Infants' salivary oxytocin and positive affective reactions to people

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Abstract

Oxytocin is a neuropeptide positively associated with prosociality in adults. Here, we studied whether infants' salivary oxytocin can be reliably measured, is developmentally stable, and is linked to social behavior. We longitudinally collected saliva from 62 U.S. infants (44% female, 56% Hispanic/Latino, 24% Black, 18% non-Hispanic White, 11% multiracial) at 4, 8, and 14 months of age and offline-video-coded the valence of their facial affect in response to a video of a smiling woman. We also captured infants' affective reactions in terms of excitement/joyfulness during a live, structured interaction with a singing woman in the Early Social Communication Scales at 14 months. We detected stable individual differences in infants' oxytocin levels over time (over minutes and months) and in infants' positive affect over months and across contexts (video-based and in live interactions). We detected no statistically significant changes in oxytocin levels between 4 and 8 months but found an increase from 8 to 14 months. Infants with higher oxytocin levels showed more positive facial affect to a smiling person video at 4 months; however, this association disappeared at 8 months, and reversed at 14 months (i.e., higher oxytocin was associated with less positive facial affect). Infant salivary oxytocin may be a reliable physiological measure of individual differences related to socio-emotional development.

Keywords: hormone, physiology, positive sociality, social interaction, longitudinal

Oxytocin is a neuropeptide involved in various physiological and psychological processes (Insel, 2010), including the formation of social bonds (Feldman, 2012). Individuals' oxytocin levels are positively associated with their social behaviors (for reviews: Crockford et al., 2014; Scatliffe et al., 2019; Shorey et al., 2023). However, little is known about the development of the oxytocin system in infants and children (Brzozowska et al., 2022; Feldman et al., 2013; Fujisawa et al., 2014; Nishizato et al., 2017). Understanding the role of the oxytocin system in early social development may help identify early protective and risk factors related to social development (Clark et al., 2013; Fujisawa et al., 2014; Lebowitz et al., 2016; Modahl et al., 1998; Torres et al., 2022). Thus, we tested whether salivary oxytocin may be an early physiological correlate of social development by longitudinally examining infants' salivary oxytocin levels, their developmental stability, and their relation to infants' affiliative social behavior.

Reliability of Salivary Oxytocin in Infancy

The measurement of oxytocin in saliva is a relatively non-invasive way to begin characterizing the development of the oxytocin system in infants (Feldman et al., 2013; Feldman et al., 2010). For example, 4- to 6-month-old infants', as well as their parent's, salivary oxytocin levels captured at two time points within the same day (~ 30 minutes apart) moderately, positively correlate with each other (r = .50 in infants; r = .55 in parents; Feldman et al., 2010), suggesting salivary oxytocin levels can be reliably measured at these ages. However, it remains unclear whether such stability extends to older infants (> 6 months) and over longer periods of time (e.g., months) in infancy, as no studies, to date, have examined developmental stability in salivary oxytocin levels with age (see Table 1 for a summary of previous studies). This is a notable gap in our understanding, especially given that early infancy is a period of rapid

42 developmental changes in the brain (Poppe et al., 2021) and in social development (Shultz et al.,
43 2018).

Table 1

Summary of previous studies of salivary oxytocin in human infants

Study	Infant Ages, Sample Size, & Term[a]	OT Level (pg/ml)[b]	OT Extraction & Assay	Within-Age Correlation	Age-related Change	Links to Social Interactions/Behaviors
Brzozowska et al. (2022)	**6-8 months:** *N* = 39 (18 Female; *M* = 232 days, *SD* = 30) **11-13 months:** N = 32 (15 Female; *M* = 371 days, *SD* = 30)	**Pre-parent interaction[c]:** 6-8 months: 8-231 (*M* = 102, *SD* = 56); 11-13 months: 25-198 (*M* = 103, *SD* = 61) **Post-parent interaction:** 6-8 months: 2-288 (*M* = 117, *SD* = 75); 11-13 months: 28-291(*M* = 145, *SD* = 89)	Extraction: N.R. Assay: enzyme immunoassay	$r = .37$, $p = .034$	N.R.	OT +,~ looking time face
Feldman et al. (2010)	**4-6 months**: *N* = 55 (sex N.R.; *M* = 157.1 days, *SD* = 11.9)	**Pre-parent interaction (pg/mg protein):** 1.3-44.4 (*M* = 16.4, *SD* = 10.7) **Post-parent interaction (pg/mg protein):** 2.4-76.4 (*M* = 21.8, *SD* = 16.9)	Extraction: No Assay: enzyme immunoassay	$r = 0.50$, $p < 0.001$	N.R.	OT ↑ after interaction Infant OT +,~ social engagement Infant OT +,~ parent-child affect synchrony
Ferera et al., (2023)	**12-16 months:** *N* = 24 (14 girls; M = 14.4 months)	**Visit 1:** **Baseline:** 1.4-304.6 (*M* = 104.4, *SD* = 95.9) **Post-parent interaction:** 7.9-12300.0 (*M* = 1943.4, *SD* = 4024.4) **End of Visit:** 0.8-41000.0 (*M* = 4362.6, *SD* = 12883.0) **Visit 2:**	Extraction: N.R. Assay: enzyme immunoassay	N.R.	N.R.	Infant OT after interaction -,~ racial categorization

		Baseline: 0.8-168.9 (*M* = 47.6, *SD* = 53.2) **Post-parent interaction:** 1.4-1308.3 (*M* = 341.5, *SD* = 477.2) **End of Visit:** 13.0-340.0 (*M* = 200.3, *SD* = 117.5)				
Filippa et al. (2021)	**1-8 days, preterm** *N* = 20 (45% Female; *M* = 3 days, *SD* = N.R.)	N.R. Estimated medians[d]: **Pre-control condition:** 0.99 **Post-control condition:** 0.81 **Pre-speaking condition:** 0.95 **Post-speaking condition:** 1.16 **Pre-singing condition:** 0.77 **Post-singing condition:** 1.38	Extraction: N.R. Assay: radio immunoassay	N.R.	N.R.	OT ↑ during speaking and singing conditions.
Fujiwara et al. (2019)	**3-10 months:** *N* = 345 (58 Female; *M*= 5.46 months, *SD* = 1.50)	0.37-59.82 (*M* = 11.14, *SD* =10.29)	Extraction: N.R. Assay: enzyme-linked immunosorbent	N.R.	No change; stats N.R.	No infant social behaviors measured.
Kommers et al. (2018)	Mean gestational age = 226.9 days (*SD* = 19.6), **preterm:** *N* = 22 (10 Female; *M* = N.R. *SD* = N.R.)	**Pre-kangaroo care:** Range: N.R. (*M* = 2.40, *SD* =1.64) **During kangaroo care:** Range: N.R. (*M* = 1.39, *SD* = 0.58)	Extraction: Yes Assay: radio immunoassay	N.R.	N/A	OT -,~ parent-infant interaction. OT ↓ during kangaroo care.
Markova et al. (2018)	**4 months:** *N* = 43 (24 Female*; M* = 139.43 days, *SD* = 19.415)	**Upon arrival:** 35.13-503 (*M* = 169.5, *SD* = 132.6) **After baseline:** 29.72-485.1 (*M* = 193.9, *SD* = 119.3) **After natural interaction:** 11.5-	Extraction: No Assay: enzyme immunoassay	*r*s = 0.39 - 0.68, *p*s < .001-.060	N/A	**After natural interaction:** OT -,~ total number of games, & -,~ time spent playing games during the

		441 (*M* = 182.1, *SD* = 116.8) **After modified interaction[e]:** 52.04-320 (*M* = 159.5, *SD* = 76.09)				interaction
Markova et al. (2019)	**4 months:** *N* = 43 (24 Female; *M* = 139.43 days, *SD* = 19.415)	**Upon arrival:** 35.13-503 (*M* = 169.5, *SD* = 132.6) **After baseline:** 29.72-485.1 (*M* = 193.9, *SD* = 119.3) **After natural interaction:** 11.5-441 (*M* = 182.1, *SD* = 116.8)	Extraction: No Assay: enzyme immunoassay	*r*s = 0.39 - 0.62, *p*s < .001-.060	N/A	OT reactivity +,~ looking time to mothers when maternal affect attunement is high
Moussa et al. (2021)	**2-6 months:** *N* = 37 (17 Female; *M* = 4 months, *SD* = 1.3)	**Pre-massage:** 68-360 (*M* = 300.86, *SD* = 86.64) **Post-massage:** 104-355 (*M* =297.70, *SD* = 75.11)	Extraction: N.R. Assay: enzyme-linked immunosorbent	*r* = 0.18, *p* = .301	N.R.	Male infant OT ↓ post-massage. Female infant OT ↑ post-massage.
Nishizato et al. (2017)	**5-90 months:** *N* = 149 (73 Female; *M* = 33.6 months, *SD* = 24.3)	30-350 (*M* = N.R., *SD* = N.R.)	Extraction: No Assay: enzyme immunoassay	N.R.	OT ↓ with age (*r* = -.44, *p* < .010)	OT +,~ fixation time on eye region OT -,~ fixation time on mouth region
Vittner et al. (2018)	**3-10 days, preterm:** *N* = 28 (sex N.R.; *M* = N.R., *SD* = N.R.)	**Pre-SSC:** *M* = 134.71 (*SD* = 104.69) & *M* = 130.71 (*SD* = 143.67) **During SSC:** *M* = 306.72 (*SD* = 275.48) & *M* = 346.87 (*SD* = 291.63) **Post-SSC:** *M* = 223.44 (*SD* = 214.02) & *M* = 260.98 (*SD* = 232.08)	Extraction: Yes Assay: enzyme immunoassay	N.R.	N.R.	OT +,~ parent-child synchrony & parental responsiveness

Vittner et al. (2020)	**3-10 days, preterm:** *N* = 28 (sex N.R.; *M* = N.R., *SD* = N.R.)	N.R.	Extraction: N.R. Assay: N.R.	N.R.	N/A	**After maternal SSC:** Infant OT +,~ self-regulatory scores & excitability scores. **After paternal SSC:** Infant OT +,~ self-regulatory scores & -,~ self-regulatory scores.
White-Traut et al. (2022)	**12-24 hours:** *N* = 102 (57 Female; *M* = N.R., *SD* = N.R.)	**Pre-multisensory intervention[f]:** 4.67-7.64 (*Median* = 5.16, *SD* = N.R.) **Post-multisensory intervention:** 4.59-7.07 (*Median* = 5.13, *SD* = N.R.)	Extraction: N.R. Assay: enzyme immunoassay	N.R.	N.R.	Infant OT did not change pre- and post-intervention.

