

Biological Correlates of Parental Engagement on Socioemotional Development in
Infancy

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Abstract

Introduction: Infants rely on social cues from their parents to help guide interactions with their external contextual world. One of the biological correlates of the parent-infant relationship is the oxytocin system, which has been shown to play a role in bonding and attachment. Oxytocin may aid in orienting our attention to external social cues, but that it is dependent on individual differences. One such difference may be the oxytocin receptor gene DNA methylation (*OXTRm*), which aids in regulating endogenous oxytocin use. We hypothesize that parental engagement will help regulate *OXTRm*, predict neurological processing of social cues, and influence infants' social-orienting behavior.

Methods: Sixty-four four-month-old infants and their parents underwent a video-recorded free play interaction, which was behaviorally coded for levels of parent engagement and infants' social-orienting cues. Infants then provided a small saliva sample, which was assayed for *OXTRm* at site -924; a previously identified site within the oxytocin receptor gene (*OXTR*) shown to undergo epigenetic modification in response to parental care. Lastly, infants underwent electroencephalography (EEG) while they listened to sounds of running water and infant-directed speech. Event-related potentials were extracted to assess neurological processing of social auditory information.

Results: We found a positive association between parental engagement and *OXTRm* at site -924. Additionally, we found a strong positive relationship between parental

engagement in the form of vocalizations and mood, and infants' social-orienting behavior. However, we did not find any evidence of neurological correlates of variability in *OXTRm*, parental engagement, or child behavior.

Discussion: This study further quantifies social behavioral correlates of epigenetic modulation of the oxytocinergic system. While past literature has focused broadly on parental engagement, this study narrows the focus to the effects of parental vocalizations and mood as a potential contributor of *OXTRm* regulation. Additionally, this study provides further evidence of the importance of parent-infant dyadic relationships on infant socioemotional development beginning as early as 4 months of age.

Keywords: Infant development, *OXTRm*, oxytocin, ERP, EEG, Sociality

Introduction

The first, and potentially most influential, social relationship individuals have is that with their parent or guardian. Maternal engagement early in infancy contributes to the child's soothability, low-intensity pleasure, and inhibitory control and reduces the infant's negative affect, signaling that dyadic interactions may regulate infants' socioemotional development (Kotila et al., 2014). Positive socioemotional development in infancy has been correlated with better regulatory control and academic competence in early childhood (Valiente et al., 2008), signifying a longitudinal trajectory of the importance of early life relationships.

However, the mechanisms by which parental interactions with their infants facilitate socioemotional development throughout childhood remain relatively unknown. One theory is that infants possess an innate attention orienting response to social cues, which directs their focus to social interactions and behaviors. Once the event is interpreted as social, the infant uses a myriad of behaviors to capture and direct their caregiver's attention to the event, and the caregiver's response is then observed. The infant then adjusts their representation of the event such that the caregiver's reaction is now encoded as the proper social response to the situation (Goldberg, 1977). This speaks to the importance of both the caregiver's interaction with the child and the use of innate social-attention orienting responses seen early in development.

Recent literature has focused on the role of oxytocin in social-attention orienting networks. Oxytocin is a neuromodulatory hormone that plays a role in social behaviors

like pair-bonding and child rearing (Galbally et al., 2011). Theorists have proposed in The Social Salience Hypothesis of Oxytocin which posits that oxytocin regulates the salience of social information via its interaction with the dopaminergic system by modulating attention orienting behavior to external social cues, but that it is contingent on baseline individual differences (Shamay-Tsoory & Abu-Akel, 2016).

One potential individual difference is in the genetic expression of the oxytocin receptor gene (*OXTR*). *OXTR* methylation (*OXTRm*) is an epigenetic mechanism that consists of adding a methyl group to the 5th carbon of a cytosine directly adjacent to a guanine on the DNA strand. The addition of this methyl group to *OXTR* site -924 on the DNA strand leads to decreases in gene expression and less endogenous oxytocin use (Gregory et al., 2009). Interestingly, methylation at this site is highly variable among the developing population, with studies showing differences of 20% methylation at this site among five month old infants (Krol, Puglia, et al., 2019). Thus, this may be one of the individual differences contributing to population-level variability in social processing and attention-orienting.

Studies have found negative correlations between parental engagement (specifically in the forms of proximity, parental affect, and attentiveness to the infant) and *OXTR* methylation (*OXTRm*) at site -924 in both human and animal models (Krol, Moulder, et al., 2019; Perkeybile et al., 2019). This further that implicates that parental behavior may be contributing to infants' socioemotional development, but that it may be doing so through the oxytocin system.

While parental behavior may be modulating *OXTR*m, the downstream effects of gene expression may also be influencing infant behavior. *OXTR*m predicts infants' behavioral responses (i.e., orienting, attending, to and processing) to social auditory cues (Puglia et al., 2020) and has been correlated with low sociability and fewer prosocial behaviors (Insel et al., 1993, 1997; Insel & Shapiro, 1992). Additionally, research has found that *OXTR*m levels predict infants' neurological processing of social auditory stimuli (Puglia et al., 2020), further implicating its role in physiologically modifying social-attention orienting.

Social-attentional networks have been studied in both adults and infants as they observe socially relevant information (Bristow et al., 2007; Walbrin et al., 2018). Infants as young as six months of age show increased attention to socially engaging interactions compared to parallel play (Chawarska et al., 2013), while additional research has found that auditory stimuli evoke specific event-related potentials (ERPs), a measure of changes in electrical neurological activity, associated with social-attentional information processing. In infants, the N100 ERP responses has been correlated with word onset priming and emotional processing of vocalizations (Becker et al., 2014; Crespo-Llado et al., 2018), while the P350 ERP response has been found to be correlated with processing of socially relevant auditory information (Purhonen et al., 2005). These neural correlates of social-attention will allow for the exploration of the effects of the regulation of the oxytocin system on social-attention processing.

Given the role of parental engagement in epigenetic regulation and the population-level variation in methylation values in *OXTR* site -924 during infancy, this study aims to replicate the finding that parental engagement effects methylation levels at site -924 and to assess whether this epigenetic variability is associated with the way individuals' brain differentially attends to social information and may regulate infants' behavioral responses to overt social cues.

