Maternal Perinatal Distress and Child Behavior:
Unpacking Psychobiological Mechanisms

Nina Bruinhof

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Chapter 1.

General introduction

Introduction

The perinatal period represents a transformative transition often framed as a positive and exciting life event. Despite its joyful aspects, pregnancy and the postpartum period are also frequently accompanied by substantial challenges that can contribute to or trigger mental health problems (Saxbe et al., 2018). Critically, these mental health issues affect not only the mother but can also affect her (unborn) child (Monk et al., 2019; Van den Bergh et al., 2020). The first 1000 days of a child's life, from conception through age two, are recognized as the most plastic and influential period in human development, making maternal mental health during this window particularly consequential (Wachs et al., 2014). Indeed, perinatal depression, anxiety, and stress have been linked to adverse child outcomes including difficult temperament, lower cognitive scores, neurobiological alterations, and later mental health problems (Slomian et al., 2019; Tarabulsy et al., 2014). While evidence on the role of maternal perinatal distress in child development is accumulating, the mechanisms by which maternal distress impacts child outcomes are not well understood. Understanding these underlying mechanisms is crucial for deepening our knowledge of developmental processes and for designing effective interventions. The goal of the current thesis is to further unravel how maternal distress affects infant and child behavioral development.

The definition of maternal perinatal mental health problems or 'distress' differs across studies. In this thesis it is defined as symptoms of depression, anxiety, and/or stress, occurring during pregnancy and the postpartum period (Miller et al., 2006). During pregnancy, approximately 30% of Dutch women report experiencing daily life stress (Loomans, et al., 2013). Moreover, approximately 1 in 5 pregnant Dutch women experience clinically relevant mental health symptoms (i.e. anxiety or depression; Browne et al., 2021). In the postpartum period, prevalence remains high with around 8 to 15% of Dutch mothers experiencing serious anxiety or depression complaints (Van Der Zee-Van Den Berg et al., 2021). For women with pre-existing mental health conditions, the perinatal period represents a particularly high-risk window for symptom recurrence (Leppers et al., 2022). International data indicate relapse rates of 20.8% for depression and 56% for anxiety among women with prior diagnoses (Martini et al., 2015). A Dutch study reveals similar patterns: two out of five pregnant women with a history of major depression experience at least one depressive episode during pregnancy or the postpartum period, with these episodes often being more severe than those occurring outside the perinatal window (Meltzer-Brody et al., 2013).

These high prevalence rates of perinatal mental health issues translate into a substantial societal and economic burden. In the UK, maternal mental health problems during pregnancy and the first postpartum year are estimated to cost £8.1 billion annually (Bauer et al., 2016). In the USA the costs are even higher, estimated at $14 billion per annual birth cohort (Luca et al., 2020). Notably, these costs extend far beyond costs related to maternal healthcare and lost productivity. A considerable amount, 72% of the estimated £8.1 billion UK costs, are due to negative long-term effects on the child's health and wellbeing (Bauer et al., 2016). Thus, intergenerational transmission of maternal distress represents the primary driver of this economic burden.

Despite this substantial impact, the mechanisms underlying the association between maternal perinatal distress and child behavioral outcomes remain poorly understood. To address this critical gap in our understanding, this thesis employs innovative research designs to examine how maternal distress affects child behavioral development. The thesis is structured according to the following order. First, the focus will be on the role of prenatal distress on child behavior, examining methods of measurement, theoretical frameworks and proposed mechanisms. Second, the focus will turn to postpartum stress, exploring its impact on children along with the underlying mechanisms and existing evidence. Lastly, maternal childhood experiences will be considered as a potential source of vulnerability for worsening children's behavioral problems.

Maternal Prenatal Distress

The prenatal phase represents a critical developmental window as the fetus undergoes rapid neurodevelopment (Harris, 2022), developing billions of neurons and trillions of synaptic connections (Gao et al., 2017; Thomason, 2020). The pace and complexity of these developmental processes, combined with the establishment of a foundational neural architecture, create heightened vulnerability to maternal physiological and psychological states. Intrauterine disruptions, such as those resulting from prenatal stress, can have lasting effects across the lifespan (Bock et al., 2015; DiMartino et al., 2014; Dufford et al., 2021). Indeed, prenatal distress has been associated with altered child brain development and long-lasting effects on the child's later outcomes, such as temperament, cognition, physiology and mental health (Van Den Bergh et al., 2017; 2020; Monk et al., 2019). These effects on child development have been named 'programming effects' because of their persistent, long-term nature and the fact that they result from gene-environment interactions in early life (Gluckman et al., 2008). To explain these associations between prenatal distress and offspring outcomes, theoretical frameworks have emerged. The 'fetal

programming hypothesis', the 'developmental programming hypothesis' and the 'Developmental Origins of Health and Disease (DOHaD) hypothesis' all theorize that the maternal intrauterine environment can have biological effects on the fetus, programming the fetus for the postnatal environment (Barker, 1990; Gluckman et al., 2005; Gluckman & Hanson, 2004; Langley-Evans, 2006; Seckl & Holmes, 2007).

Given the established links between prenatal distress and child outcomes, accurate assessment of maternal distress during pregnancy is essential. Next to the use of general distress questionnaires, several pregnancy-specific questionnaires have been developed to capture the range of emotions, worries, and experiences characteristic of the prenatal period (Van den Bergh, 1990; DiPietro et al., 2004). However, qualitative research reveals that many prenatal worries center on preparation for the postpartum period, including financial arrangements, childcare logistics, anticipated relationship changes and time management concerns (Hansen et al., 2025; Ladekarl et al., 2022; Mahaffey et al., 2022). Despite this evidence, no validated instrument specifically assessed maternal worries during pregnancy about the postnatal period until the recent development of the Baby Preparation and Worry Scale (Baby-PAWS) in the United States (Erickson et al., 2020). As this questionnaire was only validated in the US, new validation was needed to extend its applicability to a different cultural context, such as the Netherlands. To address this, in **Chapter 2** the validation of the Dutch version of the Baby-PAWS and its associations with later infant temperament are presented. Moreover, responses between Dutch and American pregnant women were compared to advance the understanding of perinatal distress across cultural contexts.

One of the proposed mechanisms associating maternal stress during pregnancy and child outcomes is exposure to heightened intrauterine glucocorticoids (Seckl, 2004). When an individual experiences a psychological or biological stressor, the hypothalamic-pituitary-adrenal (HPA) axis becomes activated, resulting in an increase in concentrations of the hormone cortisol, the end product of the HPA-axis (Sapolsky et al., 2000). While short-term elevations in cortisol are adaptive for restoring homeostasis, chronic stress may lead to sustained overproduction of cortisol (McEwen, 1998). The fetal programming hypothesis suggests that in pregnant women, elevated maternal cortisol can cross the placental barrier and reach the fetus (Seckl & Holmes, 2007). In turn, elevated intrauterine cortisol concentrations may program fetal HPA-axis functioning through epigenetic modifications of the glucocorticoid receptor gene and alterations in receptor sensitivity, with potential downstream effects on neurotrophic factors and neurotransmitters critical for brain development (Howland et al., 2017). Over time,

this could affect the child's development, behavior and wellbeing (Van Den Bergh et al., 2017).

However, empirical support for this fetal programming hypothesis has been inconsistent, with numerous studies failing to detect significant associations between elevated prenatal maternal cortisol concentrations and infant or child outcomes (Beijers et al., 2014; Zijlmans et al., 2015). Moreover, maternal psychological stress has often been found only weakly or not correlated with cortisol concentrations during pregnancy (Davis & Sandman, 2012; Werner et al., 2013). This discordance may partially stem from methodological limitations in cortisol assessments. Previous studies have predominantly relied on momentary saliva or blood samples to measure prenatal cortisol (Zijlmans et al., 2015). These measurements capture acute stress responses rather than prolonged HPA axis activity. Given that especially high and continuous levels of stress, also referred to as chronic stress, are suggested to affect the developing fetus (Graignic-Philippe et al., 2014), these momentary sampling approaches may inadequately reflect the prolonged cortisol exposure relevant to developmental programming. Hair cortisol samples may be more suitable to measure long-term and retrospective HPA axis activity (D'Anna-Hernandez et al., 2011; Kirschbaum et al., 2009; Meyer & Novak, 2021; Stalder et al., 2017; Stalder & Kirschbaum, 2012). Hair grows approximately 1 cm per month. Hence, 1 cm of hair reflects one month of cortisol exposure making this measure an attractive biomarker for chronic stress (Russell et al., 2012).

Another explanation often proposed for the weak links between psychological stress and cortisol measurements during pregnancy, are the low-risk participant samples in previous studies, with stress levels possibly too low to find associations (Lund et al., 2025; Zijlmans et al., 2015). This limitation highlights the need to examine these relationships in populations experiencing more substantial stressors. Natural disasters provide unique opportunities to examine prenatal stress effects in populations experiencing higher levels of stress (King et al., 2012). The worldwide COVID-19 pandemic represented such a context as it was shown to induce heightened stress levels in pregnant women (Gustafsson et al., 2021; Lebel et al., 2020; Perzow et al., 2021; Vacaru et al., 2021). Previous disaster studies have identified associations between elevated maternal psychological stress levels and difficult infant temperament, yet these studies lacked biological measures to elucidate underlying mechanisms (Laplante et al., 2016; Zhang et al., 2018). The logistical challenges of collecting biological samples during unpredictable disasters have historically precluded examination of physiological mechanisms in real-time. Hair cortisol analysis offers a methodological solution to this constraint,

as hair samples can be collected retrospectively, require no specialized storage conditions, and provide an integrated measure of HPA axis activity across preceding months (Russell et al., 2012). To address this gap, **Chapter 3** examined how prenatal COVID-19 related stress was associated with prenatal hair cortisol and later infant temperament, utilizing the COVID-19 stressor to examine proposed glucocorticoid programming mechanisms. Infant temperament was selected as an outcome because it has been linked to prenatal psychological stress before (Van den Bergh et al., 2020) and serves as an early indicator of later child psychological and behavioral functioning (Rothbart et al., 2000).

Maternal Postnatal Distress

After birth, elevated maternal psychological distress continues to be associated with adverse child outcomes. The current literature predominantly focuses on postpartum depression, demonstrating negative and long-term effects on children's cognitive, socioemotional, and physiological development (Field, 2018; Kingston et al., 2012; Rogers et al., 2020). Infants exposed to maternal postpartum depression exhibit heightened cortisol reactivity, compromised self-regulatory capacities, and disrupted attachment formation (Slomian et al., 2019; Stein et al., 2014). Postpartum anxiety and (parenting) stress are equally or even more prevalent than postpartum depression but remain understudied in comparison. Nonetheless, studies focusing on postpartum anxiety and stress show similar associations with adverse infant outcomes as those on postpartum depression, including emotional dysregulation and negative behavioral outcomes (Field, 2018; Glasheen et al., 2010; Goodman et al., 2016; O'Connor et al., 2003).

One mechanism through which postnatal maternal distress can affect children's development is via alterations in maternal caregiving behavior. High quality of caregiving behavior is characterized by high levels of sensitive, cooperative and positive caregiving behaviors and low levels of negative behavior. Sensitive care refers to how adequately a caregiver responds to the infant's cues, while cooperative care is how the caregiver adjusts their behavior to the infant and does not interfere with the infant's ongoing activity (Ainsworth & Bell, 1974). Maternal distress can undermine a mother's ability to provide high quality caregiving. Research has shown mothers experiencing depressive symptoms display reduced affect and emotional availability, alongside greater irritability and withdrawal (see review by Trussell et al., 2018). Mothers with postpartum anxiety are found to be more vigilant and intrusive (Beebe et al., 2011), particularly when their children are in anxiety-provoking contexts such as interactions with strangers or exposure to novel or ambiguous toys and objects (Aktar & Bögels, 2017). These

mental complaints reduce the mother's ability to engage in sensitive, cooperative and positive caregiving behavior (Booth et al., 2018), which may hinder children's development of effective emotion regulation skills (Bernier et al., 2010; Cooke et al., 2022). Additionally, when maternal care is of lower quality the child can develop an insecure attachment style, which has been linked to later relational and emotional problems (Deans, 2020). While these associations are well-documented, the supporting evidence derives predominantly from observational and longitudinal studies, with few investigations examining potential causal effects of maternal distress on caregiving behavior and infant outcomes. Experimental paradigms might offer critical insights on causality by permitting controlled examination of how maternal stress acutely influences infant outcomes through changes in maternal caregiving behavior.

The stress contagion hypothesis has been tested experimentally by inducing acute maternal stress and measuring subsequent infant responses (Engert et al., 2019). When a mother experiences stress, the infant may detect this and respond with a similar physiological or behavioral response to stress. Waters and colleagues (2014; 2017) examined potential stress contagion by exposing mothers to a laboratory stressor before reuniting them with their 12- to 14-month-old infants. Maternal stressor exposure resulted in increased sympathetic activation and avoidance behavior in infants following reunion, compared to infants of control group mothers. Hence, repeated acute maternal stress could contribute to chronic infant stress exposure, possibly through alterations in maternal caregiving behavior. However, this pathway has not yet been tested experimentally.

To address this gap in our understanding of stress contagion mechanisms and the role of maternal caregiving behavior as a potential mediating pathway, **Chapter 4** presents an experimental study in which maternal distress was manipulated in mothers of 8-week-old infants. In the laboratory, mothers were separated from their infant and randomly assigned to either an experimental stressor or a non-stressful control task. This design allowed us to assess whether experimentally induced maternal stress would cause observable changes in maternal caregiving behavior, infant behavior (i.e. crying), and infant physiology (i.e. cortisol concentrations) compared to a non-stressed control group.

While postnatal research has predominantly focused on behavioral pathways linking maternal distress to child outcomes, for breastfed infants physiological signaling between mother and infant continues beyond birth. Moreover, variations in breast milk composition represent an additional mechanism through which

maternal stress may affect child outcomes. Beyond macro and micro nutrients, breast milk contains numerous bioactive components with signaling, protective, and regulatory functions, such as immunoglobulins, cytokines, human milk oligosaccharides (HMOs), microbiota and hormones (Kim & Yi, 2020). These components contribute to child neurobiology, behavior and cognition, and as such play a role in determining the child's short and long-term development, a process termed lactocrine programming (Hinde, 2013). This framework hypothesizes that milk delivers developmental signals to the offspring, with potential to shape infant health, physiology and the brain (de Weerth et al., 2023). When the infant ingests milk, bioactive components can be absorbed through the gut and enter circulation (Hinde, 2013). Importantly, maternal factors (e.g., genetics, age, parity, distress, diet) can affect the non-nutrient bioactive components in milk (de Weerth et al., 2023). For example, heightened levels of maternal distress can result in higher concentrations of glucocorticoids (i.e., cortisol and cortisone) in maternal circulation, which can pass through the mammary gland into the breast milk (Aparicio et al., 2020; Lustermans et al., 2025; Romijn et al., 2021). In turn, ingested glucocorticoids from breast milk may affect infant behavior and later development.

However, evidence for an association between milk glucocorticoid concentrations and infant behavior is mixed. While some studies have found that heightened concentrations of milk cortisol are associated with more difficult temperament in infants (Grey et al., 2013; Nolvi et al., 2018), others have not found associations with infant crying or sleep outcomes (Hechler et al., 2018; Toorop et al., 2020). These inconsistencies may stem from a combination of differences in study designs and the inherent characteristics of both milk glucocorticoids and infant behavior. Previous studies have primarily focused on how a single cortisol sample from breast milk predicts infant behavior over a longer time frame, such as in the following days or even months. However, milk cortisol follows a 24-hour circadian rhythm and fluctuates across days (van der Voorn et al., 2016), making it challenging to establish clear links between a single cortisol sample and later infant behavior. Like cortisol, infant behavior is also highly variable on a daily basis, with substantial fluctuations in behaviors such as crying and sleeping, complicating the accurate representation of an infant's behavior based on only momentary assessments (de Weerth & Van Geert, 2001; Wolke et al., 2017). Finally, bidirectional links might be in play as infant behavior may very well impact maternal wellbeing and in turn milk glucocorticoids, but this has not been studied before. Indeed, increased infant crying and reduced infant sleep have been associated with decreased maternal mental well-being (de Barbaro et al., 2023; Brand et al., 2014; Hughes et al., 2015; Joronen & Kaunonen, 2019; Petzoldt, 2018). This suggests that when an infant cries more and sleeps less,

maternal stress may increase, leading to higher milk glucocorticoid concentrations. Subsequently, elevated milk glucocorticoids could further contribute to increased infant crying and reduced sleep, perpetuating a self-sustaining cycle of maternal and infant distress. **Chapter 5** examined the moment-to-moment bidirectional associations between maternal milk glucocorticoids and infant crying and sleep in the home environment and over the course of a day. This naturalistic daily environment helped us to untangle moment-to-moment and bidirectional associations between mother milk glucocorticoids and infant behavior.

Maternal Childhood Experiences

While maternal distress can directly affect child development through multiple mechanisms, maternal vulnerabilities stemming from her own childhood may also shape developmental pathways. Importantly, maternal childhood experiences can become especially salient during the transition to parenthood, a period marked by increased demands and potential reactivation of early relational patterns (Christie et al., 2017). Maternal vulnerabilities rooted in early experiences, such as adverse childhood experiences (ACEs) and an insecure attachment style, can compromise maternal parenting behavior and the quality of mother-child interactions (Adam et al., 2004; Doinita & Maria, 2015; Rowell & Neal-Barnett, 2022; Weistra et al., 2025). When confronted with a stressor, mothers with these vulnerabilities may find it particularly challenging to keep on responding sensitively to the infant.

ACEs are defined as experiences of emotional, physical, or sexual abuse, or emotional and physical neglect, occurring before age 18 (Bernstein et al., 2011; Felitti et al., 1998). These experiences are common, affecting 60% of adults (Madigan, et al., 2023), with women experiencing higher prevalence and severity of ACE's than men (Haahr-Pedersen et al., 2020; Swedo et al., 2025). ACEs can increase the risk for developing later problems such as depression, anxiety, substance abuse, and compromised health across the lifespan (Felitti et al., 1998; Hughes et al., 2017). The effect of maternal ACEs can extend intergenerationally through compromised parent–child interaction quality (Weistra et al., 2025), thereby increasing the risk for behavioral problems in offspring (Zhang et al., 2023).

Another maternal vulnerability stemming from childhood is an insecure attachment style. Adult attachment styles develop from early childhood experiences through repeated interactions with caregivers, which shape internal working models of relationships and emotional regulation (Cassidy, 2000; Hazan & Shaver, 1987). Insecure attachment styles reflect negative models of the self and others and are typically divided into two dimensions: avoidant attachment, characterized

by distrust of others' intentions and dismissal of close relationships, and anxious attachment, characterized by high dependency on others and fear of rejection and abandonment (Bartholomew & Horowitz, 1991). Insecure attachment styles characterize approximately 48% of the adult population (Madigan, Fearon, et al., 2023) and can negatively affect emotion regulation, relationships and parenting (Doinita & Maria, 2015; Rholes et al., 1995; Simpson, 1990). Children of mothers with insecure attachment are at increased risk for developing their own insecure attachment and experiencing relationship and behavioral problems, creating cyclical patterns of difficulties (Verhage et al., 2016).

