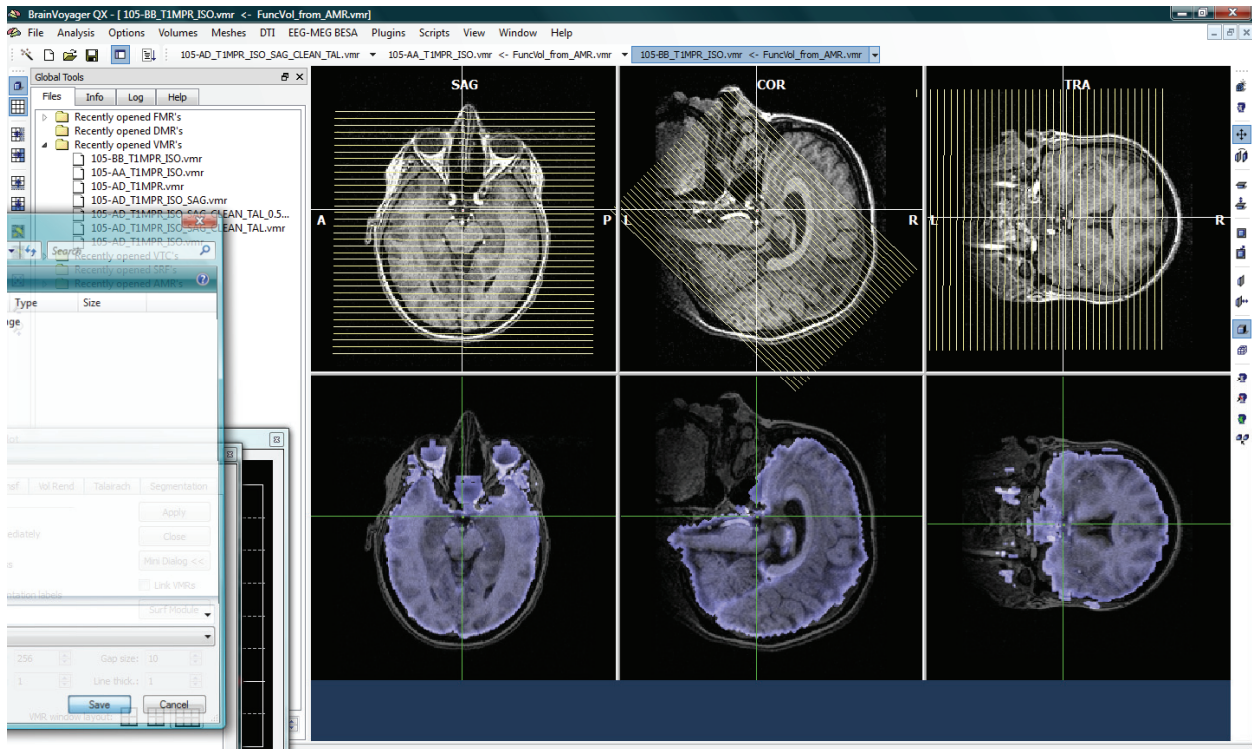
3. "In discussing anterior insula activation when dismissing mothers viewed their own infant's sad faces, (page 17), the authors state, "Thus, in contrast with reward/motivation responses seen in mothers with secure attachment, sad infant affect may inhibit normal caregiving responses in dismissing mothers, and predispose to the development of insecure attachment in infancy." However, the anterior insula is also implicated in responding empathically to a loved one's distress (Singer et al 2004), and this is of course a crucial component of successful mothering. The insula has also been implicated in aversive conditioning and could be part of the mechanism by which mothers are taught how to meet their infants' needs more effectively. Therefore, the assumption that the insula activation in the dismissive mothers is dysfunctional seems premature."
 - The valuable point that "anterior insula activation has also been linked with empathic responses to a loved one's feeling of physical pain" has now been included in the discussion, along with the caveat that "dismissing individuals score much lower on a scale of emotional empathy, making this interpretation less likely" (page 17). The premature conclusion quoted by the reviewer has now been deleted.
4. "Please explain why a fixed effects rather than a random-effects model was used when comparing brain activation across attachment groups."
 - Thank you for identifying this issue which is in need of clarification. The analysis was, in fact, a random effects analysis, in which the subjects were treated as a random factor in all ANOVA models. However, the experimental factors of the multi-factorial design were "fixed", as they were not a sample from a larger population of potential factor levels, and we were not interested in drawing inferences about the population of factor levels. This has now been clarified in the manuscript (page 10).
5. "On page 16, the authors state, '... our findings suggest that for securely attached mothers, infant cues (whether positive or negative in affect) may act as an important learning signal (Schultz 1998) to reinforce and motivate responsive maternal care'. This is an interesting idea, but if this were true then viewing a picture of one's own infant crying should deactivate the ventral striatum to teach the mother to avoid whatever behavior precipitated the infant's negative reaction."
 - Although prior studies have suggested that activation of the ventral striatum is related primarily to positive reward activation, more recent work has shown that negative—but highly salient—cues also activate this region (e.g. Faure, 2008; Berridge, 2007), suggesting a role of the striatum in "incentive salience". This point has been clarified in the discussion (page 16).
6. "Please describe the kinetics of OT release into peripheral blood. How long after an OT-releasing event does it show up in blood? Are the levels following the maternal-infant interaction reflecting that interaction or something that happened earlier?"
 - Oxytocin is rapidly released into the peripheral circulation following a stimulus such as infant suckling. Studies that have used frequent sampling of blood (every 1 to 3 min) during a bout of nursing have identified a pulsatile pattern of oxytocin release in breastfeeding women (Lucas, 1980; Johnston, 1986). Oxytocin pulses also have been reported to occur in nursing women before the onset of infant suckling, usually in response to the sight or sound of the baby (McNeilly, 1983). Thus

