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RESEARCH ARTICLE

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# Early maternal and paternal caregiving moderates the links between preschoolers' reactivity and regulation and maturation of the HPA-immune axis

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## Abstract

While early caregiving and child's temperamental dispositions work in concert to shape social-emotional outcomes, their unique and joint contribution to the maturation of the child's stress and immune systems remain unclear. We followed children longitudinally from infancy to preschool to address the buffering effect of early parenting on the link between temperamental dysregulation and hypothalamic–pituitary–adrenal (HPA)-immune axis in preschool-aged children. Participants included 47 typically developing children and their 94 parents in both mother-father and two-father families followed across the first 4-years of family formation. In infancy, we observed parent-infant synchrony and measured parental oxytocin; in preschool, we observed temperamental reactivity and self-regulation and assessed children's cortisol and secretory Immunoglobulin A (s-IgA), biomarkers of the stress and immune systems. Greater self-regulation and lower negative emotionality were associated with lower baseline s-IgA and cortisol, respectively. However, these links were defined by interactive effects so that preschoolers with low self-regulation displayed higher s-IgA levels only in cases of low parent-infant synchrony and negative emotionality linked with greater baseline cortisol levels only when parental oxytocin levels were low. Results emphasize the long-term stress-buffering role of the neurobiology of parental care, demonstrate comparable developmental paths for mothers and fathers, and delineate the complex developmental cascades to the maturation of children's stress-management systems.

**KEYWORDS**
longitudinal studies, oxytocin, self-regulation, stress, synchrony, temperament

## 1 | INTRODUCTION

Decades of theoretical and empirical research have underscored the role of the early social environment, as interacting with temperamental dispositions, in shaping psychological adaptation across development (Gallagher, 2002; Kiff et al., 2011). It has been suggested that research on the interaction of *proximal* (e.g., parenting) and *individual* (e.g., temperament) processes considers more precisely the complexity of normative and pathological development and their mutual influences (Bronfenbrenner & Morris, 1998). Over the years, several models have been formulated to describe how children's inborn dispositions interact with parenting behavior and have all suggested that temperament and parenting work in concert shape child development and adaptation (Belsky & Pluess, 2009; Chess & Thomas, 1991; Wachs & Gruen, 1982).

Evidence from the animal kingdom and from anthropological accounts of human societies suggests that the "traditional" nuclear family, consisting of primary-caregiving mother, secondary-caregiving father, and children, represent neither a universal nor an exclusive format for optimal child development (Hrdy, 2009). Still, most studies on the joint contributions of children's dispositions and early caregiving have focused on maternal behavior as the first and most important social experience (Bates & Pettit, 2007), while ignoring the major contribution of fathering and allomothering to socio-cognitive and emotional outcome and maturation of the stress response (Abraham & Feldman, 2018; Braun & Champagne, 2014; Feldman et al., 2019; Rosenbaum & Gettler, 2018). Further, studies on parenting and child temperament rely primarily on main-effect models in which mothering or child temperament directly predict the child's physiological stress response. The few studies which considered the joint contributions of temperament and parenting to HPA activity focused on maternal caregiving and infant temperament and suggested that the mother's "here and now" active regulation of the infant's internal state buffered the tendency of inhibited infants to show elevated stress responses (Gunnar & Donzella, 2002; Kertes et al., 2009; Nachmias et al., 1996).

To our knowledge, no study has tested the interactive associations of early caregiving and child temperament and their links to the maturation of the stress-immune axis in preschool-aged children, a developmental period when children develop more complex self-regulation strategies (Eisenberg et al., 2010; Feldman, 2009). Thus, the current 4-year prospective longitudinal study sought to examine the moderating role of the two arms of the neurobiology of affiliation in infancy, *parental synchrony* and *parental oxytocin* (OT), in buffering the links between preschooler's reactivity and regulation and salivary cortisol (CT) and secretory Immunoglobulin A (s-IgA) levels, biomarkers of the hypothalamic–pituitary–adrenal (HPA) and immune systems.

### 1.1 | Cortisol and s-IgA as biomarkers of HPA and immune systems

The hypothalamic–pituitary–adrenal (HPA) system marks a central pathway in the mammalian stress response, in which a cascade of stressful events leads to the release of glucocorticoid hormones. In response to a stressor, the hypothalamus releases corticotropin-releasing hormone (CRH) into the anterior pituitary gland, which responds by producing and releasing adrenocorticotropic hormone (ACTH), which, in turn, stimulates the adrenal gland to produce cortisol (CT). Higher basal CT is thought to reflect a failure to effectively regulate physiological and emotional arousal and has been linked with a variety of social impairments and psychopathological conditions across the lifespan (Dietrich et al., 2013; Dorn et al., 2009; El-Sheikh et al., 2008; Halevi et al., 2017; Lopez-Duran et al., 2009).

Several studies in animal models and humans addressed the impact of the early environment on the developing immune system (Coe & Laudenslager, 2007; O'Connor et al., 2013; O'Mahony et al., 2009). Studies have repeatedly shown that psychological distress alters immune reactivity (Engeland et al., 2016; Herbert & Cohen, 1993), and that stress-induced hormonal alterations have been linked with changes in the immune system as well as with behavioral, emotional, and cognitive processes that lead to physical illness, behavioral problems, and psychiatric symptoms (De Bellis & Zisk, 2014; Lupien et al., 2009). Secretory immunoglobulin A (s-IgA) plays a key role in protection from pathogens. Given that most infectious agents enter the body via mucosal surfaces, s-IgA serves as a critical first line of defense against infection (Mazanec et al., 1993). Research addressing s-IgA responses has yielded inconclusive findings regarding cross-system linkages; however, most studies on s-IgA have been conducted in adults and adolescents and very few examined the effects of chronic or acute stress on s-IgA levels in young children. Whereas acute psychological stress is typically associated with reduced s-IgA levels (Deinzer et al., 2000; Ng et al., 1999), chronic stress is linked with increased s-IgA (Shirtcliff et al., 2009; Ulmer-Yaniv et al., 2018; Ulmer-Yaniv, Djalovski, Yirmiya, et al., 2018; Yirmiya et al., 2018), albeit results are inconsistent (Phillips et al., 2006; Segerstrom & Miller, 2004). Here, we wished to examine for the first time the interplay between early maternal and paternal caregiving and the child's temperamental reactivity and regulation in predicting the maturation of the HPA-immune axis in a unique sample of preschoolers reared in mother-father or two-father households, in which the mother or fathers assume the primary responsibility for child care, respectively.

### 1.2 | Child temperament and the HPA-immune axis

Temperament is defined as relatively stable, physiologically based individual differences in emotional reactivity and regulation (Rothbart & Derryberry, 2002). Reactivity taps responsiveness to change in the external and internal environments, includes physiological and emotional reactions, and is detectable early in life. Studies of temperamental reactivity have typically focused on indicators of negative emotionality such as frustration, anger, sadness, and fear (inhibition, withdrawal). Regulation refers to orienting and executive control of attention and behavior that operates to modulate reactivity, facilitating or inhibiting physiological, affective, or behavioral response (Rothbart, 2012). Regulatory processes mature across the first years of life from physiological to emotional to attentional and to self-regulatory processes by the preschool years (Feldman, 2009). By that stage, children acquire more complex regulatory strategies, such as shifting attention toward active engagement and reappraisal of the situation (Feldman et al., 2011; Morris et al., 2011). Such complex strategies allow children to exercise coping and regulatory abilities and gradually move from the external regulatory function of caregivers. The regulation of negative emotions is particularly important when attempting to predict emotional, behavioral, and social development throughout life (Calkins, 2002; Eisenberg et al., 2000; Rothbart & Posner, 2006). Notably, while individual differences in

reactivity and regulation have a genetic basis, they are also critically molded by the early social environment.

Evidence suggests that temperament moderates the individual's response to stress and threat (e.g., Eisenberg et al., 1994; Wertlieb et al., 1987) and child temperament has been associated with CT reactivity and recovery (Wu & Feng, 2020). The earliest age when the interaction of CT and temperament has been demonstrated is 32 hr after birth (Gunnar et al., 1987). Young children who showed high negative emotionality, reacting to novelty with fear, anger or distress, also exhibited greater CT response to stressful events (Blair et al., 2008; Gunnar & Vazquez, 2006; Kertes et al., 2009; Steptoe et al., 2007) ,and had higher baseline CT levels (Davis et al., 2001). Only one study tested the association between child temperament and s-IgA levels and found that increases in negative affect predicted an increase in concurrent s-IgA in a sample of children and adolescents (Laurent et al., 2015).

### 1.3 | Parenting and the maturation of children's HPA-immune axis

A plethora of studies has shown that variations in the early social environment carry lasting effects on both HPA-axis reactivity (Clarke, 1993; Sanchez, 2006) and the immune system (Ulmer-Yaniv, Djalovski, Yirmiya, et al., 2018). The developing HPA axis is under strong social regulation during infancy and early childhood and is vulnerable to perturbations in the absence of sensitive and responsive caregiving. Animal studies indicate that maternal proximity, touch, and contact carry a life-long organizing effect on the offspring's stress management systems and function to regulate the stress response (Champagne, 2008), while early maternal separation results in long-term disruptions to the HPA-axis, resulting in increased baseline CT and altered CT reactivity (Levine et al., 2007). In humans, sensitive and responsive parenting attenuates the child's CT response to social stressors (Albers et al., 2008; Berry et al., 2016; Feldman et al., 2010). In contrast, parental psychopathology, insensitive and intrusive parenting and low quality of parental care alter the development of the child's stress response and threat-detection neurobiological circuits (Hostinar et al., 2014), and correlates with greater CT levels (Feldman et al., 2009; Vreeburg et al., 2010), with higher CT production (Marceau et al., 2015) and compromised immune functioning (DeVries et al., 2007). Notably, social interactions and co-regulatory experiences during moments of high negative arousal with both mother and father in preschool moderate the child's CT response (Ostfeld-Eztion et al., 2015). Chen et al. (2010) reported that adults growing up in low socioeconomic status households but experienced maternal warmth were protected from the pro-inflammatory states, and Byrne et al. (2017) reported that higher scores on the poor parental scale were associated with higher levels of both inflammation (C-reactive protein) and immune (s-IgA) activation in school children.

According to our conceptual model, the early parental care envelope that defines the neurobiology of affiliation comprises two major components; biobehavioral synchrony—the process by which the mature parental brain externally regulates the infant's immature brain and tunes it to social life and the oxytocin (OT) system—which sustains mammalian affiliation and crosstalk with the stress, immune, and reward systems (Feldman, 2016, 2017, 2020).

