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Supplementary Materials for

Epigenetic dynamics in infancy and the impact of maternal engagement

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Supplementary Results

Association between *OXTR* CpG sites -924 and -934
The primer set used in the current study amplifies and sequences another CpG site, termed -934, located ten nucleotide basepairs from site -924. *OXTR* DNA methylation (*OXTR*m) at this site is correlated with site -924 in infants and mothers at both visits (infants, 5 months: $r(98) = 0.681$, $p < 0.001$; infants, 18 months: $r(80) = 0.766$, $p < 0.001$; mothers, 5 months: $r(95) = 0.703$, $p < 0.001$; mothers, 18 months: $r(92) = 0.697$, $p < 0.001$).

Association between mothers and infants at *OXTR* CpG site -934
Maternal and infant *OXTR*m$_{-934}$ levels were positively correlated with each other to a similar extent at both visits (5 months: $r(94) = 0.239$, $p = 0.020$; 18 months: $r(79) = 0.414$, $p < 0.001$; *Fisher's r to Z* = -1.27, $p = 0.20$).

Association between 5-month and 18-month visits at *OXTR* CpG site -934
*OXTR*m$_{-934}$ levels are positively correlated between visits in both infants and mothers (infants: $r(80) = 0.775$, $p < 0.001$; mothers: $r(90) = 0.938$, $p < 0.001$), though maternal *OXTR*m$_{-934}$ correlated most strongly over time (*Fisher's r to Z* = -4.40, $p < 0.001$).

DNA methylation at *OXTR* CpG site -934 is not statistically dynamic from 5 to 18 months
Implementing the same latent multigroup path models reported for site -924 in the current manuscript, we find that infant *OXTR*m$_{-934}$ distributions are not statistically different from 5 to 18 months, neither by variance ($\chi^2(1) = 1.19$, $p = 0.28$) nor by mean ($\chi^2(1) = 0.26$, $p = 0.61$). Likewise, there is no statistical difference in *OXTR*m$_{-934}$ over time in the mothers, neither by variance ($\chi^2(1) = 0.17$, $p = 0.68$) nor by mean ($\chi^2(1) = 0.04$, $p = 0.84$).

Though infant *OXTR*m$_{-934}$ does not statistically change from 5 to 18 months as it does at CpG site -924, we repeated our Actor-Partner Interdependence Model to investigate whether maternal engagement might impact infant change in *OXTR*m$_{-934}$ in a similar way. We find that maternal engagement does not significantly impact infant *OXTR*m$_{-934}$ change, though findings reflect a similar pattern to the reported -924 data: $ß = -0.22$, $\chi^2(1) = 2.99$, $p = 0.08$.

Fig. S1.