in the present study, it seems plausible that the release of oxytocin likely relates to emotional cues triggered by mother-infant interaction. This has been explained in detail on pages 3-4.

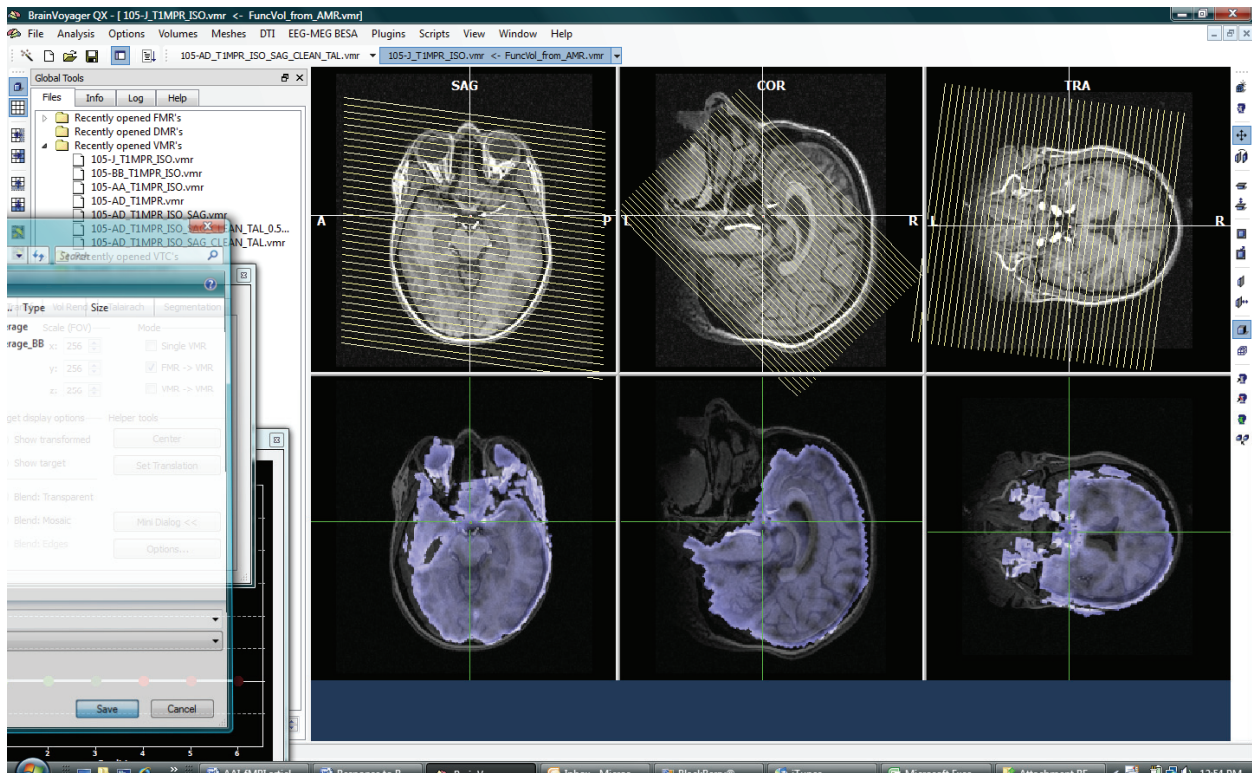
7. "Type A mothers activated DLPFC more than type B mothers when viewing their own infant's face. What is the authors' interpretation of this?"
 - In the model proposed in our original paper (Figure 6, Strathearn, 2008), we suggested that DLPFC activation in relation to attachment stimuli were indicative of more cognitive, and less affect focused, responses to infant face cues. In line with this model, we now propose in the paper that DLPFC activation in insecure/dismissing mothers is related to a more cognitive, impersonal response triggered by seeing their own baby's face (page 16-17).
8. "Please indicate what was happening to the infants when they were separated from their mother?"
 - During the separation of mother and infant, smiling, neutral and crying faces were elicited while the infant was videotaped while in a car seat. Smiling faces were elicited using age-appropriate toys and blowing bubbles. If the infant did not cry during this period, he/she was videotaped while being briefly left alone in the room, but observed from behind a one-way mirror. The infant was not left to cry for more than 30 seconds before being picked up and pacified. The infant was constantly in the supervision and care of the study investigator or a trained research assistant. These details are referenced on page 6 to the methods section of a prior paper (Strathearn, 2008).
9. "The Talairach coordinate listed for the hypothalamic activation in figure 3b is off the brain in my atlas, or perhaps on the optic chiasm. Nevertheless, considering smoothing and registration errors, it is plausible that the activation reflects hypothalamic activity. However, please show the hypothalamic/pituitary activation on an EPI functional image to verify that it is on the brain in a region with good signal. Also, a close-up view of the activation with relevant structures labeled would be helpful."
 - We recognize that this region of activation does not precisely correspond with the hypothalamus and pituitary in some brain atlases. However, when localized onto an averaged structural brain image created from all of the study subjects, the activation corresponds well with this brain region (see Figure 3b). On the recommendation of this reviewer, we have confirmed that the hypothalamic/pituitary activation does, in fact, lie within the functional maps of individual subjects, as illustrated below. A close-up, labeled view of these regions has now been incorporated into Figure 3b, as suggested by Reviewer 2.