Note: None of the studies tested/reported stability in salivary oxytocin with age. [a]All infants were healthy and full-term unless otherwise stated. [b]Units are pg/ml unless otherwise indicated. [c]Parent-child interactions consisted of naturalistic free-play and touch interactions. [d]Descriptive statistics were not reported. Medians were estimated based on data visualization. [e]Mothers were instructed to change their interaction style with their infant (e.g., use adult-directed speech). [f]Multisensory intervention included infant-directed speech, eye-to-eye-contact, and massage. N.R. = not reported. OT = salivary oxytocin levels. SSC = skin to skin contact. *N* = Sample size, *M* = mean, *SD* = standard deviation, n.s. = not statistically significant, +,~ = positively associated; -,~ = negatively associated, ↓ = decreased, ↑ = increased. ***p<.001, **p<.01, *p<.05.

Salivary Oxytocin Changes with Age: Group Level Development

In addition to the need to track developmental stability in *individual differences* in salivary oxytocin, there is also a need to understand *group level* changes with age (i.e., average pattern across individuals) to establish normative models of healthy infant development. Age-related changes in salivary oxytocin levels have been observed, but only using cross-sectional methods and linear data analysis, yielding somewhat inconsistent findings. For example, a cross-sectional study in children across a wide age range—from 5 months to 7 years old—reported declines in salivary oxytocin with age (Nishizato et al., 2017). In contrast, a cross-sectional study across a narrower age range—from 3- to 10-month-old infants—reported no change with age in salivary oxytocin (Fujiwara et al., 2019). Thus, existing studies have reported inconsistent findings regarding changes in oxytocin over time. In comparison to cross-sectional studies, longitudinal studies may offer a complementary and more sensitive approach to capture age-related changes in infants' salivary oxytocin levels while controlling for inter-individual variability (Louis et al., 1986). By characterizing the longitudinal developmental pattern of salivary oxytocin levels of healthy infants, we can better understand the development of the endogenous oxytocin system.

Salivary Oxytocin is Positively Associated with Social Behaviors

There is preliminary support for a link between infants' salivary oxytocin levels and their social interactions. Prior experimental studies in infants, 1 week to 6 months old, report that salivary oxytocin levels may be increased by a variety of parent-infant social interactions, including receiving parental affectionate contact, play and touch interaction, maternal massage, infant-directed vocalizations, and skin-to-skin contact (Feldman et al., 2010; Filippa et al., 2021; Moussa et al., 2021; Vittner et al., 2018). However, it remains unclear how the peripheral

oxytocin system reacts to social interactions as some studies report a decrease in salivary oxytocin levels in infants (Kommers et al., 2018; Moussa et al., 2021). For example, preterm infants show a decrease in salivary oxytocin levels during a parent-infant intervention that requires skin-to-skin contact from the baseline levels (Kommers et al., 2018). Moreover, male 2- to 6-month-old infants show a decrease in salivary oxytocin after receiving maternal massage, while female infants show a decrease after a massage (Moussa et al., 2021). These findings suggest that the link between oxytocin and social interaction may vary.

While these findings suggest short-term changes (i.e., within minutes) in infants' salivary oxytocin levels after social interactions, it remains unclear whether there may be natural variability in infants' baseline salivary oxytocin levels associated with their social development. While these manipulations measure how reactive infants' oxytocin systems are to social interactions initiated by parents, it is untested whether infants' natural baseline oxytocin levels are associated with individual differences in their own social behaviors beyond the parent-interaction context.

Indeed, emerging cross-sectional evidence suggests associations between baseline levels of salivary oxytocin and social behavior early in development. For example, in newborn macaques—a species often used as a model for human infants—baseline salivary oxytocin levels are positively correlated with the time infants spend in proximity to a human social partner during a structured assessment in the first two weeks after birth, suggesting salivary oxytocin may reflect meaningful individual variability in sociality (Simpson et al., 2014). A series of cross-sectional studies also support this hypothesis in humans. Higher baseline salivary oxytocin levels in human infants are positively correlated with greater social orienting, such as longer looking time to images of faces relative to nonsocial objects at 6- to 13 months of age

(Brzozowska et al., 2022), and longer looking time to the eye region of dynamic, affect-neutral human faces making eye-contact between 5 months and 7 years of age (Nishizato et al., 2017). These findings suggest that infants and children with higher salivary oxytocin levels may show greater social interest. Moreover, at 3 years of age, children's baseline salivary oxytocin levels positively correlate with their social reciprocity (i.e., give-and-receive interactions based on communicative cues) with a friend ($r = .37$, $N = 48$; Feldman et al., 2013), suggesting that salivary oxytocin levels may be linked to prosocial behavior in childhood. Furthermore, higher baseline salivary oxytocin levels in 4-year-olds are associated with greater attention to socially salient objects to which their social partners point and look ($r = .27$, $N = 58$; Fujisawa et al., 2014). Together, these cross-sectional findings in infants and children suggest that higher levels of salivary oxytocin may serve as an indicator of positive social behavior. However, longitudinal studies are necessary for uncovering potential links between the oxytocin system and social behavior. A longitudinal design allows for examination of individual developmental trajectories of oxytocin levels and social behavior, expanding our understanding of whether and how the oxytocin-sociality link changes across infancy.

Infants' Positive Affect During Social Interactions

One fundamental component of infants' sociality is the display of positive affect towards other people. Infants' positive affective expression conveys joy and affiliation during social interaction, facilitating social connection with adult social partners (Barrett, 1998; Campos et al., 1994). Over the first year of life, infants begin to coordinate their positive facial affect with gazes at their parents during face-to-face interactions (Jones & Hong, 2001; Lavelli & Fogel, 2005; Yale et al., 2003). This gaze-affect coordination becomes stronger over the first year after birth (Messinger & Fogel, 2007; Messinger et al., 2001). For example, from 2 to 5 months, infants

increase the frequency with which they express reciprocal social smiles during face-to-face interactions (Messinger & Fogel, 2007). Therefore, infants' positive affect in the first year is a crucial aspect of early social communication.

Moreover, there is substantial interindividual variability in infants' temperamental positive affect reported by parents, which is stable between 3 and 12 months of age (Putnam & Stifter, 2002; Rothbart, 1986). Such individual differences in positive affectivity in infancy appear to be an early predictor of later social development and life outcomes. For example, infant positive affectivity predicts social competence including empathy, prosocial peer interactions, and pretend play in childhood (Parlade et al., 2009), and higher levels of education attainment, life satisfaction, and optimism into adulthood (Coffey, 2020; Coffey et al., 2015). Combined, these findings suggest that individual differences in positive affect in infancy may be linked to developmental outcomes across numerous domains. While the mechanisms of these associations are unclear, these findings underscore the importance of tracking infants' positive affective response to other people as a potential marker of infants' well-being (Simpson et al., 2019).

Given that positive affect appears to be a fundamental component of human sociality (Barrett, 1998; Campos et al., 1994), we theorized that infants' baseline salivary oxytocin levels would positively correlate with their levels of positive affect directed towards other people. A previous study suggests that 4- to 6-month-olds' salivary oxytocin levels are positively associated with their social engagement, including positive facial affect, during interactions with their parents (Feldman et al., 2010). To further investigate the role salivary oxytocin may play in positive affective responses to people, there is a need to examine whether this previous finding can be extended to older infants and interactions with people other than caregivers. As such,

salivary oxytocin levels may provide an objective measure to inform our understanding of the mechanisms underlying infants' emotions in social contexts.

Current Study

In the current study, we aimed to characterize the development of the oxytocin system in infancy and investigate its relationship with the development of infants' positive affect to other people. We tracked human infants longitudinally across three ages: 4, 8, and 14 months. At each age, we measured infants' salivary oxytocin levels twice and recorded their facial affect while they watched a 77-second video of an unfamiliar woman smiling, simulating a common social experience (Zeng et al., in 2022). In addition, at the age of 14 months, we measured infants' affective reactions during live social interaction episodes of the Early Social Communication Scales (ESCS)—a structured assessment of early social skills (Mundy et al., 2003). Infants' facial affective valence was coded offline to track their positive facial affect. These two social assessments enabled us to track infants' positive affect across different contexts (video-based and live-interaction).

We tested three hypotheses regarding the development of the oxytocin system: Similar to reports of stable oxytocin levels at younger infant ages (4-6 months: Feldman et al., 2010), we hypothesized that our sample, which included older infants, would display individual stability in salivary oxytocin levels within visits (Hypothesis 1). While there are, to our knowledge, no studies of long-term stability of individual differences in oxytocin levels with age in infancy, we hypothesized that long-term stability may be present in infancy (Hypothesis 2). Given previous inconsistent findings (Fujiwara et al., 2019; Nishizato et al., 2017), we hypothesized that there may be group-level increase with age in oxytocin levels (Hypothesis 3). Furthermore, we tested three hypotheses related to the development of positive affect: Given the previous reports of

stability in infants' temperamental positive affect (Putnam & Stifter, 2002; Rothbart, 1986), we also hypothesized that individual stability in infants' positive affect to other people would be stable with age (Hypothesis 4a) and across contexts (Hypothesis 4b). We hypothesized an increase in infants' positive affect with age (Hypothesis 5), in line with previous findings of rapid development in positive affect in the first year after birth (Messinger et al., 2001). Finally, given the reported positive associations between infants' prosocial behaviors and baseline oxytocin levels (Brzozowska et al., 2022; Feldman et al., 2010), we hypothesized that infants' salivary oxytocin levels would be positively associated with their positive affect to people, both in a simulated social video (Hypothesis 6a) and in a live interaction (Hypothesis 6b).

Methods

Participants

We recruited healthy (no parent-reported medical problems), full-term (≥37 weeks gestation) infants to participate in our study longitudinally at 4, 8, and 14 months of age. Families were recruited through local events (e.g., baby expos and fairs) and local community and professional centers (e.g., classes for pregnant women). Our sample included a total of 62 healthy infants who contributed saliva samples (44% female; 56% Hispanic or Latino, 18% non-Hispanic White, 24% Black, 11% multiracial, 6% unknown). However, not all infants participated in all three visits (4-month visit: $N = 56$; 45% female; age range: 15.57-20.57, $M = 17.83$, $SD = .88$ weeks; 8-month visit: $N = 53$; 43% female; age range: 33.14-36.71, $M = 34.98$, $SD = .94$ weeks; 14-month visit: $N = 44$; 45% female; age range: 56.71-63.29, $M = 59.98$, $SD = 1.66$ weeks). See Table 2 for detailed demographics. Infants were recruited from the Miami-Dade area. The University of Miami Institutional Review Board ethics committee approved the study.