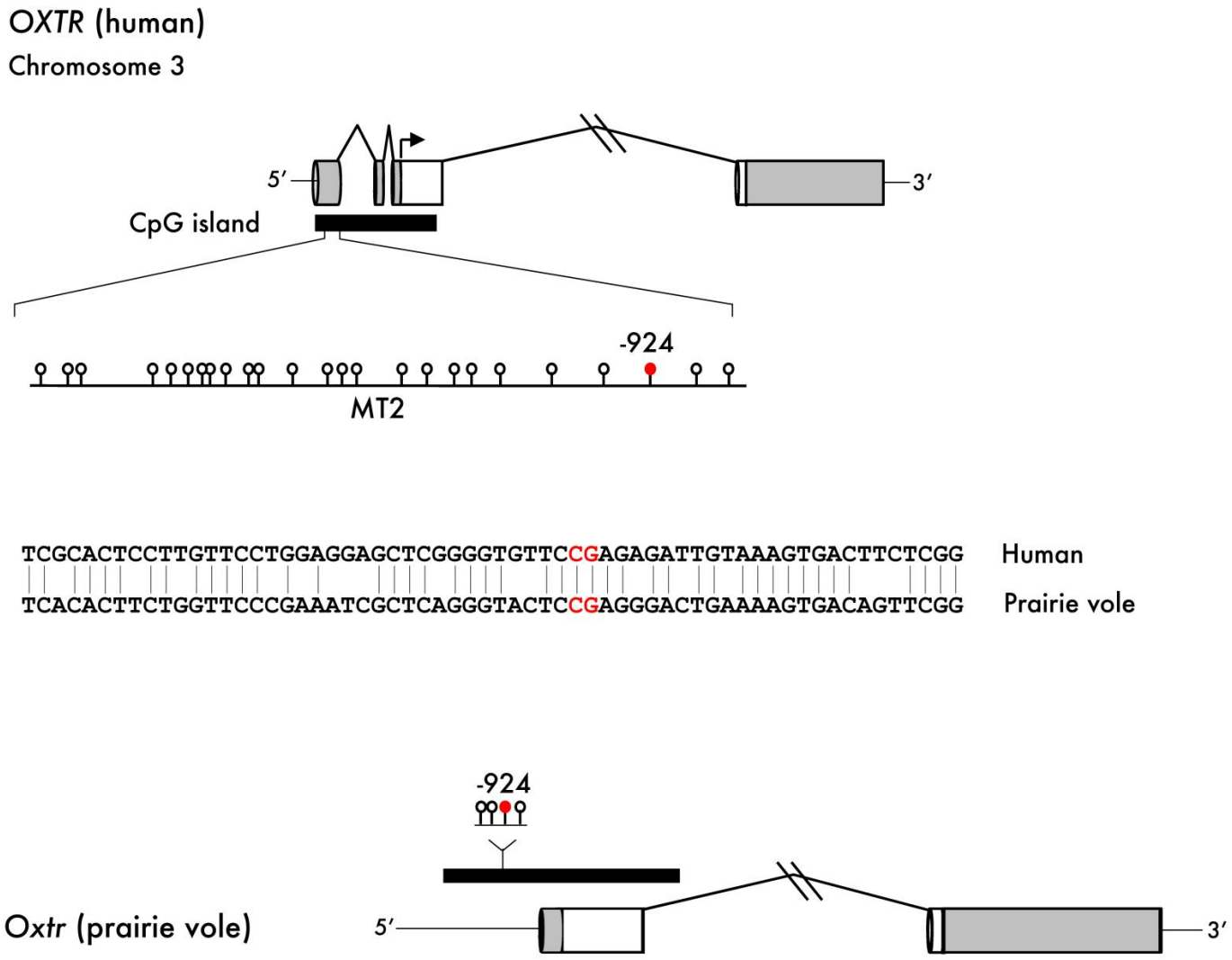


Fig. S1. Regulatory CpG site –924 in the promoter region of human *OXTR* is directly conserved in prairie vole *Oxtr*. Illustrated are human *OXTR* (above) and prairie vole *Oxtr* (below). Both contain promoter CpG islands that contain CpG site -924 (highlighted in red). That -924 is directly conserved suggests the phylogenetic importance of this site in regulating mammalian social behavior, and allows us to conduct and compare human–prairie vole translational work. Exons are displayed as boxes (coding, white; untranslated, gray) and introns are solid lines. CpG islands are illustrated with thick black bars. CpG dinucleotides are indicated with sticks and circles. Partial alignment of conserved human and prairie vole gene sequence is shown in the center, with conserved nucleotides linked with a vertical line (|).

Fig. S2.