*Biobehavioral synchrony* describes the coordination of the parent and child's physiological response during moments of social contact (Feldman, 2012, 2016). During moments of synchrony in the gaze, affect, vocal, and touch modality, parents organize the child's stress-management systems and CT activity (Feldman et al., 2009, 2010). In a study spanning from birth to 10 years, we found that parent-child synchrony at an earlier time-point shaped the child's regulatory capacities at the next developmental node (Feldman, 2015), and parental synchrony in early childhood predicted regulatory outcomes at 10 years (Priel et al., 2019), highlighting the role of early synchrony for the development of regulatory outcomes. Also, maternal and paternal synchrony are associated with the parent's OT levels, as measured in plasma, saliva, and urine (Abraham et al., 2014; Feldman et al., 2011).

Oxytocin (OT), a nine-amino-acid neuropeptide synthesized in the hypothalamus, provides the foundation for maternal- and paternal-infant bonding (Abraham et al., 2014; Gordon et al., 2010). OT is considered as an anti-stress hormone, mediating anxiolytic and relaxing effects that are associated with the calm state of social affiliation (Feldman, 2012; Latt et al., 2018). Animal and human studies have highlighted the intergenerational transmission of OT via a biobehavioral feedback loop: parental OT supports parental caregiving, which in turn shapes the infant's OT system through the provision of species-typical parenting behavior (Champagne, 2008), and synchronous caregiving in humans (Feldman, 2012). Research has shown that OT administration increased social affiliation and reduced CT levels (Brown et al., 2016; Ditzen et al., 2009; Meinlschmidt & Heim, 2007), and buffered CT responses to stress in adults with impaired emotion regulation abilities (Quirin et al., 2011). Parent-child synchrony and the OT system are shaped during the transition to parenthood and carry long-term effects on child's brain, behavior, and psychopathology (Abraham et al., 2020), hence are defined as markers of resilience and are related to child's greater adaptation in the context of adversity (Feldman, 2020).

### 1.4 | The current study

Although research on the impact of early social experiences on offspring neurohormonal development has traditionally focused on mothers (Meaney, 2001), studies in biparental species provide insight into the specific role of fathers in shaping the phenotype, brain, neuroendocrine and immune systems of offspring (Saltzman et al., 2017); however, there remains limited research on human fathers' contribution to children's psychobiological development (Abraham & Feldman, 2018; Storey et al., 2020), and no investigation into the direct effect of father parenting on the child maturation of the HPA-immune axis, or its interaction with child's inborn traits.

The limited inquiry into early human paternal caregiving effects on children's neuroendocrine systems regulating social behavior and immune response is particularly noteworthy, given recent sociocultural changes and technological advantages in Western societies that redefined the family unit in flexible ways, increased the numbers of fathers that are involved in direct childcare, and created new types of families, including households in which fathers assume the primary responsibility for infant care and raise infants with no maternal involvement since birth.

Here, we tested for the first time the role of parent-infant synchrony and parental OT in primary-caregiving mothers and primary- and secondary-caregiving fathers as moderators of the links between preschoolers' reactivity and regulation and children's salivary baseline CT and s-IgA concentration. We recruited 47 children and their 94 first-time parents from two types of families; mother-father families and two-father families of couples raising their first infant within a committed relationship with no maternal involvement since birth. In infancy (T1), we visited families at home, videotaped parent-infant interactions which were coded offline for parental synchrony and measured parental OT. Four years later, we revisited families when children were approximately 4 years old (T2). Experimenters, whom child's have never met before, visited families at home. The visit involved a good deal of child's interaction with strangers (the experimenters), which may potentially act as social stressors during the beginning of the visit (arrival effect), while coming in close contact with unfamiliar adult and experiencing a novel or challenging event. We measured children's CT and s-IgA at three time-points across the home visit, and children participated in structured fear and joy tasks aimed to index temperament (LAB-TAB), which were micro-coded for emotionality and self-regulation. In light of the literature underscoring the buffering role of early caregiving on youth's internal and environmental risk factors during childhood (e.g., Abraham et al., 2018; Greence et al., 2020; Hazel et al., 2014; Pilakouta et al., 2015), we expected to find associations between early caregiving (parental synchrony and OT) -temperament (self-regulation and negative emotionality) interactions and stress and immune biomarkers in preschoolers. Specifically, we expected that parental synchrony and parental OT in infancy would buffer the links between temperamental dysregulation (low self-regulation and high negative emotionality) and baseline CT and s-IgA levels. We postulated that under conditions of less optimal caregiving, preschoolers with low regulation and high negative emotionality would show higher baseline salivary CT and s-IgA levels, but such links would be buffered in contexts of greater early synchrony and higher parental OT.

## 2 | METHODS

### 2.1 | Participants

A total of 47 typically developed children and their 94 first-time parents (47 couples) participated in the study (for demographic information, see Table 1). These included 24 heterosexual primary-caregiving biological mothers and their partners—24 heterosexual secondary-caregiving biological fathers (24 couples), and 46 homosexual primary-caregiving fathers (23 couples) raising their infant without maternal involvement since birth through surrogacy. In each father's couple, one father was the biological father and the other was the adoptive father. Two families chose not to participate in the follow-up home visits and we could not locate three families; therefore, we excluded their data. Infants were all born at term and healthy since birth. In all families, parents were healthy, with no history of mental illness, including postpartum depression, and both parents shared housework and childcare responsibilities. All families were predominantly middle-class, educationally advantaged, and Caucasian. Parents were recruited through advertisements in various parenting online forums. Before home visits, a short phone interview was conducted in which we asked each parent: "*who is the primary caregiver responsible for infant care and providing more direct daily caregiving to the infant since birth*." In mother-father families, we recruited only families where parents agreed unanimously that the mother was the primary-caregiver, provided more direct daily caregiving, and spent more time with the infant compared with father, and the father was the secondary caregiver. In the two-father family group, we recruited both partners when both indicated that they equally shared all caregiving activities since the infant's birth and when both partners shared the 12-week mandatory paternity leave. To determine the parent's caregiving responsibilities and primary caregiving role, we conducted a structured interview, and parents completed a self-report questionnaire. The 30-question structured interview can be found in Abraham et al., 2014, Table S6. Interview data indicated all primary-caregiving fathers in the two-father families provided significantly more daily caregiving to their infants and spent more time alone with the child compared to secondary-caregiving fathers in the mother-father families, whereas no differences emerged between biological and nonbiological fathers (for more details about the interview, see Abraham et al., 2014, Table S7). Participants received compensation for their time and gave written informed consent. The study received approval from the Institutional Review Board.

### 2.2 | Procedure

The experimental procedure included two sessions with each family. Families were visited at home twice between 4:00 and 7:00 p.m. to control for hormonal variability. In the first, we visited families at home (T1: Infancy), salivary samples were collected for parental OT, and each parent was videotaped interacting with the infant for 7 min. Instructions were: "*Play with your child as you typically do*." In the second session, a few years later, (T2: Preschool), when children reached preschool age, we revisited families at home. Three saliva samples were collected from a child during the home visit. Ten minutes after arrival at home and following child acquaintance with the RA, a baseline saliva sample was collected from each child by

**TABLE 1** Demographic information

| Number of participants | Total sample 94 parents | Two-father families Fathers ($n = 46$) | Mother-father families Mothers ($n = 24$) | Mother-father families Fathers ($n = 24$) |
|---|---|---|---|---|
| **Parents** | | | | |
| Age (T1)—years | 36.3 ± 4.47 (range: 29–45) | 37.80 ± 4.80 | 33.79 ± 4.80 | 35.97 ± 3.20 |
| Education | | | | |
| Completed high school | | 12.3% | 9.1% | 36.3% |
| Beyond high school (college or university) | | 87.7% | 90.9% | 63.7% |
| Monthly salary | | | | |
| <10,000 NIS | | 11% | 24.3% | 13.2% |
| >10,000 NIS | | 89% | 75.7% | 86.8% |
| **Children** | | | | |
| Number of participants | 47 children | 23 | 24 | |
| Age (T1-infancy)—months | 10.05 ± 6.28 (range: 5–29) | 10.01 ± 6.54 | 10.09 ± 6.01 | |
| Age (T2-preschool)—months | 40.41 ± 4.82 (range: 36–55) | 41.24 ± 5.17 | 39.76 ± 6.28 | |
| Gender | | | | |
| Females | 21 (44.7%) | 10 | 11 | |
| Males | 26 (55.3%) | 13 | 13 | |

experimenter placing a Salivette (Sarstedt, Rommelsdorft, Germany) in the child's mouth for one minute. The second CT sample was collected 20 min after the end of the fear of evocative ER paradigms (masks). The final CT sample was collected 15 min after the second sample. To assess preschoolers' self-regulation and emotionality, we administrated a Fear episode—"Mask," and a "Joy/Pleasure" episode—"Bubble," both adapted from the Laboratory Temperament Assessment Battery (LAB-TAB; Goldsmith & Rothbart, 1996) as well as a "Pick-up" task, which were all videotaped for later coding when the parent was in the room. The parent was relegated by the research protocol to a passive role in which she (or he) was asked to respond minimally to the child's signals. The Lab-TAB is a leading observational measure of childhood temperament, with considerable support for its validity and clinical and predictive value (Rothbart et al., 2000). As the interest of the current study was on task children's reactivity and regulation to negative emotion-eliciting tasks, we used data from the "Mask" procedure. In the "Mask" procedure, the child sits in front of the experimenter who puts on four increasingly fear-eliciting masks: rabbit, lion, alligator, and monster. The experimenter wore each mask for 15 s. Each time, the experimenter would speak the child's name, while moving her head slowly from side to side and then lean toward the child. We assessed children's negative emotionality and self-regulation abilities during moments of increased stress. The Joy/Pleasure bubble game task was administered after the Fear task principally to provide a more positive interaction experience at the end of the assessment; data from this task are not presented. At this point, parents and children separated and completed different tasks.

## 2.3 | Measures

### 2.3.1 | Parent-infant interaction (T1—Infancy)

Interactions were coded using our well-validated Coding Interactive Behavior Manual (CIB) (Feldman, 1998). The CIB is a global rating system, including multiple scales each raged from 1 to 5 that are integrated into theoretically meaningful constructs. The CIB has been validated in multiple studies across numerous cultures, ages, and psychopathologies with good psychometric properties (for review: Feldman, 2012). In infancy (T1), we used the *parental synchrony* construct to index the central behavioral expression of attuned human caregiving. Codes describe the expression of the human species-typical parental behavior (parent gaze, positive affect, "motherese" vocalization, affectionate touch) and their coordination with the infant's signals (mutual adaptation, dyadic reciprocity, fluency of the interaction and the degree to which it provides a supportive presence for infant play and exploration (Feldman, 2007). Inter-rater reliability, measured on approximately 20% of the sample, was intraclass, $r = .90$.