Both maternal ACE's and insecure attachment style may become particularly salient as vulnerabilities when exposed to stressors or elevated caregiving demands, such as when caring for an infant with colic. Infant colic, characterized by excessive crying in otherwise healthy infants during the first months of life, affects approximately 10-25% of infants. Children with a history of infant colic were found to be at increased risk for later elevated behavioral problems (Sammallahti et al., 2023; Smarius et al., 2017; Valla et al., 2021; Zeevenhooven et al., 2022). Moreover, parents of infants with colic often experience heightened levels of depression, anxiety, stress, and tiredness (Landgren & Hallström, 2011; Stifter & Bono, 1998). These challenges for parents of colicky infants may be particularly pronounced for mothers with vulnerabilities. Higher maternal ACEs are associated with negative and more aggressive reactions to infant crying (Buisman et al., 2018) and lower sensitivity to infant distress (Girod et al., 2023). Similarly, maternal attachment insecurity is related to overall negative parenting and lower sensitivity, reduced tolerance, and more aversion to offspring crying (Ablow et al., 2013; Jones et al., 2014; Strathearn et al., 2009). Thus, infant colic can be conceptualized as a stressor that pushes maternal caregiving resources to their limits, particularly for mothers with pre-existing vulnerabilities who demonstrate greater difficulties in demanding caregiving situations (Cooke et al., 2019). No previous research has examined whether maternal ACEs and insecure attachment styles amplify the effects of infant colic on child outcomes, while this could inform interventions designed to support at-risk families. **Chapter 6** addresses this by examining the moderating role of maternal ACEs and insecure attachment style in the association between infant colic and child behavior from early childhood to adolescence.

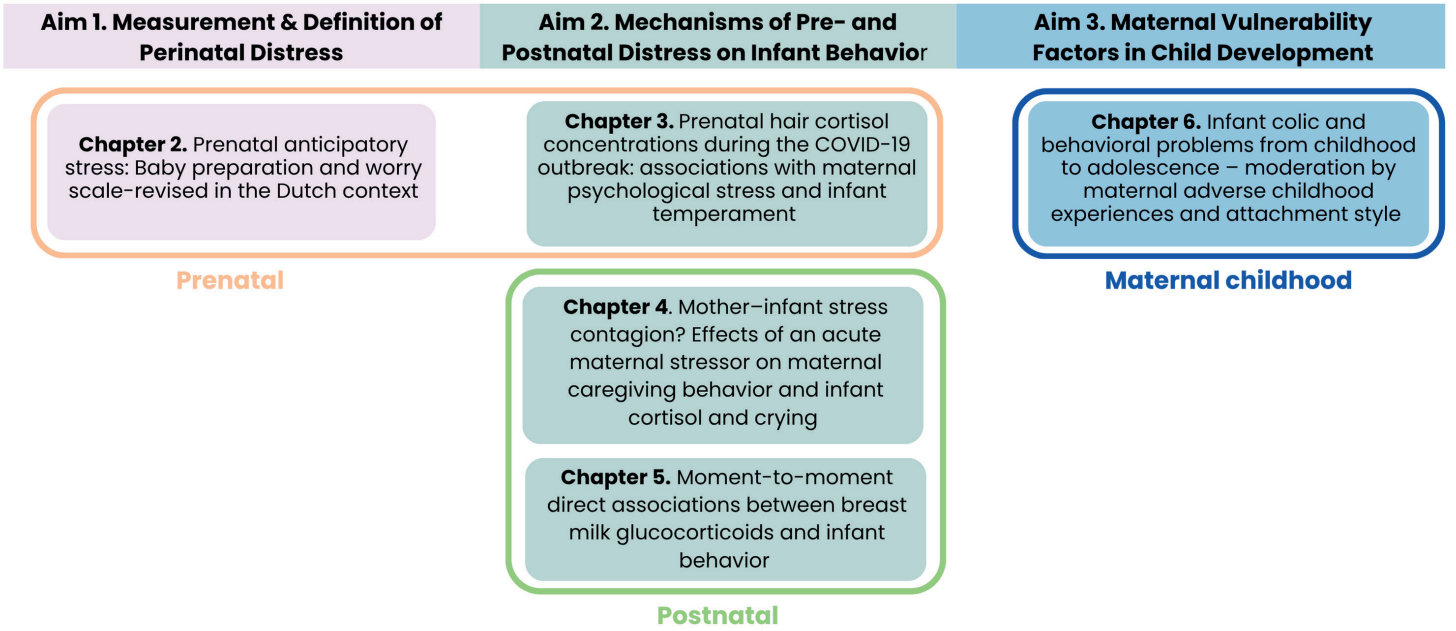

Figure 1. Overview of aims and chapters of the current thesis.

Goals of the current thesis

The main goal of this thesis is to advance understanding of the mechanisms through which maternal perinatal distress can influence children's behavioral development by employing innovative research designs. The thesis is organized around three aims (see Figure 1): (1) to examine how perinatal distress can be measured and defined in **Chapter 2**, (2) to investigate the biobehavioral mechanisms by which perinatal distress can affect infant behavioral development in **Chapters 3 to 5**, and (3) to explore how maternal vulnerabilities, particularly maternal childhood factors, can play a role in children's behavioral development in **Chapter 6**. To study the three aims, biological, psychological and observational data were combined. More broadly, the thesis emphasizes the significance of maternal mental wellbeing as a public health priority and seeks to advance our understanding of how maternal distress can impact the developing child.

Description of studies used in this thesis

This thesis made use of data of multiple longitudinal cohorts and one cross-sectional online study. Each study is described in the following sections.

Online study

A Dutch sample of healthy pregnant women (N = 240) was recruited specifically for the Baby-PAWS validation (**Chapter 2**). This was a onetime cross-sectional online survey (around 15 minutes) for women in the third trimester of pregnancy focusing on prenatal mental wellbeing of mothers. Only healthy women with a complete pregnancy questionnaire were included. Recruitment was fully online and took place in 2022.

SKIPPY

The SKIPPY study is a randomized controlled trial examining the effects of a skin-to-skin contact intervention in full-term healthy infants. Recruitment and intervention delivery occurred between April 2016 and February 2018. Participants were enrolled at 34–36 weeks of gestation, with mothers in the intervention group being instructed to perform the skin-to-skin contact during the first five weeks postpartum. Longitudinal follow-up assessments were conducted for both intervention and control groups, with the latest wave at 6 years of age. The intervention was not utilized in the current thesis. Instead, for the analyses presented in **Chapter 2**, data was used from prenatal questionnaires filled in prior

to randomization by the full sample (n=127), and postnatal questionnaire data exclusively from the control group (n=63).

SMILEY

The SMILEY study (Study of Microbiota and Lifestyle in the Early Years) is a longitudinal study following 160 mother-infant dyads from 18 weeks pregnancy to 12 weeks postpartum, with follow-up rounds at 8 months and 2 years postpartum. Pregnant women were recruited between December 2019 and May 2021. The SMILEY cohort offers unique insights through its longitudinal design, combined with multiple assessment methods, including questionnaires, experiments, diary data, observations, and biomarkers. For the current thesis SMILEY data was used in three out of five chapters. The prenatal and 12 weeks postpartum questionnaires were used for **Chapter 2**. The postnatal experimental visit between 6-11 weeks postpartum, was used for **Chapter 4**. This visit included an experimental stressor, multiple saliva samples for mother and baby and a mother-infant interaction that was scored on maternal caregiving behavior and infant crying. Moreover, the postpartum mental health questionnaires were also part of **Chapter 4**. For **Chapter 5**, the 24-hour infant behavior diary and milk samples at 6 weeks postpartum were used.

COPE

The COPE-study (Covid-19 and Perinatal Experiences) followed parents across different stages of the perinatal period during the COVID-19 pandemic, including preconception, pregnancy, and the first six months postpartum. This study started in April 2020 and included a follow-up of a subset of parents for a short prenatal home visit and longitudinal questionnaires. For **Chapter 3**, this subset of 100 mothers was used. Their data included pregnancy questionnaires, a hair sample provided during a home visit, and questionnaires from the 6-month follow-up.

BIBO

The BIBO Study (Basale Invloeden op de Baby Ontwikkeling, Dutch for Basal Influences on the Baby's Development) is an ongoing longitudinal project that began with 193 women in the third trimester of pregnancy. Children were born between 2006 and 2007. Similarly to the previously mentioned studies, participants were healthy pregnant mothers and their infants. BIBO data collection has been ongoing for two decades, with the 18-year-old wave completed at the end of 2025. For **Chapter 6**, the following data were used: a 4-day diary filled in by mothers at 6 weeks postpartum and questionnaire data from late pregnancy to 16 years.

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Chapter 2.

Prenatal anticipatory stress: Baby preparation and worry scale-revised in the Dutch context

Based on: Bruinhof, N., Sehic, E., Hancock, G. R., Gartstein, M. A., & de Weerth, C. (2024). Prenatal anticipatory stress: Baby preparation and worry scale-revised in the Dutch context. *Comprehensive Psychiatry*, *128*, 152437. https://doi.org/10.1016/j.comppsych.2023.152437

Abstract

Background. Prenatal distress encompasses a range of different emotions, worries, and experiences of stress. The Baby Preparation and Worry Scale (Baby-PAWS) was recently developed to target anticipatory worries during pregnancy about the postnatal period. However, the Baby-PAWS questionnaire was only examined in the United States of America, limiting the questionnaire's generalizability to different countries. To address this issue, we performed a psychometric evaluation of the questionnaire in a Dutch sample and examined associations between the Baby-PAWS questionnaire and established measures of maternal distress (i.e., EPDS, STAI, PRAQ-R) and infant temperament (i.e., IBQ-R).

Methods. Healthy pregnant women (N = 521) completed questionnaires during their third trimester and postnatally, including the Baby-PAWS and distress measures. A subsample of mothers (N = 194) also reported on infant temperament at 12 weeks postpartum.

Results. Exploratory factor analysis suggested a four-factor structure for the 16-item questionnaire in our Dutch sample, as compared to the expected three-factor structure found in the original psychometric evaluation with the American sample. The total Baby-PAWS score was related to pre-and postnatal depression, anxiety, stress, and specific scales of infant temperament. American women scored higher on the Baby-PAWS items than Dutch women.

Limitations. Our participants had higher-than-average socioeconomic status, limiting the generalizability of the findings.

Conclusion. The current analyses indicate good validity of the Baby-PAWS in a Dutch sample. Furthermore, our results highlight cross-cultural differences in perinatal mental health and show the importance of examining instrument structure of context-dependent constructs, such as prenatal worries.

Introduction

Pregnancy can be seen as a major life transition that includes unique physical and psychological changes, and often, anticipatory worries. During pregnancy, women commonly experience increased levels of stress and anxiety, with the prevalence of clinically significant forms of prenatal distress ranging between 20 and 30% (Browne et al., 2021; Fontein-Kuipers et al., 2015; Loomans, van Dijk, et al., 2013). The importance of assessing prenatal distress has been highlighted by studies repeatedly showing how maternal prenatal distress may negatively affect the developing fetus (for a review, see Van den Bergh et al., 2020). For example, heightened psychological stress levels during pregnancy have been linked to increased risk for offspring, such as more difficult temperament (Bush et al., 2017; Pluess et al., 2011) less advanced cognitive functioning (Koutra et al., 2013; Tarabulsy et al., 2014), altered brain connectivity (Humphreys et al., 2020; Scheinost et al., 2020), and later mental health problems (Capron et al., 2015; Pearson et al., 2013). An increased understanding of prenatal stress can be expected to result in more effective preventive services and clinical interventions (Monk et al., 2022).

Prenatal distress encompasses a range of emotions, worries, and experiences of stress. Instruments used to capture prenatal distress assess depression, anxiety, or perceptions of stress, with some addressing pregnancy-specific distress, such as the Pregnancy Anxiety Questionnaire-Revised (PRAQ-R; Huizink et al., 2016), Cambridge Worry Scale (CWS; Green et al., 2003), and the Pregnancy Experience Scale (PES and PES-BRIEF; DiPietro et al., 2004, 2008). Until recently, however, an instrument assessing specific pregnancy worries about the postnatal period was missing.

The Baby Preparation and Worry Scale (Baby-PAWS; Erickson et al., 2020) was developed to address this gap, specifically targeting anticipatory worries during pregnancy about the transition to parenthood. This questionnaire includes items related to common anticipatory pregnancy worries, including those concerning the future balance of adequate infant and self-care, the partner's participation, non-parental care arrangements, and the mother's own postpartum wellbeing. Erickson et al.'s (2020) validation of the original 16-item Baby-PAWS questionnaire with a sample of 276 mothers in the US resulted in an 11-item questionnaire, with factor analyses supporting a three-factor structure: Self and Partner Worry, Nonparental Childcare Worry, and Baby Caregiving Worry. The importance of measuring these anticipatory worries was highlighted by the three factors, as well as the total score, being associated with higher pre- and postnatal general anxiety and depression. At the same time, associations were modest, indicating that the Baby-PAWS taps

into worries and distress that are not addressed in general anxiety and depression questionnaires. Moreover, higher prenatal concerns, as measured by the Baby-PAWS, were also associated with infants displaying more distress, sadness, and difficulties being soothed, demonstrating the questionnaire's predictive value. To date, the Baby-PAWS factor structure has only been examined in the United States of America (US), limiting the questionnaire's generalizability. To address this limitation, the current study investigated the psychometric properties and predictive validity of the Dutch version of the Baby-PAWS in a population of pregnant women in the Netherlands. The findings serve to broaden our knowledge regarding prenatal worries in different countries, as well as help evaluate the cultural generalizability of the Baby-PAWS.

The latter is particularly important given that prenatal worries are potentially culturally-dependent, due to contextual factors such as differences in health-care availability and parental leave arrangements. For example, a previous study shows that compared to a Dutch sample, US women report higher prenatal distress (Gartstein et al., 2020). Moreover, US infants display more negative affectivity compared to Dutch infants (Sung et al., 2015). With awareness for differences noted in cultural contexts, the current study aims to validate the Dutch version of the Baby-PAWS in a healthy sample of expectant mothers in their third trimester of pregnancy. Furthermore, we examined a revised version of the questionnaire by assessing the possible psychometric and predictive improvement resulting from inclusion of four additional items to the original 16-item Baby-PAWS questionnaire. These items were added to expand the content of the original version, and thus they were hypothesized to improve the questionnaire's psychometric and predictive properties.

In this preregistered study, we have four sets of goals and hypotheses. First, we performed a psychometric evaluation of the original 16-item questionnaire in a Dutch sample to assess the factor structure of the Baby-PAWS. We expected to observe a similar factor structure to that of the US Baby-PAWS. Moreover, we tested the psychometric properties of the 20-item revised questionnaire, hypothesizing that the 20 items would yield a more definitive factor structure with stronger loadings compared to the original 16 items. Second, we assessed the association between the Baby-PAWS scores (i.e., total and subscales) and established measures of prenatal depression, anxiety, and pregnancy-specific stress to provide evidence of concurrent validity. In line with the US validation results (Erickson et al., 2020), we hypothesized positive associations between the Baby-PAWS and the other prenatal distress instruments. Third, we evaluated the predictive value of the Baby-PAWS

by assessing associations between the Baby-PAWS questionnaire and postnatal depression, anxiety, and infant temperament. Specifically, based on Erickson et al. (Erickson et al., 2020), we expected the Baby-PAWS (total and subscale scores) to be positively associated with maternal postnatal depression and anxiety, as well as with specific infant temperament factors as follows: higher negative affectivity and lower orienting/regulation. Fourth, we compared US and Dutch Baby-PAWS scores. Based on our earlier work (Gartstein et al., 2020), we expected the Baby-PAWS scores to be higher for US pregnant women compared to Dutch women. We achieved these aims, addressing hypotheses of this preregistered study by utilizing merged data from four different investigations: one cross-sectional and two longitudinal studies of healthy Dutch pregnant women, and one longitudinal study of healthy US pregnant women (Erickson et al., 2020).

Methods

Participants and Procedure

The total Dutch sample ($N = 572$) includes data from three studies approved by The Ethics Committee of the Faculty of Social Sciences from the Radboud University (SKIPPY study: ECSW2015-2311-358; SMILEY study: SW2017-1303-497; online study: ECSW2022-1303-497). This study was preregistered on the Open Science Framework (https://osf.io/gqk8j). Participants were recruited via our participant database (https://babyandchild.nl/en/), midwifery practices, and social media (Table 1).

Table 1. Descriptive statistics for total and individual studies.

	Total (*N* = 521)		**SKIPPY (*N* = 127)**		**SMILEY (*N* = 154)**		**Online (*N* = 240)**	
	***M* (*SD*)/ Mode/%**	**Range**	***M* (*SD*) / Mode/%**	**Range**	***M* (*SD*) / Mode/%**	**Range**	***M* (*SD*) Mode/%**	**Range**
Maternal age (years)	31.71 (3.83)	22-51	31.71 (3.56)	22-42	31.64 (3.62)	22-41	31.76 (4.09)	22-51
Gestational age (weeks)	33.41 (3.39)	27-44	36.46 (1.36)	32-41	31.32 (1.20)	29-35	33.14 (3.90)	27-44
Maternal Education[1]								
Low/Medium	22%		20%		14%		27%	
High	78%		80%		86%		72%	
Years of Education	18.56 (2.5)	9-25.5	-----[2]		-----		18.56 (2.5)	9-25.5
Total family income	€60.000 - €80.000		-----		-----		€60.000 - 80.000	
Partner status								
Married/cohabiting	97.5%		100%		98%		95.8%	
Single	2.3%		0%		2%		3.8%	
Parity (primiparous)	56.2%		44.4%		50.6%		65.8%	
Baby-PAWS Total	30.72 (9.1)	12-64	30.74 (7.63)	13-49	28.18 (8.72)	12-59	32.38 (9.67)	13-64
Baby-PAWS Scale 1	5.41 (2.27)	2-14	5.96 (2.36)	2-14	4.84 (2.14)	2-13	5.50 (2.23)	2-14
Baby-PAWS Scale 2	8.46 (3.19)	18	8.13 (2.95)	3-18	8.13 (2.98)	3-19	8.85 (3.41)	3-21
Baby-PAWS Scale 3	13.03 (4.55)	3-21	13.59 (4.5)	5-24	11.92 (4.35)	5-28	13.45 (4.59)	5-29
Baby-PAWS Scale 4	4.06 (2.27)	5-14	3.52 (1.68)	2-9	3.42 (1.86)	2-12	4.74 (2.57)	2-14
Prenatal EPDS	8.64 (4.33)	3-24	7.74 (3.81)	3-20	8.25 (4.12)	3-24	13.91 (3.59)	8-20
Prenatal STAI	33.57 (9.42)	20-76	29.99 (7.31)	20-64	32.89 (8.71)	20-75	35.88 (10.19)	20-76

Table 1. Continued

	Total (*N* = 521)		SKIPPY (*N* = 127)		SMILEY (*N* = 154)		Online (*N* = 240)	
	***M* (*SD*)/ Mode/%**	**Range**	***M* (*SD*) / Mode/%**	**Range**	***M* (*SD*) / Mode/%**	**Range**	***M* (*SD*) Mode/%**	**Range**
PRAQ Total	21.60 (6.5)	10-49	19.40 (5.44)	10-35	------[3]	-----	22.73 (6.72)	10-49
PRAQ Scale 1	9.43 (2.88)	4-20	8.57 (2.70)	4-17	------	-----	9.88 (2.88)	4-20
PRAQ Scale 2	6.31 (2.26)	3-15	5.67 (1.95)	3-11	6.06 (2.14)	3-12	6.81 (2.38)	3-15
PRAQ Scale 3	5.74 (2.38)	3-14	5.17 (2.13)	3-11	------	-----	6.04 (2.45)	3-14
Infant sex (% boys)	49.8%		57.1%		46.8%		------[4]	
Infant age (weeks)	12.59 (.97)	11.86-17.43	13.24 (1.36)	11.86-17.43	12.59 (.97)	11.86-17.43	------	------
Postnatal EPDS	8.07 (4.78)	3-26	13.62 (4.07)	8-21	7.75 (4.63)	3-26	------	------
Postnatal STAI	31.40 (9.44)	20-79	30.43 (8.30)	20-54	31.78 (9.85)	20-79	------	------
IBQ-R negative affect	2.92 (.77)	1.52-5.67	3.03 (.78)	1.9-5.67	2.89 (.77)	1.52-5.43	------	------
IBQ-R orienting/regulation	4.96 (.69)	1.83-6.90	5.09 (.59)	3.88-6.09	4.91 (.72)	1.83-6.90	------	------

Note. Baby-PAWS scales: Scale 1 "*Separation from Infant Worry*", Scale 2 "*Self and Partner Worry*", Scale 3 "*Baby Caregiving Worry*", Scale 4 "*Non-parental Childcare Worry*." PRAQ scales: Scale 1 "*Fear of Giving Birth*", Scale 2 "*Worries of Bearing a Handicapped Child*", Scale 3 "*Concern about Own Appearance*." [1] Education = low/medium: secondary education or vocational education; high: bachelor's or master's degree or higher (i.e. PhD) [2] Years of education and income were not obtained for the SKIPPY and SMILEY samples.[3] Not all items were administered; only Scale 2 "*Worries of Bearing a Handicapped Child*" was thus computed.[4] Postnatal variables were not measured in the Online sample.