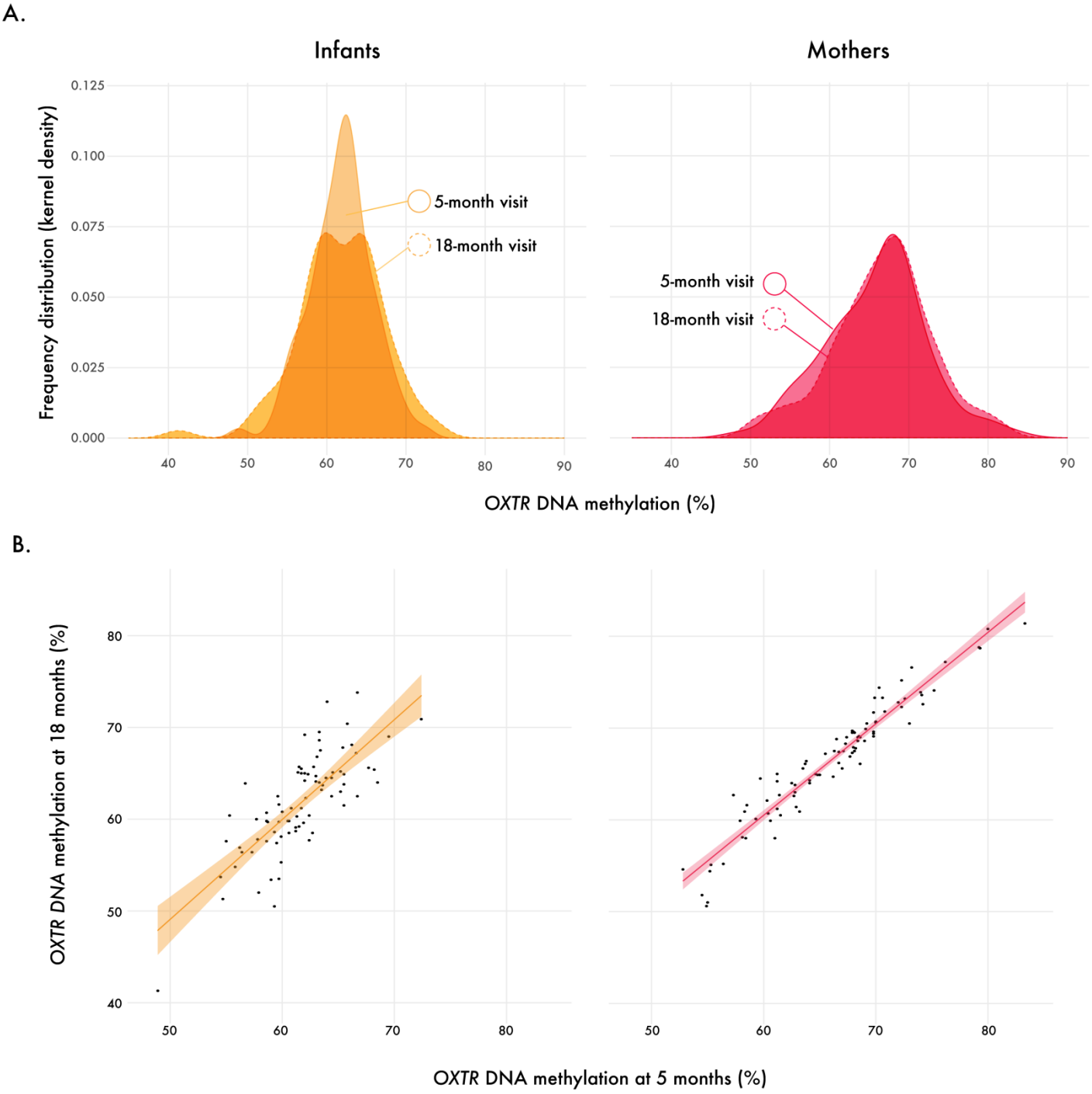


Fig. S2. Infant and maternal *OXTR*m from 5 to 18 months. A) Frequency distribution plots visualizing *OXTR* DNA methylation distributions at 5 months (solid lines) and 18 months (dashed lines) for infants (orange) and mothers (magenta), respectively. Note that only participants who provided saliva at both visits are included in this visualization. **B**) Scatterplots representing the positive association between *OXTR* DNA methylation at 5 months and 18 months in infants and mothers, respectively. Infants: $r(81) = 0.774$, $p < 0.001$; mothers: $r(90) = 0.960$, $p < 0.001$. Shaded bars represent 95% Confidence Intervals.

Fig. S3.