Table 2

Demographics for Participated Infants and Families

	N	*%*
Total		
Infant Sex		
Male	35	56.45
Female	27	43.55
Infant Ethnicity		
Hispanic or Latino	35	56.45
Other	27	43.55
Infant Race		
Black/African American	15	24.19
Other (unknown/not reported)	4	6.45
White	36	58.06
Asian and White	1	1.61
Asian, White, and Other	1	1.61
Black/African American and Asian	1	1.61
Black/African American, Asian, and White	2	3.23
Black/African American and Other	1	1.61
Black/African and White	1	1.61
Maternal Education		
≤ High School	3	4.84
Some College	11	17.74
2-year College	6	9.68
4-year College	19	30.65
Advanced/Professional Degree	23	37.10
Paternal Education		

≤ High School	14	22.58
Some College	8	12.90
2-year College	7	11.29
4-year College	23	37.10
Advanced/Professional Degree	10	16.13
Annual Household Income		
$5,000 - $9,999	2	3.23
$10,000 - $19,999	3	4.84
$20,000 - $29,999	5	8.06
$30,000 - $39,999	5	8.06
$40,000 - $49,999	3	4.84
Over $50,000	43	69.35
Not Reported	1	1.61

	Mean	**SD**
Infant Birth Weight (lbs.)	7.35	0.87
[a]Household Size (individuals)	3.32	1.69

Note. SD = Standard Deviation. [a]Household size indicates the number of individuals living in the home, including the parent(s), infant, and any siblings.

Materials

Saliva Collection and Assay

We collected infant saliva using SalivaBio Infant Swabs (Salimetrics; State College, PA). Samples were then placed into Swab Storage Tubes (Salimetrics; State College, PA), centrifuged using CF-800-1 Centrifuge (Hardware Factory Store; Covina, CA), then pipetted using Mini-Pipettes (Globe Scientific Inc; Mahwah, NJ) and transferred into CryoTube Vials (Thermo Fisher Scientific; Waltham, MA). They were then stored in Ultra Cold Chest Freezer 86-01

(Scientemp Corp.; Adrian, MI) at −80°C until they were processed. For assays, we used the commercially available ELISA kits (Enzo Life Sciences; Farmingdale, NY). In batches, we conducted duplicate assays of each sample.

Smiling Person Video

Infants observed a 77-second silent video of an unfamiliar White woman (18 years old; Figure 1A), who looked into the camera and shifted between smiling and neutral facial expressions, periodically tilting her head for a short period (i.e., smiling person video). The woman's head was sized 23.52 cm (height) × 16.58 cm (width) displayed at a visual angle of 22.18° × 15.73°. This video was designed to be similar to a common infant social experience (an adult greeting).

We validated the video stimulus by confirming that adult viewers perceived smiles and eye contact from the actress while watching the video (see Supplementary Materials). We used a single, longer video in the current study (rather than multiple short videos) to elicit more natural responses from infants, and avoid habituation, fatigue, and boredom from watching multiple similar videos. Previous studies have shown that a single-trial dynamic stimulus can provide reliable information about individual differences in infants' affectivity and social behaviors (Maylott et al., 2020; Paukner et al., 2014; Zeng et al., 2022).

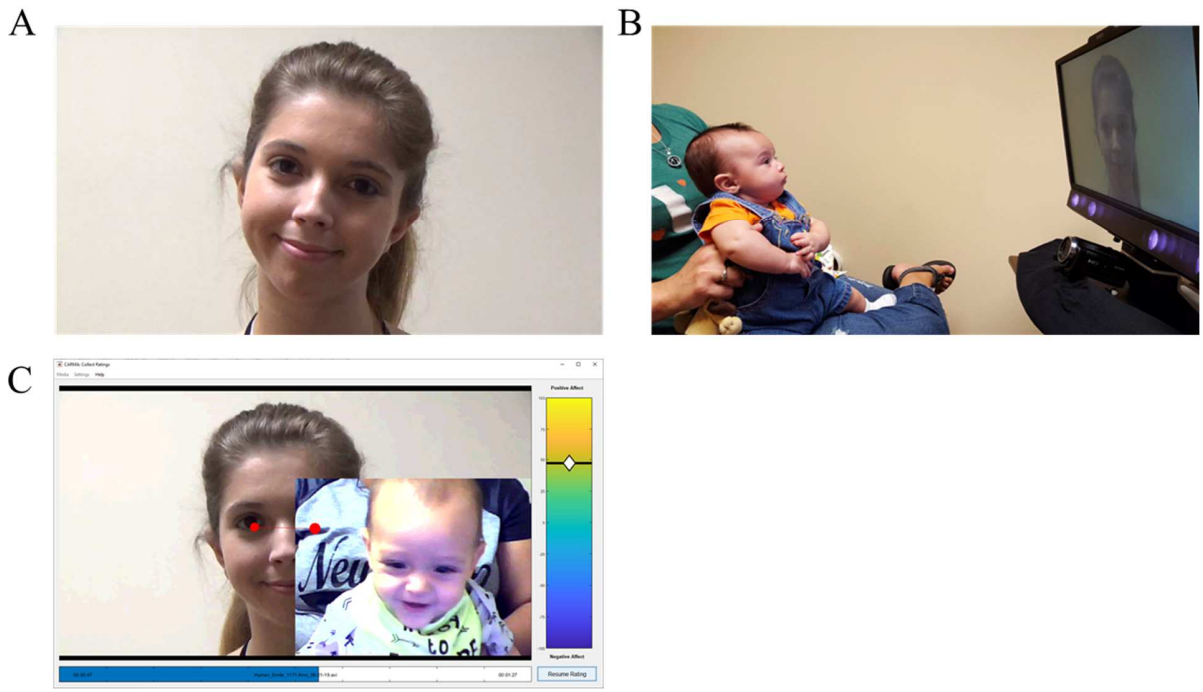


Figure 1. (A) Still-frame from the smiling person video stimulus. (B) Experimental setup showing an infant participating in the eye tracking task. (C) Interface of the Continuous Affect Rating and Media Annotation software (CARMA) for facial affective valence coding.

Display Screen and Extraction of Infant Videos

We recorded videos of infants using a Tobii TX300 eye tracker (Tobii Technology, Danderyd, Sweden), which had a remote 58.4 cm monitor (51 cm in width × 28 cm in height) with integrated dark pupil eye tracking technology (Figure 1B). Silent videos of infants recorded by the Tobii user camera were extracted from Tobii Studio (640 × 480 pixels) with a frame rate of 15 frames per second.

Procedure and Measures

We longitudinally collected human infant saliva at 4, 8, and 14 months of age, between September 2017 and March 2020. Parents gave informed consent at their first lab visit. At each visit, we collected the infant's first saliva sample upon the family's arrival. Following the first

saliva collection, the infant participated in an experimental task that involved viewing pictures and videos, including the smiling person video, for approximately 15 minutes. We then collected a second saliva sample from the infant. Given that the infants were allowed to take a break as they needed, the intervals between collections of saliva sample 1 and 2 varied by how long it took the infant to complete the experimental task (M = 38.48 minutes, SD = 14.56). At 14 months of age, infants also participated in two episodes of the Social Interaction Task in the ESCS assessment after the second saliva collection (Mundy et al., 2003). During the ESCS assessment, the infant and an experimenter sat face to face across a table. In each episode of the task, the experimenter clapped and sang the Baby Bumblebee song for about 10 seconds. After the singing part, the experimenter then gently ran their fingers across the table while softly making "Bzzzz" sound concluding with a brief, gentle tickle of the infant, which was repeated two to three times (see Supplementary Video S1). Finally, parents reported demographics (e.g., infant date of birth, ethnicity, race, family income, and parents' highest levels of education) using the Research Electronic Data Capture (REDCap), a secure web-based system for online surveys and databases (https://www.project-redcap.org; Harris et al., 2009; Harris et al., 2019). Families were compensated $50 for each visit. In total, each visit took approximately one hour.

Saliva Collection Procedure

The infants were not fed at least 15 minutes prior to saliva collection. To collect saliva samples, a research study staff member wearing a glove held one end of a salivary swab and placed the other end of the swab inside the infant's buccal mucosa or sublingually to absorb saliva, allowing infants to suck or chew on the dental cotton swab (Figure 2). The swabs were placed in infants' mouths for 5 minutes, until they were saturated or until the infant refused the swab. The infants could be placed in any position during saliva collection for their comfort. If an

infant refused the swab, the research staff initiated a game by "teasing" the infant by offering and then withdrawing the swab a few times. The saliva samples were centrifuged immediately after they were collected and were stored in a -80°C freezer immediately after centrifuging to optimize sample quality.

Figure 2. Saliva collection from an 8-month-old with the dental cotton swab.

Saliva Purification and Assay

Oxytocin was measured in saliva after extraction. We extracted the entire volume of saliva using a solid-phase extraction procedure previously described in detail (Simpson et al., 2014; Holt-Lunstad et al., 2008) with a slight modification of additional washes (Szeto et al., 2011) to improve sample quality based on a pilot study in our lab. We performed solid-phase extraction of samples using 200mg C18 Sep-Pak columns (Waters Technologies, Milford, MA). The columns were equilibrated with 3 ml of acetonitrile, then twice with 3 ml of 0.1% trifluoroacetic acid (TFA). Up to 1 ml of saliva was mixed with an equal volume of 0.1% TFA, centrifuged at 14,000g for 20 minutes at 4°C. The acidified and clarified saliva was then applied to the column. We discarded the flow-through fraction and washed the columns once with 3 ml of 0.1% TFA, then twice with 3 ml of water. Oxytocin was eluted with 3ml of 60% acetonitrile.