Subject AA



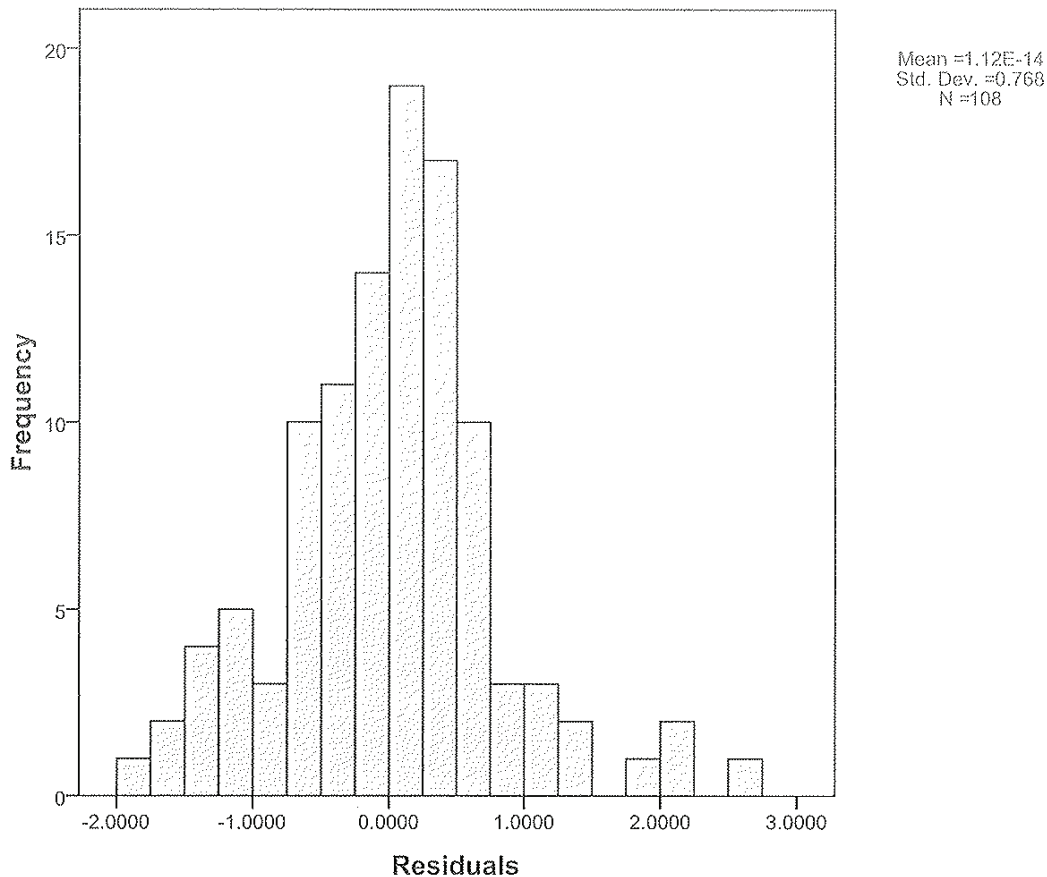