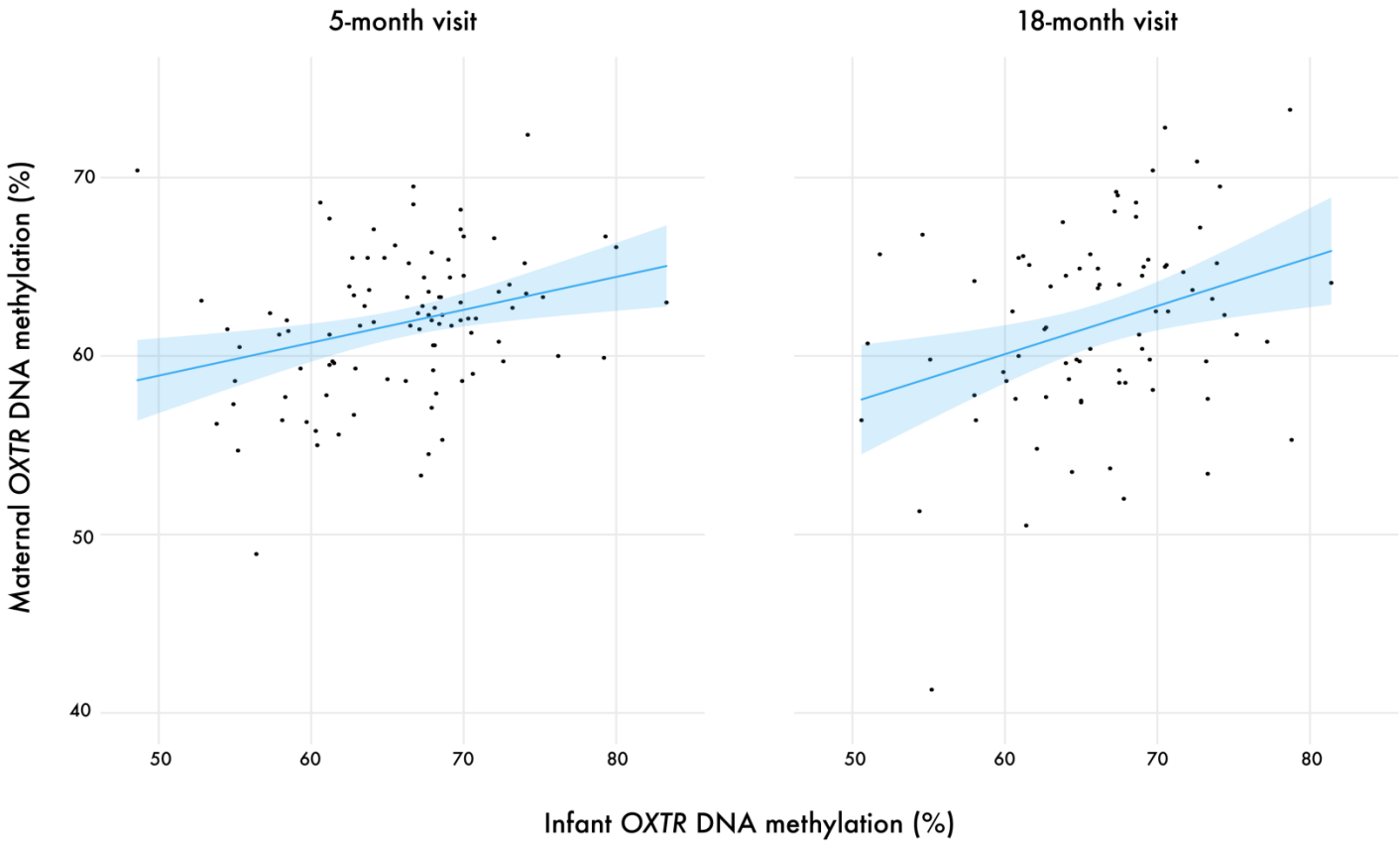
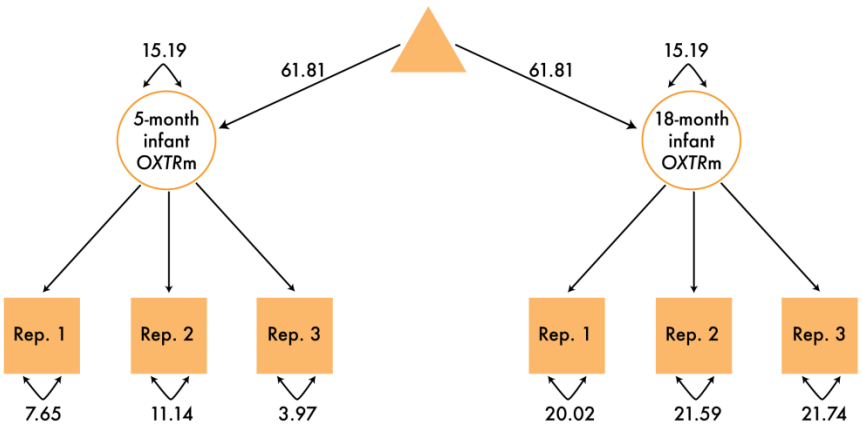


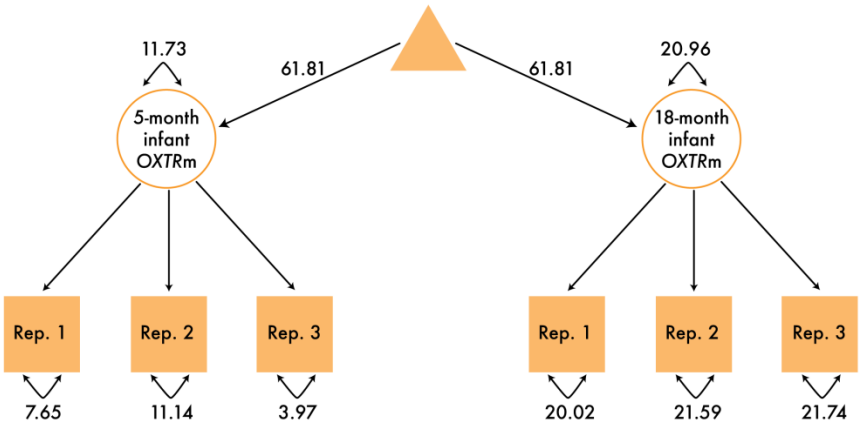
Fig. S3. Infant and maternal *OXTR*m is correlated at both visits. Maternal and infant *OXTR* DNA methylation levels were positively correlated with each other at both visits (5 months: $r(97) = 0.293$, $p = 0.004$; 18 months: $r(79) = 0.320$, $p = 0.004$). Shaded bars represent 95% Confidence Intervals.