The solvent was evaporated under a stream of nitrogen gas, and the sample completely dried by lyophilization. For immunoassay, the samples were reconstituted in 0.12 ml of assay buffer provided by the EIA kit. Sample extraction before assay may improve accuracy and reliability in measuring endogenous oxytocin concentrations by eliminating the effect of potentially interfering molecules, reducing sample matrix effects, and concentrating and enriching the analyte of interest from the sample before analysis (Szeto et al., 2011; Tabak et al., 2022). In Table 3, we report the volume of saliva that we extracted, which varied across infants. Oxytocin immunoreactivity was assayed using a commercially available ELISA kit (Enzo Life Sciences; Farmingdale, NY; Enzo Oxytocin ELISA Kit, Category number: ADI-900-153A-0001; Ordered on 5/10/19; Received 5/14/19) that had a lower limit of detection of 15.6 pg/ml and following manufacturer instructions for performing the assay.

Table 3

Descriptives of Saliva Samples for Oxytocin Extraction

		Saliva Volume (mL)					# of Not Usable Samples		
Age (months)	**Sample**	**Min**	**Max**	***M***	***SD***	**Usable (%)**	**OT Outlier[a]**	**OT Not Detected**	**Not Enough Saliva**
4	Sample 1	0.45	1.7	1.14	0.35	47 (84%)	1	8	0
	Sample 2	0	1.7	1.08	0.46	43 (77%)	0	10	3
8	Sample 1	0.1	1.8	0.87	0.43	38 (72%)	2	10	3
	Sample 2	0	1.8	0.83	0.48	33 (62%)	0	13	7
14	Sample 1	0	1.8	0.72	0.48	27 (61%)	1	11	5
	Sample 2	0	1.6	0.68	0.35	33 (75%)	0	8	3

Note. OT = oxytocin, *M* = mean, *SD* = standard deviation. [a]Samples with salivary oxytocin levels that fell more than 3 standard deviations from the mean at each age (4 months: > 9.95 pg/ml; 8 months: > 12.70 pg/ml; 14 months: > 13.33 pg/ml).

Infants' Facial Affective Valence During Smiling Person Video

We extracted video recordings of the infants while they watched the smiling person video stimulus from Tobii Studio. Infants' facial expressions to the smiling person video were coded

offline with the Continuous Affect Rating and Media Annotation software (CARMA; Girard, 2014; Figure 1C) on a Dell Windows computer with a 27-inch monitor. Three adult raters who were blind to the study hypotheses independently scored how they perceived the affective valence of each infant's facial expressions in each test session. They rated infants' facial affective valence continuously on a scale from -100 (very negative) to +100 (very positive), with 0 being neutral, by moving a Thrustmaster T16000M FCS joystick (https://www.thrustmaster.com, Carentoir, France) while watching the infants' video recordings in real-time. CARMA exported the valence ratings at 1-second intervals as the unit of analysis. All three raters established inter-rater reliability on 27% of all videos (41 out of 150 videos) based on the average intraclass correlation coefficient (ICC) of a two-way mixed effects model for absolute agreement (k = 3; Koo & Li, 2016). We used ICC for absolute agreement to examine the extent to which the raters rated on facial affective valence identically. The average ICC was .92 (95% CI = [.91, .93]). We used the rater-averaged valence ratings for the duplicate-coded videos (27% of the videos) and used the single-rater-coded valence ratings for the remaining 73% of the videos.

We captured infants' positive facial affect to the smiling person video using three measures (see Supplementary Materials for details). We measured infants' positive affect duration (i.e., the total number of seconds in which the infant was perceived with a positive facial affect), positive affect intensity (i.e., highest perceived positive facial affective valence), and a composite measure that captured both of these components: we calculated the area under the curve of infants' positive affective valence (positive affect AUC) using the trapezoidal method for each infant at each age (Haines et al., 2019). AUC is commonly used to quantify physiological reactions (Pruessner et al., 2003); our study used AUC of infants' continuous

variation of valence over time as a measure of positive facial affect. We performed a cubic root transformation to the positive affect AUC for data scaling and normalization. We noted that infants might not display any positive facial affect during a testing session (32.6% of visits). A zero positive affect AUC might reflect the absence of positive facial affect in the infant (i.e., a true zero) and therefore be interpreted as indicative of low sociality. Therefore, we decided to include these meaningful zeros in our analyses.

Infants' Affective Reactions During Live Interaction

Infants' affective reactions to the experimenter during the Social Interaction Task of the ESCS were coded from video recordings of the infants during the task (~ 20-30 seconds per episode). Four adult coders independently rated the infants' affective reactions on a scale from -3 (very aversive/fearful) to +3 (very joyful/excited), with 0 as neutral, separately for the singing part and each tickling part in each episode, generating 8 ratings in total (i.e., (1 singing + 3 tickling) * 2 episodes). All four raters had an excellent inter-rater agreement on their ratings for all videos with an average ICC of .94 (95% CI = [.93, .95]). Thus, we calculated the average ratings across the four raters. Furthermore, the affective reaction ratings were positively correlated between the singing and tickling parts, $r(95) = .45$, $p < .001$) and between the two episodes of the Social Interaction Task, $r(94) = .74$, $p < .001$. Therefore, we averaged the ratings between the singing and tickling part and between the two episodes to generate one overall rating (ESCS positive affect scores) for each infant at each age for further analyses.

Missing Data and Data Exclusion Criteria

Not all infants contributed usable data for all measures (salivary oxytocin, facial affect to the smiling person video, and positive affect during live interaction) at all ages.

We excluded samples of infants' salivary oxytocin levels that fell more than 3 standard deviations from the mean at each age (4 months: > 9.95 pg/ml; 8 months: > 12.70 pg/ml; 14 months: > 13.33 pg/ml) due to the low reliability of those samples (Fujiwara et al., 2019). This resulted in 4 oxytocin samples from 4 visits being excluded (1 from 4 months, 2 from 8 months, and 1 from 14 months). Moreover, non-detectable levels of oxytocin were due to low saliva volume used for extraction 7% of the time (21 samples out of 306 total) or undetectable levels of oxytocin 20% of the time (60 samples) and were therefore excluded from analyses (see Table 3 for details). These rates of undetectable salivary oxytocin samples are comparable to the rates reported previously in 1- to 3-month-olds (Grewen et al., 2010). Together, 21 visits from 17 infants had no usable oxytocin data and were excluded from analyses of only salivary oxytocin.

Smiling person video data were missing due to technical errors (no infant view was recorded from within Tobii) for 2% of test sessions (3 out of 153 total sessions) and the infant being too fussy (1 session), one at 4 months, two at 8 months, and one at 14 months of age. Moreover, affective reaction data during live interaction were missing from two 14-month sessions (out of 44) due to the infants being too fussy.

As a result, the analyses involving only salivary oxytocin included 58 infants (4 months: $N = 51$, 8 months: $N = 44$, 14 months: $N = 37$). The analyses involving only positive facial affect to the smiling person video included 62 infants (4 months: $N = 55$, 8 months: $N = 51$, 14 months: $N = 43$). The analyses involving both oxytocin and positive facial affect to the smiling person video included 57 infants (4 months: $N = 50$, 8 months: $N = 42$, 14 months: $N = 37$). For the 14-month visit, the analyses involving both oxytocin and affective reactions during live interaction included 36 infants (see Table 4 for details).

Table 4

Numbers and Percentages[a] of Infants Included and Excluded in Each Analysis

Inclusion/Exclusion Criteria	*Infants*[b] *Included*	*N (%)* 4 months	8 months	14 months
For analyses of salivary OT only	**58**			
No usable samples (excluded)		5 (8.9%)	9 (17.0%)	7 (15.9%)
Had only usable sample 1		8 (14.3%)	11 (20.8%)	4 (9.1%)
Had only usable sample 2		4 (7.1%)	6 (11.3%)	10 (22.7%)
Had both usable samples		39 (69.6%)	27 (50.9%)	23 (52.3%)
For analyses of positive facial affect to video only	**62**			
No affect data (excluded)		1 (1.8%)	2 (3.8%)	1(2.3%)
Had usable affect data		55 (98.2%)	51 (96.2%)	43 (97.7%)
For analyses of salivary OT and positive facial affect to video	**57**			
No OT or affect data (excluded)		0	0	1 (2.3%)
Had only usable OT data		1 (1.8%)	2 (3.8%)	0
Had only usable affect data		5 (8.9%)	9 (17.0%)	6 (13.6%)
Had both usable OT and affect data		50 (89.3%)	42 (79.2%)	37 (84.1%)
For analyses of salivary OT and affective reaction in live interaction	**36**			
No OT or affect data (excluded)		-	-	1 (2.3%)
Had only usable OT data		-	-	1 (2.3%)
Had only usable affect data		-	-	6 (13.6%)
Had both usable OT and affect data		-	-	36 (85.7%)

Note. OT = oxytocin. [a]Percentages of sessions excluded were calculated based on the number of infants who came in but failed to contribute data of interest out of the total number of infants who came in for a visit at each age (56 at 4 months, 53 at 8 months, and 44 at 14 months). [b]Number of infants who were included in the analyses in at least one visit.

Data Analysis

All data analyses were conducted using R (version 4.0.2) and RStudio (version 2021.09.0). Given the nested structure of our data (multiple ages nested within individual infants), we conducted linear mixed effects models using "lme4" (Bates et al., 2015) and "lmerTest" (Kuznetsova et al., 2017) packages, as well as generalized linear mixed effects models (to account for zero-inflation in positive facial affect measures) using the "GLMMadaptive" package (Rizopoulos, 2022).

Preliminary Analysis

Prior to the primary analysis, we conducted a series of preliminary analyses to explore whether infants' salivary oxytocin levels varied by their sex. We then examined the correlations between our three measures of positive facial affect (duration, intensity, and AUC) to further validate the measures. Given the amount of missingness in our oxytocin data, we also examined whether the missingness contributed to differences in our primary outcome variable (i.e., positive affect AUC).

Infant Salivary Oxytocin

Hypothesis 1: Within-Age Reliability of Oxytocin. To examine the inter-sample reliability within each age, we first calculated bivariate Pearson correlations between levels of oxytocin in sample 1 and sample 2 within each age. We next confirmed there were no statistically significant differences in oxytocin levels between samples 1 and 2 with paired-samples *t* tests within each age. After confirming they were correlated and not statistically different from one another, we averaged oxytocin levels across the two samples for each infant at each age for subsequent analyses.

Hypothesis 2: Developmental Stability in Oxytocin. Using these average oxytocin levels, we then examined whether there was developmental stability with age by testing for bivariate Pearson correlations among 4, 8, and 14 months. We also examined the average ICC for consistency of oxytocin levels across all three ages to examine the overall developmental stability. We used ICC for consistency to examine developmental stability while allowing for intraindividual developmental changes.