We hypothesize that as early as four months of age, parental engagement will be associated with the level of methylation at *OXTR* site -924. Additionally, we expect the level of methylation to be related to the child's external social behavior, specifically in the domain of attention-orienting. Lastly, we expect neurological attentional response

networks to be activated during interpretations of social cues compared to non-social cues and to be associated with the levels of methylation at site -924.

Methods

General Overview

The current study assesses the association between parental behavior and infants’ epigene-brain-behavior paradigm. We assessed parental and infant engagement via a 5-minute free play interaction between the dyad and assessed the correlation between parent behavior and *OXTR*m in the infant. The regulation of the *OXTR* gene was then used to predict infant neurological processing of social information via event related potentials (ERPs) using an electroencephalogram (EEG). We analyzed data as a secondary analysis of study 2 from Puglia et al., 2020.

	Response, % (N=64)
Sex of Infant	
Female	48.4
Male	51.6
Race of Infant	
Caucasian	79.7
Black	4.7
Asian/Pacific Islander	1.6
Other	14.1
Ethnicity of Infant	
Not Hispanic/Latino	96.9
Hispanic/Latino	3.1
Number of adults in the home	
1	1.6
2	82.8
3	9.4
4	3.1
5	3.1
Number of children in the home other than the participant	
0	6.3
1	17.2
2	48.4
3	20.3
4	7.8
Household Income	
<\$20,000	4.7
\$20,000-\$44,999	10.9
\$45,000-\$139,999	59.4
\$140,000-\$149,999	3.1
\$150,000-\$199,999	7.8
\$200,000+	10.9
Not provided	3.1

Table 1. Demographics of parent-child dyads who participated in the study.

Participants

Sixty-four parent-infant dyads participated in the study when the infant was four months of age ($M = 131.78$ days; $SD = 11.60$ days). Parents averaged 32 years old ($M = 32.11$ years; $SD = 4.52$ years). Infants were born full-term (36 or more weeks gestation), did not have any uncorrectable auditory or visual impairment, and were raised in a home where English was the primary language spoken. Demographics of the participants are provided in Table 1. Written informed consent was obtained from the parent prior to participation and families were given a \$50 visa gift card for participating. Study procedures were approved by the University of Virginia Health and Human Sciences Institutional Review Board.

DNA collection and isolation

Infants had their mouths gently swabbed with a sponge from the OG-250 collection kit (DNA Genotek, Ottawa, Canada) to collect passive drool. The samples were stored at room temperature until DNA isolation occurred. The samples were incubated at 50 °C for one hour, prior to centrifuging at 200 rcf for 10 minutes to remove the saliva from the sponges. Researchers isolated the DNA from 500 μ L of saliva using the DNA Genotek provided purification protocol. DNA was reconstituted in Hydration Solution (10 mM Tris, 1 mM EDTA, pH 7-8, Qiagen Valencia, CA), quantified using a nanodrop, then frozen at -20°C.

Epigenetic analysis

Bisulfite conversion, polymerase chain reaction (PCR), and pyrosequencing were used to determine *OXTR*m. The above processes were performed in triplicate to ensure reliability of the measurements. The average variation of the triplicates from the mean was $\pm 1.86\%$. The quantification methods are identical to those used in Puglia et al., 2018. Infant *OXTR*m levels at site -924 averaged 63.65% (SD = 3.90).

Behavior

During the visit, the parent and infant underwent a five-minute video-recorded naturalistic free-play interaction. Parents and their infant were led into a room that consisted of a circular blue carpeted rug, a book, two rattles, and a plush toy arranged equidistant around the carpet. Parents were instructed to play with their child as they would at home and that the researcher will retrieve them after five minutes. There were two cameras mounted in two diagonal corners of the room to record both the behavior and facial expressions of the parent and child. Parents were made aware of the location of the cameras. No further instructions were provided to keep the interaction naturalistic. After five minutes, the recordings were stopped and the dyads were removed from the room.

The dyadic interactions were then behaviorally coded using Mangold Interact software (*Mangold Interact*, 2020), which time locks the two recorded videos. All of the videos were coded by trained research assistants using an in-house coding scheme

(Grossmann et al., 2018). A secondary trained research assistant then randomly coded

25% (n=17) of the 64 behavioral videos. Krippendorff's alpha was used to assess the reliability of the two coders. The coding scheme consists of durational codes and one-to-five likert scales to assess infant and parent affect, touch, vocalizations, attention, and proximity. The first minute of the interaction was discarded to account for any behaviors that may be related to the transition into the room or discomfort from being recorded. The remaining four minutes were used to quantify dyadic behavior. Parental behaviors included how talkative the mother was (likert scale where one indicated very little vocalizations and five indicated non-stop speech, $M = 3.46$, $SD = 1.09$), what was the general mood of the parent (likert scale where one indicated a negative mood and five indicated a joyful, positive mood, $M = 4.21$, $SD = 0.77$), how much did they modulate their speech (likert scale where one indicated they utilized adult-directed speech and five indicated they used infant-directed speech, $M = 3.90$, $SD = 1.04$), the proximity of the infant to their parent (likert scale where one indicated maximum distance from parent and five indicated that the infant and parent were touching for the majority of the time, $M = 3.95$, $SD = 0.91$), total duration of operational touch (defined as when the parent was adjusting or relocating the child, $M = 65.09$ seconds, $SD = 60.43$ seconds), total duration of loving touch (defined as stroking, cuddling, or kissing the child, $M = 28.27$ seconds, $SD = 32.20$ seconds), total duration of positive affect (defined as smiling, laughing, or a positive deviation from the parent's neutral expression, $M = 12.36$ seconds, $SD = 18.60$ seconds), total duration of negative affect (defined as crying,