### 2.3.2 | Parental OT collection and determination (T1—Infancy)

Saliva samples were collected twice—at baseline and following parent–infant interactions—by sallivette (Sarstedt, Rommelsdorf, Germany). Samples were stored at −20°C until centrifuged twice, two days apart, at 4°C at 1,500 *g* for 20 min. Liquid samples were kept at −80°C, lyophilized for ten days, and stored at 20°C. On the assay day, the dry samples were reconstituted in water and concentrated × 4, before immunoassay. OT was assayed by ELISA (EnzoVR (NY, USA) with careful sample preparation; samples were centrifuged twice; delicate lyophilization maintained constant refrigeration to slows the drying, and samples were reconstituted in water prior to assay. Measurements were performed in duplicate, and the concentrations of samples were calculated using MATLAB-7 according to relevant standard curves. The intra-assay and inter-assay coefficients of variability are <19.1%.

### 2.3.3 | Fear eliciting task ("Mask") (T2—Preschool)

The Masks paradigm was micro-coded for child's self-regulatory skills and expression of negative emotionality (negative affect, withdrawal, crying/yelling, and protest), consistent with our prior research (Abraham et al., 2016, 2018; Hirschler-Guttenberg et al., 2015; Ostfeld-Etzion et al., 2015). In light of these studies on preschool-aged children we focused on more complex, age-appropriate regulatory behaviors that are not inherently self-regulatory but may be used for emotion regulation during moments of increased stress, such as substitutive-symbolic play (e.g., "dolly's hungry"), functional play (for example, moving a toy train back-and-forth), and the use of executive skills to divert attention and orienting regulation, or talking to parent or experimenter. Coding was conducted on a computerized system (The Observer, Noldus Information Technology, Wageningen, The Netherlands). Two blind trained observers coded, while the tape progressed at normal speed, shifting to slow motion when the shift in behavior occurred. Coders were trained to 90% reliability. Inter-rater reliability, measured on 20% of the sample, was intraclass, $r = .86$ for the masks. Proportion variables were used.

### 2.3.4 | Child's salivary collection (T2—Preschool)

Saliva was collected during home visits at the preschool period between 3 p.m. and 6 p.m. Preschoolers were asked to chew a roll of cotton for 1 min until it became saturated and then was placed in a Salivette (Sarstedt, Rommelsdorft, Germany). Saliva samples were collected at three time-points during the home visit: At arrival, 20 min after the end of the fear paradigm (masks), and 15 min thereafter. Parents were instructed to make sure children were not involved in exceptional physical activities and were not exposed to any stressors prior to home visits. Parents were instructed to make sure children were not involved in exceptional physical activities and were not exposed to any stressors prior to home visits. Salivates were kept cooled and then stored at −20°C until centrifuged at 4°C at 1,500× *g* for 20 min. Cortisol levels were then assayed using a commercial ELISA kit (Assay Design, MI). Measurements were performed according to the kit's instructions. CT levels were calculated by MATLAB-7 according to relevant standard curves. The intra-assay and inter-assay coefficients are less than 10.5% and 13.4%, respectively. In line with previous studies (for review, see Gunnar et al., 2009), the fear evocative paradigm has failed to produce a sufficient stress to elicit physiological stress response in preschool (a repeated measure ANCOVA-time1: mean = 435.27 *SD* = 322.34; time2: mean = 305.31 *SD* = 370.62; time3: mean = 227.93 *SD* = 169.04; $F_{(1,46)} = 5.65$, $p = .02$). It may be that the young children's first encounter with unfamiliar adults (the experimenters) during the home visit (arrival effect) acted as a social stressor, while experiencing a novel or challenging event, thus preschoolers exhibited decreasing levels of CT over the home visit. Higher CT levels should reflect, in part, an exaggerated response to such naturalistic social challenge (e.g., Hastings et al., 2011). However, the lack of a baseline measure of salivary CT collected prior to the first encounter with the adult strangers could not allow us to determine the extent to which the novel interaction with unfamiliar adults reflected baseline versus reactive CT. Therefore, in the current study, the current measure of CT is conceptualized as an index of a baseline salivary cortisol concentration, and not of stress reactivity.

*Child S-IgA*

Determination of s-IgA was performed, using a commercial s-IgA ELISA kit (EUROIMMUN AG; Luebeck, Germany). The kit provides a quantitative in vitro assay for s-IgA in human saliva. On the assay day, all samples were thawed completely, and s-IgA levels were measured according to the kit's instructions. Samples preparation was performed by Freedom-Evo (Tecan Group, Ltd.) an automatic liquid handler, and the readings and calculations were conducted by Magellan V.7 software (Tecan Austria GmbH). The intra-assay coefficient of samples and controls was 5.7%, and inter-assay coefficients for samples and controls were less than 10.83%.

*Child CT*

The concentration of CT was determined by a commercial ELISA kit (Salimetrics, USA). Measurements were performed according to the kit's instructions. In addition to the manufacture low and high controls: 1,060 ± 270, 9,700 ± 2,430 pg/ml, three in-house controls were included in each plate (250, 900, 1,200 pg/ml), thus, to correlate between plates measured in different periods. Concentration of CT calculated by MATLAB-7 according to relevant standard curves. The intra-assay and inter-assay coefficients are less than 10.5% and 13.4%, respectively.

## 2.4 | Statistical analysis

All analyses were conducted with the SAS statistical packages (SAS Institute, NC, USA; version 9.4). Consistent with prior research, CT

and s-IgA were measured by computing area under the curve with respect to the ground (AUCg; Pruessner et al., 2003), to accurately assess total overall production across the evening period of the home visit. Prior to the main analysis, bivariate correlations between study variables were examined. Next, we used generalized estimating equations (GEE) approach to control for correlations within families. GEE models can handle a variety of correlated measure models that arise from family research or correlated data that arise from longitudinal studies (Homish et al., 2010). To facilitate the interpretation of the results, all variables were z-standardized prior to analysis. We performed GEE analyses to test the prediction of a child's immune and stress biomarkers (s-IgA and CT) by parental caregiving (parental synchrony and OT) in infancy and by child temperament (Model 1). We also added the following interactions: "parent gender × parental synchrony" and "parent gender × parental OT" (Model 1A), "parent biological status × parental synchrony" and "parent biological status × parental OT" (Model 1B) and "parent caregiving role × parental synchrony" and "parent caregiving role × parental OT" (Model 1C). Next, to assess the interactive effects of early parental caregiving and child temperament, we added "parental synchrony × child self-regulation" and "parental synchrony × child negative emotionality" interactions to the other predictors already in the models (Model 2). Finally, we added "parental OT × child self-regulation" and "parental OT × child negative emotionality" interactions to the other predictors already in Model 1 (Model 3). Since our sample size was relatively small, we could not include all four interactions and covariates in the same model. Therefore, we analyzed the interaction effects in two separate models (Models 2 and 3). To illuminate the nature of significant interactions, we plotted regression slopes of a child's temperament on the child's outcomes for those with high and low early caregiving and conducted simple slopes analysis. Socioeconomic status (SES) parents' and child's age (in infancy and in preschool) and gender were all included in the analyses as potential confounding variables. The level of significance for all analyses was set at $p < .05$.

## 3 | RESULTS

### 3.1 | Preliminary analysis

As shown in Table 2, mothers and primary-caregiving fathers (from the two-father families) have significantly higher levels of parent-infant synchrony than fathers (from the mother-father families), and no differences in parental OT emerged between the three groups (as previously shown in Abraham et al., 2014). No differences were found between biological and adoptive parents. Since no differences emerged between preschoolers reared by two fathers versus a mother and a father on both reactivity and regulation as well as levels of stress and immune biomarkers, we collapsed the two groups.

#### 3.1.1 | Bivariate correlations

As shown in Table 3, parental synchrony was positively correlated with levels of parental OT in infancy and with children's self-regulation and was negatively linked to children's negative emotionality. Children's self-regulation was negatively correlated with levels of s-IgA, and with negative emotionality in preschool. Negative emotionality was positively correlated with levels of CT.

**TABLE 2** Means and *SD* for study variables: full sample and by family type

| | Total sample | | Two-father families ($n = 23$ couples, 23 children) | | | | Mother-father families ($n = 24$ couples, 24 children) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Parents ($n = 94$) | | 1. Biological father ($n = 23$) | | 2. Adoptive father ($n = 23$) | | 3. Mother ($n = 24$) | | 3. Father ($n = 24$) | | |
| **T1: Infancy** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Test statistic, *p*-value** |
| Parental synchrony (CIB) | 3.56 | 0.87 | 3.78 | 0.76 | 3.81 | 0.73 | 3.60 | 0.89 | 3.08 | 0.88 | $F_{(90)} = 3.95$; $p = .01$ Scheffe: 1,2,3 > 4 |
| Parental OT | 31.81 | 19.88 | 30.69 | 17.08 | 34.67 | 20.43 | 30.93 | 17.7 | 31.06 | 23.9 | $F_{(90)} = 0.23$; $p = .87$ |
| | Preschoolers ($n = 47$) | | 1. Preschoolers ($n = 23$) | | 2. Preschoolers ($n = 24$) | | | | | | |
| **T2: Preschool** | **Mean** | ***SD*** | **Mean** | ***SD*** | **Mean** | ***SD*** | | | | | **Test statistic, *p*-value** |
| Child s-IgA (AUCg) | 3,422.9 | 2,799 | 3,249.71 | 2,691.5 | 3,589.04 | 2,863.3 | | | | | $F_{(45)} = 0.35$; $p = .68$ |
| Child CT (AUCg) | 179 | 117.9 | 182.97 | 118.28 | 175.20 | 117.83 | | | | | $F_{(45)} = 0.49$; $p = .48$ |
| Child Self-regulation | 8.35 | 13.0 | 9.22 | 14.41 | 7.53 | 11.09 | | | | | $F_{(45)} = 0.61$; $p = .47$ |
| Child negative emotionality | 2.88 | 3.58 | 2.09 | 1.95 | 3.64 | 4.26 | | | | | $F_{(45)} = 1.74$; $p = .25$ |

**TABLE 3** Bivariate correlations of the study variables

| Variable | 1 Parent synchrony | 2 Parent OT | 3 Child s-IgA | 4 Child CT | 5 Child Self-regulation |
|---|---|---|---|---|---|
| 1. Parent synchrony (T1 = Infancy) | | | | | |
| 2. Parent OT (T1 = Infancy) | 0.325** | | | | |
| 3. Child s-IgA (T2 = Preschool) | −0.106 | −0.082 | | | |
| 4. Child CT (T2 = Preschool) | −0.104 | −0.115 | 0.124 | | |
| 5. Child Self-regulation (T2 = Preschool) | 0.280* | 0.017 | −0.312** | −0.15 | |
| 6. Child negative emotionality (T2 = Preschool) | −0.277* | −0.047 | 0.101 | 0.427*** | −0.325** |

*Note:* $N = 94$ parents and 47 children.
*$p < .05$;
**$p < .01$;
***$p < .001$.