Hypothesis 3: Developmental Change in Oxytocin with Age. We examined whether infants' oxytocin levels, averaged across sample 1 and sample 2, changed with age (categorical) with a 2-level mixed effects ANOVA model. We further explored the effect of age by comparing oxytocin levels with planned repeated contrasts between 4 and 8 months, as well as between 8 and 14 months.

Infants' Positive Affect to a Smiling Person

Hypothesis 4: Stability of Individual Differences in Infants' Positive Affect. We first examined whether there was developmental stability with age in infants' positive facial affect while watching the smiling person video by testing for bivariate correlations among the positive affect AUC at 4, 8, and 14 months using Spearman ρ to account for the non-normal distribution of the positive affect AUC (de Winter et al., 2016). We also examined the average ICCs for consistency of positive affect AUC across all three ages to examine the overall developmental stability. In addition, we conducted a Spearman correlation to examine whether there was stability in infants' positive affect across the two tasks (smiling person video vs. live interaction) at 14 months of age.

Hypotheses 5 and 6: Positive Affect Associated with Age and Oxytocin. We then investigated how infants' positive affect changed with their age and levels of salivary oxytocin

using three 2-level generalized hurdle mixed effects models, each for one of our positive affect measures as the outcome variable (a hurdle Poisson model for positive affect duration and hurdle lognormal models for positive affect intensity and positive affect AUC). The models included fixed effects of salivary oxytocin levels (mean centered), age (continuous; recoded as 0 = 4 months old; 2 = 8 months old; 5 = 14 months old), and their interaction, as well as random intercepts and random slopes of salivary oxytocin levels at the infant-level. The model also included zero-inflated terms of the fixed intercept and fixed effect of oxytocin levels to account for the zero-inflated distribution of these measures (see Supplementary Materials). We centered the infant's age at 4 months (i.e., 0 = 4 months old) to simplify the interpretation of results as the main effect of OT level on positive affect AUC reflects the effect of OT level at 4 months of age.

By 3 months of age, infants already show differential social perception and preference based on racial and ethnic background of the social partner, including preferential attention to own-race faces, greater likelihood to approach strangers of their own race than of other races, and attaching more positive affective valence to own-race than other-race faces (see Quinn et al., 2018 for a review). Hence, we included, relative to the stimulus (which was White, Non-Hispanic/Non-Latino), infants' race (matching, own-race: White vs. mismatching, other-race: Non-White) and ethnicity (matching, own-ethnicity: Non-Hispanic/Non-Latino vs. mismatching, other-ethnicity: Hispanic/Latino), as well as their interactions with oxytocin levels and age, as control variables in the model. Even though our study was not designed to systematically investigate ethnicity and race effects, we included them as variables in our analyses to control for potential effects of race/ethnicity matching/mis-matching on infant affective reactivity.

To further test our final hypothesis, we also examined the ESCS positive affect scores, with higher scores indicating more positive affective reactions to the live person at 14 months to

438 determine if it is associated with their salivary oxytocin levels at 14 months using a Pearson
439 correlation.

440 The de-identified raw data, as well as the pre-processed data and R code file for main
441 analyses, are available at Open Science Framework repository:
442 https://osf.io/y8m6f/?view_only=e9f973b7cf26448ea29a30b8cd51a22b.

443 **Results**

444 **Preliminary Analysis**

445 We detected no significant sex differences or differences in infants' attention to the
446 stimuli in relation to infants' salivary oxytocin levels (see Supplementary Materials); therefore,
447 we combined males and females into one group for subsequent analyses (see Supplementary
448 Materials with main analysis including infants' sex).

449 We next examined whether our measures of infants' positive facial affect during the
450 smiling person video (i.e., positive affect duration, positive affect intensity, and positive affect
451 AUC) were capturing the same underlying construct using Spearman correlations. We found that
452 the three measures of infants' positive affect were positively correlated with each other: between
453 positive affect AUC and positive affect duration, $\rho(127) = .94$, $p < .001$, between positive affect
454 AUC and positive affect intensity, $\rho(127) = .95$, $p < .001$, and between positive affect duration
455 and positive affect intensity, $\rho(127) = .90$, $p < .001$. Therefore, we focused on positive affect
456 AUC as our primary dependent measure; a series of parallel analyses using positive affect
457 duration and positive affect intensity as outcome variables revealed the same pattern of primary
458 findings (see Supplementary Materials).

459 We also detected no difference in the positive affect AUC to the smiling person video
460 between infants with ($M = 4.56$, $SD = 4.05$) and without ($M = 3.60$, $SD = 3.37$) usable salivary

oxytocin data, $t(147) = 1.10$, $p = .271$. Moreover, there was no difference in infants' affective reactions to the live social partner between infants with ($M = .77$, $SD = .78$) and without ($M = .70$, $SD = .80$) usable salivary oxytocin data, $t(40) = .83$, $p = .413$. Together, these findings suggest that infants who had missing salivary oxytocin data did not appear to differ in their positive affect from those who had usable data. However, we acknowledge that the absence of differences in positive affect due to missingness in oxytocin data does not preclude an impact on their associations. Therefore, this remains an unavoidable limitation of our study.

Infant Salivary Oxytocin

Hypothesis 1: Within-Age Reliability of Oxytocin

We found positive correlations between infants' salivary oxytocin levels in the first and second samples taken within each visit: at 4 months, $r(37) = .49$, $p = .002$, 8 months, $r(25) = .82$, $p < .001$, and 14 months, $r(21) = .50$, $p = .015$ (Figure 3). Furthermore, we confirmed that such within-age stability was unlikely confounded by the lengths of intervals between sampling by calculating the partial correlation between sample 1 and sample 2 at each age with the sampling intervals as a control variable (4 months: $r(36) = .49$, $p = .002$; 8 months: $r(25) = .81$, $p < .001$; 14 months: $r(21) = .47$, $p = .026$). These results together suggest a moderate to high level of measurement reliability. We additionally confirmed that there were no statistically significant differences between samples 1 and 2 at any of the ages: 4 months, $t(38) = -.53$, $p = .600$, 8 months, $t(26) = .35$, $p = .730$, 14 months $t(22) = -.99$, $p = .331$. Given this consistency, in subsequent analyses we report the average of these two samples, for each infant at each age.

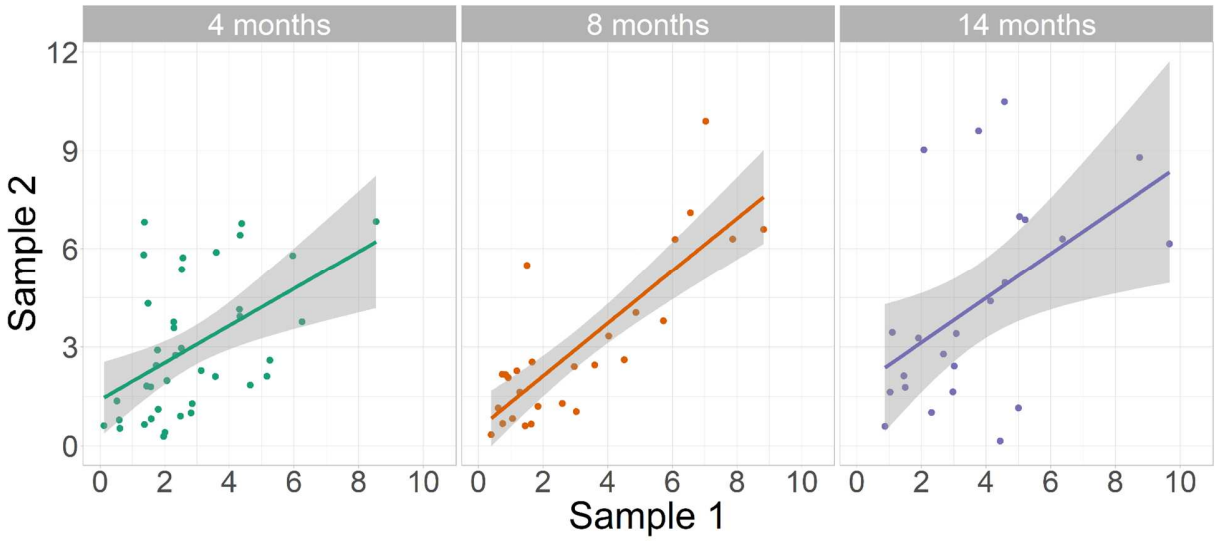


Figure 3. Infants' salivary oxytocin levels (pg/ml), measured from saliva sample 1 and sample 2, were positively correlated with each other (*p*s ≤ .015) within each age visit (4, 8, and 14 months). Key: lines = regression lines, gray shadow = standard errors of the regression, dots = data points for individual infants.

Hypothesis 2: Developmental Stability in Oxytocin

Infants' salivary oxytocin levels were positively correlated between 4 and 8 months, $r(36) = .43$, $p = .007$ (Figure 4A), between 8 and 14 months, $r(29) = .59$, $p < .001$ (Figure 4B), and between 4 and 14 months, $r(33) = .57$, $p < .001$ (Figure 4C). Moreover, across all three ages, the average ICC for consistency of a two-way mixed effects model was .75 (95% CI = [.62, .84]), suggesting a moderate to high level of stability with age, across 4, 8, and 14 months. Together, these findings suggest that infants have moderately to highly stable individual differences in their salivary oxytocin levels across infancy.