frowning, disciplining, or negative demeanor, $M = 0.19$ seconds, $SD = 0.73$ seconds), total duration of parental pointing (defined as a hand gesture used to direct attention, $M = 7.29$ seconds, $SD = 10.87$ seconds), total duration of their vocalizations (defined as time the parent spent talking or emitting noise from their mouth towards the baby, $M = 90.05$ seconds, $SD = 51.28$ seconds), and the number of times the parent switched activities (defined as when the parent changed the goal of the activity, $M = 5.30$, $SD = 6$). Infant behaviors included the general attentiveness they had to their parent (likert scale where one indicated they ignored their caregiver and five indicated they paid sole attention to the caregiver, $M = 2.97$, $SD = 1.12$), and their overall mood (likert scale where one indicated a negative mood and five indicated a joyful, positive mood, $M = 3.14$, $SD = 0.72$), the total duration of eye contact with their parent (defined as mutual attention held to the eye region from both parent and infant, $M = 30.42$ seconds, $SD = 38.67$ seconds), total duration of positive affect (defined as smiling, laughing, or a positive deviation from the infant's neutral expression, $M = 5.72$ seconds, $SD = 10.71$ seconds), total duration of negative affect (defined as crying, frowning, or negative demeanor, $M = 2.90$ seconds, $SD = 11.86$ seconds), total duration of vocalizations (defined as time the infant was cooing, burbling, or otherwise making noise from their mouth, $M = 9.91$ seconds, $SD = 11.86$ seconds), total duration of the child pointing (defined as a hand gesture meant to guide attention, $M = 4.06$ seconds, $SD = 10.90$ seconds), and the number of times the child switched activities (defined as when the child changed the goal of the activity, $M = 0.30$, $SD = 1.12$).

EEG data and preprocessing

Infants were placed on their caregivers' laps about 100 cm from the presentation monitor throughout the EEG. Infants were presented with a series of auditory and visual stimuli that were social and non-social in nature, resulting in a 2x2

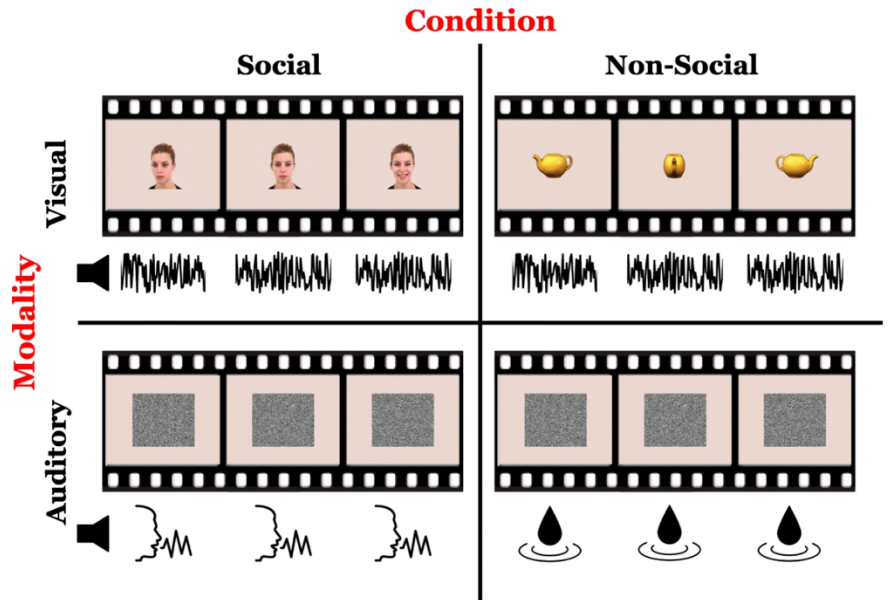


Figure 1. From: [Epigenetic tuning of brain signal entropy in emergent human social behavior](#); example of EEG paradigm displaying the 2x2 block design with context (social vs. non-social) and modality (auditory or visual). Only auditory social and non-social stimuli were analyzed in this study.

factor (auditory or visual) by modality (social or non-social) design (figure 1). For the current project, only social and non-social auditory stimuli was analyzed.

Non-social auditory stimuli included sounds of running water, which were downloaded from <http://www.findsounds.com>. Social auditory stimuli included infant-directed speech (high-pitched tonal everyday words) that had been pre-recorded from seven English-speaking mothers (Brent & Siskind, 2001). The recordings were obtained from the Child Language Data Exchange System (*The CHILDES Project (3rd Ed.)*, n.d.). Short phrases and single-word remarks were isolated from the recordings. Auditory social and non-social clips were matched on mean fundamental frequency ($M_{\text{social}} =$

311.16 Hz, $M_{\text{non-social}} = 335.29$ Hz, $t = -1.29$, $p = 0.200$), standard deviation of fundamental frequency ($M_{\text{social}} = 66.85$ Hz, $M_{\text{non-social}} = 57.82$ Hz, $t = -1.35$, $p = 0.179$), and duration ($M_{\text{social}} = 0.87$ seconds, $M_{\text{non-social}} = 0.97$ seconds, $t = -1.35$, $p = 0.179$) via Praat v6.0.36 (*Praat: Doing Phonetics by Computer*, n.d.) and internal MATLAB scripts. In total, there were 60 distinct social and 60 distinct non-social auditory sound bites. Recordings were grouped into social and non-social conditions into six 10-clip, 18 second trials, in which no word or water sound bit was repeated in any given trial. The inter-stimulus interval was randomly assigned and ranged between 500 and 1000 milliseconds between clips. The order of the condition and the clips within the condition was randomized across subjects. Static TV noise created by MATLAB was presented during the auditory stimuli to create a controlled visual stimulus, allowing for the auditory and visual conditions to be comparable.

Auditory and visual stimuli were projected to the infant using PsychToolBox v3.0.14 in MATLAB (Brainard, 1997). During the stimuli presentation and EEG, parents were instructed not to interact or speak with their child. Trials within blocks were pseudo-randomized to ensure alternation between visual and auditory domains. In between blocks, a pulsating colorful shape combined with an attention-grabbing noise was presented to reorient the infant's attention back to the screen. Once they were focused on the stimuli, a researcher would begin the next block. The experimenter was able to observe the parent and infant through a camera in the control area and could pause between trials to reorient the child's attention or adjust if the infant was

distressed. The task was ended either after 24 blocks or if the infant became too distracted or irritable. Infants completed an average of 7.03 (SD = 3.00) blocks of the experiment.

During the stimuli presentation, the infant was hooked up to an EEG to measure brainwave activity. The EEG cap consisted of 32 Ag/AgCl active actiCAP slim electrodes (Brain Products GmbH, Germany) positioned and affixed in the 10-20 electrode placement system. The vertical electrooculogram (EOG) was recorded from Fp1 and Fp2, which lie on the supraorbital ridge of the eyes. The horizontal EOG was taken from F7 and F8, which sit at the outer canthi of the eyes. The infant's head circumference was measured to determine the correct cap size. The infant was then capped and gelled. Impedances were inspected using the actiCAP Control Box before beginning EEG recording. Impedances were less than 60 kOhms for all electrodes. The EEG was amplified using a BrainAmp DC Amplifier and recorded with BrainVision Recorder software. Data was sampled at a rate of 5000 Hz, band-pass filtered 0.1-1000 Hz, and referenced to FCz during collection. Post collection, EEG was downsampled to 500 Hz for further analysis.