### 3.2 | Main analysis

To explore the independent contribution of early parental caregiving (T1) and child temperament (T2) to child's immune and stress systems in preschool years (T2), we performed two GEE analyses to examine the association between parental synchrony and OT and child's self-regulation and negative emotionality with child s-IgA and baseline CT levels, controlling for SES, parents' and children age and gender. As shown in Table 4 (Model 1), above and beyond child temperamental traits, parental synchrony, but not parental OT levels, predicted child s-IgA in preschool, indicating that reduced parental synchrony in infancy was associated with higher child s-IgA levels a few years later. As shown in Table 5 (Model 1), above and beyond child temperament, both parental synchrony and OT predicted child baseline CT in preschool, indicating that lower levels of parental OT and reduced parental synchrony in infancy were associated with higher child baseline CT levels a few years later. As shown in Table 4 (Model 1), above and beyond parental caregiving, child's high levels of self-regulation were associated with lower s-IgA levels. No association was found between negative emotionality and s-IgA levels. Also, as shown in Table 5 (Model 1), above and beyond parental caregiving, child's high levels of negative emotionality were associated with elevated child's baseline CT levels. No association was found between child's self-regulation and baseline CT levels. No interactions between parental measures (synchrony and OT) and parent's gender, biological relation to the child (biological vs. adoptive parent), and caregiving role (primary vs. secondary caregiver) were found (Tables 4 and 5, Models $1_{A,B,C}$). Finally, to test the associations between early parental caregiving—child temperament interactions and child's biomarkers, we added interactions to the other predictors already in the models. We found a significant interaction between early parental synchrony and child's self-regulation associated with child's s-IgA levels (Table 4, Model 2). To illuminate the nature of the interaction, we plotted regression slopes of child's self-regulation on the child's s-IgA for those with high and low early parental synchrony and conducted simple slopes analysis (Figure 1a). GEE analyses revealed that the children with low self-regulation had higher s-IgA levels compared to those with better regulation skills, but only in the context of low early parental synchrony (Low parental synchrony: $\beta = -0.42$, $SE = 0.18$, CI = −0.77 to −0.06, $Z = -2.3$, $p = .01$; High parental synchrony: $\beta = -0.05$, $SE = 0.09$, CI = 0.23–0.12, $Z = -0.62$, $p = .5$). We also found a significant interaction between early parental OT and child's negative emotionality linked with child's baseline CT levels (Table 5, Model 3). As shown in Figure 1b, children with high negative emotionality exhibited higher baseline CT levels compared to those with lower levels, but only in the context of low early parental OT (Low early parental OT: $\beta = 0.366$, $SE = 0.08$, CI = 0.19–0.53, $Z = 4.27$, $p < .0001$; High early parental OT: $\beta = 0.15$, $SE = 0.23$, CI = −0.16–0.75, $Z = 1.27$, $p = .3$).

## 4 | DISCUSSION

Our prospective 4-year longitudinal study provides a unique opportunity to detail the interplay between parental synchrony and functionality of parent's oxytocinergic system in the first months of parenthood and preschoolers' behavioral measures of temperament, emotional reactivity and regulation, in the development of HPA-axis and immune systems among preschool-aged children in diverse families. Several findings emerged from our longitudinal study. First, we found that high parent-infant synchrony predicted lower child s-IgA levels and lower child baseline CT in preschool, and high parental OT in infancy predicted lower child baseline CT levels in preschool. Second, we found associations between stress and immune biomarkers and children temperament; higher self-regulation linked with low s-IgA levels and high negative emotionality with higher child baseline CT levels. Finally, we found significant associations between parents' early markers of the neurobiology of affiliation—child's temperamental traits interactions and child's later stress and immune biomarkers. Simple slope analyses confirmed our

**TABLE 4** GEE Regressions models predicting child s-IgA (AUCg)

| | *β* | *SE* | 95% CI | *Z* | *p* |
|---|---|---|---|---|---|
| Model 1 | | | | | |
| **Parent synchrony (Infancy)** | **−0.12** | **0.06** | **−0.25–0.006** | **−0.20** | 0.03 |
| Parental OT (Infancy) | −0.002 | 0.04 | −0.12–0.06 | −0.43 | 0.53 |
| **Child's self-regulation (preschool)** | **−0.29** | **0.04** | **−0.40 to −0.01** | **−2.58** | **0.001** |
| Child's negative emotionality (preschool) | 0.03 | 0.07 | −0.11–0.16 | 0.36 | 0.72 |
| Gender—parent | 0.02 | 0.06 | −0.09–0.17 | 0.68 | 0.66 |
| Gender—child | 0.21 | 0.11 | −0.01–0.44 | 1.80 | 0.07 |
| Age—parent (years) | 0.02 | 0.09 | −0.16–0.20 | 0.23 | 0.81 |
| Age—child (months) | 0.01 | 0.07 | −0.13–0.16 | 0.17 | 0.86 |
| Age—infant (months) | 0.03 | 0.07 | −0.19–0.1 | 0.57 | 0.57 |
| SES (education + monthly income) | −0.005 | 0.03 | −0.04–0.07 | −0.11 | 0.65 |
| Model 1A | | | | | |
| Parental synchrony × parent gender | 0.05 | 0.04 | −0.02–0.13 | 1.13 | 0.18 |
| Parental OT × parent gender | 0.03 | 0.04 | −0.05–0.12 | 0.82 | 0.41 |
| Model 1B | | | | | |
| Parental synchrony × parent's biologically related status (bio vs. adoptive parent) | −0.02 | 0.02 | −0.07–0.02 | −1.06 | 0.28 |
| Parental OT × parent's biologically related status (bio vs. adoptive parent) | 0.007 | 0.01 | −0.01–0.03 | 0.58 | 0.56 |
| Model 1C | | | | | |
| Parental synchrony × parent's caregiving role (primary vs. secondary caregiver) | −0.46 | 0.03 | −0.10–0.01 | −1.50 | 0.13 |
| Parental synchrony × parent's caregiving role (primary vs. secondary caregiver) | −0.03 | 0.02 | −0.08–0.01 | −1.20 | 0.23 |
| Model 2 | | | | | |
| **Parent synchrony × Child self-regulation** | **0.13** | **0.04** | **0.03–0.21** | **2.80** | **0.006** |
| Parent synchrony × Child negative emotionality | −0.04 | 0.03 | −0.10–0.01 | −1.62 | 0.10 |
| Model 3 | | | | | |
| Parent OT × Child self-regulation | 0.04 | 0.03 | −0.10–0.13 | 1.72 | 0.12 |
| Parent OT × Child negative emotionality | −0.02 | 0.04 | −0.11–0.06 | −0.59 | 0.42 |

*Note:* $N = 94$ parents. GEE = Generalized Estimation Equation. All models included SES, parents and children age and gender as covariates. Gender: 0 = female 1 = male; Gender: 0 = female 1 = males; Biological relation: 0 = adoptive 1 = biological; Parental role: 0 = secondary-caregiver 1 = primary-caregiver.

Bold indicates significant values ($p < 0.05$).

**TABLE 5** GEE regressions models predicting child cortisol (AUCg)

| | *β* | *SE* | 95% CI | *Z* | *p* |
|---|---|---|---|---|---|
| Model 1 | | | | | |
| **Parent synchrony (Infancy)** | **−0.18** | **0.08** | **−0.27 to −0.005** | **−1.35** | 0.04 |
| **Parent OT (Infancy)** | **−0.16** | **0.05** | **−0.30 to −0.06** | **−3.18** | **0.003** |
| Child's self-regulation (preschool) | −0.16 | 0.05 | −0.14–0.22 | −1.55 | 0.11 |
| **Child's negative emotionality (preschool)** | **0.25** | **0.06** | **0.12–0.38** | **3.87** | **0.0001** |
| Gender—parent | −0.03 | 0.08 | −0.19–0.13 | −0.38 | 0.67 |
| Gender—child | −0.001 | 0.09 | −0.18–0.17 | −0.05 | 0.96 |
| Age—parent (years) | 0.08 | 0.10 | −0.12–0.29 | 0.76 | 0.44 |
| Age—child (months) | 0.04 | 0.07 | −0.09–0.18 | 0.59 | 0.55 |
| Age—infant (months) | 0.02 | 0.09 | −0.21–0.16 | 0.28 | 0.76 |
| SES (education + monthly income) | −0.04 | 0.10 | −0.10–0.20 | −0.30 | 0.54 |
| Model 1A | | | | | |
| Parental synchrony × parent gender | −0.01 | 0.08 | −0.18–0.15 | −0.16 | 0.87 |
| Parental OT × parent gender | −0.15 | 0.09 | −0.34–0.03 | −1.55 | 0.12 |
| Model 1B | | | | | |
| Parental synchrony × parent's biologically related status (bio vs. adoptive parent) | −0.11 | 0.09 | −0.3–0.07 | −1.17 | 0.24 |
| Parental OT × parent's biologically related status (bio vs. adoptive parent) | 0.03 | 0.06 | −0.08–0.15 | 0.61 | 0.54 |
| Model 1C | | | | | |
| Parental synchrony × parent's caregiving role (primary vs. secondary caregiver) | −0.03 | 0.18 | −0.33–0.40 | −0.20 | 0.53 |
| Parental synchrony × parent's caregiving role (primary vs. secondary caregiver) | −0.20 | 0.17 | −0.13–0.50 | −1.19 | 0.22 |
| Model 2 | | | | | |
| Parent synchrony × Child self-regulation | 0.05 | 0.05 | −0.04–0.35 | 1.03 | 0.30 |
| Parent synchrony × Child negative emotionality | −0.04 | 0.04 | −0.26–0.10 | −1.01 | 0.31 |
| Model 3 | | | | | |
| Parent OT × Child self-regulation | 0.09 | 0.05 | −0.01–0.45 | 0.98 | 0.26 |
| **Parent OT × Child negative emotionality** | **−0.31** | **0.04** | **−0.41 to −0.02** | **−3.48** | **0.0009** |

*Note:* 94 parents. GEE = Generalized Estimation Equation. All models included SES, parents and children age and gender as covariates. Gender: 0 = female 1 = male; Gender: 0 = female 1 = males; Biological relation: 0 = adoptive 1 = biological; Parental role: 0 = secondary-caregiver 1 = primary-caregiver.