SKIPPY Study

A randomized controlled trial (SKIPPY) of a skin-to-skin postnatal intervention for full-term infants was conducted (*N* = 127; Cooijmans et al., 2017). At 34-36 weeks of gestation, prior to randomization, participants completed questionnaires addressing demographics and prenatal distress. At 12-weeks postpartum, participants were asked to respond to an online questionnaire, including surveys with items inquiring about maternal mental health and infant temperament. Data collection took place between April 2016 and February 2018.

Inclusion criteria for this cohort were: singleton pregnancy, no substance use, fluent in Dutch, ≥ 18 years of age, no severe physical/mental health issues, and no concurrent participation in other studies. Infant inclusion criteria included: full-term status (≥ 37 weeks); birthweight ≥ 2,500 g; no congenital anomalies; and a 5-minute Apgar score of ≥ 7. Due to the intervention, only control group (*N* = 63) participants contributed to postnatal analyses, with a total of 55 meeting inclusion criteria and completing the questionnaire. Exclusion was due to birth complications (*n* = 4), personal circumstances (*n* = 2), or missing the 12-weeks postpartum questionnaire (*n* = 2).

SMILEY Study

Women in the second Dutch sample were part of the ongoing SMILEY study (Study of Microbiota and Lifestyle in the Early Years), following 160 mothers and their infants from pregnancy onward. Participants responded to online questionnaires addressing prenatal distress at 18-weeks (not used in the current study) and 32-weeks of pregnancy. Questionnaires at 12-weeks postpartum addressed maternal distress and infant temperament. Data were collected from December 2019 to February 2022.

Inclusion criteria were: singleton pregnancy, mastery of the Dutch language, ≥ 18 years of age, no severe physical/mental health issues, enrollment at < 21 weeks of gestation, and a pre-pregnancy BMI < 30. The final sample included 154 participants for prenatal analyses at 32-weeks of pregnancy. Exclusion was due to obstetric complications (*n* = 2), stillbirth (*n* = 1), medication use (*n* = 1), and being unable to contact participants (*n* = 2). Infant inclusion criteria at birth were the same as for the SKIPPY study. Data from 139 participants were available for the predictive postpartum analyses. Exclusion was due to birthweight < 2500 (*n* = 1), delivery < 37 weeks gestation (*n* = 3), Apgar 5min < 7 (*n* = 2), infant health issues (*n* = 1), maternal medication use (*n* = 2), obstetric complications in late pregnancy (*n* = 2), personal reasons (*n* = 2), or missing the 12-week postpartum questionnaire (*n* = 2).

Online Study

The third Dutch sample of pregnant women (*N* = 285) was recruited specifically for the current validation study. Online questionnaires were administered in the third trimester (27-41+ weeks) between April and June 2022. From 285 completed responses, 240 participants met inclusion criteria. Exclusions were due to serious health complaints (*n* = 41): (gestational) diabetes *n* = 26; (gestational) hypertension *n* = 7; fibromyalgia *n* = 3; rheumatoid arthritis *n* = 2; miscellaneous *n* = 3; or gestational age less than 27-weeks (*n* = 4).

Three samples of pregnant women (*N* = 521) were included in prenatal analyses. For the postnatal/predictive analyses, SKIPPY and SMILEY samples (*N* = 194) were used.

US Sample. The US sample included expectant mothers in their third trimester (i.e., 27-40 weeks gestation). Women (*N* = 276) were recruited through community flyers, social media, birthing classes, and hospitals in Eastern Washington/Northern Idaho. Women were mostly in their late 20s or early 30s and primarily White. Although the average income was relatively high, the range was also considerable, with most women reporting working and living with a spouse/partner (Erickson et al., 2020).

Measures

Baby Preparation and Worry Scale (Baby-PAWS)

The Baby Preparation and Worry Scale (Baby-PAWS; Erickson et al., 2020) displayed acceptable internal consistency for both the subscales and the total score in the US (Self and Partner Worry α = 0.90, ω = 0.91; Non-parental Childcare Worry α = 0.77, ω = 0.79; Baby Caregiving Worry α = 0.74, ω = 0.75; sum score of the 11 items that loaded onto subscales α = 0.89, ω = 0.90). The questionnaire administered to participants in the original study consisted of 16 items. All items loading <.40 or presenting with substantive loading (i.e., >.40) on more than one factor were excluded from the scales, resulting in 11 items considered in subsequent analyses.

In the current study we used the revised questionnaire, which consisted of the original 16-item questionnaire, with four additional items, resulting in the final 20 items. These four additional items were: 17. Not being able to sooth the baby when he/she is crying; 18. Having a baby who doesn't sleep well at night; 19. Not loving the baby enough; and 20. Not having enough time to maintain relationships with others because you're busy with the baby. Analyses for the revised 20-item questionnaire were carried out with the SMILEY and Online study Dutch samples

(*N* = 394). These four items were introduced based on discussions with prenatal stress researchers and Baby-PAWS developers (Erickson et al., 2020). Participants were instructed: "When you think about your life after the baby is born, how often do you worry, feel nervous or uneasy about the following?" A 7-point Likert scale (Never, Very rarely, Less than half the time, Half the time, More than half the time, Almost always, Always) was used with corresponding scores ranging from 1 to 7. "Does not apply" was an answer option and higher scores indicated greater worries. The questionnaire was translated to Dutch by two research assistants. These translations were back-translated to English by an English native speaker; incongruities were discussed and resolved by the Baby-PAWS researchers.

Edinburgh Postnatal Depression Scale (EPDS)

The Edinburgh Postnatal Depression Scale (EPDS; Cox et al., 1987) is widely used to measure perinatal depression. The questionnaire consists of 10 items, with a 4-point scale ranging from 0 to 3. A total score is a sum ranging from 0 to 30, with higher scores indicating greater depression. Internal consistency in the current study was good (α = .81, ω = .71).

The State Trait Anxiety Inventory (STAI)

Anxiety was measured by the State subscale of the State Trait Anxiety Inventory (STAI*;* Spielberger, 1985), with 20 items on a 4-point scale ranging from 1 to 4. Scores are based on a sum of all items range from 20 to 80, with higher scores reflecting greater anxiety. Internal consistency in the present sample was excellent (α = .93, ω = .93).

Pregnancy-Related Anxieties Questionnaire – Revised (PRAQ-R/PRAQ-R2)

In the SKIPPY study, the original PRAQ-R was administered, while the newer version of the questionnaire (PRAQ-R2) was administered in the SMILEY and online studies (Huizink et al., 2016). The PRAQ-R2 differs in rephrasing of one item, making the questionnaire applicable for multiparous women (Huizink et al., 2016). Both questionnaires include 10 items forming three subscales: 1) Fear of giving birth, 2) Worries of bearing a handicapped child, and 3) Concern about one's own appearance. Only 7 of the 10 items were administered in the SMILEY study, thus only the "Worries of Bearing a Handicapped Child" scale was computed. Internal consistency in the current study was as follows: (1) Fear of giving birth (α = .59, ω =.66); (2) Worries of bearing a handicapped child (α = .56, ω = .58); and (3) Concern about own appearance (α = .62, ω =.68); Total score (α = .81, ω = .75).

Infant Behavior Questionnaire-Revised, Short Form (IBQ-R)

Infant temperament was measured with the Dutch version of the Infant Behavior Questionnaire-Revised Short (IBQ-R Short; Gartstein & Rothbart, 2003). The IBQ-R Short Form has 91 items rated on a 7-point scale, which yield 14 scales and 3 factors serving as valid and reliable indicators of infant temperament (Gartstein & Marmion, 2008). For this study, we were particularly interested in the factors of Negative Emotionality, Orienting/Regulation, and their subscales. The Negative Emotionality factor consists of the subscales Distress to Limitations, Fear, Sadness, and Falling Reactivity/Rate of Recovery from Distress (reversed). Infant Orienting/Regulation consists of the subscales Low Intensity Pleasure, Cuddliness, Duration of Orienting, and Soothability. Internal consistency in the current study were as follows: (1) Distress to Limitations (α = .79, ω = .54); (2) Fear (α = .67, ω = .80); (3) Sadness (α = .76, ω = .73); (4) Falling Reactivity/Rate of Recovery from Distress (α = .83, ω = .84); (5) Low Intensity Pleasure (α = .75, ω = .72); Cuddliness (α = .68, ω = .74); Duration of Orienting (α = .78, ω = .73); and Soothability (α = .87, ω = .84).

Statistical analyses

We first assessed mean differences among the three data sets using independent group *t*-tests. Descriptive statistics (means, SDs) for the original 16-item and revised 20-item questionnaire were also considered.

Exploratory Factor Analyses (EFA)/Exploratory Structural Equation Modeling (ESEM) using maximum likelihood estimation with robust corrections for potential nonnormality and goemin oblique rotation within Mplus Version 8.6 (Muthen & Muthen, 2017) were performed. Results were also used to compute model-based estimates of omega reliability (see, e.g., McNeish, 2018).

To establish concurrent validity of the Dutch Baby-PAWS, bivariate correlations were computed between subscales and the total score, identified as optimal on the basis of EFA/ESEM results, with prenatal anxiety and depression indicators: EPDS, STAI (state), PRAQ-R Fear of giving birth, Fear of bearing a handicapped child, Appearance concerns and PRAQ-R total score. Subsequently, multiple regression models were examined to determine the contribution of Baby-PAWS subscales and total scores to postpartum maternal distress (EPDS, STAI), fine-grained negative emotionality and regulation dimensions (IBQ-R Negative Emotionality and Orienting/Regulation scales). When predicting postpartum depression and anxiety, prenatal anxiety and depression scores were considered as covariates, along with maternal age, weeks of gestation, and parity. A total of 8 regression equations was considered for infant temperament, with Distress to Limitations, Fear, Sadness, and

Falling Reactivity (Negative Emotionality) and Low Intensity Pleasure, Cuddliness, Duration of Orienting, and Soothability (Orienting/Regulation) as dependent variables, controlling for maternal age, weeks of gestation, and parity.

Results

Questionnaire Structure and Internal Consistency

EFA results conducted with the 16-item version of Baby-PAWS provided support for a 4-factor structure. Fit indices for solutions with one through four factors were considered; 1-factor: AIC (Akaike Information Criterion) = 25351.62, BIC (Bayesian Information Criterion) = 25555.80, RMSEA (Root Mean Square Error Of Approximation) = 0.08, CFI (Comparative Fit Index) = 0.81, SRMR (Standardized Root Mean Square Residual) = 0.07; 2-factor: AIC = 25160.85, BIC = 25428.84, RMSEA = .07, CFI = 0.87, SRMR = 0.05; 3-factor: AIC = 25044.34, BIC = 25371.88, RMSEA = .06, CFI = 0.92, SRMR = 0.04; and 4-factor: AIC = 24973.54, BIC = 25356.39 , RMSEA = .06, CFI = 0.95, SRMR = 0.03.

Overall, superiority of fit indices (i.e., lowest AIC and BIC values, lowest RMSEA and SRMR values, and highest CFI values) supported the 4-factor structure in the Dutch sample, with the latter implemented in the remaining analyses. The obtained four factors were readily interpretable: Factor 1 reflected the theme of "*Separation from Infant Worry;*" Factor 2 was consistent with the previously reported "*Self and Partner Worry*" subscale; Factor 3 paralleled the original "*Baby Caregiving Worry"* factor, including an additional item addressing bonding; Factor 4 items were from the original "*Non-parental Childcare Worry*" subscale. These four factors were indicated by the totality of 12 items with clear primary loadings (Table 2).

Additional items included in the 20-item version of Baby-PAWS did not contribute to a more conclusive or interpretable factor structure. That is, support for the 4-factor structure of Baby-PAWS was mixed, with RMSEA (3-Factor = .07; 4-Factor = .08) and CFI/TLI (3-Factor = .88/.83; 4-Factor = .84/.74) favoring the 3-factor solution, and SRMR (3-Factor = .05; 4-Factor = .04) supporting the 4-factor structure. The fourth factor was also associated with a single uniquely-loading item. Thus, evidence indicating additional four items benefit the Baby-PAWS factor structure was not obtained.

For the remaining analyses we hence proceeded with the 12 items for the total score and the 4 factors, obtaining the following internal consistency for each of the factors/

scales as well as the total score: (1) Separation from Infant Worry (ω = .69)[1]; (2) Self and Partner Worry (α = .83, ω = .81); (3) Baby Caregiving Worry (α = .66, ω = .74); (4) Non-parental Childcare Worry (α = .66, ω = .67); and (5) Total score (α = .85, ω = .83).

Concurrent Questionnaire Associations

All bivariate correlations between four Baby-PAWS subscales, total score, and prenatal maternal anxiety/depression indicators were statistically significant (Table 3). These associations were in the predicted direction, with higher transition to parenthood worries captured by Baby-PAWS related to greater prenatal anxiety and depression.

Table 2: Primary loadings for the 4-Factor Baby-PAWS

Baby-PAWS Item	Factor 1 (SIW)	Factor 2 (SPW)	Factor 3 (BCW)	Factor 4 (NpCW)
2. Transitioning back to work, because it will be difficult to separate from the baby*.	.99			
6. Leaving my baby with others*.	.42			
4. Finding quality time to be with my partner once we have the baby.[1]		.83		
5. Having "me time" to relax and enjoy hobbies after the baby is born.[1]		.83		
7. Changes in the relationship with my romantic partner.[1]		.57		
1. Not being able to figure out why the baby is crying.[3]			.48	
8. Breastfeeding and/or the baby's diet.[3]			.48	
9. Knowing what to do if the baby is sick or injured.[3]			.55	
15. Bonding with the baby.*			.50	
16. Feeling exhausted/sleep-deprived and stressed-out after having the baby.[1]			.55	
11. Not finding adequate childcare for my baby.[2]				.54
12. The costs of daycare and other financial needs of the child.[2]				.67

Note. SIW = Separation from Infant Worry; SPW = *Self and Partner Worry*; BCW = *Baby Caregiving*; NpCW = *Non-parental Childcare Worry*; * Item was not associated with a factor in the previously identified 3-Factor solution for a US sample (Erickson et al., 2020). [1]Item loaded onto Factor SPW in the US sample solution. [2]Item loaded onto Factor NpCW in the US sample solution. [3]Item loaded onto Factor BCW in the US sample solution. Confirmatory Factor Analysis (CFA) was conducted for the 3-factor solution obtained with the US sample, yielding indicators of poor fit. Cronbach's α could not be computed for Separation from Infant Worry scale, which contains two items.

[1] Cronbach's α could not be computed for Separation from Infant Worry scale that contains only two items

Table 3. Concurrent associations between Baby-PAWS, prenatal anxiety/depression indicators.

	1	2	3	4	5	6	7	8	9	10	11
1. Baby-PAWS Factor 1	---										
2. Baby-PAWS Factor 2	.33**	---									
3. Baby- PAWS Factor 3	.44**	.55**	---								
4. Baby- PAWS Factor 4	.36**	.36**	.34**	---							
5. Baby-PAWS Total	.67**	.78**	.86**	.62**	---						
6. EPDS	.18**	.35**	.37**	.31**	.40**	----					
7. STAI-State	.10*	.22**	.24**	.18**	.25**	.46**	----				
8. PRAQ-R/Fear of giving birth	.21**	.34**	.42**	.31**	.48**	.31**	.28**	----			
9. PRAQ-R/Fear of bearing handicapped child	.17**	.37**	.32**	.31**	.44**	.42**	.23**	.74**	----		
10. PRAQ-R/ Appearance concerns	.13*	.30**	.20**	.23**	.33**	.43**	.22**	.60**	.44**	----	
11. PRAQ-R Total	.18**	.39**	.35**	.33**	.47**	.52**	.29**	.93**	.85**	.79**	----

Note. **$p < .01$; *$p < .05$; #$p < .10$. Factor 1: "*Separation from Infant Worry*", Factor 2: "*Self and Partner Worry*", Factor 3: "*Baby Caregiving Worry*", Factor 4: "*Non-parental Childcare Worry*".

Predictive Analyses

Baby-PAWS total score reliably predicted postpartum anxiety and depression scores controlling for covariates, including prenatal symptom indicators (Table 4a). However, only Factor 3 (*Baby Caregiving Worry*) approached significance (p = .07) in relation to the STAI State subscale. All related regression coefficients indicated that, as expected, high Baby-PAWS scores were predictive of more pronounced postpartum anxiety and depression, after accounting for prenatal symptoms.

The Baby-PAWS total score as well as the score based on Factor 4 (*Nonparental Childcare Worry*) predicted higher Distress to Limitations (Table 4b). Factor 3 (*Baby Caregiving Worry*) was associated with higher Sadness. Fear and Falling Reactivity effects approached significance. Fear was marginally predicted by the Baby-PAWS total score (p = .053), as well as Factor 2 (*Self and Partner Worry*; p = .09), with greater worry related to higher infant fear. The Baby-PAWS total score was marginally associated (p = .054) with lower Falling Reactivity, or lower ability to recover from distress.

For the regulation-related dimensions, Factor 3 (*Baby Caregiving Worry*) predicted lower duration of orienting and less cuddling. Higher cuddling was marginally predicted (p = .09) by the newly identified Factor 1 (*Separation from Infant Worry)*. Baby-PAWS scores corresponding to Factors 1, 3, and 4 made significant contributions to the Soothabililty scale, wherein lower soothability was predicted by greater *Baby Caregiving Worry* and *Non-parental Childcare Worry*. However, greater *Separation from Infant Worry* was associated with having a more soothable infant. No significant effects were noted for Low Intensity pleasure.

Comparison of US and Dutch Women

Because the 4-factor structure identified for the Dutch sample differed from the 3 factors obtained with the US sample, comparisons of US and Dutch Baby-PAWS scores were limited to item-level independent groups *t*-tests focused on the original 16 items (Table 5 and Figure 1). Multiple statistically significant differences emerged, most indicating higher levels of worry about transition to parenthood in the US.