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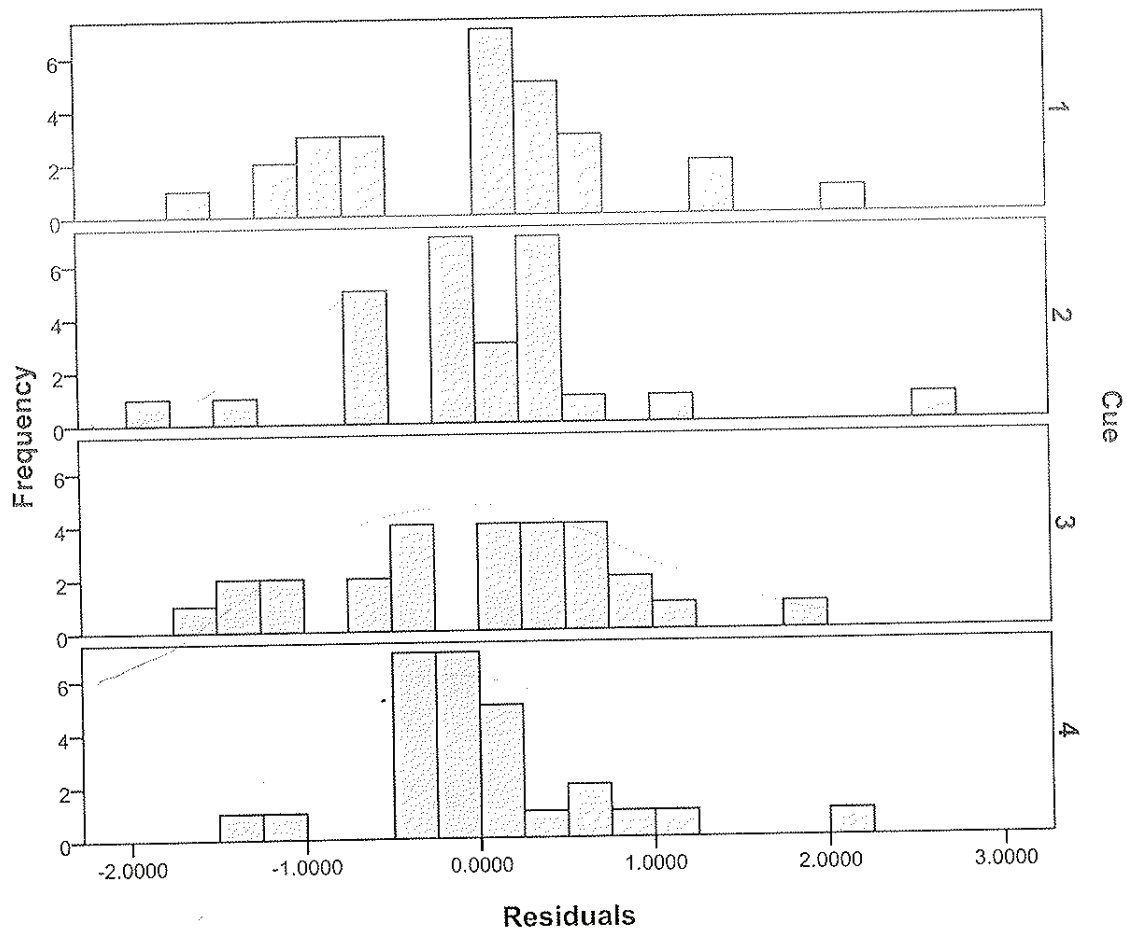