Bold indicate significant value ($P<0.05$)

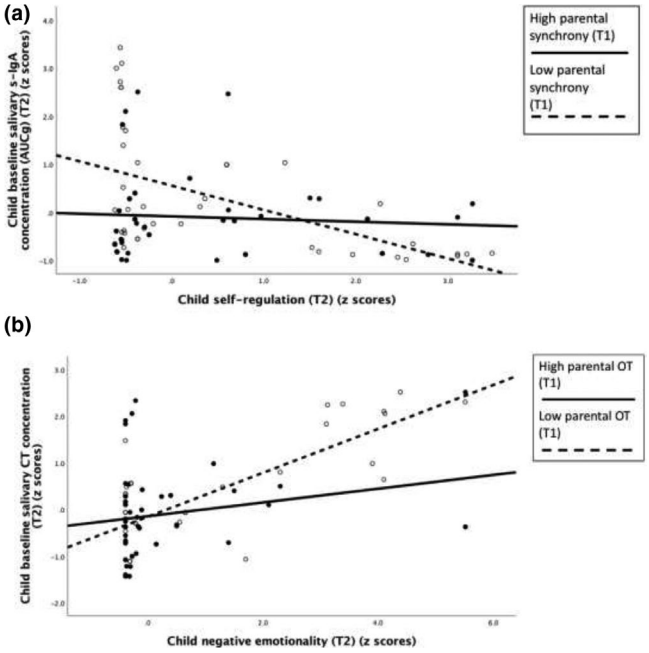


**FIGURE 1** Interactive effects of early parental caregiving and child temperament on child's stress and immune biomarkers in preschoolers

*Notes*. $N = 94$ parents. (a) Graphical depiction of association between preschooler's self-regulation and levels of preschooler's s-IgA by early parental synchrony (high vs. low using median split), with larger difference in preschooler's s-IgA between negative emotionality under low early parental synchrony, than under high levels of parental synchrony. (b) Graphical depiction of association between preschooler's negative emotionality and levels of preschooler's CT by early parental OT (high vs. low using median split), with larger difference in preschooler's CT between negative emotionality under low early parental OT, than under high levels of early parental OT. Controlling for SES, parent and child age and gender.

hypothesis on the role of early parental caregiving as a protective buffer against the links between child's temperamental dysregulation and stress-immune axis. Children with low self-regulation had higher s-IgA levels compared to those with better regulatory skills, but only in the context of low early parental synchrony. Also, children with high negative emotionality exhibited higher baseline CT levels compared to those with lower levels, but only in the context of low parental OT in infancy.

Since the turn of the 19th century in Western society, mothers have been considered the most influential force in shaping and preserving children's physical and mental lives (Dye & Smith, 1986). The mother-infant relationship has been seen as the epitome of human-bonding and research has stressed the mother's primary and exclusive childrearing responsibility (Badinter, 1981). Elucidating the contribution of different caregivers is particularly important nowadays when caregivers other than the biological mother, including fathers, are displaying greater and more equal participation in their children's lives. In fact, this is the first period in human history when fathers are raising infants within a partnered relationship with no maternal involvement since birth. Importantly, and consistent with research indicating no differences in developmental outcomes between children raised by two same-sex parents and those raised by two opposite-sex parents (Abraham et al., 2014, 2016; Crowl et al., 2008; Golombok, 2015), we found no differences on both stress and immune biomarkers, as well as on temperamental traits in children reared in these two family types. This study is the first, to our knowledge, to provide empirical evidence of the role of parent-infant synchrony and parental OT in first-time mothers and fathers as moderators of the links between preschoolers' reactivity and regulation and the maturation of HPA-immune axis in two-father families setting, in which fathers are the primary-caregivers, as well as in the "traditional" mother-father families, in which males are cooperative coparents and involved secondary-caregiver fathers, and mothers are the primary-caregivers. Our findings highlight the stress-buffering role of early human caregiving and its associated neurobiology, whether provided by women or men, biological or adoptive parents, and demonstrate their long-lasting associations, together with the complex developmental cascades, with the maturation of children's stress-management systems which support the ability to enter the social group and parent the next generation. Our findings highlight the importance of considering child development from a complex system perspective and widening the lens on human caregiving for child development.

Overall, our results provide evidence for the links between early maternal and paternal caregiving and child's stress-neurobiology 4 years later, as well as for the associations between child reactivity and regulation and markers of the stress and immune systems. Our findings extend prior developmental research by showing that dispositional and contextual factors function in a synergistic fashion and are associated with physiological response to stress in preschool-aged children, such that relations are manifest only under specific conditions or when factors are considered in combination. It is important to note that our findings describe a pattern of associations and such intercorrelations do not in any way imply causality and may be impacted by other unmeasured variables, such as genetic and epigenetic influences.

Over the last decades, studies of the HPA axis have burgeoned, examining the adrenal end-product CT as an indicator of stress regulation. Adrenal glucocorticoids released during stress-induced HPA axis activation exert profound effects on immune functioning (Miller et al., 2007; Sapolsky et al., 2000). Still, while a plethora of studies suggests that acute and chronic stress consistently increase CT levels, the same cannot be said about S-IgA levels, and the mechanisms behind stress and immune functioning may be more complex than expected and are still not fully understood (Benham et al., 2009). Our finding that child negative emotionality was associated with higher baseline CT levels is in line with previous studies showing that young children who were more temperamentally dysregulated, reacting to novelty with fear, anger or distress, had higher baseline CT levels and showed greater CT elevations to stressful events (Blair et al., 2008; Kertes et al., 2009; Talge et al., 2008). For example, in 2-year-old children, both salivary baseline and post-task (Lab-TAB battery) CT was positively associated with negative affect (Fortunato

et al., 2008). In another study, high child temperamental negative affectivity observed in a standardized set of Lab-TAB tasks at the age of 3 years predicted higher salivary waking and evening CT at the age of 6 years (Dougherty et al., 2013). To our knowledge, our study is the first to examine the associations between self-regulation and s-IgA activity in young children. Our findings that greater regulation linked with lower s-IgA levels corroborates previous studies that identified some personality variables that reduced the immune-suppressive impact of stressors, such as internal locus of control (Kubitz et al., 1986) and coping efficiency (Bandura et al., 1985) in adults. In another study (Farnè et al., 1994), the authors reported associations between greater conscious regulatory behaviors and lower s-IgA levels in adults.

The parent-child relationship is among the central contributors to children's physiology and behavior, particularly the maturation of the HPA-immune axis (Gunnar et al., 2015). Our study showed that early parental synchrony and OT predicted child baseline CT levels in preschool. These findings are consistent with prior research showing that insensitive parenting is associated with child's lower baseline CT levels (Conradt et al., 2016; Ulmer-Yaniv, Djalovski, Yirmiya, et al., 2018), attenuated child CT response to social stressors (Ahnert et al., 2004; Albers et al., 2008), that greater connectivity in mothers' and fathers' brain network that support embodied simulation processes predicted lower baseline CT concentration in their children a few years later (Abraham et al., 2018), and that intranasal OT administered to fathers altered the infant's physiological and behavioral stress response as a function of father-child synchrony (Weisman et al., 2013). Our findings are also supported by animal studies which showed that in marmoset monkeys, a cooperative breeding species, early caregiving from multiple classes of caregivers, including fathers, offspring that received higher rates of rejections from the mother, father, and older siblings showed higher CT responses to social separation (Birnie et al., 2013). We also found a significant association between parental synchrony and child s-IgA activity. This finding is supported by two previous studies reporting significant associations between parenting and child s-IgA levels in late childhood which found links between maternal sensitivity (Yirmiya et al., 2018) and self-reported parenting (Byrne et al., 2017) with child s-IgA levels. Still, much further research is required to understand the links between early parenting and markers of the immune system in young children.

Of special interest are our findings that parental synchrony in infancy buffered the association between low child regulation and s-IgA levels, while parental OT in infancy buffered the link between child negative emotionality and baseline CT levels in the preschool years. These findings may accord with the theory of *environmental specificity* (Wachs, 1987), which suggests that developmental outcomes vary as a function of different environmental variables, including specific parenting behaviors. Moreover, our findings are in line with previous research investigating the risk-buffering model, in which parenting, classified as a risk factor (e.g., harsh or intrusive parenting) amplified the relations between child's risk factors and adjustment, while sensitive, responsive, and warm parenting buffered this association (Gunnar et al., 2015). For example, we previously showed that the link between child OT and later somatic symptoms was moderated by parental OT; when parent OT was high the child's somatic symptoms were not impacted by their own OT levels (Abraham et al., 2019). Similarly, we showed that high maternal OT in chronically depressed mothers provided a protective buffer against the effects of maternal depression on their 6-year-old children's OT response (Pratt et al., 2015), and that sensitive paternal behavior buffered the negative effects of maternal depression on child psychopathology (Vakrat et al., 2018). Another study reported that nurturing mother-child relationship buffered the impact of maternal PTSD on children's depression and anxiety symptoms, disruptive behavior, and stress-related symptom (Greene et al., 2020). Recently, Wu and Feng (2020) found an association between emotion regulation strategies and higher CT production among infants of intrusive mothers. The authors suggested that for infants of intrusive mothers, relying on daily co-regulation with their mothers may increase stress levels due to lack of mother's appropriate supportive presence in the time of distress. Several other studies reported that among inhibited children, those who were insecurely attached had higher CT compared to those who were securely attached (Gunnar & Donzella, 2002), and extremely inhibited 3- to 6-year-old children showed a larger CT increase in response to the social challenge, but only in the context of low-quality parenting (Kertes et al., 2009).

Several study limitations should be considered. First, the sample size was modest and included typically developing children from low-risk, educationally advantaged, middle-class, and Caucasian families and studies in high-risk samples, and families from other races should be conducted before the findings can be generalized. Another limitation of our study is that we did not measure parental CT and s-IgA and any other stress or immune biomarkers. Generally, s-IgA is relevant to inflammation in the sense that the endocrine and sympathetic nervous systems are connected to the immune system, but it is not a direct biomarker of inflammation as it is associated with both pro-inflammatory (Wu et al., 2007) and anti-inflammatory (Ben Mkaddem et al., 2013) processes. Finally, the fear evocative tasks used in this study (Lab-TAB) did not provoke significant elevations in CT for most of the children. The majority of the children exhibited decreasing levels of CT over the home visit. This task may not have been optimal to detect associations with child's temperament and the role of parental caregiving in moderating HPA axis reactivity to stress. However, it may be that the young children's first encounter with unfamiliar adults (the experimenters) during the home visit (arrival effect) acted as a social stressor, and this was the reason for preschoolers' highest CT levels were at first assessment (a few minutes after arrival). However, we can not determine the extent to which the novel interaction with unfamiliar adults reflected baseline versus reactive CT, since no salivary CT was collected prior to the first encounter with the adult strangers.

Still, our results support the complex and interactive nature of early rearing experiences and individual differences in temperamental reactivity and regulation in the developing stress and immune systems in preschool children. Our findings highlight the need to

construct individually tailored early life family system and caregiver-child interventions, which may improve mother-child and father-child interactions and assist parents with their own parental responses and ways to build and enhance child's self-regulation skills and strategies which may disrupt the continuity of the effects of adversity across generations.