Fig. S4.

A. Null model: Locked variance, locked means



B. Hypothesized model: Free variance, locked means, X^2 (1) = 4.04, p = 0.04, Cramer's V = 0.22



C. Hypothesized model: Locked variance, free means, X^2 (1) = 1.60, p = 0.99, Cramer's V = 0.001

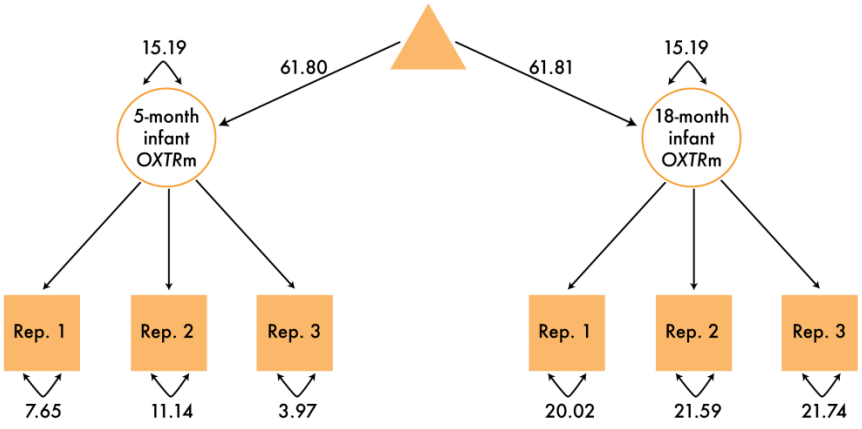
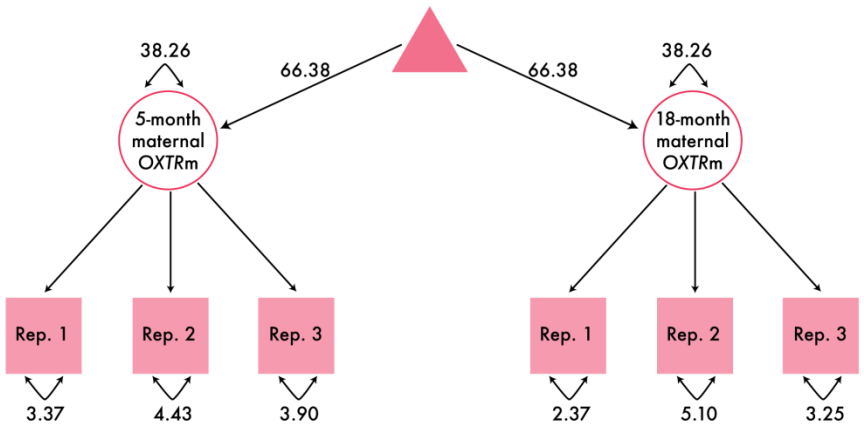


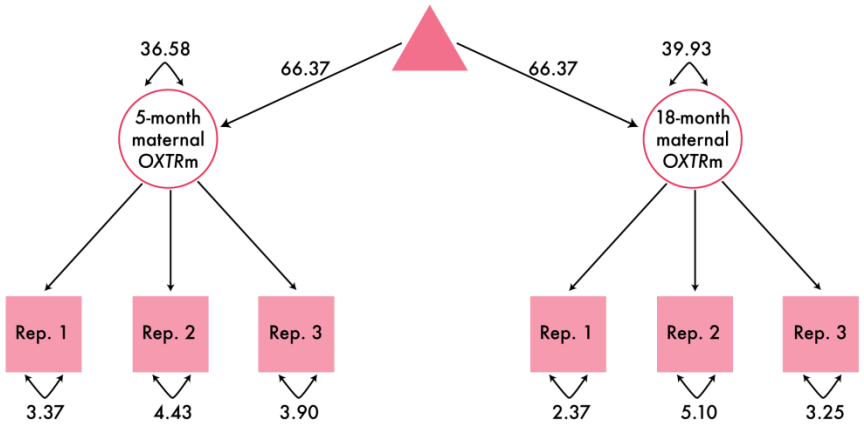
Fig. S4. The variance in infant *OXTR*m increases from 5 to 18 months. Latent multigroup path analysis was employed to compare methylation distributions from the 5 and 18 month visits. Distributions were estimated using the methylation values generated from three technical replicates from each individual. Circles represent the latent variables, and squares represent the observed variables (the technical methylation replicates, labeled Reps. 1, 2, and 3). The triangles represent constants, and paths from these triangles estimate the mean of the latent variables. All replicate paths were constrained. Double-headed, curved error paths pointing to each respective variable represent the residual variance within each variable. **A**) A null model was created in which both visit distributions were constrained in variance and mean, rendering the two distributions equal. This model was compared to models in which either **B**) means were constrained, or **C**) variances were constrained. Findings from this analysis demonstrate that Model B (constrained means) does not differ from the null model (A), suggesting that the two distributions do not differ by mean. In contrast, Model C significantly differs from the null (A), demonstrating that the data better fit a model in which the two distributions differ in variance, $\chi^2(1) = 4.04$, $p = 0.04$. *OXTR*m = *OXTR* DNA methylation.