Subject J

Reviewer #2:

1. "A primary concern is the way that stats were handled for the group differences in plasma oxytocin levels in response to the environmental manipulations. An ANOVA was used, but the data were non-parametric so that between-group comparisons at specific time points were done with a Mann-Whitney-U. Wouldn't it be more appropriate to use a Friedman test and then examine post-hoc between differences? My concern is that using a separate test to look at between-group differences at specific time points leads to a spurious increase in power and perhaps a false positive result."
- We appreciate the opportunity to clarify and refine our analyses in this area. We first confirmed that the oxytocin concentration residuals were normally distributed, which was the case for all values combined and for each individual time point (see histograms). Thus the Friedman test for non-parametric data was not necessary. We used a linear mixed model to assess the effects of interaction between attachment security and time point, while controlling for breastfeeding status, and found that the interaction was significant at $P=0.04$. We then compared the oxytocin response at each time point while correcting for multiple comparisons (Bonferroni), and found a significant difference between attachment groups after the first episode of mother-infant interaction ($P=0.0124$). These changes have been noted accordingly in the text (pages 8-9, 13).





2. "On page 14 you have one result as non-significant at $p=0.05$ and another as significant at the same alpha level. A choice should be made one way or the other."
 - We apologize for the lack of clarity in the original version. This has now been corrected to say that no significant results were seen "using an alpha of 0.05" as a statistical threshold (page 14).
3. "I think it is important that it is made more clear that the scanning and oxytocin results are separated by 5 months. This has strengths and weaknesses--it suggests that the correlations may be long lasting or that one might predict the other, but it also decouples potential causality to some degree."
 - We have now clarified in the paper the time lag between the oxytocin response measure and fMRI scanning (which was actually 4 months on recalculation; Figure 1 and pages 5-6, 9). This limitation, as noted by the reviewer, is now added to the discussion (page 18). Also noted is the point that this correlation between oxytocin response and brain activation may also "reflect an enduring trait difference associated with attachment security".
4. "Looking at Table 1 I am struck by the fact that differences between groups seemed to have a left-right assymetry. If there is anything to this "eyeballed" finding it might be worth a comment."

- Thank you for this interesting observation. Although we are uncertain about the significance of these post-hoc differences, the observation has now been noted in the results (page 15).

Reviewer #3:

1. "Introduction could be more detailed and rationale better developed - at present this is very methodological e.g. discussion of AAI would be more appropriate for methods- also the focus on oxytocin in humans needs greater explanation - exploring its effects on the encoding for happy faces (e.g., gustella et al., 2008)- this seems particularly relevant for both intro and discussion."
 - As suggested, we have restructured the Introduction to include a more detailed review of oxytocin's role in face processing and maternal behavior (pages 3-4). The discussion of the AAI has now been moved to the Methods section, as suggested.
2. "At present, the focus on oxytocinergic and dopaminergic systems appears selective, considering complex interactions between multiple systems and brain regions - this could be better addressed by providing more detailed background and rationale."
 - We now acknowledge that "there is likely to be a complex interaction of multiple neuroendocrine systems" involved in maternal behavior (page 3), but that the dopaminergic and oxytocinergic systems have been most consistently shown to be involved. Additional background and rationale has now been added to the introduction (pages 3-4).
3. "Page 5, how was the AAI modified - assuming cited reference relates to the original version."
 - The cited reference does in fact refer to the modified version of the AAI. However, we have now also added a citation to the original version of the AAI. "The modified version was chosen because of its theoretical links with patterns of information processing in the brain" (page 5).
4. "Although page 4 indicates that AAI was administered prior to birth to ensure that it was not influenced by infant temperament or behaviour, it is unclear why the AAI was not administered at each assessment to examine this issue specifically. What is the impact of state factors on this measure? What is the reliability and validity of this measure? Rationale here might be helpful. Furthermore, presumably fMRI responses would also be influenced by infant temperament/behaviour. Would it not be better to assess AAI and fMRI responses on the same assessment? Again- rationale needed."
 - These questions have been addressed on page 7, as follows: The AAI "categorizes the mother's capacity to form secure attachment relationships on the basis of a narrative of her own attachment experience... From both cross-sectional and prospective longitudinal studies, adult attachment has been shown to reliably predict maternal behavior patterns, the development of infant attachment (van IJzendoorn 1995), and infant social and emotional development (Sroufe et al. 2005). We chose to measure attachment during pregnancy using a longitudinal design to preclude the possibility that the infant's temperament or mother-infant interaction patterns might influence the way the mother discusses her own attachment experiences". The reason why the interview was not repeated on subsequent visits is because it relies on the interviewee

being “surprised” by the questions, such as requests to provide episodic memories in relation to generalizations that they had previously made (George and Main, 1996). Although fMRI responses may indeed be influenced by infant temperament, there were no significant differences between the two attachment groups, based on the Infant Temperament Questionnaire (page 12; Supplementary table 1).

5. “Why was screening for depression only conducted at visit 1? Depression levels may have changed dramatically between visit 1 and visit 3 (~10 months delay between two visits).”
 - As noted in Figure 1, Supplementary Table 1 and the Methods section (page 5), the Beck Depression Inventory (BDI) was measured at each of the 4 study visits. No differences in depression were seen between attachment groups in either pre- or post-natal assessments of depression, as shown in Supplementary Table 1. In addition, no differences in depression were seen between attachment groups at Visit 2, 3 or 4 individually. This has now been added to the Results section (page 12).
6. “What was the rationale for why videotaping and OT sampling took place 3 months prior to fMRI scanning - at present this seems a little problematic.”
 - See response to Reviewer #2, Point 3. This limitation is now noted on page 18, although having the oxytocin sampling and fMRI scanning separated by several months also suggests that oxytocin may be a marker of stable trait differences, whereas concurrent measurements would only establish state differences between the two groups (page 18).
7. “Re methods, it is unclear why a non-pregnant/mother control group was not used as an additional control group (this should be mentioned as a limitation and discussed).”
 - Only mothers could be used in this study, because the design was focused on brain responses on viewing one’s own infant’s face. The actual fMRI scanning visit took place after the pregnancy, when the infant was around 11 months old.
8. “Why was fixed effects analysis rather than a random effects analysis used? Fixed effects analysis does not allow for findings to be generalized beyond current sample. Random effects analyses should be conducted to examine whether Group (Type A, B) X category findings are able to be replicated. Why was random effects analysis used for within-group analyses, but only a fixed effects analysis for between-group ANOVA?”
 - See response to Reviewer #1 Point 4.
9. “My major concern really is about possible confounds of differences in other variables e.g. depression and parenting stress, at fMRI assessment. While page 11 indicates no differences between groups, this data was collected ~10 months prior to fMRI assessment. Can the authors confirm that no differences in these variables were present at fMRI assessment? If not, this is a serious concern for the present study, which seeks to examine differences between attachment groups rather than groups differing on mood. For instance, if there was a significant difference in mood between the two attachment groups at the time of fMRI scanning, an alternative explanation for finding reduced activation of brain reward pathways in mothers with insecure attachment could be associated anhedonia.”

- As noted in the response to Reviewer #3 Point 5 (above), no difference in depression between attachment groups was noted at the time of the fMRI scanning (page 12). We acknowledge the limitation that parenting stress was not measured at the time of fMRI scanning, although this visit was only 4 months (not 10 months) after the prior videotaping visit (see Figure 1). We would also predict that the other measures (infant and adult temperament, personality and SES) would be relatively stable between these 2 study visits, which were separated only by 4 months. As seen in Supplementary Table 1, breastfeeding status did not differ between groups at the time of the scanning visit.