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## REFERENCES

Abraham, E., & Feldman, R. (2018). The neurobiology of human allomaternal care; implications for fathering, coparenting, and children's social development. *Physiology & Behavior*, *193*, 25–34. https://doi.org/10.1016/j.physbeh.2017.12.034

Abraham, E., Hendler, T., Shapira-Lichter, I., Kanat-Maymon, Y., Zagoory-Sharon, O., & Feldman, R. (2014). Father's brain is sensitive to childcare experiences. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(27), 9792–9797. https://doi.org/10.1073/pnas.1402569111

Abraham, E., Hendler, T., Zagoory-Sharon, O., & Feldman, R. (2016). Network integrity of the parental brain in infancy supports the development of children's social competencies. *Social Cognitive and Affective Neuroscience*, *11*(11), 1707–1718. https://doi.org/10.1093/scan/nsw090

Abraham, E., Hendler, T., Zagoory-Sharon, O., & Feldman, R. (2019). Interoception sensitivity in the parental brain during the first months of parenting modulates children's somatic symptoms six years later: The role of oxytocin. *International Journal of Psychophysiology*, *136*, 39–48. https://doi.org/10.1016/j.ijpsycho.2018.02.001

Abraham, E., Posner, J., Wickramaratne, P. J., Aw, N., van Dijk, M. T., Cha, J., Weissman, M. M., & Talati, A. (2020). Concordance in parent and offspring cortico-basal ganglia white matter connectivity varies by parental history of major depressive disorder and early parental care. *Social Cognitive and Affective Neuroscience*, *15*(8), 889–903. https://doi.org/10.1093/scan/nsaa118

Abraham, E., Raz, G., Zagoory-Sharon, O., & Feldman, R. (2018). Empathy networks in the parental brain and their long-term effects on children's stress reactivity and behavior adaptation. *Neuropsychologia*, *116*, 75–85. https://doi.org/10.1016/j.neuropsychologia.2017.04.015

Ahnert, L., Gunnar, M. R., Lamb, M. E., & Barthel, M. (2004). Transition to child care: Associations with infant–mother attachment, infant negative emotion, and cortisol elevations. *Child Development*, *75*(3), 639–650. https://doi.org/10.1111/j.1467-8624.2004.00698.x

Albers, E. M., Marianne Riksen-Walraven, J., Sweep, F. C., & Weerth, C. D. (2008). Maternal behavior predicts infant cortisol recovery from a mild everyday stressor. *Journal of Child Psychology and Psychiatry*, *49*(1), 97–103. https://doi.org/10.1111/j.1469-7610.2007.01818.x

Badinter, E. (1981). *Mother love: Myth and reality: Motherhood in modern history*. Macmillan.

Bandura, A., Taylor, C. B., Williams, S. L., Mefford, I. N., & Barchas, J. D. (1985). Catecholamine secretion as a function of perceived coping self-efficacy. *Journal of Consulting and Clinical Psychology*, *53*(3), 406. https://doi.org/10.1037/0022-006X.53.3.406

Bates, J. E., & Pettit, G. S. (2007). Temperament, parenting, and socialization. *Handbook of Socialization*, *2*, 153–177.

Belsky, J., & Pluess, M. (2009). Beyond diathesis stress: Differential susceptibility to environmental influences. *Psychological Bulletin*, *135*(6), 885. https://doi.org/10.1037/a0017376

Benham, G., Nash, M. R., & Baldwin, D. R. (2009). A comparison of changes in secretory immunoglobulin A following a stress-inducing and stress-reducing task. *Stress and Health: Journal of the International Society for the Investigation of Stress*, *25*(1), 81–90. https://doi.org/10.1002/smi.1225

Berry, D., Blair, C., Granger, D. A., & Family Life Project Key Investigators (2016). Child care and cortisol across infancy and toddlerhood: Poverty, peers, and developmental timing. *Family Relations*, *65*(1), 51–72.

Birnie, A. K., Taylor, J. H., Cavanaugh, J., & French, J. A. (2013). Quality of maternal and paternal care predicts later stress reactivity in the cooperatively-breeding marmoset (*Callithrix geoffroyi*). *Psychoneuroendocrinology*, *38*(12), 3003–3014. https://doi.org/10.1016/j.psyneuen.2013.08.011

Blair, C., Granger, D. A., Kivlighan, K. T., Mills-Koonce, R., Willoughby, M., Greenberg, M. T., Hibel, L. C., & Fortunato, C. K. (2008). Maternal and child contributions to cortisol response to emotional arousal in young children from low-income, rural communities. *Developmental Psychology*, *44*(4), 1095. https://doi.org/10.1037/0012-1649.44.4.1095

Braun, K., & Champagne, F. A. (2014). Paternal influences on offspring development: Behavioural and epigenetic pathways. *Journal of Neuroendocrinology*, *26*(10), 697–706. https://doi.org/10.1111/jne.12174

Bronfenbrenner, U., & Morris, P. A. (1998). The ecology of developmental processes. *Handbook of Child Psychology*, *1*(5), 993–1028.

Brown, C. A., Cardoso, C., & Ellenbogen, M. A. (2016). A meta-analytic review of the correlation between peripheral oxytocin and cortisol concentrations. *Frontiers in Neuroendocrinology*, *43*, 19–27. https://doi.org/10.1016/j.yfrne.2016.11.001

Byrne, M. L., Badcock, P. B., Simmons, J. G., Whittle, S., Pettitt, A., Olsson, C. A., Mundy, L. K., Patton, G. C., & Allen, N. B. (2017). Self-reported parenting style is associated with children's inflammation and immune activation. *Journal of Family Psychology*, *31*(3), 374. https://doi.org/10.1037/fam0000254

Calkins, S. D. (2002). Does aversive behavior during toddlerhood matter? The effects of difficult temperament on maternal perceptions and behavior. *Infant Mental Health Journal: Official Publication of the World Association for Infant Mental Health*, *23*(4), 381–402. https://doi.org/10.1002/imhj.10024

Champagne, F. A. (2008). Epigenetic mechanisms and the transgenerational effects of maternal care. *Frontiers in Neuroendocrinology*, *29*(3), 386–397. https://doi.org/10.1016/j.yfrne.2008.03.003

Chen, E., Cohen, S., & Miller, G. E. (2010). How low socioeconomic status affects 2-year hormonal trajectories in children. *Psychological Science*, *21*(1), 31–37. https://doi.org/10.1177/0956797609355566

Chess, S., & Thomas, A. (1991). Temperament and the concept of goodness of fit. In J. Strelau, & A. Angleitner (Eds.), *Explorations in temperament* (pp. 15–28). Springer.

Clarke, A. S. (1993). Social rearing effects on HPA axis activity over early development and in response to stress in rhesus monkeys. *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology*, *26*(8), 433–446. https://doi.org/10.1002/dev.420260802

Coe, C. L., & Laudenslager, M. L. (2007). Psychosocial influences on immunity, including effects on immune maturation and senescence. *Brain*,

*Behavior, and Immunity*, *21*(8), 1000–1008. https://doi.org/10.1016/j.bbi.2007.06.015

Conradt, E., Hawes, K., Guerin, D., Armstrong, D. A., Marsit, C. J., Tronick, E., & Lester, B. M. (2016). The contributions of maternal sensitivity and maternal depressive symptoms to epigenetic processes and neuroendocrine functioning. *Child Development*, *87*(1), 73–85. https://doi.org/10.1111/cdev.12483

Crowl, A., Ahn, S., & Baker, J. (2008). A meta-analysis of developmental outcomes for children of same-sex and heterosexual parents. *Journal of GLBT Family Studies*, *4*(3), 385–407. https://doi.org/10.1080/15504280802177615

Davis, E. P., Donzella, B., Krueger, W. K., & Gunnar, M. R. (2001). The start of a new school year: Individual differences in salivary cortisol response in relation to child temperament. *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology*, *35*(3), 188–196. https://doi.org/10.1002/(SICI)1098-2302(199911)35:3<188:AID-DEV3>3.0.CO;2-K

De Bellis, M. D., & Zisk, A. (2014). The biological effects of childhood trauma. *Child and Adolescent Psychiatric Clinics*, *23*(2), 185–222. https://doi.org/10.1016/j.chc.2014.01.002

Deinzer, R., Kleineidam, C., Stiller-Winkler, R., Idel, H., & Bachg, D. (2000). Prolonged reduction of salivary immunoglobulin A (sIgA) after a major academic exam. *International Journal of Psychophysiology*, *37*(3), 219–232. https://doi.org/10.1016/S0167-8760(99)00112-9

DeVries, A. C., Craft, T. K., Glasper, E. R., Neigh, G. N., & Alexander, J. K. (2007). 2006 Curt P. Richter award winner: Social influences on stress responses and health. *Psychoneuroendocrinology*, *32*(6), 587–603.

Dietrich, A., Ormel, J., Buitelaar, J. K., Verhulst, F. C., Hoekstra, P. J., & Hartman, C. A. (2013). Cortisol in the morning and dimensions of anxiety, depression, and aggression in children from a general population and clinic-referred cohort: An integrated analysis. The TRAILS study. *Psychoneuroendocrinology*, *38*(8), 1281–1298. https://doi.org/10.1016/j.psyneuen.2012.11.013

Ditzen, B., Schaer, M., Gabriel, B., Bodenmann, G., Ehlert, U., & Heinrichs, M. (2009). Intranasal oxytocin increases positive communication and reduces cortisol levels during couple conflict. *Biological Psychiatry*, *65*(9), 728–731. https://doi.org/10.1016/j.biopsych.2008.10.011

Dorn, L. D., Kolko, D. J., Susman, E. J., Huang, B., Stein, H., Music, E., & Bukstein, O. G. (2009). Salivary gonadal and adrenal hormone differences in boys and girls with and without disruptive behavior disorders: Contextual variants. *Biological Psychology*, *81*(1), 31–39. https://doi.org/10.1016/j.biopsycho.2009.01.004

Dougherty, L. R., Smith, V. C., Olino, T. M., Dyson, M. W., Bufferd, S. J., Rose, S. A., & Klein, D. N. (2013). Maternal psychopathology and early child temperament predict young children's salivary cortisol 3 years later. *Journal of Abnormal Child Psychology*, *41*(4), 531–542.

Dye, N. S., & Smith, D. B. (1986). Mother love and infant death, 1750–1920. *The Journal of American History*, *73*(2), 329–353. https://doi.org/10.2307/1908225

Eisenberg, N., Fabes, R. A., Guthrie, I. K., & Reiser, M. (2000). Dispositional emotionality and regulation: Their role in predicting quality of social functioning. *Journal of Personality and Social Psychology*, *78*(1), 136. https://doi.org/10.1037/0022-3514.78.1.136

Eisenberg, N., Fabes, R. A., Nyman, M., Bernzweig, J., & Pinuelas, A. (1994). The relations of emotionality and regulation to children's anger-related reactions. *Child Development*, *65*(1), 109–128. https://doi.org/10.2307/1131369

Eisenberg, N., Spinrad, T. L., & Eggum, N. D. (2010). Emotion-related self-regulation and its relation to children's maladjustment. *Annual Review of Clinical Psychology*, *6*, 495–525. https://doi.org/10.1146/annurev.clinpsy.121208.131208

El-Sheikh, M., Erath, S. A., Buckhalt, J. A., Granger, D. A., & Mize, J. (2008). Cortisol and children's adjustment: The moderating role of sympathetic nervous system activity. *Journal of Abnormal Child Psychology*, *36*(4), 601–611. https://doi.org/10.1007/s10802-007-9204-6

Engeland, C. G., Hugo, F. N., Hilgert, J. B., Nascimento, G. G., Junges, R., Lim, H.-J., Marucha, P. T., & Bosch, J. A. (2016). Psychological distress and salivary secretory immunity. *Brain, Behavior, and Immunity*, *52*, 11–17. https://doi.org/10.1016/j.bbi.2015.08.017

Farnè, M. A., Boni, P., Corallo, A., Gnugnoli, D., & Sacco, F. L. (1994). Personality variables as moderators between hassles and objective indications of distress (S-IgA). *Stress Medicine*, *10*(1), 15–20. https://doi.org/10.1002/smi.2460100104

Feldman, R. (1998). *Mother-newborn coding system manual*. Bar-Ilan University Press.