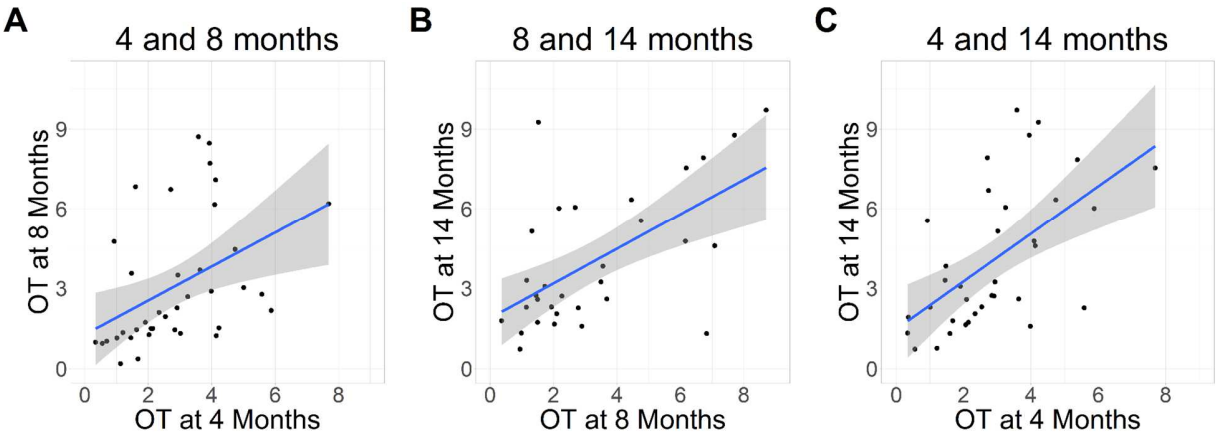


Figure 4. Individual differences in infants' salivary oxytocin levels (OT; pg/ml) at younger ages positively correlated with their levels at older ages (*p*s ≤ .007). Key: lines = regression lines, gray shadow = standard errors of the regression, dots = data points for individual infants.

Hypothesis 3: Developmental Changes in Oxytocin with Age (Hypothesis 3)

The mixed effects ANOVA model, controlling for infant-level variances, revealed that infants' salivary oxytocin levels changed with age, $F(2, 85.41) = 7.05$, $p = .001$ (Figure 5), $\eta_p^2 = .13$. More specifically, while we detected no change from 4 months ($M = 2.53$, $SD = 1.67$; range: .34 - 7.69) to 8 months ($M = 2.91$, $SD = 2.32$; range: .19 - 8.71), $t(86.94) = .98$, $p = .328$, $d = .21$, there was an increase from 8 to 14 months ($M = 4.00$, $SD = 2.58$; range: .73 - 9.71), $t(85.16) = 2.65$, $p = .010$, $d = .57$. These findings suggest a non-monotonic developmental pattern of oxytocin across the first year after birth, which is characterized by an early stability followed by a later increase in salivary oxytocin around the infants' first birthday.

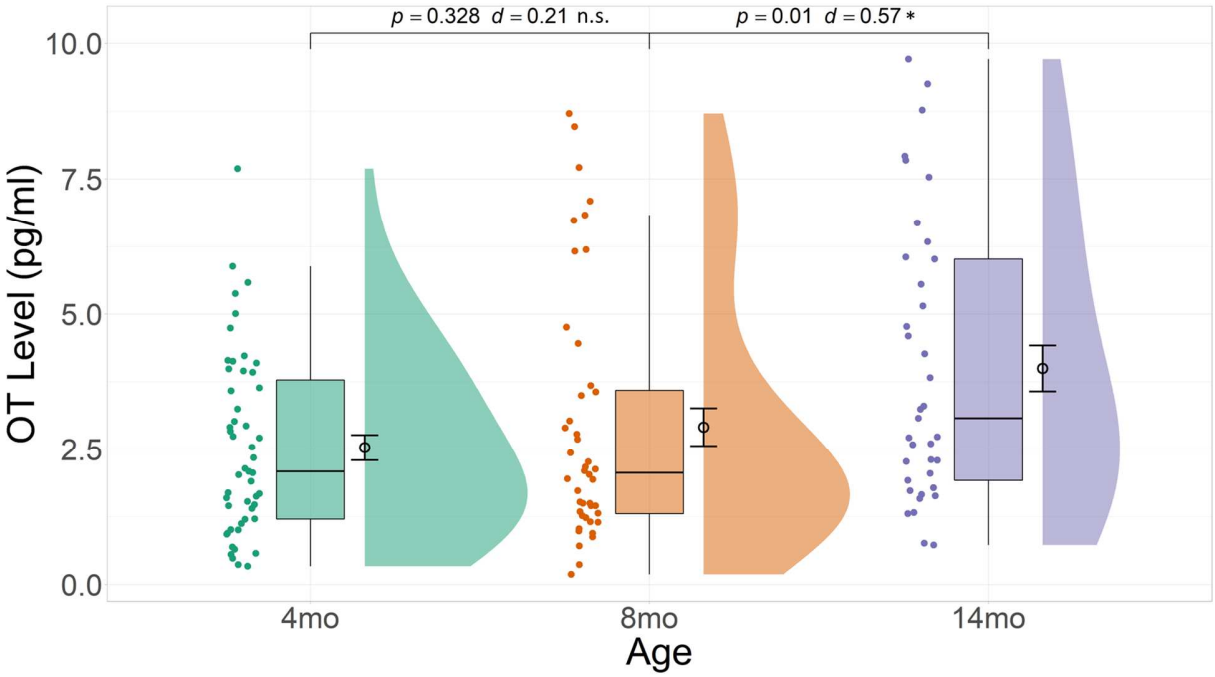


Figure 5. Infants' salivary oxytocin levels (OT) at each age. We detected no change from 4 to 8 months (p = .328), then an increase with age, from 8 to 14 months (p = .010). Boxplots: Lines within the boxplots indicate the medians. Hinges of the boxplots show the first (bottom) and third (top) quartiles. The whiskers extend up to 1.5 × Interquartile Range (IQR; distance between top and bottom hinges), above and below the hinges. Colored jittered dots to the left of the boxplots show the oxytocin levels for individual infants. Density plots to the right of the boxplots show the distributions of oxytocin levels. Open dots and error bars on the left edge of the density plots show the means and standard deviations.

Infants' Positive Affect to a Smiling Person

Hypothesis 4: Stability of Individual Differences in Infants' Positive Affect

We did not detect correlations in infants' positive affect AUC between 4 and 8 months ((44) = .09, p = .543; Figure 6A) or 4 and 14 months (ρ(37) = .25, p = .127; Figure 6B), but a positive correlation between 8 and 14 months (ρ(36) = .39, p = .015; Figure 6C), suggesting increasingly stable individual differences in infants' positive affect with age. In addition, across all three ages, the average ICC of a two-way mixed effects model for consistency was .54 (95%

CI = [.31, .71]), suggesting a small to moderate level of stability across 4, 8, and 14 months of age.

Furthermore, at the age of 14 months, the infants' positive affect AUC to the smiling person video correlated positively with infants' affective reactions during the live interaction, $\rho(40) = .31$, $p = .045$. This finding suggests stability of individual differences in positive affect across different contexts.

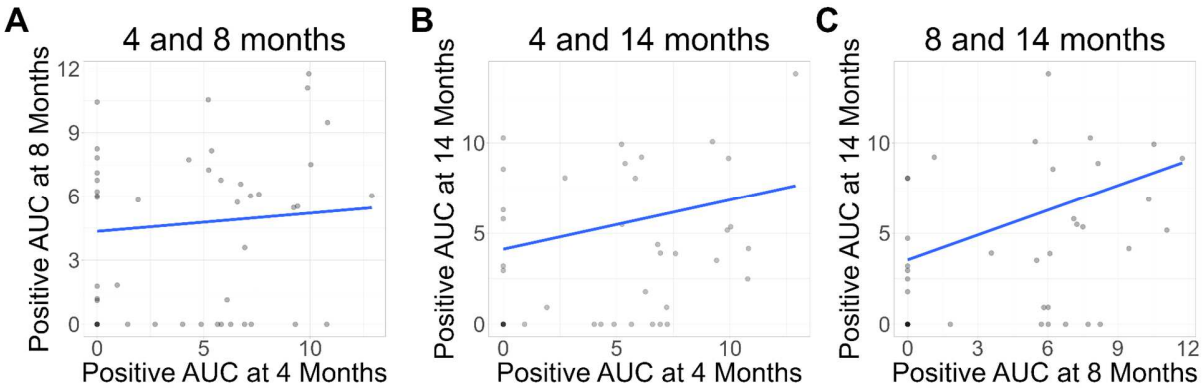


Figure 6. Individual differences in infants' positive affect AUC at 8 months positively correlated with their levels at 14 months (right plot; $p < .05$). Key: blue lines = fitted line on the rank-ordered positive affect AUC measures to show the Spearman correlation, dots = data points of positive affect AUC of individual infants (darker = denser distribution).

Hypothesis 5 & 6: Positive Affect Changed with Age and Oxytocin Levels

We did not detect a main effect of age in positive affect AUC, $b = -.04$, 95% CI = [-.10, .01], $SE = .03$, $z = -1.49$, $p = .136$, $\beta = -.12$, suggesting that infants' positive facial affect to a smiling person may not change with age at the group-level. However, we found that the effect of age varied by the infants' ethnic and racial background (Figure 7). There was a statistically significant interaction between ethnicity and age, $b = .06$, 95% CI = [.00, .11], $SE = .03$, $z = 2.03$, $p = .043$, $\beta = .17$. Further, the simple slopes analysis revealed that infants whose ethnicity matched the stimulus (Non-Hispanic/Non-Latino) had stable positive affect AUC with age ($\beta =$

552 .05, p = .690), but those whose ethnicity did not match the stimulus (Hispanic/Latino) showed
553 declining positive affect AUC with age (β = -.29, p = .010; Figure 7A). We also found an
554 interaction between infants' race and age that approached but did not reach statistical
555 significance, b = .06, 95% CI = [.00, .12], SE = .03, z =1.91, p = .057, β = .18; however, the
556 patterns were consistent with those we found for ethnicity: Infants whose race matched the
557 stimulus (White) had stable AUC with age (β = .05, p = .650), but those whose race did not
558 match the stimulus (Non-White) showed declining AUC with age (β = -.30, p = .023; Figure
559 7B).
560