Fig. S5.

A. Null model: Locked variance, locked means



B. Hypothesized model: Free variance, locked means, X^2 (1) = 0.16, p = 0.69, Cramer's V = 0.04



C. Hypothesized model: Locked variance, free means, X^2 (1) = 0.25, p = 0.61, Cramer's V = 0.05

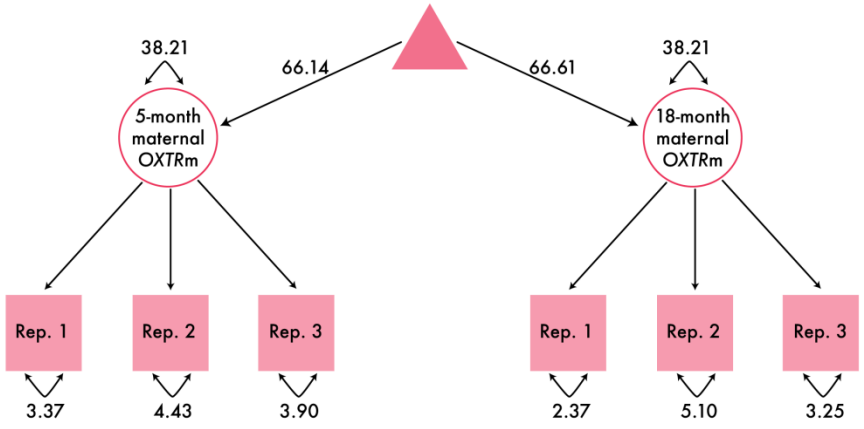
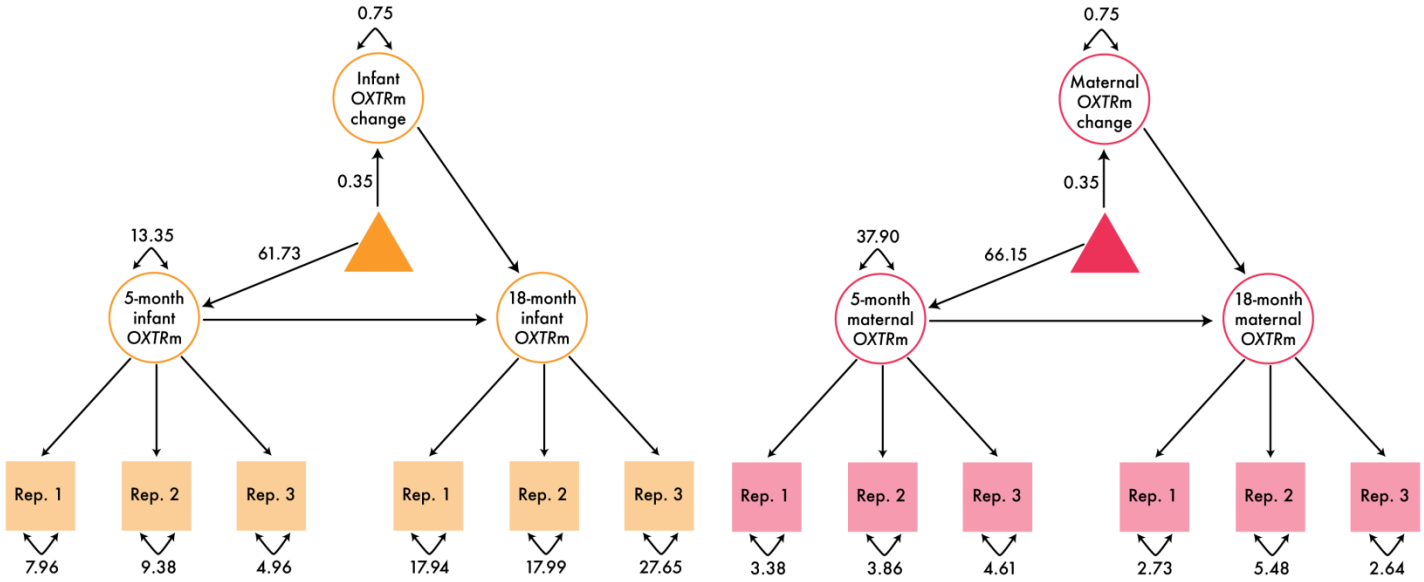