Table 4. Predictive multiple hierarchical regression models

4a. Outcome: postnatal maternal symptoms						
Variable	***R***	***R*²**	**Δ*R*²**	***β***	**B**	**95% CI**
EPDS						
Predictor: Baby-PAWS Total Score						
Final Model	55	.30	.04**			
Maternal Age				.12	.16	[-.05, .37]
Gestational Age				.12	.36	[-.08, .79]
Parity				-.19*	-1.85	[-3.35, -.34]
Prenatal EPDS				.39**	.47	[.28, .65]
Baby-PAWS Total				.21**	.12	[.03, .20]
Predictors: Baby-PAWS Subscale Scores						
Final Model	.55	.31	.05#			
Maternal Age				.14	.19	[-.04, .41]
Gestational Age				.11	.32	[-.12, .77]
Parity				-.19*	-1.78	[-3.32, -.25]
Prenatal EPDS				.39**	.46	[.27, .65]
Baby-PAWS Factor 1				.06	.13	[-.32, .57]
Baby-PAWS Factor 2				-.01	-.01	[-.34, .32]
Baby-PAWS Factor 3				.12	.13	[-.10, .37]
Baby-PAWS Factor 4				.13	.34	[-.15, .83]
STAI-State						
Predictor: Baby-PAWS Total Score						
Final Model	.47	.22	.11**			
Maternal Age				-.03	-.07	[-.47, .33]
Gestational Age				-.07	-.25	[-.78, .28]
Parity				.00	.03	[-2.72, 2.78]
Prenatal STAI-State				.26**	3.58	[1.62, 5.54]
Baby-PAWS Total				.34**	.37	[.22, .52]
Predictors: Baby-PAWS Subscale Scores						
Final Model	.49	.24	.13**			
Maternal Age				-.01	-.03	[-.44, .39]
Gestational Age				-.08	-.28	[-.81, .25]
Parity				.03	.47	[-2.29, 3.23]
Prenatal STAI-State				.24**	3.30	[1.30, 5.30]
Baby-PAWS Factor 1				-.00	-.00	[-.68, .67]
Baby-PAWS Factor 2				-.04	-.15	[-.75, .46]
Baby-PAWS Factor 3				.33**	.70	[.31, 1.10]
Baby-PAWS Factor 4				.19*	1.05	[.20, 1.89]

Table 4. Continued

4b. Outcome: infant temperament						
Variable	***R***	***R2***	**ΔR^2**	***β***	**B**	**95% CI**
Negative Emotionality						
IBQ-R Distress to Limitations						
Predictor: Baby-PAWS Total Score						
Final Model	.28	.08	.04**			
Maternal Age				-.19*	-.06	[-.10, -.01]
Gestational Age				-.02	-.01	[-.07, .05]
Parity				.01	.03	[-.29, .35]
Baby-PAWS Total				.21**	.02	[.01, .04]
Predictors: Baby-PAWS Subscale Scores						
Final Model	.32	.10	.06*			
Maternal Age				-.17*	-.05	[-.10, -.00]
Gestational Age				-.00	.00	[-.06, .06]
Parity				.02	.04	[-.28, .36]
Baby-PAWS Factor 1				-.05	-.02	[-.10, .06]
Baby-PAWS Factor 2				.11	.04	[-.03, .11]
Baby-PAWS Factor 3				.09	.02	[-.03, .06]
Baby-PAWS Factor 4				.16*	.10	[.00, .20]
IBQ-R Fear						
Predictor: Baby-PAWS Total Score						
Final Model	.20	.04	.02#			
Maternal Age				-.07	-.02	[-.06, .03]
Gestational Age				.07	.03	[-.03, .08]
Parity				.10	.18	[-.12, .48]
Baby-PAWS Total				.15#	.02	[.00, .03]
Predictors: Baby-PAWS Subscale Scores						
Final Model	.27	.07	.06*			
Maternal Age				-.03	-.01	[-.05, .04]
Gestational Age				.09	.03	[-.02, .09]
Parity				.08	.15	[-.15, .45]
Baby-PAWS Factor 1				.08	.03	[-.04, .11]
Baby-PAWS Factor 2				.17#	.06	[-.01, .12]
Baby-PAWS Factor 3				-.12	-.03	[-.07, .02]
Baby-PAWS Factor 4				.14	.08	[-.02, .17]

Table 4. Continued

IBQ-R Sadness						
Predictor: Baby-PAWS Total Score						
Final Model	.17	.03	.01			
Maternal Age				-.13	-.04	[-.09, .01]
Gestational Age				-.00	.00	[-.06, .06]
Parity				.02	.04	[-.30, .37]
Baby-PAWS Total				.10	.01	[-.01, .03]
Predictors: Baby-PAWS Subscale Scores						
Final Model	.25	.06	.04#			
Maternal Age				-.14	-.04	[-.09, .01]
Gestational Age				.01	.00	[-.06, .07]
Parity				.04	.09	[-.25, .43]
Baby-PAWS Factor 1				-.16#	-.07	[-.15, .01]
Baby-PAWS Factor 2				-.05	-.02	[-.09, .05]
Baby-PAWS Factor 3				.23*	.05	[.01, .10]
Baby-PAWS Factor 4				.13	.08	[-.03, .18]
IBQ-R Falling Reactivity						
Predictor: Baby-PAWS Total Score						
Final Model	.29	.09		.02#		
Maternal Age				.22**	.07	[.02, .11]
Gestational Age				.14#	.06	[-.00, .12]
Parity				.00	.01	[-.32, .33]
Baby-PAWS Total				-.14#	-.02	[-.04, .00]
Predictors: Baby-PAWS Subscale Scores						
Final Model	.29	.09	.02			
Maternal Age				.23**	.07	[.02, .12]
Gestational Age				.14#	.06	[-.00, .12]
Parity				.00	.00	[-.33, .33]
Baby-PAWS Factor 1				-.01	-.00	[-.08, .08]
Baby-PAWS Factor 2				-.05	-.02	[-.09, .05]
Baby-PAWS Factor 3				-.09	-.02	[-.07, .03]
Baby-PAWS Factor 4				-.03	-.02	[-.12, .09]
Orientation/Regulation						
IBQ-R Low Intensity Pleasure						
Predictor: Baby-PAWS Total Score						
Final Model	.25	.06	.00			
Maternal Age				.13	.04	[-.01, .08]

Table 4. Continued

Gestational Age				.18*	.07	[.01, .13]
Parity				.08	.16	[-.16, .49]
Baby-PAWS Total				.01	.00	[-.02, .02]
Predictors: Baby-PAWS Subscale Scores						
Final Model	.29	.09	.03			
Maternal Age				.17*	.05	[.00, .10]
Gestational Age				.17*	.07	[.01, .13]
Parity				.07	.13	[-.19, .46]
Baby-PAWS Factor 1				.14	.06	[-.02, .14]
Baby-PAWS Factor 2				-.01	-.00	[-.07, .07]
Baby-PAWS Factor 3				-.14	-.03	[-.08, .01]
Baby-PAWS Factor 4				.07	.05	[-.05, .15]
IBQ-R Cuddliness						
Predictor: Baby-PAWS Total Score						
Final Model	.30	.09	.01			
Maternal Age				.18*	.03	[.00, .07]
Gestational Age				.08	.02	[-.02, .06]
Parity				.15#	.20	[-.01, .41]
Baby-PAWS Total				-.08	-.01	[-.02, .01]
Predictors: Baby-PAWS Subscale Scores						
Final Model	.36	.13	.05#			
Maternal Age				.19*	-.04	[.01, .07]
Gestational Age				.08	.02	[-.02, .06]
Parity				.12	.16	[-.05, .37]
Baby-PAWS Factor 1				.15#	.04	[-.01, .09]
Baby-PAWS Factor 2				.12	.03	[-.02, .07]
Baby-PAWS Factor 3				-.28**	-.04	[-.07, -.01]
Baby-PAWS Factor 4				-.09	-.04	[-.10, .03]
IBQ-R Duration of Orientation						
Predictor: Baby-PAWS Total Score						
Final Model	.18	.03	.00			
Maternal Age				.08	.03	[-.03, .09]
Gestational Age				.05	.03	[-.05, .10]
Parity				-.18*	-.46	[-.87, -.05]
Baby-PAWS Total				-.06	-.01	[-.03, .01]
Predictors: Baby-PAWS Subscale Scores						
Final Model	.28	.08	.05#			

Table 4. Continued

Maternal Age				.13	.05	[-.01, .11]
Gestational Age				.07	.03	[-.04, .11]
Parity				-.20*	-.51	[-.91, -.10]
Baby-PAWS Factor 1				.04	.02	[-.07, .12]
Baby-PAWS Factor 2				.08	.04	[-.05, .12]
Baby-PAWS Factor 3				-.25*	-.07	[-.13, -.01]
Baby-PAWS Factor 4				.13	.10	[-.03, .22]
IBQ-R Soothability						
Predictor: Baby-PAWS Total Score						
Final Model	.19	.04	.01			
Maternal Age				.13	.04	[-.01, .09]
Gestational Age				.07	.03	[-.03, .09]
Parity				.02	.05	[-.29, .38]
Baby-PAWS Total				-.11	-.01	[-.03, .00]
Predictors: Baby-PAWS Subscale Scores						
Final Model	.31	.10	.07*			
Maternal Age				.13	.04	[-.01, .09]
Gestational Age				.04	.02	[-.04, .08]
Parity				-.01	-.01	[-.34, .32]
Baby-PAWS Factor 1				.23*	.10	[.02, .18]
Baby-PAWS Factor 2				.01	.01	[-.07, .08]
Baby-PAWS Factor 3				-.23*	-.05	[-.10, -.01]
Baby-PAWS Factor 4				-.18*	-.11	[-.21, -.01]

Note. $^{**}p < .01$; $^{*}p < .05$; $\#p < .10$. Factor 1: "Separation from Infant Worry"; Factor 2: "Self and Partner Worry"; Factor 3: "Baby Caregiving Worry"; Factor 4: "Non-parental Childcare Worry".

Table 5. Independent samples T-tests and factor loading for Baby-PAWS items for US and Dutch samples.

Items English	Items Dutch Translation	US		Dutch		*t* (df)	*p*	Factors US	Factors NL
		M	*SD*	*M*	*SD*				
1. Not being able to figure out why the baby is crying.	1. Er niet achter kunnen komen waarom de baby huilt.	2.18	1.14	2.75	1.34	5.19 (286)	.00**	BCW	BCW
2. Transitioning back to work, because it will be difficult to separate from the baby.	2. Weer aan het werk gaan omdat het moeilijk is om gescheiden te zijn van de baby.	2.97	2.19	2.75	1.30	1.16 (184)	.25	x	SIW
3. Having a strong social support network I can rely on to help with childcare.	3. Het hebben van een sterk sociaal netwerk waar je op terug kan vallen met opvang van de baby.	2.67	1.82	2.28	1.48	2.48 (215)	.01*	NpCW	x
4. Finding quality time to be with my partner once we have the baby.	4. Het vinden van quality time met mijn partner als de baby geboren is.	3.16	1.66	2.86	1.22	2.09 (202)	.04*	SPW	SPW
5. Having "me time" to relax and enjoy hobbies after the baby is born.	5. Het vinden van quality time voor mijzelf om tot rust te komen en te genieten van hobby's als de baby is geboren.	3.10	1.62	2.98	1.32	.83 (214)	.41	SPW	SPW
6. Leaving my baby with others.	6. Het achterlaten van de baby bij anderen.	3.24	1.80	2.68	1.33	3.56 (203)	.00**	x	SIW
7. Changes in the relationship with my romantic partner.	7. Veranderingen in de relatie met mijn partner.	2.80	1.64	2.61	1.23	1.33 (203)	.18	SPW	SPW
8. Breastfeeding and/ or the baby's diet.	8. (Borst)voeding van de baby.	3.34	1.82	2.62	1.32	4.58 (201)	.00**	BCW	BCW
9. Knowing what to do if the baby is sick or injured.	9. Weten wat je moet doen als de baby ziek of gewond is.	2.40	1.33	2.57	1.20	1.48 (671)	.14	BCW	BCW

Table 5. Continued

Items English	Items Dutch Translation	US		Dutch		*t* (df)	*p*	Factors US	Factors NL
		M	*SD*	*M*	*SD*				
10. Having friends whom I can talk to about parenting.	10. Het hebben van vrienden waarmee ik kan praten over het ouderschap.	2.08	1.32	1.88	1.11	1.73 (219)	.09#	x	x
11. Not finding adequate childcare for my baby.	11. Het niet kunnen vinden van goede opvang voor mijn baby.	2.31	1.60	1.89	1.25	3.04 (210)	.00**	NpCW	NpCW
12. The costs of daycare and other financial needs of the child.	12. De kosten van opvang en andere financiële benodigdheden voor de baby.	3.03	2.09	2.18	1.42	4.75 (195)	.00**	NpCW	NpCW
13. Having the time to complete household tasks (e.g., cooking, cleaning, laundry) once the baby is born.	13. Het hebben van tijd om huishoudelijke taken te doen (bijvoorbeeld, koken, schoonmaken, de was doen) als de baby geboren is.	3.52	1.59	2.65	1.34	6.13 (219)	.00**	x	x
14. Sharing tasks like feeding and changing our child with my partner.	14. Het verdelen van taken zoals eten geven en het verschonen van de baby met mijn partner.	2.63	1.56	2.23	1.22	2.91 (210)	.00**	SPW	x
15. Bonding with the baby.	15. Hechten aan de baby.	2.22	1.61	1.84	1.18	2.72 (201)	.01**	x	BCW
16. Feeling exhausted/sleep-deprived and stressed-out after having the baby.	16. Uitgeput en gestrest voelen en het hebben van een slaapgebrek na het krijgen van de baby.	3.82	1.71	3.26	1.49	3.69 (225)	.00**	SPW	BCW

Note. BCW = *Baby Caregiving Worry (NL factor 3*, US factor 3); SIW = Separation from Infant Worry (new NL factor 1); SPW = *Self and Partner Worry* (NL factor 2, US factor 1); NpCW = *Non-parental Childcare Worry (NL factor 4, US factor 2)*; x = not loading on a factor. **$p < .01$; *$p < .05$; #$p < .10$. Differences in degrees of freedom stem from missing item-level data on the Baby-PAWS questionnaire.

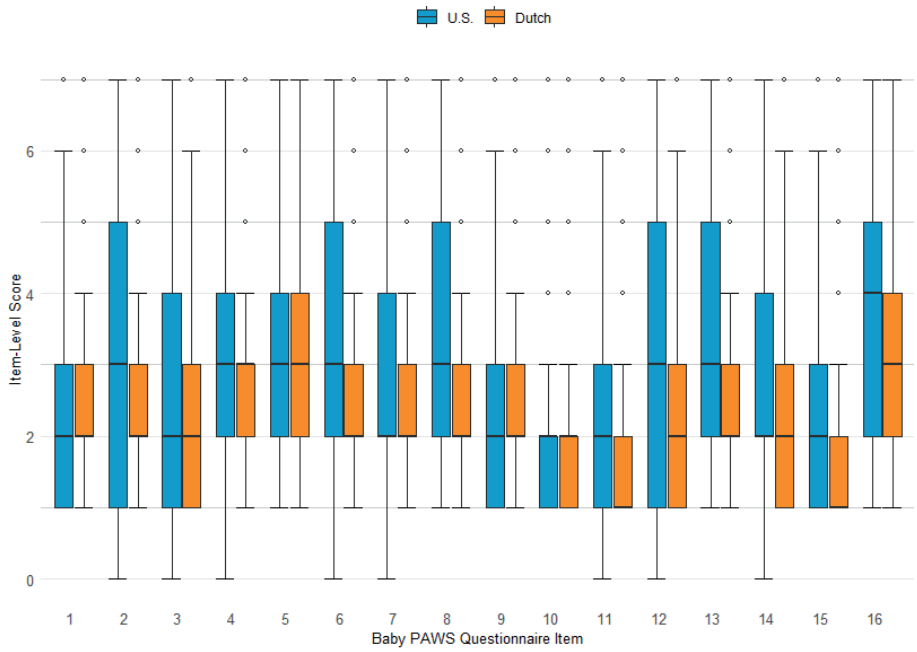

Figure 1. US and Dutch comparison of Baby PAWS item-level means

Discussion

We aimed to validate the Dutch version of the newly developed Baby Preparation and Worry Scale (Baby-PAWS) designed to measure the worries regarding the anticipated transition to parenthood. Furthermore, we examined associations between the Baby-PAWS questionnaire and established measures of maternal distress and infant temperament, providing evidence of concurrent validity and predictive value. We identified a four-factor structure for the 16-item questionnaire in our Dutch sample, as compared to the expected three-factor structure found in the original psychometric evaluation with the US sample (Erickson et al., 2020). These factors were demonstrated as internally consistent, with three of the four factors consistent with the three-factor US solution: 1) Self and Partner Worry, 2) Baby Caregiving Worry, and 3) Non-parental Childcare Worry. However, in the Dutch sample an additional fourth factor characterized as Separation from Infant Worry emerged. Higher levels of prenatal worries, as captured by Baby-PAWS factors, were related to higher scores on established measures of prenatal anxiety, depression, and pregnancy-specific stress. These correlations were generally moderate in strength, indicating that Baby-PAWS scores did not substantially overlap with the established constructs. This is not surprising given the Baby-PAWS

was designed to address distinct worries of pregnant women about the transition to parenthood, and is thus conceptually distinct from general depression and anxiety targeted by existing instruments. Ascertaining these specific worries is crucial for a deeper understanding of psychological challenges associated with becoming a parent. A more precise understanding of women's psychological challenges during this period can lead to the development of more effective (preventive) interventions in the future. The Baby-PAWS total score (sum of the original 12 items that loaded on factors in the Dutch sample) also predicted postnatal maternal depression, anxiety, and infant distress to limitations and sadness. Together, our findings provide evidence of good psychometric properties and validity for the Dutch Baby-PAWS and add to the existing literature indicating links between prenatal worries and postpartum psychological wellbeing, as well as infant temperament (Bush et al., 2017; Pluess et al., 2011; Van den Bergh et al., 2020).

In contrast to the findings from the original Baby-PAWS validation study, we identified a factor structure consisting of four rather than three factors. This divergence in factor structure is likely a function of considerable differences in perinatal policies and access to care in the US and the Netherlands. Importantly, maternity leave policies differ greatly between the Netherlands and the US. The US lacks a national paid maternity leave policy, at best allowing for up to 12 weeks of unpaid leave for eligible employees (FMLA, 2022). Alternatively, the Netherlands has a minimum of 16 weeks of fully paid leave, with additional days available (OECD, 2021). The anticipation of paid maternity leave decreases stress levels in families in the prenatal period (Van Niel et al., 2020). In contrast, the absence of paid maternity leave leads to higher employment rates during pregnancy and earlier return to work after birth, linked to poorer maternal mental health and infant outcomes (Brito et al., 2022; Heshmati et al., 2023). In the Netherlands, the mother's partner also receives one week of fully paid leave, with the possible addition of five weeks of 70% paid leave, shown to decrease maternal distress (Cardenas et al., 2021).