EEG preprocessing was done using EEGLab, v.2020.0. EEG data was bandpass filtered between 0.3 and 20 Hz to remove muscle tension artifacts and slow drift, before being re-referenced to the average of all electrodes. Data was then divided into stimulus-specific epochs between 0 milliseconds and 500 milliseconds after the stimulus onset with an averaged pre-stimulus baseline correction window occurring 100

milliseconds before presentation for each of the averaged trials. A 200-millisecond moving peak-to-peak window was used to identify and

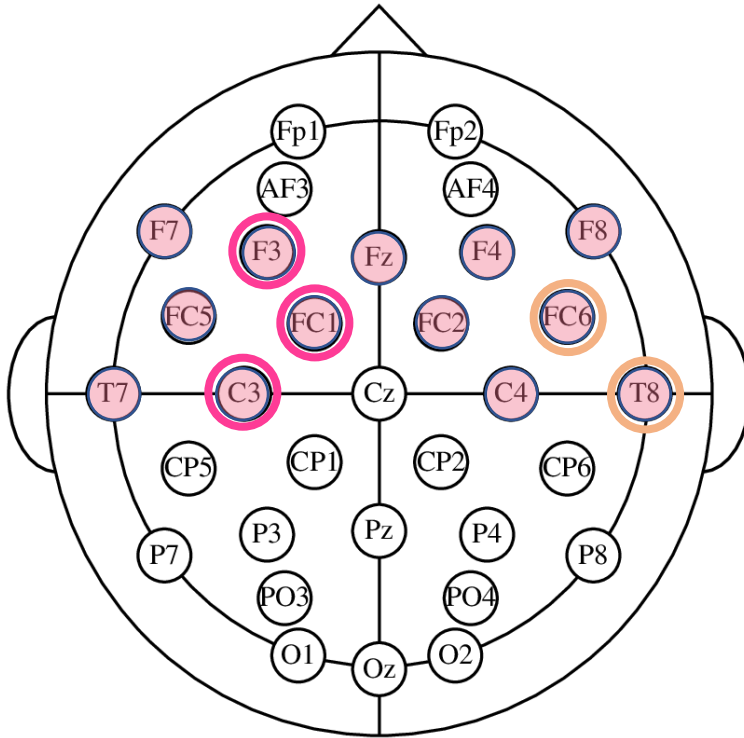


Figure 2. 10-20 electrode sites analyzed are highlighted in light pink. For the final P350 model (which shows the most differentiation between social and non-social ERP responses), the electrode sites circled in magenta (F3, FC1, and C3) loaded most strongly onto the left hemisphere latent variable, while those circled in orange (FC6 and T8) loaded most strongly onto the right hemisphere latent variable.

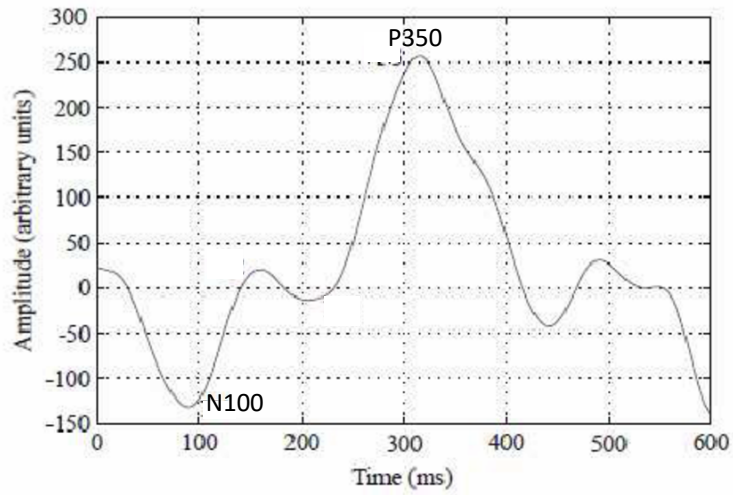


Figure 3. Figure adapted from Al-Ani & Trad, 2010. Figure displays the two ERP components that were analyzed for differences between social and non-social neural responses before being fed into the partial least squares model.

remove any remaining artifacts that exceeded 200 mV. Data was then visually inspected for noisy data that may have been missed by the artifact rejection algorithm. Epochs were removed if a channel was representative of electrical noise, movement, or muscle tension using channel data scroll artifact rejection in ERPLab. All participants had at least ten trials from all conditions by modalities. There were no differences in the number of epochs visually-removed between the social (mean= 4.08, sd= 5.11) and non-social (mean= 5.02, sd= 5.83) condition ($t(60)= -1.92, p= 0.06$).

Univariate analyses for mean peak amplitude (MPA) event-related potentials were calculated for both the social and non-social auditory response using ERPLab in Matlab. Past research suggests that most auditory processing occurs fronto-centrally and temporally (Becker et al., 2014; Daliri & Max, 2016). Therefore, mean peak

amplitude at sites F7, F8, F3, F4, Fz, FC5, FC1, FC2, FC6, C3, C4, T7, and T8 in the 10-20 system were analyzed at N100 (100-300 milliseconds after stimulus