Fig. S5. Maternal *OXTR*m does not change from 5 to 18 months. Latent multigroup path analysis was employed to compare methylation distributions from the 5 and 18 month visits. Distributions were estimated using the methylation values generated from three technical replicates from each individual. Circles represent the latent variables, and squares represent the observed variables (the technical methylation replicates, labeled Reps. 1, 2, and 3). The triangles represent constants, and paths from these triangles estimate the mean of the latent variables. All replicate paths were constrained. Double-headed, curved error paths pointing to each respective variable represent the residual variance within each variable. **A)** A null model was created in which both visit distributions were constrained in variance and mean, rendering the two distributions equal. This model was compared to models in which either **B)** means were constrained, or **C)** variances were constrained. Findings from this analysis demonstrate that neither model (constrained means, constrained variance) differed from the null model (A), suggesting that the two distributions do not differ by mean or variance. *OXTR*m = *OXTR* DNA methylation.

Fig. S6.

A. Null model: Locked variance, locked means



B. Hypothesized model: Free variance, locked means, X^2 (1) = 2.90, p = 0.08, Cramer's V = 0.13

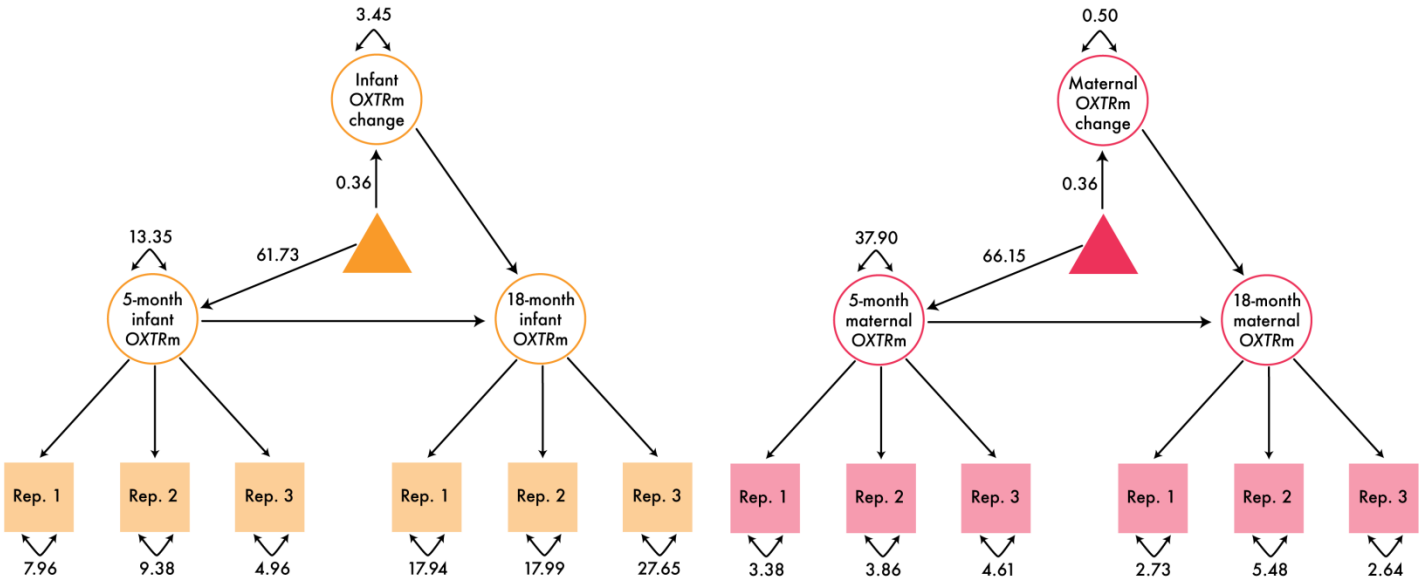


Fig. S6. Exploratory latent difference score model. Though underpowered, we created latent difference score models to test whether infant *OXTR* DNA methylation change differed in variance from maternal *OXTR* DNA methylation change. Similar to figs. S4 and S5, distributions for the 5- and 18-month visits were estimated using the methylation values generated from three technical replicates from each individual (labeled Reps. 1, 2, and 3). We then created latent difference scores for infants (orange) and mothers (magenta), respectively, by subtracting the 5-month latent variable from the 18-month latent variable. Circles represent the latent variables, and squares represent the observed variables. The triangles represent constants, and paths from these triangles estimate the mean of the latent variables. All other paths were constrained. Double-headed, curved error paths pointing to each respective variable represent the residual variance within each variable. **A)** A null model was created by constraining the means and variances of both infant and maternal latent difference scores. **B)** We compared the null model to a hypothesized model in which means remained free, but variance was constrained. There was a trend for unequal variance between mothers and infants with a small to medium effect, χ^2 (1) = 2.90, p = 0.088, Cramer's V = 0.13.