Second, the Netherlands also provides free postpartum care for all families with daily visits 8-10 consecutive days after birth. Maternity care assistants guide families through the transition after birth by educating them about postnatal care and providing help with household chores (Wiegers, 2006), which are all domains of worries reflected in the Baby-PAWS questionnaire. Third, perinatal health care is covered entirely by government insurance in the Netherlands, unlike the US which lacks a universal healthcare system, often resulting in financial stress because of steadily increasing costs around pregnancy and birth (U.S. Department of Services Health and Human, 2022). Together, these differences in paid maternity leave,

postnatal professional care, and health care costs between the Netherlands and the US may contribute to lower prenatal worries in the Netherlands, which our post-hoc item-level findings supported. Importantly, these policy differences may also be responsible for the observed discrepancy in the structure of anticipatory worries between Dutch and US mothers. For example, with the additional perinatal supports in the Netherlands, Dutch mothers expect to spend more time with their infants postpartum, and separating from the infant becomes a more concentrated area of worry.

As predicted, experiences of anticipatory worries indexed by Baby-PAWS were associated with increased pre- and postnatal distress symptoms. These findings align with the existing understanding of associations between pregnancy-specific worries and perinatal depressive and anxiety symptoms (Mudra et al., 2020; Osborne et al., 2021; Walker et al., 2021). In contrast with the Baby-PAWS total scores, the subscale scores were not related to either postnatal depression or anxiety. It may be that the combination of all four subscales, or the totality of anticipatory worries, is required to adequately predict postpartum anxiety or depression for Dutch mothers.

We expected positive associations between Baby-PAWS total/subscale scores and infant negative affectivity and inverse associations with orienting/regulation, and our findings supported some of these relationships (i.e., Baby-PAWS total and Factor 4 [Nonparental Childcare Worry] and Distress to Limitations; Factor 3 [Baby Caregiving Worry] and Sadness; Factor 3 and Cuddliness; Factor 3 and Duration of Orientation; and Factor 1 [Separation from Infant Worry], Factor 3, and Factor 4 each with Soothability). Similar to the relationship between concurrent and postnatal distress reported by mothers, Factor 3 (Baby Caregiving Worry) emerged as a significant predictor of infant temperament. One potential reason may be that mothers with heightened prenatal caregiving worries might also experience increased, or stable, parenting-related anxiety after the infant is born, which has been associated with a more difficult infant temperament (Fernandes et al., 2021; Oddi et al., 2013).

Notably, in the US validation of Baby-PAWS (Erickson et al., 2020), Factor 2 (Self and Partner Worry) instead of Factor 3 (Baby Caregiving Worry) emerged as the primary significant predictor of both maternal mental health and infant temperament. The present findings further highlight the need for cross-cultural examinations of parental psychology and maternal mental health, even for countries characterized as "European American". Pregnancy and the first months of life are periods characterized by transition and heightened plasticity that provide a

unique window of opportunity for interventions (Monk et al., 2022; WHO, 2023). An improved understanding of prenatal worries across different cultures/countries can provide the foundation for designing tailored preventive interventions and clinical treatments. Moreover, related research can be expected to result in identification of contextual factors contributing to maternal prenatal worries, such as policies on parental leave and perinatal support. This will deepen our understanding of complexity that exists between (and within) cultural groups as a result of policies, support, and norms (Lansford, 2022).

Our study has multiple strengths. We had a large sample size of more than 500 Dutch pregnant women, with adequate power for the factor analysis. Moreover, the longitudinal design in a subsample of pregnant women allowed us to discern that higher prenatal anticipatory worries, as measured by the Baby-PAWS, were indeed associated with higher postnatal levels of depression and anxiety. However, several limitations should also be noted. The participants had a higher socioeconomic status (SES) than average in the Netherlands, and we studied a relatively healthy community sample of pregnant women. Thus, our findings are not generalizable to all worries of Dutch pregnant women. Future research with a more diverse sample would allow for an examination of Baby-PAWS across socioeconomic strata and women experiencing perinatal health issues.

The fact that our large sample was obtained through different studies, with questionnaires completed at different time points between 2016 to 2022, represents another limitation. In particular, the SMILEY sample completed questionnaires during the peak of COVID-19, which may have resulted in some variability in the pattern of worries given the uncertainty and restrictions that occurred as a function of the pandemic. We accounted for differences between samples, controlling for the origin of data statistically in all analyses; however, the latter still warrants acknowledgement as a limitation. A further limitation is that no content validity checks were carried out for the translation of the questionnaire. In addition, our findings relied on self-report of one primary caregiver (i.e., mothers), and we did not obtain longitudinal data from all respondents. It should also be noted that although internal consistency analyses for established measures (e.g., EPDS, PRAQ) provided satisfactory alpha and omega estimates, the examination of factor structure required to obtain the latter estimates indicated relatively poor fit and potential deviations from prescribed solutions, which require further evaluations. Finally, although we controlled for prenatal levels of depression and anxiety, additional factors might have played a role in shaping postpartum depressive and

anxiety symptoms. As the present study does not allow for tests of causality, results speak to associations instead.

Despite these limitations, our results provide an initial validation of Baby-PAWS in a community sample of Dutch pregnant women. In the future, validation of the questionnaire could be expanded by considering other sources of information (e.g., observations of temperament) as well as inclusion of other, especially non-Western countries, to establish generalizability of Baby-PAWS. Moreover, our results indicate that factor structure of assessment tools widely considered to be established should nonetheless be examined when applied in different cultural contexts. We also recommend assessing associations between the Baby-PAWS scores and later maternal and infant outcomes. Before we can determine the clinical value of the Baby-PAWS, future research needs to address factors responsible for elevated Baby PAWS scores and greater worries about transition to parenthood. For example, research with clinical/"high-risk" samples (e.g., women with clinical diagnoses, such as Major Depressive Disorder, and those with trauma exposure) should be conducted to ascertain characteristics of women endorsing greater worry and obtaining higher Baby-PAWS scores. It will also be important to consider whether the Baby-PAWS scores are predictive of relevant postnatal function, such as impairments in mother-infant bonding, or lower quality of care. Lastly, future research should explore the relation between the Baby-PAWS scores and maternity leave (i.e., fully or partly paid), as well as access to perinatal care, which could clarify the potential impact of these contextual factors on prenatal stress and child outcomes.

In conclusion, we found a four-factor structure of the Baby-PAWS to provide generally reliable and valid indicators of anticipatory worry regarding transition to parenthood in a Dutch population. Baby-PAWS scores were associated with pre- and postnatal depression/anxiety, and infant temperament. However, a different factor structure in our Dutch sample, relative to the US analyses, emerged. The current validation study contributes to a better understanding of anticipatory prenatal worries and general cross-cultural differences in perinatal mental health. A better understanding of prenatal anticipatory worries can aid policymakers in supporting expecting families. The awareness of anticipatory worries and their relation to postpartum mental health can guide future interventions to reduce maternal perinatal distress.

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Supplementary material Chapter 2

Supplementary Table S1

Baby-PAWS Factor Loadings: Three-Factor Confirmatory Factor Analysis.			
Baby-PAWS Item	**SPW**	**BCW**	**NpCW**
4. Finding quality time to be with my partner once we have the baby.[1]	.87		
5. Having "me time" to relax and enjoy hobbies after the baby is born.[1]	.87		
7. Changes in the relationship with my romantic partner.[1]	.59		
6. Leaving my baby with others.*		.51	
11. Not finding adequate childcare for my baby.[2]		.57	
12. The costs of daycare and other financial needs of the child.		.67	
1. Not being able to figure out why the baby is crying.[3]			.43

Note. Chi-Square=227.68 (p<.01); RMSEA (Root Mean Square Error of Approximation)=0.06; CFI (Comparative Fit Index)= 0.91; TLI (Tucker–Lewis Index)=0.86; SRMR (Standardized Root Mean Square Residual)=0.039.SPW = Self and Partner Worry; BCW = Baby Caregiving; NpCW = Non-parental Childcare Worry; * Item was not associated with a factor in the previously identified 3-Factor solution for a US sample (Erickson et al., 2020). [1]Item loaded onto Factor SPW in the US sample solution. [2]Item loaded onto Factor NpCW in the US sample solution. [3]Item loaded onto Factor BCW in the US sample solution.

Chapter 3.

Prenatal hair cortisol concentrations during the COVID-19 outbreak: Associations with maternal psychological stress and infant temperament

Based on: Bruinhof, N., Vacaru, S. V., van den Heuvel, M. I., de Weerth, C., & Beijers, R. (2022). Prenatal hair cortisol concentrations during the COVID-19 outbreak: Associations with maternal psychological stress and infant temperament. *Psychoneuroendocrinology*, *144*, 105863. https://doi.org/10.1016/j.psyneuen.2022.105863

Abstract

Background. Maternal psychological stress during pregnancy, including stress resulting from disasters and trauma, has been linked to temperamental difficulties in offspring. Although heightened cortisol concentrations are often hypothesized as an underlying mechanism, evidence supporting this mechanism is not consistent, potentially because of methodological issues and low stress in the population.

Aim. To address these issues, this preregistered study investigated the following associations between: 1) prenatal psychological stress and hair cortisol, as a biomarker for chronic stress, during the COVID-19 outbreak (i.e., as a major worldwide psychological stressor), and 2) maternal hair cortisol during the COVID-19 outbreak and later infant temperamental negative affectivity and orienting/regulation. Additionally, we explored whether associations were different for women with low versus high socioeconomic status (SES; maternal education and annual household income) and at different stages of pregnancy.

Method. Pregnant women (N = 100) filled out online questionnaires during the first COVID-19 lockdown. Six months later, when most mothers were still pregnant or had just given birth, maternal hair samples were collected during home visits. When infants were six months old, mothers reported on their infant's temperament.

Results. Although hierarchical regression analyses revealed no associations between prenatal COVID-19 psychological stress and hair cortisol during the COVID-19 outbreak, SES proved to be a moderator in this association. Only pregnant women with higher levels of SES, not lower levels, showed a positive association between work-related and social support-related COVID-19 worries and hair cortisol. Finally, prenatal hair cortisol was not associated with later infant temperamental negative affectivity and orienting/regulation.

Conclusion. Although the COVID-19 outbreak proved to be a major psychological stressor worldwide, the physiological impact of the crisis might be different for pregnant women with higher SES as compared to lower SES.

Introduction

According to the *fetal programming hypothesis*, a stressful prenatal environment biologically 'programs' offspring's behavior (Seckl & Holmes, 2007). For example, research showed that maternal psychological stress during pregnancy forecasts infant behavioral development, including having a difficult temperament (for a review see: Van den Bergh et al., 2017). Specifically, elevated prenatal psychological stress has been linked to higher infant temperamental negative affectivity (i.e., fear, sadness, distress, and anger) and lower infant self-regulation (Huizink et al., 2002; Bush et al., 2017; van den Heuvel et al., 2015). The biological mechanisms underlying the associations between maternal prenatal psychological stress and infant outcomes remain unclear. Studies often focus on the maternal hypothalamus-pituitary-adrenal (HPA) axis, which becomes activated when confronted with stressors and results in the production of cortisol. However, evidence has been inconsistent about this link between psychological stress, prenatal cortisol, and infant behavior (Glover et al., 2010; Beijers et al., 2014). The nature of cortisol measurements (i.e., mostly saliva) and participant samples (i.e., low-risk pregnant women) used in previous studies might have obscured the possible role of cortisol in the association between prenatal psychological stress and infant outcomes (Zijlmans et al., 2015). To address this issue, the current study aims to investigate hair cortisol in pregnant women exposed to the COVID-19 crisis and its associations with psychological stress and infant temperament. The COVID-19 crisis was chosen because it led to heightened stress in the majority of the population, including low-risk pregnant women (e.g., Lebel et al., 2020; Vacaru et al., 2021). Because the COVID-19 pandemic is considered a chronic stressor, hair cortisol was chosen as a biomarker of maternal prenatal psychological stress capturing cortisol secretion over longer periods (Kirschbaum et al., 2009). The focus on infant temperament is due to 1) its associations with prenatal psychological stress (van den Bergh et al., 2017) and 2) having shown to be an early indicator for later psychological functioning (Rothbart et al., 2000; Gartstein et al., 2012).

Heightened levels of maternal cortisol are hypothesized to impact the intrauterine environment and affect the infant's developing neural system, with implications for later behavioral developmental outcomes (Bale et al., 2010; Bock et al., 2015; van den Bergh et al., 2018). However, psychological stress has often been found only weakly or not correlated with cortisol concentrations during pregnancy (Davis & Sandman, 2012; Werner et al., 2013; van den Heuvel et al., 2018). Cortisol is frequently measured with momentary saliva or blood samples (Zijlmans et al., 2015), reflecting acute stress and this may partly explain these weak links. In

contrast, especially high and continuous levels of stress (i.e., chronic stress) are suggested to affect the developing infant (Graignic-Philippe et al., 2014). As such, hair cortisol, as a biomarker for chronic stress, would be more suitable to measure long-term and retrospective HPA axis activity (Kirschbaum et al., 2009; D'Anna-Hernandez et al., 2011). Consequently, the methodological advantages of using hair cortisol (i.e. its chronic and retrospective nature) may address some of the limitations of the previously used cortisol measures. Yet, results for hair cortisol are mixed as well (Mustonen et al., 2018). Some studies have shown that hair cortisol is positively linked to prenatal psychological stress (Kalra et al., 2007; Hoffman et al., 2016) and to maternal emotion dysregulation (albeit not to episodic and chronic stress; Conradt et al., 2020), yet others did not replicate these findings (Kramer et al., 2009; Galbally et al., 2019; Orta et al., 2019). To date, only one study investigated the associations between maternal prenatal cortisol, when measured in hair, and infant temperament (Enlow et al., 2017), revealing that maternal prenatal hair cortisol was negatively correlated with infant rate of recovery from distress. Another explanation often proposed for the weak links between psychological stress and cortisol measurements during pregnancy is that low-risk samples were used in previous studies, with stress levels that were too low to find associations (Zijlmans et al., 2015). In this study, we therefore use hair cortisol as a measure of cortisol concentration in a sample of highly stressed pregnant women during a worldwide pandemic.

Studies that assess high prenatal stress due to exposure to disasters (e.g., ice storm; King & Laplante, 2005) or severe trauma (e.g., 9/11; Yehuda et al., 2005), can be seen as natural experiments in which all participants experience the same independent stressor (King et al., 2012). Although there is some evidence for an association between elevated maternal psychological stress levels, as caused by exposure to a disaster, and difficult infant temperament, these studies missed biological measures (Laplante et al., 2016; Zhang et al., 2018). The worldwide COVID-19 outbreak is a unique disaster situation different from previously studied disasters, with high uncertainty and social distancing policies. The crisis induced heightened stress levels in pregnant women (Lebel et al., 2020; Gustafsson et al., 2021; Perzow et al., 2021; Vacaru et al., 2021). COVID-19-related psychological stress during pregnancy included worries about altered social support (e.g., not seeing friends and family), work or finances (e.g., losing one's job), and general COVID-19-related aspects (e.g., getting sick; Vacaru et al., 2021). The current study examined two preregistered associations between these three types of COVID-19 worries and hair cortisol concentrations during pregnancy.

The current study tested two preregistered associations: 1) between COVID-19-related prenatal psychological stress levels (social support-, work- and general COVID-19-related worries) and prenatal hair cortisol, and 2) between prenatal hair cortisol during the COVID-19 outbreak and infant temperament (i.e. negative affectivity and orienting/regulation). We hypothesized that higher concentrations of prenatal hair cortisol during the COVID-19 outbreak were associated with 1) higher maternal psychological stress and 2) a more difficult infant temperament, specifically higher levels of negative affectivity and lower levels of orienting/regulation. In addition, two potential moderators were studied for hypothesis 1. Because of a lack of previous studies on these moderators, these analyses were carried out in an exploratory fashion. First, pregnant women might be differently impacted by the outbreak depending on their SES. On the one hand, low SES has been observed to be a risk factor for elevated stress during the COVID-19 crisis, due to lower financial and social resources (Berthelot et al., 2020). On the other hand, women with low SES have been found to be physiologically more resilient to an uncontrollable stressor due to already having experienced or experiencing more stress and adversity throughout their daily life (Glazier et al., 2004; Goyal et al., 2010). This previously experienced adversity can result in blunted cortisol (re)activity (Desantis et al., 2015; Khoury et al., 2019; Misiak et al., 2022). Second, maternal psychological stress and hair cortisol concentrations may be different at different stages of pregnancy as cortisol is known to increase over the course of gestation (de Weerth & Buitelaar, 2005), and pregnant women progressively exhibit dampened cortisol stress reactivity (Kammerer et al., 2002; Buss et al., 2009). Therefore, we will study the potential moderating roles of maternal SES and gestational age on the association between COVID-19-related psychological stress and hair cortisol during pregnancy. The results of our study may aid our understanding of the possible impact of the COVID-19 crisis on the next (unborn) generation.

Methods

Participants

This study is part of the international COPE study (COVID-19 and Perinatal Experiences) and the CovGen Alliance (http://covgen.org). The COPE study longitudinally followed COVID-19 related experiences of women and men attempting to conceive, expecting a child or with an infant younger than 6 months. Materials and procedures for the international COPE study were preregistered and shared on the Open Science Framework (https://osf.io/uqhcv/). Additionally, the two main hypotheses and statistical analyses were pre-registered on the Open

Science Framework (https://osf.io/bs45c/). The first wave of data was collected during the first COVID-19- lockdown in the Netherlands (April-May 2020). This lockdown entailed the closing of schools, restaurants, sports centers, and daycare. Stores were still open, but people were strongly advised to stay at home, work remotely and practice social distancing.

Pregnant participants of the COPE study (N=1421) were recruited through social media (60%), word of mouth (15%), midwifery centers (14%), and others (11%). A subsample was contacted again in the summer of 2020 for the collection of hair samples. Due to time constraints related to lockdown restrictions before July and washout effects of hair cortisol concentrations after September (Kirschbaum et al., 2009), there was a short time window to collect these data. Therefore, we scheduled home visits that could be geographically combined to reduce driving time. These took place in different regions in the Netherlands in August and September 2020 until reaching a convenience sample of 100 participants. These participants were all pregnant during the COVID-19 outbreak and when filling in the lockdown questionnaire. We compared our subsample with the total sample of 1421 pregnant women with Welch's t-tests and found no significant difference in SES between the groups (t (129.99) = -1.68, p = .09). However, our subsample had lower work (t (100.93) = 2.08, p < .05) and COVID-19 related worries (t (82.08) = 2.41, p < .05) but not social support-related worries (t (103.97) = 1.14, p = .26).

Of the 100 participants, 3 participants had to be excluded. Exclusion criteria were excessively high hair cortisol concentrations (surpassing known prenatal hair cortisol reference ranges, see Marceau et al., 2020) due to glucocorticoid medication use (n=1), and participants being less than 6 weeks pregnant during the first online COVID-19 outbreak questionnaire (n=2). These last participants were excluded because they were a small group and their COVID-19-related worries were expected to be different from those of women further along in pregnancy. This resulted in a final sample of 97 participants. At first contact the mothers had an average age of 31.89 years (SD = 3.43; range = 22 – 41) and were on average 26.44 weeks pregnant (SD = 8.40; range = 7.43 – 40.86).

Participation in the study was voluntary and participants were provided with information about data collection and -storage, before giving informed consent. The online study was approved by The Ethics Review Board of Tilburg University (RP2019-143). The collection of biological materials, such as the hair samples, was separately approved by The Ethics Committee Faculty of Social Sciences of Radboud University (ECSW-2020-056).