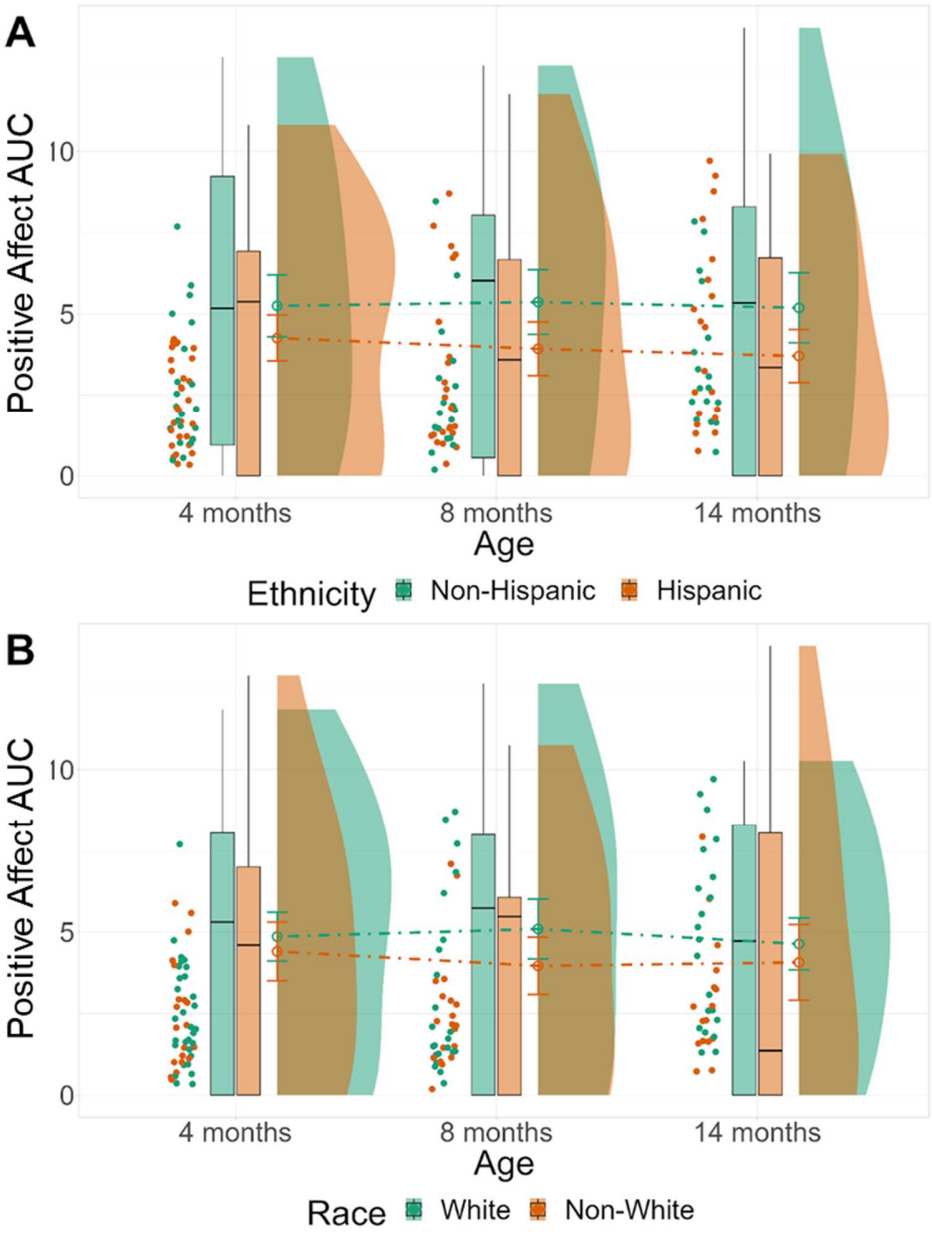
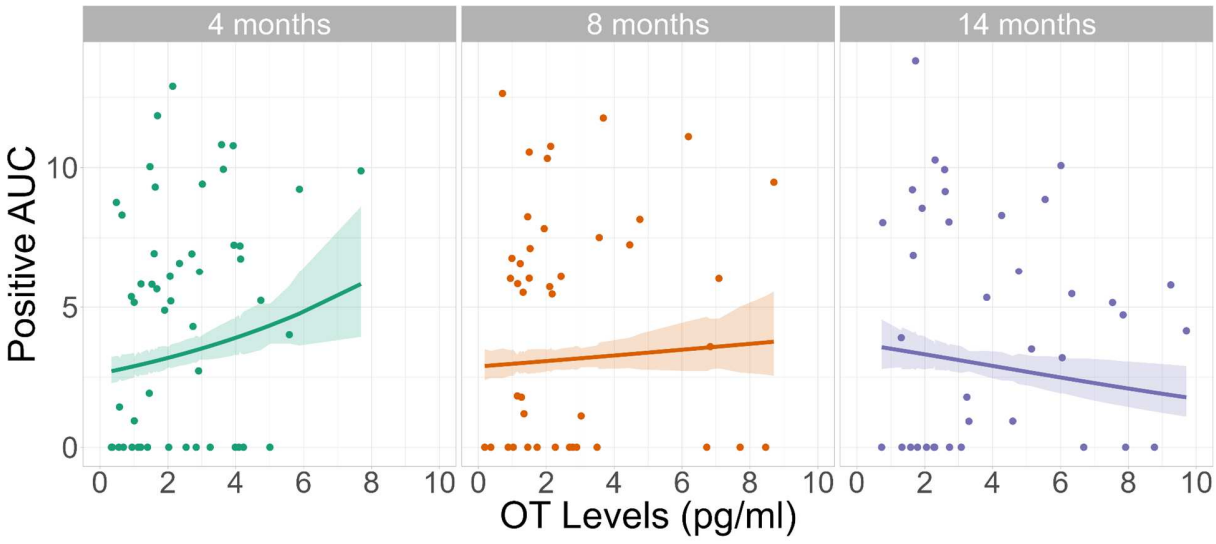


Figure 7. Infants with different (A) ethnic and (B) racial backgrounds show differential age-related changes in the positive affect AUC to the smiling person video (green = Non-Hispanic/Non-Latino, White infants; orange = Hispanic/Latino, Non-White infants) at 4, 8, and 14 months. Hinges of the boxplots show the first (bottom) and third (top) quartiles. The whiskers extend up to 1.5 × Interquartile Range (IQR; distance between top and bottom hinges), above and below the hinges. Dots to the left of the boxplots show the positive affect AUC for individual infants. Density plots to the right of the boxplots show the distributions of the positive affect AUC. Open dots and error bars on the left edge of the density plots show the means and standard errors respectively.

572 Moreover, there was a main effect of oxytocin levels on infants' positive affect AUC, b =
573 .11, 95% CI = [.01, .21], SE = .05, z = 2.11, p = .034, β = .18, which reflected that the infants
574 with higher oxytocin levels exhibited more positive facial affect at the age of 4 months. The
575 main effects of oxytocin and age were further qualified by an oxytocin × age interaction, b = -
576 .05, 95% CI = [-.08, -.02], SE = .01, z = -3.38, p < .001, β = -.12, suggesting that the association
577 between oxytocin levels and positive affect AUC varied with age (Figure 8). Simple slope
578 analyses revealed that there was a positive association between infants' positive affect and their
579 oxytocin levels at 4 months (β = .18, p = .034), but no association at 8 months (β = .01, p =
580 .852), and then a negative association at 14 months (β = -.25, p = .011). We detected no
581 interactions between oxytocin levels and infants' races or ethnicities, including no 2-way
582 interactions between oxytocin and race/ethnicity, and no 3-way interactions among age,
583 oxytocin, and race/ethnicity (ps > .10).



584

585 ***Figure 8***. Infants' salivary oxytocin levels (OT; x-axis) were associated with their
586 positive affect AUC (y-axis), which changed with age. Key: lines = predicted regression
587 functions; colored shadow = standard errors for the predictions; dots = observed data
588 points for individual infants.
589

Furthermore, we also found that, at the age of 14 months, infants' ESCS positive affect scores during live interactions were negatively correlated with their salivary oxytocin levels, $r(34) = -.41$, $p = .012$. That is, infants with lower oxytocin levels responded to the experimenters' singing and tickling with greater positive affective reactions, suggesting that the negative association between oxytocin levels and positive affect at 14 months may generalize across different social contexts.

Discussion

The current study aimed to characterize typical, normative longitudinal developmental patterns of salivary oxytocin levels in infancy and its association with social development. We examined infants' salivary oxytocin levels over time and in relation to their positive affect to a smiling woman. We found that infants' salivary oxytocin levels were a stable and reliable measure of individual differences and were linked to their socioemotional development. Understanding the normative development of infants' endogenous oxytocin levels may facilitate earlier identification of atypical deviations in physiological systems predictive of later social disruptions (Fujisawa et al., 2014; Lebowitz et al., 2016).

Reliability and Stability of Infant Salivary Oxytocin Across Samples and with Age

As we hypothesized, infants' salivary oxytocin levels were moderately to highly stable over minutes within a lab visit (Hypothesis 1; Figure 3). This finding is consistent with reports that infants between the age of 4 and 13 months show moderate stability in salivary oxytocin levels over brief periods (Brzozowska et al., 2022; Feldman et al., 2010; Markova, 2018). Moreover, we used a sample extraction procedure—a process that is recommended for eliminating potential interfering molecules—which enhances measurement accuracy of oxytocin concentrations in infants' saliva (Tabak et al., 2022). Our samples revealed higher short-term

(within-visit) stability (*r*s = .49-.82; Figure 3) compared to previous studies that did not use (or report) an extraction procedure (11/13 studies in Table 1; within-visit stability: *r*s = .18-.68).

We also found intraindividual stability of salivary oxytocin levels across longer intervals, from 4 to 14 months (Hypothesis 2; Figure 4). To our knowledge, the present study is the first to test for developmental stability in salivary oxytocin levels over such a long (i.e., 10-month) time span in infancy. Our findings suggest baseline salivary oxytocin may be a stable trait that can be reliably measured in infancy.

Age-Related Patterns of Overall Oxytocin Levels

We found that infants' salivary oxytocin levels did not change between 4 and 8 months, but increased from 8 to 14 months, which partially supports our hypothesis of increase with age (Hypothesis 3; Figure 5). Furthermore, our finding of consistent levels of salivary oxytocin from 4 to 8 months is in line with research on a cross-sectional sample of 3- to 10-month-olds (Fujiwara et al., 2019). However, another cross-sectional study reported salivary oxytocin levels and age were negatively correlated across a wider age range, from 5 months to 7 years old (Nishizato et al., 2017). Together, these findings suggest that salivary oxytocin levels may not change significantly in early infancy (e.g., first ~ 8-10 months), but may show increases through their first birthday, possibly followed by later decreases in early childhood.