Table S1.

Table S1. Infant and maternal *OXTR* methylation at 5- and 18-month visits. A total of 101 dyads participated in this study. Sample sizes (Ns) listed indicate how many infants and mothers were successfully epigenotyped. d = days; y = years.

	Infants			Mothers		
Visit	5 months	18 months	Change	5 months	18 months	Change
Mean Methylation (SD) (%)	61.81 (3.97)	61.71 (5.38)	0.11 (3.44)	65.86 (6.35)	66.63 (6.33)	.48 (1.79)
Range (%) (Min – Max)	23.50 (48.90 – 72.40)	32.50 (41.30 – 73.80)	17.60 (-8.80 – 8.80)	34.70 (48.60 – 83.30)	30.80 (50.60 – 81.40)	9.70 (-4.30 – 5.40)
Mean Age (SD)	147.62 d, (14.57)	556.80 d, (13.45)		31.27 y, (4.53)	32.08 y, (4.35)	
N	100 (50 F, 50 M)	82 (43 F, 39 M)	81 (42 F, 39 M)	98	93	90

Table S2.

Table S2. Table displaying Pearson's *r* coefficients for each *OXTR* methylation measurement and demographic variable. All correlations were non-significant (*p*-values > 0.05). *OXTR*m = oxytocin receptor gene DNA methylation; EBF = exclusive breastfeeding duration; PBF = percentage of breastfed meals; mo = months.

	Infant *OXTR*m		Maternal *OXTR*m	
	5 months	18 months	5 months	18 months
Infant age (5 mo)	.078	.003	.144	.147
Infant age (18 mo)	-.185	-.148	-.161	-.103
Maternal age (5 mo)	-.182	-.092	-.033	.049
Maternal age (18 mo)	-.139	-.060	-.040	.029
Maternal parity	-.109	-.112	.003	.045
Maternal education	-.156	-.192	.075	.073
PBF (5 mo)	-.115	-.021	-.016	-.063
EBF (5 mo)	.045	.003	.057	.093
EBF (18 mo)	-.125	-.079	-.072	-.028

Table S3.

Table S3. Mother-reported questionnaire descriptives and demographics. EPDS = Edinburgh Postnatal Depression Scale; EBF = exclusive breastfeeding duration; PBF = percentage of breastfed meals; y = years; mo = months.

	Mean (SD)
Postnatal Depressive Symptoms (EPDS)	5.32 (3.58)
Parenting Sense of Competence	70.24 (7.80)
Social Support: Quantity	3.86 (1.46)
Social Support: Quality	5.29 (0.65)
Parity	0.49 (0.66)
Education (y)	16.61 (3.70)
PBF (5 mo) (%)	79.11 (34.33)
EBF (5 mo) (days)	128.70 (41.73)
EBF (18 mo) (days)	159.88 (55.35)

Table S4.

Table S4. Descriptives for each subscale of the free-play analysis coding scheme.

	Mean (SD)
Maternal talkativeness	3.36 (1.02)
Maternal proximity	3.66 (0.96)
Maternal attention/engagement	4.04 (1.01)
Maternal positive mood	3.68 (0.73)
Infant attention/engagement	3.75 (1.03)
Infant positive mood	3.36 (1.00)
Duration of infant laughter (s)	0.90 (2.41)
Duration of infant smiling (s)	9.75 (14.08)
Duration of active touch (s)	38.23 (39.26)
Duration of passive touch (s)	5.85 (9.76)