Procedure

Psychological stress was assessed through an online questionnaire during the first COVID-19 lockdown in the Netherlands (April 4 - May 10, 2020). After giving informed consent, participants answered questions about their current COVID-19 situation, including questions about experienced worries due to COVID-19. The complete questionnaire took 30 to 45 minutes for participants to fill in. Participants received 10 euros for completing the questionnaire.

Around 6 months after the start of the COVID-19 crisis (i.e., in August and September 2020), maternal hair was collected during a home visit by a trained researcher. A hair sample of around 100 to 150 hairs was cut close to the scalp. Each sample was wrapped in aluminum foil and stored at room temperature. In October 2020, all hair samples were sent to the Dresden LAB Service, Germany to determine the cortisol concentrations using a column-switching LC-APCI-MS/MS method. Participants were compensated with 5 euros and a small present for their participation in the home visits. When infants were 6 months old, mothers filled in another online questionnaire about maternal wellbeing and infant development, including temperament. Filling in the questionnaire took around 30 minutes and mothers received 10 euros reimbursement.

Measures

Internal consistency of the questionnaires was assessed using Revelle's omega total (ω_t; McDonald, 2013). Omega as a measure of internal consistency is especially useful for questionnaires with items varying in how strongly they relate to the construct being measured and thus in which tau equivalence is not assumed (McNeish, 2018).

Psychological Stress

Maternal prenatal COVID-19-related stress was measured with the COVID-19 and Perinatal Experiences Questionnaire (COPE questionnaire, Thomason et al., 2020). This questionnaire was created to assess the impact of the COVID-19 pandemic, such as COVID-19-related symptoms, restrictions on daily life, social support, and experienced stress. The questionnaire consists of 8 slider-questions ranging from 0.0 to 10.0. A higher score on an item indicated higher psychological stress. From these 8 items, 3 latent variables were identified by a factor analysis and principal component analysis (PCA; Vacaru et al., 2021). These variables were deemed to represent the major types of COVID-19 worries in this population and were hence chosen as the psychological stress measures in our study. The three identified factors were: 1) work and financial-related worries (worries related to current and

future work and financial situation), 2) social support-related worries (worries related to changes in partner and social support), and 3) general COVID-19 related worries (worries of COVID-19 symptoms for oneself and others, caring for family, and general worries related to the COVID-19 outbreak). The components were calculated by averaging the respective items, but only when there was one or no missing values on the items. Omega for the current study population was .92 for work-related worries, .64 for social support-related worries, and .87 for general COVID-19-related worries (see Vacaru et al., 2021 for the questionnaire items and detailed PCA description).

Hair cortisol

The hair samples were split into 3 segments of 3 cm each, as measured from the scalp (see Figure 1). The first segment (furthest from the scalp) reflects the cortisol secretion from December 2019 to February 2020 (pre-COVID-19), the second segment reflects cortisol secretion from March to May 2020 (first COVID-19 lockdown), and the third segment reflects the cortisol from June to August 2020 (post-COVID-19 lockdown). For the analyses, we only used the first (pre-COVID-19) and second (COVID-19 lockdown) hair cortisol segment. To control for potential washout effects on the cortisol of the oldest segments (Kirschbaum et al., 2009), and following the assumption that the washout effect occurs for everyone, we exploratorily assessed the individual change by controlling for pre-COVID-19 hair in our hierarchical model. During the collection of the hair samples, 42.3% of the women were pregnant. Because women were at different stages of pregnancy when filling out the COVID-19 questionnaire, we controlled for gestational age in the analyses.

Infant temperament

Infant temperament was measured with the Dutch version of the Infant Behavior Questionnaire-Revised Short (IBQ-R Short; Gartstein & Rothbart, 2003), which is a reliable and valid method of measuring infant temperament (Gartstein & Marmion, 2008). The IBQ-R has 91 items on a 7-point Likert scale. For this study, we assessed the temperamental factors of infant Negative Affectivity and infant Orienting/Regulation. Negative Affectivity consisted of the scales Sadness, Distress to Limitations, Fear, and Falling Reactivity/Rate of Recovery from Distress (reversed). Higher scores on this factor mean more instances of fear, sadness, distress, anger, and discomfort. Orienting/Regulation, consisted of the scales Low Intensity Pleasure, Cuddliness, Duration of Orienting, and Soothability. Higher scores on this factor mean infants are harder to soothe and display less attentional orienting behavior. Omega of both orienting/regulation (.88) and negative affectivity (.93) was excellent.

Moderators

Maternal socioeconomic status (SES) was assessed by calculating a composite score of the standardized values of maternal education (highest achieved education) and annual household income (total estimated yearly income; one missing allowed). Gestational age in weeks was reported when filling out the COPE Questionnaire during the first COVID-19 outbreak and was linked to the corresponding COVID-19 lockdown hair cortisol segment (March-May 2020 period).

Covariates

During the home visit, participants filled in a questionnaire that included four potential hair cortisol confounders: hair product use, frequency of hair washing, heat-based/chemical hair treatments (e.g., perms, dyes, bleaching, chemical straightening), and glucocorticoid use of the past 3 months (e.g., tablets, inhalation, cream, injections). For the temperament analyses infant sex was used as a covariate.

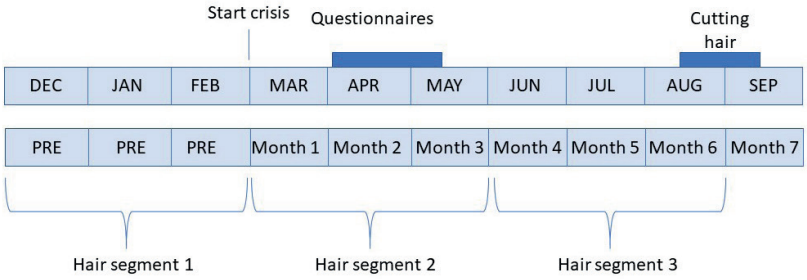

Figure 1. Timeline of the 9cm hair strand and COVID-19 outbreak

Missing data

For the first hypothesis, of the 97 participants three had missingness on work-related worries, and one on social support-related worries, resulting in a remaining sample of 93 participants. For the second hypothesis, 20 participants did not provide complete data for the temperament measurement at 6 months postnatal follow-up. Of the remaining 77 participants, four participants did not fill out infant sex. As a result, the sample consisted of 73 participants for hypothesis 2. Lastly, there were 3 missing values of hair cortisol pre-COVID-19 outbreak, due to the undetectability of cortisol levels in the hair sample.

A statistical sensitivity power analysis performed in G*Power showed that with 73 participants (minimum number of participants in the analyses with hair cortisol concentrations and infant temperament), a significance level of .05, we have enough power (.80) to detect an effect of .29 (small to moderate effect size).

Statistical analyses

First, all study variables were checked for outliers (greater or smaller than 3 SD from the mean). Two outliers were detected: hair cortisol COVID-19 outbreak (N=1), hair cortisol pre-COVID-19 outbreak (N=1). These outliers were winsorized (i.e., replaced by a value of 3 SD from the mean). The potential hair cortisol covariates were tested with multiple one-way ANOVAs. When significant, they were added as a covariate, as based on a recent hair cortisol meta-analysis (Marceau et al., 2020).

For the main analyses, hierarchical regression was performed. For hypothesis 1, we conducted a regression with maternal psychological stress during pregnancy as independent variable and prenatal hair cortisol concentrations during the COVID-19 outbreak as dependent variable. The covariates were added in step 1 and the three subscales of COVID-19 psychological stress were added in step 2. For hypothesis 2, two regression models were computed (i.e., one with infant temperamental negative affectivity as dependent variable and one with temperamental orienting/regulation as dependent variable). The covariates were added in step 1, and the hair cortisol concentrations were added in step 2. The exploratory analyses consisted of interactions of SES and the three subscales of psychological stress, and gestational age and the three subscales of psychological stress, on maternal hair cortisol. Finally, to check the cortisol stress response to the unexpected COVID-19 outbreak we reran the three hierarchical regression models of our main analyses with a relative difference score of pre-COVID-19 and COVID-19 outbreak cortisol concentrations, by additionally controlling for pre-COVID-19 hair cortisol concentrations in step 1. Potentially, not the absolute hair cortisol levels during the lockdown but increases in cortisol from pre-lockdown to lockdown, are a better representation of maternal stress. Because there are no studies yet on prenatal hair cortisol difference scores, these analyses were exploratory. All analyses were performed in R (R Core Team, 2019).

Results

Descriptive analyses

Descriptive statistics are shown in Table 1. Correlations between the study variables are presented in Table 2. Hair cortisol during the COVID-19 outbreak was not correlated to work-, social support- and general COVID-19-related worries. Moreover, hair cortisol during the COVID-19 outbreak was not correlated to infant negative affectivity and orienting/regulation.

The hair cortisol covariates analysis showed that hair cortisol was significantly correlated to heat-based/chemical hair treatments (e.g., perms, dyes, bleaching, chemical straightening; $F(2,94) = 4.09$, $p < .05$). Therefore, the variable hair treatment was used as a covariate in all main analyses. The other three hair cortisol covariates were not significantly associated with hair cortisol concentrations: frequency of hair washing, ($F(5, 91) = 1.40$ $p = .23$), glucocorticoid use of the past 3 months, (e.g., tablets, inhalation, cream, injections; $F(2,94) = .34$, $p = .72$), and hair product use, ($F(2,94) = 3.05$, $p = .052$). We followed our preregistration by only including covariates with a *p*-value smaller than .05. However, due to the almost significant *p*-value of hair product use, we re-ran the main analyses with this variable included and found similar results. Log-transformations were performed on hair cortisol concentrations since both pre-COVID-19 and COVID-19 hair cortisol variables were positively skewed. After transformation, the distributions of the hair cortisol variables met the normality assumption.

Table 1. Demographic characteristics ($N = 97$)

	***M (SD)* / % (N)**	**Range**
Infant sex (% boys)	53.93%	
Parity (nulliparous)	38.14%	
Hair treatment use	39.18%	
Hair product use	23.96%	
Glucocorticoid use	18.56%	
Hair washing per week (<1/1-2/3-4/>4)	4/51/34/11%	
Dutch background	97%	
Gestational age (in weeks, April-May 2020)	26.44 (8.40)	7.43 – 40.86
1st trimester	8.25% (8)	
2nd trimester	42.27% (41)	
3rd trimester	49.48% (48)	
Education		
Low	23.71%	
High	76.29%	
Annual household income*		
< €40,000	18.39%	
€40,000 to €100,000	65.52%	
> €100,000	16.09%	
Pre-COVID-19 hair cortisol	2.16 (2.63)	.12 – 17.59
COVID-19 hair cortisol	3.70 (3.96)	.06 – 17.84
Work Worries	4.08 (2.70)	0 – 9.5
Social Support Worries	2.63 (2.41)	0 – 8.65
General COVID-19 Worries	5.34 (2.08)	0 – 9.1
Infant Negative Affectivity	2.71 (.74)	1.35 – 4.90
Infant Orienting/ Regulation	5.45 (.51)	4.33 – 6.46

Note. Raw hair cortisol values (pg/mg) before log transformation, hair treatment use = heat-based/ chemical hair treatments (e.g., perms, dyes, bleaching, chemical straightening), hair product use = temporary products present in hair at the moment (e.g., hair spray, mousse, wax), hair washing per week divided in categories: less than once a week, one to two times a week, 3 to 4 times a week and more than 4 times a week. Education = low: secondary education or vocational education, high: bachelor or master's degree or higher (i.e. PhD). *N* = total sample, *M* = mean, *SD* = standard deviation. *10% preferred not to answer the item on annual household income.

Table 2. Pearson Correlation Matrix among all variables in the study (*N*= 97)

	1.	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**	**10**
1.COVID-19 hair cortisol	-	.08	.07	- .02	.06	- .17	-.01	.00	.10	-.10
2. Difference hair cortisol		-	-.05	-.11	-.08	-.14	-.02	.21*	-.02	.01
3. Work Worries			-	.43***	.62***	.09	.05	-.06	-.16	-.21*
4. Social Support Worries				-	.48***	.06	-.03	.21*	-.11	.04
5.COVID-19 Worries					-	.06	.01	- .05	-.01	-.33***
6.Negative Affectivity						-	-.41***	-.12	-.09	.07
7. Orienting/Regulation							-	.10	.09	-.11
8. Gestational age								-	-.13	-.04
9. SES									-	.04
10. Infant sex										-

Note. *** < .001 ** < .01 * < .05. Log transformed COVID-19 cortisol, difference hair cortisol = difference score of log transformed pre- and COVID-19 hair cortisol, SES = Socioeconomic status, *N* = total sample. Infant sex: boys = 1, girls = 2.

Main analyses

Table 3 presents the results of the hierarchical regression models. Hair cortisol concentrations during the COVID-19 outbreak were significantly predicted by the covariates gestational age, SES, and hair treatment (Step 1). Adding the three maternal COVID-19 worries variables did not significantly improve the model. Adding COVID-19 outbreak hair cortisol in step 2 did not significantly improve the model. Temperamental orienting/regulation also showed no significant relations in both steps 1 and 2. Moreover, neither associations were moderated by infant sex (see supplementary materials).

Exploratory regression analyses

Moderation by SES and gestational age. Hair cortisol during the COVID-19 outbreak was significantly predicted by the interaction between SES and work-related stress (b = .05, SE= .02, p = .019) and by the interaction between SES and social support-related stress (b = .14, SE= .07, p = .034). Figure 2 present the simple slopes associated with these interactions as a function of SES (high SES= +1SD, low SES= -1SD). Post hoc analyses indicated that, while hair cortisol concentrations were positively associated with work-related worries for women with high SES (b= .06, SE= .03, p = .03), they were not associated with work-related worries for women with low SES (b= -.02, SE= .02, p =.32). The interaction between SES and social support-related worries on hair cortisol concentrations was significant, but post hoc analyses showed non-significant slopes for both low SES (b= -.04, SE= .03, p =.16) and high SES (b= .04, SE= .03, p = .23). The association between general COVID-related worries and outbreak hair cortisol was not moderated by SES (b = .03, SE= .03, p = .20). Furthermore, the interactions between gestational age and hair cortisol were not significant: work-related worries (b = -.00, SE= .00, p =.86), social support-related worries (b = -.01, SE= .01, p = .32), general COVID-19-related worries (b = -.00, SE= .00, p = .30).

Difference score for pre-and outbreak COVID-19 hair cortisol. Table 4 summarizes that the hair cortisol difference scores from pre-COVID-19 outbreak to post COVID-19 outbreak were not significantly related to psychological COVID-19-related stress (work-, social support-, COVID-19-related worries), nor to infant negative affectivity or orienting/regulation.

Table 3. Results from three main Hierarchical Regression Models of COVID-19 hair cortisol on prenatal worries and two subscales of infant temperament on COVID-19 hair cortisol.

Variables		β	R^2-model	R^2 change	*p*-value
COVID-19 outbreak hair cortisol					
Step 1			.12	.12	.02*
	SES	.09			.10
	Hair treatment	-1.38			.00**
	Gestational age	.00			.40
Step 2			.13	.01	.78
	Work Worries	.01			.63
	Social Support Worries	-.02			.43
	COVID-19 Worries	.01			.64
Infant negative affectivity					
Step 1			.05	.05	.57
	SES	-.08			.43
	Hair treatment	-.21			.29
	Gestational age	-.01			.46
	Infant sex	.01			.94
Step 2			.08	.03	.14
	COVID-19 Outbreak hair cortisol	-.30			
Infant orienting/regulation					
Step 1			.03	.03	.89
	SES	.04			.62
	Hair treatment	.05			.75
	Gestational age	.00			.66
	Infant sex	-.11			.39
Step 2			.03	.00	.82
	COVID-19 Outbreak hair cortisol	-.03			

Note. β = standardized regression coefficient, R^2-model= total explained variance by the model, R^2 change = partial explained variance by added predictors (step 2), *p*-value =significance level set at <.05. *** < .001 ** < .01 * < .05. Log transformed COVID-19 cortisol, SES = socioeconomical status, hair treatment use = heat-based/chemical hair treatments (e.g., perms, dyes, bleaching, chemical straightening).

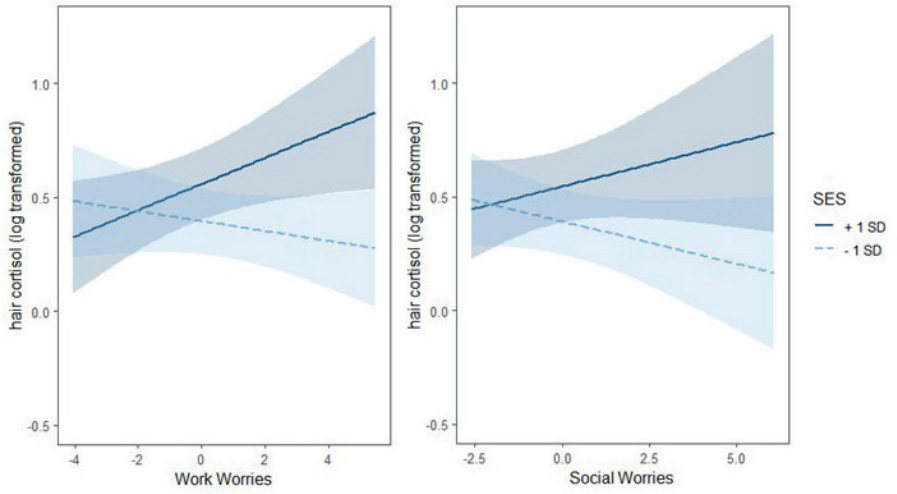

Figure 2. Moderation of socioeconomic status (SES) on hair cortisol concentrations and work- and social support-related worries

Table 4. Results from 3 exploratory Hierarchical Regression Models of difference in pre-COVID and COVID-19 hair cortisol on prenatal worries and 2 subscales of infant temperament on COVID-19 hair cortisol.

Variables		β	R^2-model	R^2 change	*p*-value
Work, social support, COVID-19 related stress					
Step 1			.03	.02	.55
	Pre-COVID-19 cortisol	1.11			.43
	SES	-1.12			.15
	Hair treatment	1.38			.66
	Gestational age	.01			.62
Step 2			.04	.00	.41
	COVID-19 Outbreak hair cortisol	-2.39			
Infant Negative Affectivity					
Step 1			.07	.07	.44
	Pre-COVID-19 cortisol	-.18			.38
	SES	-.08			.43
	Hair treatment	-.27			.20
	Gestational age	-.01			.48
	Infant sex				.69
Step 2			.09	.02	.28
	COVID-19 Outbreak hair cortisol	-.41			

Table 4. Continued

Variables		β	R^2-model	R^2 change	*p*-value
Infant Orienting/Regulation					
Step 1			.03	.03	.86
	Pre-COVID-19 cortisol	.03			.83
	SES	.02			.73
	Hair treatment	.07			.64
	Gestational age	.00			.86
	Infant sex	-.13			.29
Step 2			.03	.00	.76
	COVID-19 Outbreak hair cortisol	-.08			

Note. M=mean, SD=standard deviation, β = standardized regression coefficient, R^2-model = total explained variance by the model, R^2 change = partial explained variance by added predictors (step 2), p-value =significance level set at <.05. * < .05 ** < .001. Log transformed COVID-19 hair cortisol, SES = socioeconomical status, hair treatment use = heat-based/chemical hair treatments (e.g., perms, dyes, bleaching, chemical straightening).