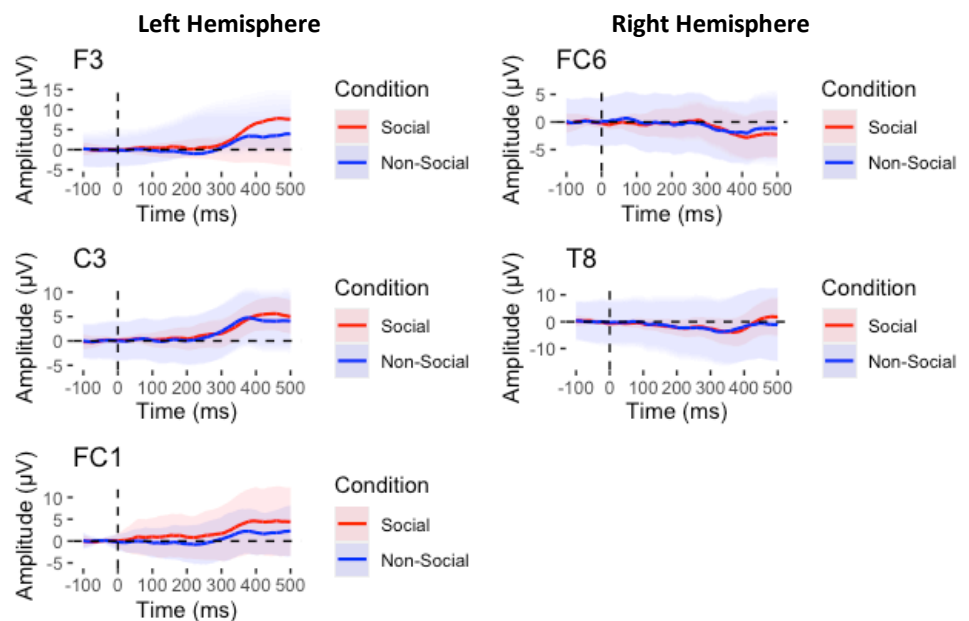


Figure 4. Visual representation of social (red line) vs. non-social (blue line) ERP response for the site comprising the left and right hemispheres, respectively, in the final P350 model. We begin to see non-significant differentiation of the social and non-social ERP response around 350 ms.

and P350 (350-500 milliseconds after stimulus onset) (figure 2), as these components have been identified as being unique to infant auditory processing (Kushnerenko et al., 2002) (Figure 3). ERP peaks (Figure 4) were visually inspected for each condition to ensure the windows were encompassing the intended peaks for a majority of the participants. ERPs for both the social and non-social stimuli were quantified and then the non-social response was subtracted from the social response. The difference score allows for the identification of the true social response by negating other background neural processes ($N100_{\text{social-nonsocial response}}$: $M = -1.11$, $SD = 10.81$; $P350_{\text{social-nonsocial response}}$: $M = -0.59$, $SD = 12.39$). These difference scores were used as manifest variables for the neurological response to social information in the final partial least-squares model.

Statistical Considerations

This study aims to assess the experience-epigene-brain-behavior relationship among parents and their infants. Thus a multivariate, predication-based method was implemented using partial least squares path modeling (PLS-PM) using Smart PLS (Ringle et al., n.d.). PLS-PM allows for the evaluation of latent and manifest variables and is optimal for investigative, hypothesis-driven research where a theory is not yet established and for studies with smaller sample sizes (*Handbook of Partial Least Squares*, n.d.; Ravand & Baghaei, 2019).

A model was created to assess associations between four latent variables- parental behavior, *OXTR*m, ERPs, and infant behavior. Manifest variables for the

parental behavior consisted of parental affect, total duration of vocalizations, how talkative the parent was, and how often they modulate their speech to be infant directed. The *OXTRm* latent variable consisted of each of the triplicate values for site - 924. Infant behavior was comprised of the total duration of eye contact, infant overall mood, and the infant's attentiveness towards the parent. Auditory ERPs were computed as the difference in mean peak amplitude between social and non-social conditions and were broken into left and right hemispheres after observing a lateralization effect within the model. Electrode sites for each component were decided based off their loading values onto the model. The N100 responses for the left hemisphere consisted of F8 and FC6, while the right hemisphere consisted of F7 and FC5. The P350 left hemisphere ERP response consisted of F4 and T8, while the right hemisphere ERP consisted of F3, F7, and FC5.

Each ERP component was modeled independently, such that a model was created for the N100 and P350 ERP response. In each of the two models, directional associations were assessed from parental behavior to *OXTRm*, left and right hemisphere neurological responses (e.g., either N100 or P350), and infant social behavior. Additionally, associations were assessed from *OXTRm* to left and right ERP response and infant behavior. Finally, the last two pathways assessed associations from left and right neural activation to infant behavior.

The convergent and discriminant validity of the model were considered in the following ways. Unidimensionality (i.e., how well the manifest variables represent their

latent variable) of the model were assessed using Cronbach’s alpha. All outer-model loadings were ensured to be >0.70 and the cross loadings of the model were assessed to ensure the indicators properly represented the correct latent variable. Reflective measures (which typically indicate causality from latent variables to its manifest variables (Hanafiah, 2020)) were recorded to investigate variance in the endogenous latent variable explained by the independent variable, rms theta was assessed for goodness of fit (>0.12) and R² (low variance is an R²<0.20, moderate is 0.20<R²<0.50, and high is R²>0.5), path coefficients, and F² were assessed to interpret magnitude of results (Benitez et al., 2020).

Results

Code	Krippendorf’s α
<i>Parent</i>	
Durational Codes	
Duration of vocalizations	0.48

Likert Scales		We hypothesized that at
Parental talkativeness	0.35	
Parental modulation of speech	0.53	four months of age, infants'
Parental Mood	0.38	
<i>Infant</i>		
Durational Codes		<i>OXTRm</i> at site -924 would be
Duration of eye contact	0.30	
Likert Scales		regulated by parental
Infant's attention to parent	0.53	
Infant Mood	0.54	engagement and that

methylation at this site would modulate infants' social orienting behavior. Following the Social Salience Hypothesis, we specifically expected that increases in parental engagement would reduce the methylation at site -924 and result in more social orienting behavior in infancy. We also predicted that less methylation at -924 would result in an increase in mean peak amplitude in neural response to social information compared to non-social information. Lastly, we hypothesized that increases in *OXTRm* would be associated with less differentiation between mean peak amplitude neural responses to social and non-social stimuli.

Analyses began by assessing interrater reliability (IRR) for the behavioral videos using Krippendorff's alpha (Krippendorff, 2004). Our IRR ranged from 0.30 to 0.54 (M=0.44, see table 2), indicating disagreement between raters in the coding paradigm.

Table 2.

Krippendorff's alpha on 25% (n=17) of the behavioral codes with 6 total raters. Demonstrates low inter-rater reliability for our coding paradigm.