Feldman, R. (2007). Parent–infant synchrony and the construction of shared timing; physiological precursors, developmental outcomes, and risk conditions. *Journal of Child Psychology and Psychiatry*, *48*(3–4), 329–354. https://doi.org/10.1111/j.1469-7610.2006.01701.x

Feldman, R. (2009). The development of regulatory functions from birth to 5 years: Insights from premature infants. *Child Development*, *80*(2), 544–561.

Feldman, R. (2012). Oxytocin and social affiliation in humans. *Hormones and Behavior*, *61*(3), 380–391. https://doi.org/10.1016/j.yhbeh.2012.01.008

Feldman, R. (2015). Mutual influences between child emotion regulation and parent-child reciprocity support development across the first decade of life: Implications for developmental psychopathology. *Development and Psychopathology*, *27*, 1007–1023.

Feldman, R. (2016). The neurobiology of mammalian parenting and the biosocial context of human caregiving. *Hormones and Behavior*, *77*, 3–17. https://doi.org/10.1016/j.yhbeh.2015.10.001

Feldman, R. (2017). The neurobiology of human attachments. *Trends in Cognitive Sciences*, *21*(2), 80–99. https://doi.org/10.1016/j.tics.2016.11.007

Feldman, R. (2020). What is resilience: An affiliative neuroscience approach. *World Psychiatry*, *19*(2), 132–150. https://doi.org/10.1002/wps.20729

Feldman, R., Braun, K., & Champagne, F. A. (2019). The neural mechanisms and consequences of paternal caregiving. *Nature Reviews Neuroscience*, *20*(4), 205–224. https://doi.org/10.1038/s41583-019-0124-6

Feldman, R., Gordon, I., & Zagoory-Sharon, O. (2011). Maternal and paternal plasma, salivary, and urinary Oxytocin and parent-infant synchrony: Considering stress and affiliation components of human bonding. *Developmental Science*, *14*, 752–761. https://doi.org/10.1111/j.1467-7687.2010.01021.x

Feldman, R., Granat, A., Pariente, C., Kanety, H., Kuint, J., & Gilboa-Schechtman, E. (2009). Maternal depression and anxiety across the postpartum year and infant social engagement, fear regulation, and stress reactivity. *Journal of the American Academy of Child & Adolescent Psychiatry*, *48*(9), 919–927. https://doi.org/10.1097/CHI.0b013e3181b21651

Feldman, R., Singer, M., & Zagoory, O. (2010). Touch attenuates infants' physiological reactivity to stress. *Developmental Science*, *13*(2), 271–278. https://doi.org/10.1111/j.1467-7687.2009.00890.x

Fortunato, C. K., Dribin, A. E., Granger, D. A., & Buss, K. A. (2008). Salivary alpha-amylase and cortisol in toddlers: Differential relations to affective behavior. *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology*, *50*(8), 807–818. https://doi.org/10.1002/dev.20326

Gallagher, K. C. (2002). Does child temperament moderate the influence of parenting on adjustment? *Developmental Review*, *22*(4), 623–643. https://doi.org/10.1016/S0273-2297(02)00503-8

Goldsmith, H. H., & Rothbart, M. K. (1996). *The Laboratory Temperament Assessment Battery (LabTAB): Locomotor version 3.0 Technical manual*. Department of Psychology, University of Wisconsin.

Golombok, S. (2015). *Modern families: Parents and children in new family forms*. Cambridge University Press.

Gordon, I., Zagoory-Sharon, O., Leckman, J. F., & Feldman, R. (2010). Oxytocin and the development of parenting in humans. *Biological Psychiatry*, *68*(4), 377–382. https://doi.org/10.1016/j.biopsych.2010.02.005

Greene, C. A., McCarthy, K. J., Estabrook, R., Wakschlag, L. S., & Briggs-Gowan, M. J. (2020). Responsive parenting buffers the impact of maternal PTSD on young children. *Parenting*, *20*(2), 141–165. https://doi.org/10.1080/15295192.2019.1707623

Gunnar, M. R., & Donzella, B. (2002). Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology*, *27*(1–2), 199–220. https://doi.org/10.1016/S0306-4530(01)00045-2

Gunnar, M. R., Hostinar, C. E., Sanchez, M. M., Tottenham, N., & Sullivan, R. M. (2015). Parental buffering of fear and stress neurobiology: Reviewing parallels across rodent, monkey, and human models. *Social Neuroscience*, *10*(5), 474–478. https://doi.org/10.1080/17470919.2015.1070198

Gunnar, M. R., Isensee, J., & Fust, L. S. (1987). Adrenocortical activity and the Brazelton Neonatal Assessment Scale: Moderating effects of the newborn's biomedical status. *Child Development*, *58*(6), 1448–1458. https://doi.org/10.2307/1130684

Gunnar, M. R., Talge, N. M., & Herrera, A. (2009). Stressor paradigms in developmental studies: What does and does not work to produce mean increases in salivary cortisol. *Psychoneuroendocrinology*, *34*(7), 953–967. https://doi.org/10.1016/j.psyneuen.2009.02.010

Gunnar, M. R., & Vazquez, D. (2006). Stress neurobiology and developmental psychopathology. *Developmental Psychopathology*, *2*, 533–577.

Halevi, G., Djalovsky, A., Kanat-Maymon, Y., Yirmiya, K., Zagoory-Sharon, O., Koren, L., & Feldman, R. (2017). The social transmission of risk; Maternal stress physiology, synchronous parenting, and well-being mediate the effects of war exposure on child psychopathology. *Journal of Abnormal Psychology*, *126*, 1087–1103. https://doi.org/10.1037/abn0000307

Hastings, P. D., Ruttle, P. L., Serbin, L. A., Mills, R. S., Stack, D. M., & Schwartzman, A. E. (2011). Adrenocortical responses to strangers in preschoolers: Relations with parenting, temperament, and psychopathology. *Developmental Psychobiology*, *53*(7), 694–710. https://doi.org/10.1002/dev.20545

Hazel, N. A., Oppenheimer, C. W., Technow, J. R., Young, J. F., & Hankin, B. L. (2014). Parent relationship quality buffers against the effect of peer stressors on depressive symptoms from middle childhood to adolescence. *Developmental Psychology*, *50*(8), 2115. https://doi.org/10.1037/a0037192

Herbert, T. B., & Cohen, S. (1993). Stress and immunity in humans: A meta-analysis review. *Psychosomatic Medicine-Washington*, *55*, 364.

Hirschler-Guttenberg, Y., Golan, O., Ostfeld-Etzion, S., & Feldman, R. (2015). Mothering, fathering, and the regulation of negative and positive emotions in high-functioning preschoolers with autism spectrum disorder. *Journal of Child Psychology and Psychiatry*, *56*(5), 530–539. https://doi.org/10.1111/jcpp.12311

Homish, G. G., Edwards, E. P., Eiden, R. D., & Leonard, K. E. (2010). Analyzing family data: A GEE approach for substance use researchers. *Addictive Behaviors*, *35*(6), 558–563. https://doi.org/10.1016/j.addbeh.2010.01.002

Hostinar, C. E., Sullivan, R. M., & Gunnar, M. R. (2014). Psychobiological mechanisms underlying the social buffering of the hypothalamic–pituitary–adrenocortical axis: A review of animal models and human studies across development. *Psychological bulletin*, *140*(1), 256.

Hrdy, B. S. (2009). *Mothers and others: The evolutionary origins of mutual understanding*. Belknap/Harvard.

Kertes, D. A., Donzella, B., Talge, N. M., Garvin, M. C., Van Ryzin, M. J., & Gunnar, M. R. (2009). Inhibited temperament and parent emotional availability differentially predict young children's cortisol responses to novel social and nonsocial events. *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology*, *51*(7), 521–532. https://doi.org/10.1002/dev.20390

Kiff, C. J., Lengua, L. J., & Zalewski, M. (2011). Nature and nurturing: Parenting in the context of child temperament. *Clinical Child and Family Psychology Review*, *14*(3), 251. https://doi.org/10.1007/s10567-011-0093-4

Kubitz, K. A., Peavey, B. S., & Moore, B. S. (1986). The effect of daily hassles of humoral immunity: An interaction moderated by locus of control. *Biofeedback and Self-Regulation*, *11*(2), 115–123. https://doi.org/10.1007/BF00999979

Latt, H. M., Matsushita, H., Morino, M., Koga, Y., Michiue, H., Nishiki, T., Tomizawa, K., & Matsui, H. (2018). Oxytocin inhibits corticosterone-induced apoptosis in primary hippocampal neurons. *Neuroscience*, *379*, 383–389. https://doi.org/10.1016/j.neuroscience.2018.03.025

Laurent, H. K., Stroud, L. R., Brush, B., D'Angelo, C., & Granger, D. A. (2015). Secretory IgA reactivity to social threat in youth: Relations with HPA, ANS, and behavior. *Psychoneuroendocrinology*, *59*, 81–90. https://doi.org/10.1016/j.psyneuen.2015.04.021

Levine, A., Zagoory-Sharon, O., Feldman, R., Lewis, J. G., & Weller, A. (2007). Measuring cortisol in human psychobiological studies. *Physiology & Behavior*, *90*(1), 43–53. https://doi.org/10.1016/j.physbeh.2006.08.025

Lopez-Duran, N. L., Kovacs, M., & George, C. J. (2009). Hypothalamic–pituitary–adrenal axis dysregulation in depressed children and adolescents: A meta-analysis. *Psychoneuroendocrinology*, *34*(9), 1272–1283. https://doi.org/10.1016/j.psyneuen.2009.03.016

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, *10*(6), 434–445. https://doi.org/10.1038/nrn2639

Marceau, K., Laurent, H. K., Neiderhiser, J. M., Reiss, D., Shaw, D. S., Natsuaki, M. N., Fisher, P. A., & Leve, L. D. (2015). Combined influences of genes, prenatal environment, cortisol, and parenting on the development of children's internalizing versus externalizing problems. *Behavior Genetics*, *45*(3), 268–282. https://doi.org/10.1007/s10519-014-9689-z