Discussion

This study examined maternal prenatal hair cortisol concentrations during the COVID-19 outbreak and its association with maternal psychological stress during pregnancy and infant temperament at 6 months of age. The analyses did not reveal the expected association between COVID-19-related prenatal psychological stress and hair cortisol levels. However, SES moderated this association with women with higher SES showing a positive association between work- and social support-related worries and hair cortisol during the COVID-19 outbreak, and women with lower SES levels not showing these associations. Our results showed no association between maternal prenatal hair cortisol during the COVID-19 outbreak and infant temperamental negative affectivity and orienting/regulation. Lastly, the explored difference between pre-COVID-19 and COVID-19 outbreak hair cortisol was not associated with maternal psychological stress or infant temperament.

The findings of the current study do not support those of studies reporting positive associations between hair cortisol concentrations and psychological stress during pregnancy (Kalra et al., 2007; Hoffman et al., 2016), or between hair cortisol concentrations and emotion dysregulation during pregnancy (Conradt et al., 2020). However, our results are in line with those of multiple other studies that do not find associations between hair cortisol and psychological stress during pregnancy

(Kramer et al., 2009; Galbally et al., 2019; Orta et al., 2019; Conradt et al., 2020). In general, these studies, as well as our current study, used psychological stress measures that reflected daily, short-term disturbances in stress, while hair cortisol is thought to reflect more chronic cortisol secretion (Mustonen et al., 2018). Therefore, hair cortisol may be only associated with more chronic psychological stress. To test this hypothesis, studies are needed that require filling out questionnaires multiple times during the corresponding period of the hair cortisol segment.

Another explanation for the absence of a direct association is that it may not be present for the whole sample, but only for a subsample of participants. Indeed, we found that SES was a moderator, with women with a high SES showing a positive association between work- and social support-related worries and hair cortisol during the COVID-19 outbreak. Pregnant women with lower SES levels may have been differently impacted by the COVID-19 crisis than women with higher SES levels. Pregnant women with lower SES, in general, may already experience more stress (Glazier et al., 2004; Goyal et al., 2010). Therefore, an environmental stressor, such as the COVID-19 crisis, might affect them less physiologically. As a history of exposure to adversity and stress is hypothesized to result in altered HPA axis functioning with overall lower cortisol levels and lower reactivity to stressors (Desantis et al., 2015; Khoury et al., 2019; Misiak et al., 2022), this could explain the lack of an association between psychological stress and hair cortisol concentrations in pregnant low SES women. In contrast, high SES women might be expected to display a more typical physiological reaction of the HPA axis, with higher cortisol levels when experiencing heightened levels of daily stress. With the current study indicating a differential impact of the COVID-19 crisis on high and low SES groups, future research on prenatal exposure to stressors or life events should consider examining the role of SES in relation to hair cortisol.

We did not find support for our second hypothesis, that prenatal hair cortisol concentrations during the COVID-19 outbreak would be related to later infant temperament. There is little evidence for the mediating role of maternal cortisol as measured in saliva or blood in the link between maternal prenatal psychological stress and infant temperament (Zijlmans et al., 2015). Therefore, we used hair cortisol as an alternative measure of maternal cortisol that is better related to chronic stress, such as that produced by a natural disaster. However, in the current study hair cortisol concentrations during the first COVID-19 lockdown were still unrelated to maternal reports of psychological stress levels and infant temperament. Potentially, other infant outcomes such as infant health might be predicted by maternal hair cortisol concentrations during the COVID-19 outbreak

and this should be further investigated. In the past, associations between maternal prenatal reported stress, cortisol and infant health, have been observed (Beijers et al., 2010; Zijlmans et al., 2017).

We additionally explored the cortisol response to the COVID-19 outbreak by examining the difference in hair cortisol levels between the pre-COVID-19 sample and the COVID-19 sample. This hair cortisol difference was not related to COVID-19-related worries and infant temperament. This exploratory analysis was based on previous studies assessing prenatal saliva cortisol changes over time. For example, a steep or blunted cortisol increase during pregnancy has been found to be associated with maternal stress, anxiety, or depression, while independent cortisol assessments were not (Kane et al., 2014). Possibly, we found no associations in the current study due to the combination of a small sample size and participants differing in gestational age. We controlled for gestational age at the time of filling in the questionnaires in the analyses, but variation in gestational age may have added noise to the natural cortisol increase during gestation (de Weerth & Buitelaar, 2005; van den Heuvel et al., 2018). Future studies with larger samples may give more power to distinguish subtle changes in chronic hair cortisol measures due to maternal psychological stress from the natural rise in cortisol over pregnancy.

This study has several strengths, such as the combination of physiological and behavioral measures, the longitudinal design, and the use of a pandemic as a global chronic stressor that induced heightened reported stress levels in pregnant women. In a previous study on the same cohort of pregnant women (N=1421), we showed that, compared to a pre-COVID-19 pregnant sample, scores above the clinical cutoff for both depression and anxiety doubled during the COVID-19 outbreak (see Vacaru et al., 2021). A post-hoc analysis showed that our subsample of women providing hair cortisol also reported higher levels of clinically relevant depression (9% versus 6% pre-pandemic) and anxiety (40% versus 24% pre-pandemic).

However, limitations also need to be mentioned. With respect to hair cortisol, the maternal hair sample was cut in 3 X 3 cm segments, which implies that there may have been a washout effect for the pre-COVID-19 hair segment. The cortisol concentrations of the oldest hair segment are generally lower than those of newer hair segments (Kirschbaum et al., 2009). Although longer lengths of hair samples have been used and validated (Manenschijn et al., 2011), authors caution against using segments beyond the first 6 cm from the scalp because of cortisol washout (Kirschbaum et al., 2009; Orta et al., 2018). We took the washout effect into account by focusing on within-person cortisol changes, and by assuming that the washout

effect occurs similarly for everyone. However, it has to be noted that, although visiting participants multiple times for hair samples collection would not have been feasible due to the then-applicable COVID-19 lockdown restrictions (e.g., social distancing), future studies should consider collecting hair segments at multiple time points, hence allowing the use of 'younger' segments (Orta et al., 2018). Moreover each hair cortisol segment reflected three months of cortisol exposure. This might have contributed to finding no associations with the difference between the pre-COVID-19 and COVID-19 samples. To assess hair cortisol trajectories, such as the impact of the COVID-19 outbreak, it could be useful to divide hair samples into smaller segments (i.e., 1 cm), to allow for more accurate measures of individual cortisol change over time.

With respect to reported worries, our subsample had lower work- and COVID-19-related worries, than the total sample of pregnant women (N=1421), suggesting that our participants may have possibly been less representative. Accordingly, it can be argued that this sample may not have been ideal in addressing the question of whether the severity of one's stress affects how hair cortisol is related to a psychological stressor. A pre-COVID-19 control group could have been more optimal for answering this question. However, distress levels in the total sample of pregnant women were significantly higher than those of pre-COVID-19 pregnant women (Vacaru et al., 2021). In a post-hoc analysis, we assessed the distress levels in our COVID-19 subsample of women that provided hair samples (N=97), and also found elevated percentages of women experiencing clinically relevant depression and anxiety (i.e. 9% and 40%, respectively). Moreover, the variance and ranges for the worries variables indicated that some women in our sample were extremely worried. In sum, the COVID-19 outbreak was a global stressor that apparently affected our sample psychologically in such a way that it need not be considered a low-risk participant sample that is inadequate for answering our research question (Zijlmans et al., 2015).

Next, the factors social support and work-related worries only consisted of two items, which goes against the general recommendation of a minimum of three items per factor (MacCallum et al., 1999). However, both these factors were found to predict anxiety and depressive symptoms in pregnancy (Vacaru et al., 2021) as well as insensitive parenting practices (van den Heuvel et al., 2022), which increases our confidence in their value. Nonetheless, we advise future researchers to use more items to assess maternal worries when possible. Additionally, our sample showed some variation in SES and educational level (i.e. 24% of the participants had completed lower education and 18% reported a low household income), but this

was not representative of the Dutch population, thus limiting the generalizability of our results. Lastly, we used a mother-reported measure of temperament, which despite providing a knowledgeable and broad view of the child's temperament, is also subject to potential reporter bias. The use of objective behavioral measures could be an alternative for future research.

In sum, though the COVID-19 crisis affected the majority of society, the current study found no evidence for general associations between maternal prenatal psychological stress, hair cortisol concentrations, and infant temperament. However, evidence was found that COVID-19 psychological stress might have physiologically impacted pregnant women with higher SES differently than pregnant women with lower SES. Women with higher SES showed higher hair cortisol when they had more psychological stress. These results warrant further research into the mechanisms through which certain subgroups of women may be more vulnerable to chronic pregnancy stressors than others.

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Supplementary Material Chapter 3

Moderation Infant sex. Furthermore, we tested the moderating role of infant sex on the association between prenatal hair cortisol during the COVID-19 outbreak and infant temperament. All predictor variables were centered. The interaction between negative affectivity and infant sex (b = .70, SE = .38, p= .07) and orienting/regulation (b = -.27, SE = .27, p= .32) on prenatal hair cortisol were not significant.

Chapter 4.

Mother–infant stress contagion? Effects of an acute maternal stressor on maternal caregiving behavior and infant cortisol and crying

Based on: Bruinhof, N., Beijers, R., Lustermans, H. and de Weerth, C. (2025). Mother–infant stress contagion? Effects of an acute maternal stressor on maternal caregiving behavior and infant cortisol and crying. *Journal of Child Psychology and Psychiatry*, *66*: 1040-1052. https://doi.org/10.1111/jcpp.14119

Abstract

Background. Postpartum maternal distress has been associated with adverse infant outcomes. A potential pathway of how maternal distress affects infant outcomes could be alterations in maternal caregiving behavior. However, the associations between maternal distress, caregiving behavior, and infant outcomes have never been tested in a controlled experiment. This preregistered study utilized an experimental design to investigate effects of an acute maternal stressor on infant cortisol and crying, and the possible mediating role of maternal caregiving behavior.

Methods. Mother-infant dyads (N = 91) participated in a lab visit at 8 weeks postpartum, where mothers were separated from their infants to either perform a Trier Social Stress Test (TSST) or a control task. The task was immediately followed by a mother-infant interaction to assess maternal caregiving behavior and infant cortisol and crying.

Results. Our structural equation model found no differences between conditions (stressor/control) on maternal caregiving behavior and infant response to maternal stress. Secondary findings revealed that higher quality of maternal caregiving behavior was related to lower levels of infant crying and lower cortisol levels at the end of the visit, but not cortisol at reunion.

Conclusion. Our findings do not support the occurrence of mother-infant stress contagion in this experimental setting, but do indicate a link between maternal caregiving behavior and infant behavioral and cortisol responses. Given the high prevalence of maternal mental health problems and their possible negative association with offspring development, further (experimental) research is needed to understand just how maternal postpartum distress affects young infants.

Introduction

The transition to parenthood is often accompanied by maternal psychological distress (i.e. symptoms of depression, anxiety and stress; Dipietro et al., 2008; Monk et al., 2022). During pregnancy, increased maternal distress predicts poorer infant outcomes, including compromised neurodevelopment and increased negative affect, via various physiological mechanisms (Bates et al., 2022; Field, 2018; Kingston et al., 2012). Following childbirth, maternal psychological distress continues to be associated with compromised infant outcomes (Bates et al., 2022; Field, 2018; Kingston et al., 2012). During the postpartum period, one mechanism through which maternal psychological distress might impact the infant is through the transmission of distress, also referred to as stress contagion. During this process of stress contagion, negative affective states are transferred from one partner to another (Engert et al., 2019). When a mother is stressed, the infant might pick up on this stress and respond with their own cortisol or behavioral stress response. Only a few studies have investigated causal effects of maternal exposure to a stressor on infant outcomes. Waters et al. (2014; 2017) investigated potential stress contagion by exposing the mother to a laboratory stressor and then reuniting her with her 12- to 14-month-old infant. Maternal exposure to a stressor resulted in more sympathetic activation and avoidance behavior in the infant after reunion, as compared to infants of control group mothers. Whether the same would be true in younger infants is yet unknown. Therefore, our first goal was to investigate whether in the first postnatal months, maternal exposure to a stressor subsequently leads to an infant cortisol and behavioral response. The relevance of this goal is given by this postnatal period being critical due to rapid infant brain development, hypothalamus-pituitary-adrenal (HPA)-axis maturation, and the early stages of the development of the mother-infant attachment bond (Evans & Porter, 2009; Hodel, 2018). Furthermore, a better causal understanding of stress transmission from mother to young infant is important for designing effective (preventive) interventions (Perlman et al., 2022).

A potential pathway for how maternal distress can affect infant outcomes is through alterations in her caregiving behavior (Choe et al., 2013). Maternal caregiving behavior often decreases in quality when struggling with postpartum distress (Booth et al., 2018). Moreover, low quality of maternal caregiving behavior has been associated with adverse infant outcomes, (Berry et al., 2017; Deans, 2020), and found to mediate the association of maternal distress to infant behavior (Choe et al., 2013). However, this relation has never been tested in a controlled experiment. Our second research goal was to investigate whether maternal

exposure to a stressor affects the quality of her caregiving behavior, which in turn mediates the association between maternal stressor exposure and infant cortisol and behavioral response.

This experimental study was carried out in a low-risk healthy sample of mothers and their 8-week-old infants. Mothers were assigned to a stressor (i.e., Trier Social Stress Test; Kirschbaum et al., 1993) or control condition. Our main hypotheses were: (1) after reunion, infants of mothers exposed to the stressor will have higher cortisol and crying responses, compared to infants from mothers in the control condition, (2) this association will be (partially) mediated by lower maternal caregiving quality. Secondary hypotheses were, first, because individuals already experiencing daily distress are more susceptible to the effects of the acute stressor (Allen et al., 2014; Chida & Hamer, 2008), and many mothers experience distress postpartum (Browne et al., 2020): (3) the effects of maternal exposure to the stressor on caregiving quality and infant cortisol and crying will be stronger for mothers with pre-existing high levels of postpartum distress. It is important to note that we refer to these general levels of postpartum distress as measured by questionnaires as "distress". Moreover, we refer to the experimental manipulation as a "stressor" and to the maternal response to this stressor with “stress”. Second, as separation from the newborn might be stressful for all postpartum mothers, regardless of condition, we hypothesized that (4) for the group as a whole mothers’ heightened cortisol concentrations and negative affect will be associated with higher levels of infant cortisol and crying, and this association will be (partially) mediated by lower caregiving quality.

Methods

Participants

The current study is a randomized controlled experiment within the ongoing longitudinal SMILEY study (Study of Microbiota and Lifestyle in the Early Years) that follows a low-risk community sample of 160 healthy women and their infants from 18 weeks of pregnancy. Participants were recruited through our Baby and Child participant database (https://babyandchild.nl/en/), midwifery practices in surrounding areas, and social media. Maternal inclusion criteria were: singleton pregnancy, mastery of the Dutch language, ≥ 18 years of age, no severe physical/mental health issues, <21 weeks of gestation at enrollment, and pre-pregnancy BMI ≤30. Infant inclusion criteria were: born at ≥37 weeks of pregnancy, birth weight ≥2500 g, 5-min Apgar score ≥7 and absence of serious malformations and

diseases. An overview of excluded participants is presented in the participants flowchart (Figure 1). In total 142 mother-infant dyads were participating at 8 weeks postpartum, of which 92 dyads visited the lab between December 2020 and January 2022. Participants gave informed consent. From inclusion until 12 weeks postpartum, they received three small gifts and 100 euros. The hypotheses and statistical analyses of the study were preregistered on the Open Science Framework (https://doi.org/10.17605/OSF.IO/N5VPS). The study procedures were approved by the Ethics Committee Faculty of Social Sciences of the Radboud University (SW2017-1303-497), including two amendments (ECSW-2019-051 and ECSW-2020-021).

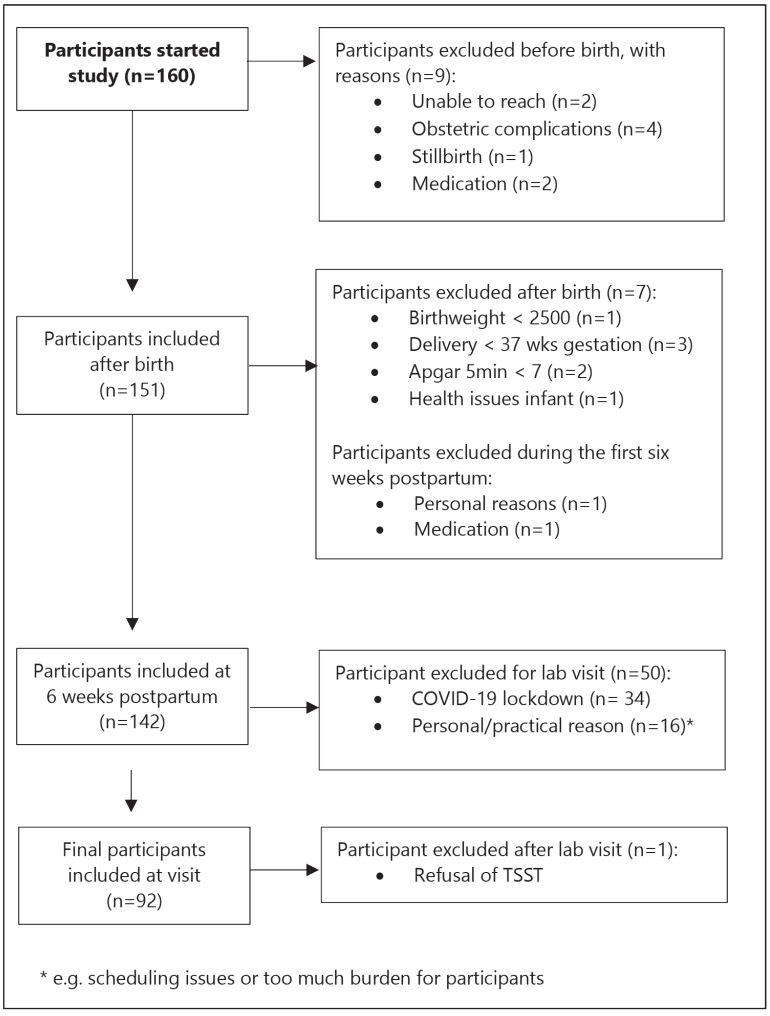

Figure 1. Participant flowchart

Procedure

Mothers and their infants were invited to visit the lab around 8 weeks postpartum (*M*= 8.21, *SD*=1.33). Mothers filled in questionnaires at home on general distress at 2 and 6 weeks postpartum. Upon arrival at the lab, mothers first received instructions. Then mothers were separated from their infant. Subsequently, the manipulation (stressor or control task) for the mother followed. In the meantime, a babysitter (i.e. a research assistant or a partner, parent, or friend of the mother) took care of the infant in a different room. After, mother and infant were reunited and a mother-infant interaction took place with the aim of observing maternal caregiving quality and infant behaviors. The infant was thus not directly exposed to the stressor or control task the mother performed. In total, the lab visit lasted around two hours (see Figure 2). Throughout the lab visit, five saliva samples of both mother and infant were collected to measure cortisol concentrations (see Table 1). Visits were scheduled in the afternoon (between 12.00h and 17.30h), to minimize the effect of natural cortisol circadian rhythm on cortisol reactivity. Mothers were instructed not to eat or drink anything other than water or caffeine-free tea, and to refrain from physical activity, for at least 1 hour before the visit.