	FC6	C3	C4	T7	T8
03	-0.72	0.34	1.06	0.19	8.26

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SD Social (μV)	7.15	8.07	5.65	8.63	11.49	5.63	5.13	11.68	4.67	10.73	6.46	64.15
Mean Non-Social (μV)	-0.81	-0.22	-1.23	-1.23	1.22	1.09	0.16	1.71	2.20	1.97	0.06	-7.34
SD Non-Social (μV)	5.76	5.39	4.76	3.70	6.23	6.20	4.35	24.99	5.15	4.50	4.47	66.26
Degrees of Freedom	62	62	62	62	62	62	62	62	62	62	62	62
F-Value	1.08	1.37	0.58	0.63	1.56	1.48	0.69	1.15	0.34	0.57	1.12	-0.05
P-Value	1	1	1	1	1	1	1	1	1	1	1	1

MPA P200												
	F3	F4	F7	F8	FC1	FC2	FC5	FC6	C3	C4	T7	T8
Mean Social (μV)	6.70	5.72	3.91	4.35	4.40	4.95	6.93	10.72	5.04	2.41	3.06	-0.93
SD Social (μV)	6.09	4.92	5.81	11.10	7.54	6.13	6.86	33.13	5.66	4.57	5.90	11.30
Mean Non-Social (μV)	3.39	3.20	1.15	2.01	1.20	2.88	3.89	7.82	4.29	1.02	0.90	-1.60
SD Non-Social (μV)	4.62	5.55	4.77	5.73	5.01	4.43	3.87	25.82	4.94	5.21	6.29	12.63
Degrees of Freedom	62	62	62	62	62	62	62	62	62	62	62	62
F-Value	3.31	2.51	2.76	2.34	2.40	2.07	3.04	2.91	0.76	1.39	2.16	0.67
P-Value	0.99	1	0.99	1	1	1	1	1	1	1	1	1

Next, a repeated measures ANOVA was implemented to compare the effect of the electrode site by the condition (social vs. non-social) to assess differences in the mean peak amplitude between the neurological responses to social and non-social

Table 3. F-values from a repeated measures Anova with Tukey HSD correction comparing social to non-social mean peak amplitude (MPA) ERP responses for each of the two components. No significant differences were found between social and non-social ERP stimuli for the N100 and P350 response. A Tukey HSD test to correct for multiple

comparisons was implemented. After performing the correction, we did not find any significant differences ($p > 0.05$) in social and non-social responses for any electrode site (see table 3 for means, standard deviations, F statistics, and p-values for each comparison).

The partial least squares model was then constructed to assess associations between parental engagement, *OXTRm* at site -924, and differences in neurological processing of social and non-social stimuli. We ensured that all latent variables were independently identified by their manifest variables by ensuring all loading values were > 0.70 for its given construct (*N100*: $M_{\text{ParentalBehavior}} = 0.82$, $M_{\text{OXTRm}} = 0.83$, $M_{\text{LeftAuditoryERP}} = 0.87$, $M_{\text{RightAuditoryERP}} = 0.90$, $M_{\text{InfantBehavior}} = 0.79$, *P350*: $M_{\text{ParentalBehavior}} = 0.83$, $M_{\text{OXTRm}} = 0.83$, $M_{\text{LeftAuditoryERP}} = 0.76$, $M_{\text{RightAuditoryERP}} = 0.88$, $M_{\text{InfantBehavior}} = 0.79$). This statistical analysis ensures that the latent variable is properly represented by its manifest variables (Benitez et al., 2020). Cross loadings were examined to assess the reflective indicators discriminant validity, or the extent to which these manifest variables do not relate to other latent constructs in the model. All manifest variables were valid, as each manifest variable showed the highest cross-loading value onto its respective latent variable compared to its cross-loading onto other latent variables (Nasution et al., 2020). *OXTRm* was represented by three triplicate assays of site -924. Parental engagement, left hemisphere neurological response, right hemisphere neurological response, and infant behavior were created by first loading all measured variables onto the latent construct. Measures for the parental engagement construct included total duration of parental

vocalizations, total duration of operational touch, total duration of loving touch, total duration of positive affect, total duration of negative affect, total duration of eye contact, total duration of parental pointing, the number of times the parent switched activities (defined as when the goal of the activity has changed), likert scales for the parent's talkativeness, modulation of speech, proximity to the infant, and general mood. Measures for the infant behavior construct included the child's total duration of positive affect, total duration of negative affect, total duration of the child's vocalization, total duration of the child pointing (defined as a hand gesture meant to guide attention), total duration of eye contact with the parent, the number of times the child switched activities, and likert scales for infant's attentiveness and general mood. Measured neural activity for the left hemisphere included peak mean amplitudes at F3, F7, FC1, FC5, C3, and T7 for each of the ERP components, while the right hemisphere included F4, F8, FC2, FC6, C4, and T8. Variables were removed one at a time based on the lowest loading values until all manifest variables remaining had a loading value greater than 0.70, meaning they adequately represent the latent variable. Removing loadings <0.70 left parental modulation of speech (N100 Model: loading = 0.83, P350 Model: loading = 0.82), parental talkativeness (N100 Model: loading = 0.86, P350 Model: loading = 0.87), total durations of vocalizations (N100 Model: loading = 0.77, P350 Model: loading = 0.78), and parental mood (N100 Model: loading = 0.83, P350 Model: loading = 0.83) as manifest variables for parental engagement. Infant behavior was defined by the infants' attention to the parent (N100 Model: loading = 0.85, P350 Model: loading = 0.85), the

infants' mood (N100 Model: loading = 0.76, P350 Model: loading = 0.74), and the duration of eye contact with their parent (N100 Model: loading = 0.76, P350 Model: loading = 0.76). Manifest variables varied for each of the three ERP component models. The N100 model consisted of F8 (loading = 0.79) and FC6 (loading = 0.095) for the left hemisphere and F7 (loading = 0.95) and FC5 (loading = 0.84) for the right hemisphere, which all lie within the topographical location for speech processing in infants, found by Becker et al. (2014). The P350 model had the left hemisphere neurological activity composed of F3 (loading = 0.70), FC1 (loading= 0.78), and C3 (loading= 0.79), while the right hemisphere consisted of FC6 (loading = 0.83), and T8 (loading = 0.93). In adults, the P350 component is found in the frontal lobe, which would encompass F3, F4, F7, and