We also noted that, overall, the levels of oxytocin we observed appeared lower than those reported in previous studies for these age groups (Feldman et al., 2010; Fujiwara et al., 2019). However, this apparent discrepancy is likely due to methodological improvements: we used a solid-phase extraction procedure (Simpson et al., 2014; Holt-Lunstad et al., 2008) with a slight modification of additional washes (Szeto et al., 2011). Such additional procedures allow for an approximately 8.3-fold concentration factor in our samples, which is useful for studies in infants

who do not always produce a large quantity of saliva. Our approach, however, is likely to give more precise oxytocin levels because it minimizes the effect of potentially interfering molecules, reduces sample matrix effects, and concentrates and enriches the analyte of interest from the sample (Szeto et al., 2011; Tabak et al., 2022). Future studies are encouraged to examine the measurement accuracy of oxytocin levels in saliva samples with and without extraction, particularly in low volumes.

Development of Positive Affect to a Smiling Person

We found a small to moderate level of stability of individual differences in infants' positive facial affect to a smiling person video with age, beginning around 4 months of age and growing stronger between 8 and 14 months of age (Hypothesis 4a; Figure 6). These findings suggest our measure is reliable, consistent with reports of developmental stability in infants' temperamental positive affect, typically measured through parent-report or observations (Bornstein et al., 2015; Putnam & Stifter, 2002; Rothbart, 1986). Moreover, we also found intraindividual stability of positive facial affect across contexts (i.e., viewing a simulated social video of a smiling person and during a live interaction; Hypothesis 4b), establishing convergent validity for our positive affect measures.

In contrast to our hypothesis that infants would exhibit increases in positive affect with age (Hypothesis 5), we did not detect overall changes from 4 to 14 months in infants' positive affect to the unfamiliar smiling person. In contrast to reports of increasing positive affect from 1 to 6 months of age during parent-infant interactions (Messinger & Fogel, 2007; Messinger et al., 2001), our findings suggest that infants may show different developmental patterns of positive affect to unfamiliar people.

Unexpectedly, among infants whose race and ethnicity matched the unfamiliar person (White and/or Non-Hispanic/Latino), infants' positive affect remains relatively high consistently from 4 to 14 months (Figure 7). However, in contrast, among infants whose race and ethnicity were different from the unfamiliar person (Non-White and/or Hispanic/Latino), infants' positive affect appeared to decline from 4 to 14 months (Figure 7). These findings are consistent with reports of increasing fear of strangers from about 6 to 12 months of age (e.g., Brooker et al., 2013). A limited number of studies reveal that, as infants grow more fearful of strangers (e.g., 5-9 months old), they may express fewer positive affect towards strangers especially of other racial and ethnic backgrounds (Berberian & Snyder, 1982; Feinman, 1980).

Although not designed to investigate race and ethnicity effects, our study comprises a racially and ethnically diverse U.S. sample, which allows for a preliminary examination of own-race/ethnicity versus other-race/ethnicity effects in the development of positive affect to unfamiliar people. For many infants in our sample, this stranger (a White female) may appear racially different from those in their family group. Given that race influences infants' social perceptions from a young age (Hwang et al., 2021; Kelsey et al., 2019; Singh et al., 2022), our findings highlight a need to examine infants' positive affective reactions to strangers of various racial and ethnic groups.

Oxytocin and Positive Affect to a Smiling Person

We found that salivary oxytocin levels of 4-month-old infants positively predicted their positive facial affect to a video of a smiling person at 4 months of age, as we hypothesized (Hypothesis 6a; Figure 8). This finding is consistent with a report that 4- to 6-month-olds' salivary oxytocin levels are positively associated with their positive facial and vocal expressions during parent interactions (Feldman et al., 2010), suggesting these effects may extend beyond

caregivers to strangers. Therefore, natural baseline levels of oxytocin in infants' saliva may be useful as an objective and unobtrusive index to augment current measurement of infant sociality, particularly if future research continues to replicate the association across studies and with a diverse variety of assessments of sociality.

However, when we examined this association between salivary oxytocin and positive facial affect to the smiling person later, at 8 months of age, we found they were not associated, and unexpectedly, at 14 months of age, were negatively associated with both positive affective measures (smiling person video and the live interaction) (Hypothesis 6b; Figure 8). These findings suggest that the association between infants' salivary oxytocin and their social behavior may change over time. To our knowledge, this is the first study of infants' salivary oxytocin in relation to their positive affect to unfamiliar people in infancy.

These age changes in the association between oxytocin and positive affect are congruent with the social salience hypothesis of oxytocin, which proposes that oxytocin may amplify the salience of social information, generally (e.g., relative to nonsocial information), amplify specific types of social cues (e.g., eye-contact, facial expression, social identity), and enhance social information processing (e.g., improved recognition) (see Shamay-Tsoory & Abu-Akel, 2016 for a review). More supporting evidence for this hypothesis comes from adult studies: For example, adults with higher salivary oxytocin levels rated positive faces as more positive compared to those with lower oxytocin levels (Bhandari et al., 2014). Oxytocin also enhances adults' perceptions of positive and negative faces, improving their emotion recognition (Bhandari et al., 2014; Gamer et al., 2010; Groppe et al., 2013) and increasing their preference for in-group over out-group members (De Dreu et al., 2011). While this theory has yet to be explored in infants, there are some empirical findings that are consistent with this hypothesis. For example, among

infants between 6 and 13 months old, higher salivary oxytocin levels are associated with greater attention captured by human faces, competing with images of other objects (Brzozowska et al., 2022).

In the current study, the social salience hypothesis would predict that the social signals in the smiling person video (i.e., eye contact, smiles) may be more salient to infants with higher levels of salivary oxytocin, which in turn elicits more positive facial affect in them, as compared to those with lower oxytocin levels, which is consistent with our findings in 4-month-olds. Further, this hypothesis could explain why this association changed with age: a socially-relevant contextual feature of the smiling person video—the social identity of the smiling video person and the live social partner during the ESCS interaction—might have become increasingly salient to the high-oxytocin infants as they grew, which might explain the high-oxytocin infants' decline with age in positive facial affect to the video, while the low-oxytocin infants maintained a consistent level of positive facial affect. With age, infants grow both more socially capable, but also more socially selective in how and with whom they interact (Bigelow et al., 2008; Morrison et al., 2021). Our adult models (video and live) were of different racial and/or ethnic backgrounds from most of our infant participants. Therefore, the social identity of our adult models as out-group members might be more salient to infants with higher oxytocin levels, which elicited fewer positive affect in them, as compared to their low-oxytocin peers. This interpretation conforms to the previous findings in adults: Oxytocin is positively associated with ethnocentrism and prosocial behaviors only towards in-group members (Bethlehem et al., 2014; De Dreu et al., 2011; Triki et al., 2022). Furthermore, a recent study suggests 14-month-olds' levels of salivary oxytocin may be related to their perception of facial race categories (Ferera et al., 2023). Nonetheless, we did not detect an interaction effect between infants' race/ethnicity

and infants' salivary oxytocin levels on infants' positive facial affect. Consistent with the social salience hypothesis, the current study provides preliminary evidence for changing relationships between salivary oxytocin and social behavior during early development. Due to the design and limited power of our study, we cannot draw definitive conclusions about the developmental changes in such a relationship, which might be subject to contextual factors including race and ethnicity of the infants and their social partner, as well as the social contexts; therefore, future studies must further examine these developmental changes in larger and more diverse samples and explore the effects of contextual variables more systematically.

Here we presented a potentially friendly stranger, reflecting a typical or common encounter for infants, as we were primarily interested in infants' positive affect as an indicator of their affiliation and sociality (Barrett, 1998; Campos et al., 1994). Our stimulus was not designed to elicit overt distress or negative affect. Therefore, we did not examine negative affect in the current study. For a more comprehensive understanding of the roles of salivary oxytocin in socio-emotional development, future research is needed to examine the relationship between salivary oxytocin and negative affect using a more appropriate design (e.g., the Still-Face paradigm; Tronick et al., 1978; or the Strange Situation procedure; Ainsworth et al., 1979).

While the current study focused on healthy infants with no known developmental delays, there remains a need to develop early indicators of disruptions to development. Individuals with Autism Spectrum Disorder (ASD) often experience disruptions to social development (Jones & Klin, 2013; Richardson et al., 2020), which may be linked to atypicality in their oxytocin system (Krol et al., 2021; Stavropoulos & Carver, 2013). Children with ASD have lower levels of endogenous oxytocin than neurotypical children, highlighting the potential of oxytocin as a promising indicator of individual differences in physiology underlying social behaviors

(Feldman et al., 2014; John & Jaeggi, 2021). Nonetheless, the associations between infants' endogenous oxytocin levels and later ASD symptoms, as well as the underlying mechanisms, remain to be explored.

Oxytocin is pleiotropic and involved in physiological processes in various locations of human bodies including the peripheral (e.g., measured in saliva or plasma) and central nervous systems (e.g., measured in cerebrospinal fluid) (Alves et al., 2015; Scatliffe et al., 2019; White-Traut et al., 2009). In the current study, we only measured salivary oxytocin; therefore, we cannot draw any conclusions about oxytocin levels centrally or in other parts of the body (e.g., blood). A number of previous studies have reported associations in oxytocin among different systems and their potential parallel effects on social behavior in adults and infants (Alves et al., 2015; Chen et al., 2022; Clark et al., 2013; Martin et al., 2018; Ross and Young, 2009; Strathearn et al., 2009). However, findings are mixed as to whether there is a reliable link between peripheral and central oxytocin levels, suggesting there may be complex relations between them (Kagerbauer et al., 2019; Scatliffe et al., 2019). Nonetheless, given the difficulty of collecting cerebrospinal fluid samples in infants and young children, saliva provides an unobtrusive way to measure oxytocin levels. Our findings provide additional evidence for the feasibility and reliability of using salivary oxytocin levels to investigate the neurobiology of infant social development. Future studies are needed to fully understand the relations between peripheral and central oxytocinergic processes.

Conclusions

Infant salivary oxytocin appears to be a stable and reliable physiological measure in early infancy, but how its levels relate to infants' emotional behaviors varies by age. These findings preliminarily indicate that salivary oxytocin may undergo developmental changes in what it

reflects. Our findings highlight new research questions about the mechanisms underlying the developmental increases in salivary oxytocin levels in infancy and the implications of the developmental changes in the associations between infants' salivary oxytocin levels and their positive affect. Further studies are needed to more fully explore whether infants' salivary oxytocin levels are associated with other aspects of infants' social and emotional development. Nonetheless, our findings help establish healthy baseline levels of salivary oxytocin in infancy. Establishing these normative levels reflects a first step towards uncovering how infants' psychophysiological systems may hold important clues about how we can support infants' health and wellbeing (Moberg et al., 2020; Norholt, 2021; Vittner et al., 2020).

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