Mazanec, M. B., Nedrud, J. G., Kaetzel, C. S., & Lamm, M. E. (1993). A three-tiered view of the role of IgA in mucosal defense. *Immunology Today*, *14*(9), 430–435. https://doi.org/10.1016/0167-5699(93)90245-G

Meaney, M. J. (2001). Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annual Review of Neuroscience*, *24*(1), 1161–1192. https://doi.org/10.1146/annurev.neuro.24.1.1161

Meinlschmidt, G., & Heim, C. (2007). Sensitivity to intranasal oxytocin in adult men with early parental separation. *Biological Psychiatry*, *61*(9), 1109–1111. https://doi.org/10.1016/j.biopsych.2006.09.007

Miller, G. E., Chen, E., & Zhou, E. S. (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychological Bulletin*, *133*(1), 25. https://doi.org/10.1037/0033-2909.133.1.25

Mkaddem, S. B., Rossato, E., Heming, N., & Monteiro, R. C. (2013). Anti-inflammatory role of the IgA Fc receptor (CD89): From autoimmunity to therapeutic perspectives. *Autoimmunity Reviews*, *12*(6), 666–669. https://doi.org/10.1016/j.autrev.2012.10.011

Morris, A. S., Silk, J. S., Morris, M. D., Steinberg, L., Aucoin, K. J., & Keyes, A. W. (2011). The influence of mother–child emotion regulation strategies on children's expression of anger and sadness. *Developmental Psychology*, *47*(1), 213. https://doi.org/10.1037/a0021021

Nachmias, M., Gunnar, M., Mangelsdorf, S., Parritz, R. H., & Buss, K. (1996). Behavioral inhibition and stress reactivity: The moderating role of attachment security. *Child Development*, *67*(2), 508–522. https://doi.org/10.2307/1131829

Ng, V., Koh, D., Chan, G., Ong, H. Y., Chia, S. E., & Ong, C. N. (1999). Are salivary immunoglobulin A and lysozyme biomarkers of stress

among nurses? *Journal of Occupational and Environmental Medicine*, *41*(10), 920–927. https://doi.org/10.1097/00043764-199910000-00013

O'Connor, T. G., Winter, M. A., Hunn, J., Carnahan, J., Pressman, E. K., Glover, V., Robertson-Blackmore, E., Moynihan, J. A., Lee, F.-H., & Caserta, M. T. (2013). Prenatal maternal anxiety predicts reduced adaptive immunity in infants. *Brain, Behavior, and Immunity*, *32*, 21–28. https://doi.org/10.1016/j.bbi.2013.02.002

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A.-M., Quigley, E. M. M., Cryan, J. F., & Dinan, T. G. (2009). Early life stress alters behavior, immunity, and microbiota in rats: Implications for irritable bowel syndrome and psychiatric illnesses. *Biological Psychiatry*, *65*(3), 263–267. https://doi.org/10.1016/j.biopsych.2008.06.026

Ostfeld-Etzion, S., Golan, O., Hirschler-Guttenberg, Y., Zagoory-Sharon, O., & Feldman, R. (2015). Neuroendocrine and behavioral response to social rupture and repair in preschoolers with autism spectrum disorders interacting with mother and father. *Molecular Autism*, *6*(1), 11. https://doi.org/10.1186/s13229-015-0007-2

Phillips, A. C., Carroll, D., Evans, P., Bosch, J. A., Clow, A., Hucklebridge, F., & Der, G. (2006). Stressful life events are associated with low secretion rates of immunoglobulin A in saliva in the middle aged and elderly. *Brain, Behavior, and Immunity*, *20*(2), 191–197. https://doi.org/10.1016/j.bbi.2005.06.006

Pilakouta, N., Jamieson, S., Moorad, J. A., & Smiseth, P. T. (2015). Parental care buffers against inbreeding depression in burying beetles. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(26), 8031–8035. https://doi.org/10.1073/pnas.1500658112

Pratt, M., Apter-Levi, Y., Vakart, A., Feldman, M., Fishman, R., Feldman, T., Zagoory-Sharon, O., & Feldman, R. (2015). Maternal depression and child oxytocin response; moderation by maternal oxytocin and relational behavior. *Depression and Anxiety*, *32*(9), 635–646. https://doi.org/10.1002/da.22392

Priel, A., Zeev-Wolf, M., Djalovsky, A., & Feldman, R. (2019). Maternal depression impairs child emotion recognition, executive functions, and social collaboration: The role of regulatory maternal care across the first decade. *Emotion*. https://doi.org/10.1037/emo000061, *20*(6), 1042–1058.

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology*, *28*(7), 916–931. https://doi.org/10.1016/S0306-4530(02)00108-7

Quirin, M., Kuhl, J., & Düsing, R. (2011). Oxytocin buffers cortisol responses to stress in individuals with impaired emotion regulation abilities. *Psychoneuroendocrinology*, *36*(6), 898–904. https://doi.org/10.1016/j.psyneuen.2010.12.005

Rosenbaum, S., & Gettler, L. T. (2018). With a little help from her friends (and family) part II: Non-maternal caregiving behavior and physiology in mammals. *Physiology & Behavior*, *193*, 12–24. https://doi.org/10.1016/j.physbeh.2017.12.027

Rothbart, M. K. (2012). Advances in temperament: history, concepts and measures. In M. Zentner, & R. L. Shiner (Eds.), *Handbook of Temperament* (pp. 3–20). New York, NY: Guilford Press.

Rothbart, M. K., & Derryberry, D. (2002). Temperament in children. *Psychology at the Turn of the Millennium*, *2*, 17–35.

Rothbart, M. K., Derryberry, D., Hershey, K., Molfese, V. J., & Molfese, D. L. (2000). *Temperament and personality development across the life span*. Lawrence Erbaum Associates, Publishers.

Rothbart, M. K., & Posner, M. I. (2006). Temperament, attention, and developmental psychopathology. In D. Cicchetti, & D. Cohen (Eds.), *Developmental psychopathology*, Vol. *2*, 2nd ed., (pp. 465–501)., Developmental Neuroscience. New York: Wiley.

Saltzman, W., Harris, B. N., De Jong, T. R., Perea-Rodriguez, J. P., Horrell, N. D., Zhao, M., & Andrew, J. R. (2017). Paternal care in biparental rodents: Intra-and inter-individual variation. *Integrative and Comparative Biology*, *57*(3), 589–602. https://doi.org/10.1093/icb/icx047

Sanchez, M. M. (2006). The impact of early adverse care on HPA axis development: Nonhuman primate models. *Hormones and Behavior*, *50*(4), 623–631. https://doi.org/10.1016/j.yhbeh.2006.06.012

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, *21*(1), 55–89.

Segerstrom, S. C., & Miller, G. E. (2004). Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. *Psychological Bulletin*, *130*(4), 601. https://doi.org/10.1037/0033-2909.130.4.601

Shirtcliff, E. A., Coe, C. L., & Pollak, S. D. (2009). Early childhood stress is associated with elevated antibody levels to herpes simplex virus type 1. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(8), 2963–2967. https://doi.org/10.1073/pnas.0806660106

Steptoe, A., Hamer, M., & Chida, Y. (2007). The effects of acute psychological stress on circulating inflammatory factors in humans: A review and meta-analysis. *Brain, Behavior, and Immunity*, *21*(7), 901–912. https://doi.org/10.1016/j.bbi.2007.03.011

Storey, A. E., Alloway, H., & Walsh, C. J. (2020). Dads: Progress in understanding the neuroendocrine basis of human fathering behavior. *Hormones and Behavior*, *119*, 104660. https://doi.org/10.1016/j.yhbeh.2019.104660

Talge, N. M., Donzella, B., & Gunnar, M. R. (2008). Fearful temperament and stress reactivity among preschool-aged children. *Infant and Child Development: An International Journal of Research and Practice*, *17*(4), 427–445.

Ulmer-Yaniv, A., Djalovski, A., Priel, A., Zagoory-Sharon, O., & Feldman, R. (2018). Maternal depression alters stress and immune biomarkers in mother and child. *Depression and Anxiety*, *35*(12), 1145–1157. https://doi.org/10.1002/da.22818

Ulmer-Yaniv, A., Djalovski, A., Yirmiya, K., Halevi, G., Zagoory-Sharon, O., & Feldman, R. (2018). Maternal immune and affiliative biomarkers and sensitive parenting mediate the effects of chronic early trauma on child anxiety. *Psychological Medicine*, *48*(6), 1020–1033. https://doi.org/10.1017/S0033291717002550

Vakrat, A., Apter-Levy, Y., & Feldman, R. (2018). Sensitive fathering buffers the effects of chronic maternal depression on child psychopathology. *Child Psychiatry & Human Development*, *49*(5), 779–785. https://doi.org/10.1007/s10578-018-0795-7

Vreeburg, S. A., Hartman, C. A., Hoogendijk, W. J. G., van Dyck, R., Zitman, F. G., Ormel, J., & Penninx, B. W. (2010). Parental history of depression or anxiety and the cortisol awakening response. *The British Journal of Psychiatry*, *197*, 180–185. https://doi.org/10.1192/bjp.bp.109.076869

Wachs, T. D. (1987). Specificity of environmental action as manifest in environmental correlates of infant's mastery motivation. *Developmental Psychology*, *23*(6), 782. https://doi.org/10.1037/0012-1649.23.6.782

Wachs, T. D., & Gruen, G. (1982). *Early experience and development*. New York: Plenum Press.

Weisman, O., Zagoory-Sharon, O., & Feldman, R. (2013). Oxytocin administration alters HPA reactivity in the context of parent–infant interaction. *European Neuropsychopharmacology*, *23*(12), 1724–1731. https://doi.org/10.1016/j.euroneuro.2013.06.006

Wertlieb, D., Weigel, C., Springer, T., & Feldstein, M. (1987). Temperament as a moderator of children's stressful experiences. *American Journal of Orthopsychiatry*, *57*(2), 234–245. https://doi.org/10.1111/j.1939-0025.1987.tb03533.x

Wu, D., Ren, Z., Pae, M., Guo, W., Cui, X., Merrill, A. H., & Meydani, S. N. (2007). Aging up-regulates expression of inflammatory mediators in mouse adipose tissue. *The Journal of Immunology*, *179*(7), 4829–4839. https://doi.org/10.4049/jimmunol.179.7.4829

Wu, Q., & Feng, X. (2020). Infant emotion regulation and cortisol response during the first 2 years of life: Association with maternal parenting profiles. *Developmental Psychobiology*, *62*(8), 1076–1091. https://doi.org/10.1002/dev.21965

Yirmiya, K., Djalovski, A., Motsan, S., Zagoory-Sharon, O., & Feldman, R. (2018). Stress and immune biomarkers interact with parenting behavior to shape anxiety symptoms in trauma-exposed youth. *Psychoneuroendocrinology*, *98*, 153–160. https://doi.org/10.1016/j.psyneuen.2018.08.016

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