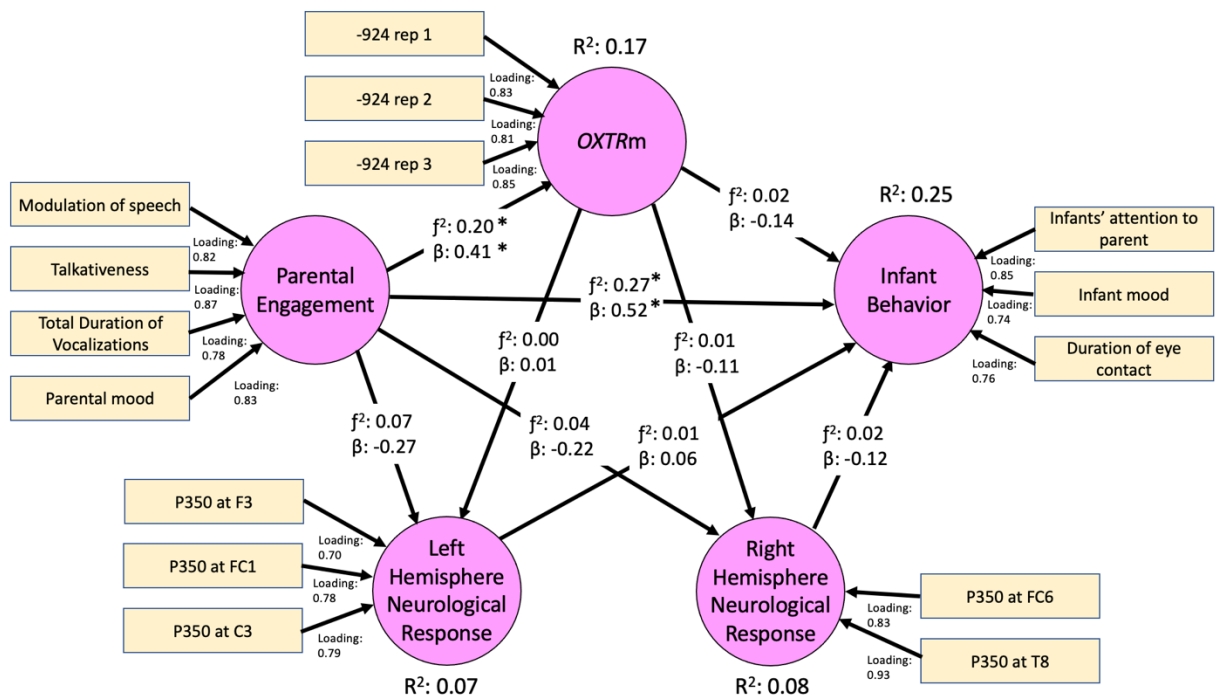


Figure 5. Partial Least Squares model for the P350 response consisting of outer model loadings running from manifest variables (yellow rectangles) to the latent variables (pink circles). R^2 is shown next to each latent variable. F^2 and path coefficients are displayed on the arrow between each latent variable. Significant pathways are indicated with *.

FC5. The T8 loading may reflect auditory processing more broadly rather than differentiation between the stimuli (Scott, 2005).

Having confirmed each of the models' construct reliability and validity and discriminant validity, this study found marginal associations between parental engagement and *OXTRm* at site -924 and that parents' behavior, specifically their vocalization and mood, positively predicts infants' social orienting behavior (eye contact, attentiveness to the parent, and their mood). However, *OXTRm* is not associated with infants' behavior or ERP response in the auditory domain either in the left hemisphere or the right hemisphere. Similarly, parental behavior is not associated with infant ERP response to social auditory information in either the left or right hemisphere. Lastly, ERP responses are not associated with infant social behavior in either the left or right hemisphere. The topographical loadings, R^2 , path coefficients (β), F^2 values and path modeling of the P350 ERP response can be seen in Figure 5. There were no statistically significant differences between the three models, as each model was configured in an identical path diagram, with the ERP responses varying based on the component. Composite reliability and R^2 values can be found in Table 4 and modeling results (path coefficients and f^2 values) for each of the three models can be found in Table 5.

Table 4 Model quality Indices

Model	Composite reliability coefficients
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	Parental Engagement	<i>OXTRm</i>	Left Hemisphere P350 ERP	Right Hemisphere P350 ERP	Infant Social Orienting Behavior
N100	0.89	0.87	0.89	0.87	0.83
P350	0.90	0.87	0.80	0.87	0.83

R² Coefficients

	Parental Engagement	<i>OXTRm</i>	Left Hemisphere P350 ERP	Right Hemisphere P350 ERP	Infant Social Orienting Behavior
N100	-	0.18	0.02	0.05	0.26
P350	-	0.17	0.07	0.08	0.25

Composite reliability coefficients show internal reliability and consistency. *R*² coefficients represent the explanatory construct for each latent variable within the model.

Abbreviations: OXTRm oxytocin receptor gene DNA methylation

N100 Response

		Variable being predicted				
		Parental Engagement	<i>OXTRm</i>	Infant Social Orienting Behavior	Left Hemisphere ERP	Right Hemisphere ERP
Variable predicting	Parental Engagement					
	β	-	0.42	0.5	0.14	0.13
	f^2	-	0.22	0.27	0.02	0.01
	<i>OXTRm</i>					
	β	-	-	-0.12	0.12	0.03
	f^2	-	-	0.02	0.01	0.00
	Left Hemisphere ERP					
	β	-	-	0.02	-	-

	f^2	-	-	0.00	-	-
Right Hemisphere ERP						
	β	-	-	0.14	-	-
	f^2	-	-	0.03	-	-

P350 Response

		Variable being predicted					
		Parental Engagement	<i>OXTRm</i>	Infant Social Orienting Behavior	Left Hemisphere ERP	Right Hemisphere ERP	
Variable predicting	Parental Engagement						
		β	-	0.41	0.52	-0.27	-0.22
		f^2	-	0.20	0.27	0.07	0.04
	<i>OXTRm</i>						
		β	-	-	-0.14	0.01	-0.11
		f^2	-	-	0.02	0.00	0.01
	Left Hemisphere ERP						
		β	-	-	0.06	-	-
		f^2	-	-	0.01	-	-
	Right Hemisphere ERP						
	β	-	-	-0.12	-	-	
	f^2	-	-	0.02	-	-	

Table 5. Path coefficients (β) and f^2 values for each latent variable within each of the three ERP models.

Discussion

In line with our hypothesis, parental vocalizing behavior and mood was associated with methylation values in four-month-old infants, as well as infant attention-orienting behavior. However, neither parental behavior nor methylation at *OXTR* site -924 was associated with infants' neurological processing of auditory stimuli at the N100 or P350 component. Additionally, neurological processing of social auditory information at each of the three ERPs did not predict infants' behavior.

Parental engagement has been shown in previous literature to modulate the change in *OXTRm* at site -924 as infants age from five to 18 months, such that more parental engagement leads to less change in methylation over the course of those 13 months (Krol, Moulder, et al., 2019). The current study found that parental engagement, specifically through vocalizations and parental mood, increases methylation at site -924 as early as four months of age. While initially our results seem contradictory to the previous literature, it is important to note that Krol, Moulder, et al., (2019) investigated the difference in methylation from five to 18 months of age. In other words, parents' engagement with their child may be actively modulating epigenetic variability at -924, such that it creates a stable level of methylation, or a homeostatic set-point. The current study is assessing one cross-sectional timepoint. Perhaps at four months of age, parental engagement is aiding in creating the methylation setpoint, thus accounting for the positive relationship that will become the maintenance pattern of methylation. The setpoint may then be modulated later in life by the parents' continued involvement. Future research should assess the longitudinal trajectory *OXTRm* levels throughout the

first few years of infancy to better understand the homeostatic regulation of the gene's expression.

Previous literature has extensively explored the positive effects of parental behavior on infants' cognitive development. Maternal caregiving activities, positive affect, responsiveness, and interactive behavior have all been associated with earlier development of attention and longer attention spans (Bornstein & Tamis-Lemonda, 1997; Bridgett et al., 2011; Gartstein et al., 2008; Swingler et al., 2017). In regards to parental vocalizations specifically, one study in preterm infants found that maternal speech elicited less motor activity and more wakefulness in the neonates (Bozzette, 2008), thus speech may aid in the development of social awareness. In at-term infants, variability in the pitch of parental vocalizations was an independent predictor of the babies' joint attention skills (e.g., declarative pointing or gaze following) at 12 months of age (Roberts et al., 2013). These studies provide evidence that infant-directed speech promotes newborns' involvement in communicative and content-sharing interactions- which are fundamental to social relationships later in life. Thus, it seems rational that parental engagement, in the form of vocalizing behavior, would positively associate with social attention orienting behavior in four-month-old infants, as found in this study.

We failed to find a relationship between either parental behavior or *OXTR*m and neurological correlates of social processing in infancy, nor did it find a relationship between neurological responses and infant behavior. However, our results showed a lack of differentiation between social and non-social ERP responses, indicating that

perhaps the neurological correlates were not predictive due to the lack of differentiation between the two conditions. While previous research has found social attention to be apparent in infants at four months of age (LoBue et al., 2017), other research has found that ERP responses to social information may be dependent on individual differences, with some showing greater ERP responses to social information, others to non-social information, and some showing no differences (Puglia et al., 2020). It could be that our aggregative methods are canceling the effects of individual differences that may be caused by parental engagement or *OXTR*m and more predictive of infant behavior. Future research will need to assess within person variation in neurological responses to better elucidate this variable's predictive power.

The single time point of this cross-sectional data does limit our ability to extrapolate developmental trajectories of the parental relationship. It is plausible that parental engagement begins modulating the epigenetic set-point even earlier than four months of age. Future research will need to follow infants from birth through early childhood to truly understand the epigenetic trajectory of site -924 and how parental engagement is affecting gene expression.

The original intent of this data was to assess differences in multiscale entropy to social and non-social stimuli (Puglia et al., 2020). Hence, the design of the study was not optimized to capture delayed onset potentials prior to the presentation of the next stimulus. Thus, it could also be that perhaps the P350 response would differentiate more given additional time to propagate.

Lastly, it is important to note that we observed low inter-rater reliability, meaning there is variability in objective measurements of social behavior of both the parent and the infant. It is conceivable that the suggested behavioral manifestations of parental vocalizations and the infants' social attention orienting responses are not true representations of the actual interaction, which could result in smaller effect sizes than currently presented. Future analyses will consist of standardizing the coding scheme to reduce bias in subjective measurement to ensure an accurate representation of these latent variables.

While previous research focused on attention, proximity, and vocalizations as driving forces of changes in methylation levels of *OXTR* (Krol, Moulder, et al., 2019), our data reflects the importance of parental mood and directed vocalizations in predicting methylation. Perhaps these behaviors are reflective of the social orienting to vocalized auditory cues, while proximity and attention are measuring slightly different constructs. It is also important to note, that both attention and proximity were measured using a five-point likert scale, which may not be capturing the intricacies of these behaviors.

While prediction of regulators for epigenetic modifications is helpful in understanding infant development, it is important to acknowledge that the behavioral and developmental consequences of decreased methylation at site -924 is not well-studied. Thus, this study is not meant to quantify parental speech or mood as propelling a positive or negative developmental trajectory for their child. Rather, it is meant to elucidate the possible genetic impacts of this domain of engagement.

Future studies will need to address the variation in the behavioral observational coding to create a more concrete and definite understanding of the parental and infant actions that may be contributing the modulation of methylation. Additionally, it will be important to follow infants from birth through the early childhood years to understand when site -924 reaches homeostasis and what environmental impacts may be altering its methylation. A longitudinal design will allow for the assessment of developmental trajectories necessary to make claims about modulating forces. Lastly, while there was not a neurological correlate to *OXTR*m, parental engagement, or infant social-orienting behavior, that does not mean we are devoid of such as infants. Our current data did not show differences between social and non-social responses on the aggregate level; however, it could be that individual variability would be more predictive. Additionally, EEG is spatially limited to the cortex; however, it has been shown that most social processing occurs in deeper brain structures (Dunbar, 2012; Frith, 2007; Kanai et al., 2012). Thus, future research should explore differences in infant neural activation patterns of deeper brain structures (e.g., medial prefrontal cortex, superior temporal sulcus, temporo-parietal junction, fusiform gyrus, amygdala, etc.), that EEG methods may not be able to access. Therefore, it is crucial to implement other neuroimaging techniques, like fMRI, to assess differentiation patterns to social and non-social stimuli.

The results of the current study speak to the importance of the dyadic model of socioemotional development early in infancy. Parental vocalizations, which may be a proxy for attention-orienting, were shown to predict *OXTR*m levels in infants and infant

social-orienting behaviors. Thus, this study shows biological correlates of dyadic interactions which had not yet been explored at such a young age.

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