Randomization

In pregnancy, participants were randomized into two conditions: a stressor condition (i.e. Trier Social Stress Test (TSST), and a control condition. The randomization was stratified using a random block randomization method to ensure a comparable distribution of primi- and multiparous women in both conditions. With a computer-generated allocation sequence, mothers were randomly assigned to either the TSST or control condition (1:1) in blocks of 4 or 6. An independent researcher, who was not involved in the study, prepared the schedule and concealed the allocation sequence in stapled envelopes. Before the prenatal visit, the main researcher opened the envelopes to allocate participants to stressor or control condition. The mothers that were randomly assigned to the TSST condition in pregnancy did the control condition postpartum and vice versa. The pregnancy visit studied effects of the acute stressor on dietary intake (see Lustermans et al., 2024). Participants were blinded for group allocation and unaware of the different conditions, until debriefing at twelve weeks postpartum.

Manipulation

In the stressor condition, participants performed the Trier Social Stress Test (TSST; Kirschbaum et al., 1993). Social evaluative lab stressors including the TSST have been shown to provoke a cortisol and subjective stress response in most participants and can be seen as the golden standard for eliciting acute stress (Zänkert et al.,

2019). Due to the ongoing COVID-19 pandemic, the jury was online through a live connection (Lustermans et al., 2024). The control condition (i.e., separation-only) participants performed a fabric feeling task (Van Strien et al., 2013).

For both conditions, the manipulation consisted of three elements: a preparation phase (5 minutes), a manipulation phase (10 minutes) and a waiting phase (5 minutes). For the stressor condition, the researcher explained the task according to the TSST protocol (Kirschbaum, Pirke, & Hellhammer, 1993). The mothers had 5 minutes to prepare for a presentation about their dream job, after which they were brought to a different room for their presentation. On a TV screen, two female judges were present wearing white lab coats. The judges were trained to look strict, give no positive feedback and take notes during the assignment. The participants performed a presentation (5 minutes) and an arithmetic task, in which participants had to count back from 1687 in increments of 13 (5 minutes). They were recorded by a visible camera. After the arithmetic task, the researcher came back to the room, instructing the participant to wait for a few more minutes for the jury to evaluate the performance. After 5 minutes of waiting the participant was told she did well on the task and brought back to be reunited with her infant.

For the control condition, the set-up mimicked the TSST set-up. Mothers were first instructed to read paper instructions about a fabric feeling task for 5 minutes. Afterward, they were brought to a different room (i.e., the same room as the TSST, however, without camera or jury) to rate different fabrics (e.g., denim, lace, satin) on different characteristics (e.g., softness, pleasantness, warmth). After 10 minutes, the mother was instructed by the researcher to stop the task and wait for the researcher to come back. After 5 minutes of waiting she was reunited with her infant.

Mother-infant interaction

After mothers had been reunited with their infants, a mother-infant interaction immediately followed with the aim of observing maternal caregiving quality and infant behaviors. To increase variability between mothers and ecological validity, mothers were instructed to undress, weigh and then dress their infants again. During this interaction, mothers and their infants were alone in the room and filmed by three corner cameras. The duration of the mother-infant interaction was 13 minutes, regardless of how far mothers were with the instructed tasks. This duration was chosen to ensure the interaction was long enough to reliably observe maternal caregiving behaviors.

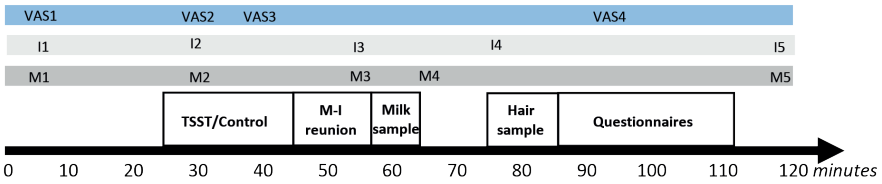

Figure 2. Timeline laboratory visit

Note. VAS1 – VAS4: Visual Analogue Scales. M1 – M5: maternal saliva samples 1 to 5, I1-I5 : infant saliva sample 1 to 5. TSST: Trier Social Stress Test. M-I reunion: mother-infant reunion. This timeline shows when the maternal and infant cortisol samples were collected. The timeline reflects the study protocol without interruptions (i.e. due to infant feeding or diaper change), however, adaptations were made to meet the infant's needs (e.g. infant feeding prior to manipulation or after sampling milk). Preregistered rules determined if deviations of the protocol were acceptable (see Table 1 for rules). Note that the hair sample, milk sample and questionnaires were not part of the current study.

Measures

Maternal response to acute stressor

Maternal cortisol reactivity

During the lab visit, mothers collected five saliva samples (M1 to M5) through passive drooling through a straw. Times of collection were dependent on the manipulation (see Table 1). Participants were instructed to collect at least 0.5 ml of saliva. After the visit, samples were stored in a freezer at –20 °C. Cortisol concentrations were determined at the Laboratory of Endocrinology at UMC Utrecht. They were measured without extraction using an in-house competitive radio-immunoassay employing a polyclonal anticortisol-antibody (K7348). [1,2-3H(N)]-Hydrocortisone (PerkinElmer NET396250UC) was used as a tracer. The lower limit of detection was 1.0 nmol/l. We calculated maternal reactivity as the maximum cortisol increase. We first selected the lowest baseline (either sample M1 or M2) and the highest peak (either sample M3 or M4) and used the formula: highest peak – lowest baseline. This method of direct comparisons between defined time points was chosen to capture the acute effects of the stressor more precisely and in accordance with previous papers to assess the individual variation in reactivity (Simons et al., 2019) We also conducted sensitivity analyses for cortisol reactivity by testing the peaks M3 and M4 separately, subtracting each from the lowest baseline (M1 and M2).

Maternal negative affect reactivity

To assess maternal negative affect in response to the stressor, mothers filled in the Dutch translation of the Visual Analogue Scales (VAS; Monk, 1989) at four time points (VAS1 to VAS4). The times of filling in the VAS reflected the timing of the saliva samples and reactivity to the stressor (see Table 1). The VAS consisted of 4 items asking about feelings of tenseness, calmness, happiness and sadness. Mothers filled in paper versions of the VAS by setting a cross on lines ranging from "not at all" on the left to "a lot" on the right. Scores were calculated by measuring the distance from the left of the line to the cross in millimeters (range 0 to 117). Total scores were calculated following the formula for global affect =[(happy)+(calm)+200-(sad)-(tense)]/4 (Monk, 1989). Total scores were reversed so that higher scores indicated more negative affect. To assess maternal negative affect reactivity, we followed a similar procedure as for cortisol reactivity by subtracting the highest peak (either VAS2 or VAS3) from baseline (VAS1). Similarly to cortisol reactivity, we also ran sensitivity analyses with VAS2 and VAS3 as peak separately.

Infant response to the mother's stressor

Infant salivary cortisol concentrations

To assess infant cortisol concentrations, five saliva samples (I1 to I5) were taken. Saliva of the infant was collected either by the mother or the researcher by gently swabbing the infant's mouth with absorbent eye sponges for approximately one minute (de Weerth et al., 2007). The timing of the infant cortisol samples corresponds to the timing of the mother samples, except for sample I4, which for the infant reflected the reunion (see Table 1). To avoid contamination of saliva samples by milk, the lab protocol prescribed that infant cortisol was not assessed immediately after feeding. After collection, the samples were centrifuged and stored at -20 °C. The laboratory analysis of the infant samples was identical to the maternal samples. Only samples I4 and I5 reflected moments after the manipulation (i.e. I4: reunion, and I5: end of the visit) and therefore expected to differ between the stressor and control group.

Infant crying

Infant crying was scored using the 13-minute videos of the mother-infant interaction. Every interval of 15 seconds was scored by the first author and a research assistant as containing infant crying or not (yes=1, no=0). Crying was defined as sounds with clear negative affect. Because videos differed slightly in duration, for every video, a percentage was calculated representing the proportion of intervals with infant crying. Thirty percent of the videos were double scored and

agreement between observers was good (92.99%). Observers of the videos were blind to the mother's condition (stressor/control) and other study variables (i.e., maternal postpartum distress levels).

Maternal caregiving behavior

Quality of maternal caregiving behavior was scored using the videos of the 13-minute mother-infant interactions. Mothers were rated on four different caregiving behavior scales: sensitivity, cooperation, positive regard for the child and negative regard for the child. High quality caregiving behavior is referred to as sensitive (i.e., caregiver responds timely and adequately to infant's needs and signals) and cooperative (i.e., caregiver adjusts behavior to infant and does not interfere with the infant's ongoing activity; (Ainsworth et al., 1978). Moreover, caregiving behavior quality often includes positive and negative regard to the child, with more positive and less negative regard promoting infant development (Brooker et al., 2016; Sheinkopf et al., 2017). Maternal sensitivity and cooperation were assessed using the Ainsworth scales (Ainsworth et al., 1978) with a 9-point scale ranging for sensitivity from 1 (highly insensitive) to 9 (highly sensitive), and for cooperation from 1 (highly interfering) to 9 (conspicuously cooperative). Positive and negative regard towards the child were scored on 7-point scales and were assessed using the Observational Record of the Caregiving Environment (ORCE) scales (NICHD Early Child Care Research Network., 1996). Higher scores indicated more positive and more negative regard towards the child, respectively. The four caregiving measures were added into the models as a latent factor. Videos were scored by the first author and two trained assistants, with 30% of the videos being double scored. Observers of maternal caregiving behavior were also blind to the mother's condition (stressor/control) and other study variables (i.e., maternal postpartum distress levels). Interrater agreement (Weighted Cohen's kappa, k) on the measures was excellent: sensitivity (k = .90), cooperation (k = .86), positive regard (k = .90), negative regard (k = .93). All measures were significantly correlated with each other, with correlations ranging from -.35 to .92.

General maternal postpartum distress

General maternal postpartum distress consisted of postpartum depressive, anxiety, and stress symptoms at 2 and 6 weeks postpartum. The questionnaires used were: the Edinburgh Postnatal Depression Scale (EPDS; (Cox, Holden, & Sagovsky, 1987), the State subscale of the State Trait Anxiety Inventory (STAI; (Spielberger, 1985) and the Perceived Stress Scale-10 (PSS-10) questionnaire (Cohen et al., 1983). The EPDS is a widely used questionnaire for perinatal depression. The questionnaire consists of 10 items, with a 4-point scale ranging from 0 to 3. The total scores range from

0 to 30, with higher scores indicating higher levels of depressive feelings. A score of 11 is advised as a cutoff score for an indication of subclinical depression (Levis et al., 2020). The STAI State consists of 20 items on a 4-point scale ranging from 1 to 4 and is often used to measure anxiety. A total score ranges from 20 to 80, with higher scores indicating higher reported anxiety levels. A score > 40 is commonly used as a cutoff score for subclinical anxiety (Dennis et al., 2013). Lastly, the PSS-10 is a questionnaire to measure stress. The questionnaire consists of 10 items with a 5-point scale, ranging from 0 and 4. Total scores range from 0 to 40, with higher scores indicating higher reported anxiety levels. While the EPDS assesses feelings of the past week and the STAI feelings of that moment, the PSS-10 scores indicate stress of the past month, therefore we only used the PSS-10 assessed at 6 weeks postpartum. Internal consistency for the questionnaires in the current study was excellent: EPDS (2 weeks ωt = .91, 6 weeks ωt = .86), STAI (2 weeks ωt = .94, 6 weeks ωt = .93) and PSS-10 (ωt = .91). The questionnaires were standardized and summed to a composite score called general maternal postpartum distress.

Covariates

No covariates were added to the analyses answering hypotheses 1 to 3. As the participants were randomized, groups are assumed not to differ systematically on variables that may confound the results (de Boer et al., 2015). For hypothesis 4, covariates are included. The selection of covariates was made with the use of a directed acyclic graph (DAG) following Cinelli et al., (2022). The covariates that subsequently were included for hypothesis 4 were: familiarity with the babysitter during separation (i.e., brought by mother versus researcher), infant cortisol at baseline (I3), breastfeeding before manipulation (yes/no), infant age (in days), and maternal food intake less than 1 hour before the visit (yes/no).

Missing data

One participant was excluded from the 92 lab visits due to refusing to perform the TSST. The final sample consisted of 91 participants, with 47 in the stressor condition and 44 in the control condition. Women who withdrew from the postnatal phase of the study did not significantly differ from those who did visit the lab on the following variables: maternal depressive, anxiety and stress symptoms at 2 or 6 weeks postpartum (all p's > .30), maternal education (p = .09) and parity (p = .22).

For the maternal cortisol samples, total missingness was 2.20% and for infant cortisol samples total missingness was 24.84 %. Missingness for infant samples was higher than maternal samples, mostly due to an insufficient volume of infant saliva or inability or refusal to collect when the infant was crying or sleeping. Sample 1

was on average taken 7 minutes after arrival (SD = 4, range = 0 - 33). Samples 2 to 5 were screened on sample time and removed when taken too late (see Table 1). Missingness of infant cortisol samples did not depend on postnatal depressive, anxiety and stress symptoms at 2 and 6 weeks (all p's > .36). Furthermore, missingness was also not related to maternal education (p= .11) nor parity (p = .27). We did find that infants with a missing cortisol sample at reunion cried more at that same moment (p < .01), and older infants had higher rates of missing data (p < .001). Our models corrected for missing values by means of imputation techniques (see section 5).

Table 1. Saliva cortisol sample information

Sample	Reflecting moment	Corresponding mother's VAS	Removing when?	N samples removed:
		Mother		
M1	Baseline*	N/A	N/A	N/A
M2	Start lab visit	VAS1	<35 min after VAS1	2
M3	Preparation manipulation	VAS2	>35 min after start manipulation	0
M4	Waiting after manipulation	VAS3	> 35 min after end manipulation	6
M5	End of visit	VAS4	<45 min after end manipulation	5
		Infant		
I1	Baseline*	N/A	N/A	N/A
I2	Start lab visit	N/A	<35 min after VAS1	2
I3	Babysitting	N/A	>35 min after start manipulation	0
I4	Reunion**	N/A	>30 min after end mother-infant interaction	0
I5	End of visit	N/A	<45 min after end manipulation	0

Note. M1 to M5 is mother sample 1 to 5, I1 to I5 is infant sample 1 to 5, VAS= Visual Analogue Scale, N/A = not applicable, min = minutes. * Reflects around 20 minutes before the visit, mostly when the mothers were traveling to the visit. ** Proposed contagion moment between mother and infant.

Statistical analyses

Before analyzing the data, cortisol values were checked for implausible high cortisol concentrations higher than 60 nmol/l (Miller et al., 2013). No implausible cortisol values for either mother or infant were detected in our data. Then, all study variables were checked for outliers (greater or smaller than 3 SD from the mean). The maternal cortisol and negative affect reactivity was tested for outliers per group (stressor/control). For the stressor group we had an outlier for M3 (n=1). For the control group we had outliers for M1 (n=1), M2 (n=1), M3 (n=1) and VAS3

(n= 1). For the whole group we had the following outliers: I5 (n=1), crying ratio (n=1), I1 (n=2), I3 (n=2), I4 (n=2), maternal negative regard (n=3), maternal depressive symptoms (n=1) and anxiety symptoms (n=1). Outliers were winsorized, meaning the value was brought back to 3 SD from the mean. Variables were normally distributed except M4, I3, I4, I5, infant crying, maternal depressive symptoms and anxiety symptoms scores. These variables were log-transformed, except maternal depressive symptoms score, which was square root transformed due to containing 0 values. As a manipulation check, we compared maternal cortisol and negative affect reactivity between the conditions (stressor/control) with a MANOVA.

For our main analyses we used structural equation modeling (SEM). To refrain from multiple testing and to be parsimonious, we used as little statistical tests as possible. Therefore, we used SEM to simultaneously test Hypothesis 1 and 2 (i.e., maternal condition (stressor/control) on infant response, mediated by caregiving quality). We performed three SEMs, one for each different outcome (i.e. infant cortisol at reunion, infant cortisol at the end of the visit, infant crying), with the two different conditions dummy coded as predictor (stressor/control; see Supplementary Figure 1A).

To investigate hypothesis 3 whether mothers with higher general postpartum distress showed a stronger association between stressor exposure and infant outcomes, we performed four regression models with maternal general postpartum distress as the moderator, the two different conditions (stressor/control) as predictor and a) infant cortisol at reunion, b) infant cortisol at the end of the visit, c) infant crying and d) maternal caregiving behavior as outcome. Due to the regression analyses, maternal caregiving behavior was added as a standardized sum score, instead of the latent factors used in SEM.

For secondary hypothesis 4 whether caregiving behavior mediated the association between maternal reactivity and infant response, we performed six SEMs with the continuous predictor: 1) maternal cortisol reactivity or 2) maternal negative affect reactivity, and with the infant outcomes: a) cortisol at reunion, b) cortisol at the end of the visit, c) crying and the mediator maternal caregiving behavior. We added the previous mentioned covariates (babysitter during separation, infant cortisol at baseline, breastfeeding before, infant age, and maternal food intake less than 1 hour before the visit) to all these models (see Supplementary Figure 1B).

All analyses were performed in R. All SEMs displayed a negative variance for maternal sensitivity in the latent factor caregiving, therefore maternal sensitivity

within the latent factor was set to 0. For the mediation analyses, we used lavaan (Rosseel, 2012) for a classic mediation set-up with the direct effect of X on Y and the indirect effect of X on Y via M. Missing data was imputed in the model with Full Information Maximum Likelihood (FIML) for the SEMs and Multiple Imputation by Chained Equations (mice; van Buuren & Groothuis-Oudshoorn, 2011) for the regression analyses.

Results

Descriptive analyses

Table 2 presents the descriptive statistics and Table 3 the correlations between the study variables. More infant crying was correlated to higher infant cortisol concentration at reunion (I4) and at the end of the visit (I5). Moreover, higher levels of general postpartum distress were correlated to more infant crying. Infant cortisol samples 4 and 5 were highly correlated.

Preliminary analyses

We examined maternal reactivity differences between the two experimental conditions (TSST/control; see Figure 3). As expected, a MANOVA revealed that mothers in the stressor condition had higher cortisol levels during the preparation for the manipulation (M3, $p < .001$), waiting after the manipulation (M4, $p < .001$) and at the end of the visit (M5, $p < .001$). Similarly for negative affect, we found that mothers in the stressor condition had higher levels of negative affect during the preparation for the manipulation (VAS2, $p < .001$) and waiting after the manipulation (VAS3, $p < .001$), but not at the end of the visit (VAS4, $p = .07$). Infant cortisol concentrations at reunion and at the end of the visit did not significantly differ between maternal conditions (stressor/control task; see Figure 3; see Table 3 for correlations).

Table 2. Demographic characteristics

	Total (*N* = 91)*.		Stressor (n = 47)		Control (n = 44)	
	M (SD) / % (N)	**Range**	**M (SD) / % (N)**	**Range**	**M (SD) / % (N)**	**Range**
Maternal age (in years)	32.56 (3.57)	22.95 – 42.27	33.22 (3.82)	22.95 – 38.38	32.54 (3.33)	25.79 – 42.27
Infant age (in weeks)	8.21 (1.33)	6.29 - 11.57	8.24 (1.23)	6.29 – 11.00	8.18 (1.23)	6.29 – 11.57
Infant sex (% boys)	50.55% (46)		54.19% (25)		47.73 (21)	
Parity (%nulliparous)	48.35% (44)		53.19% (25)		43.18% (19)	
Dutch background	91.21% (83)		89.26% (42)		93.18 (41)	
Education						
Low/medium	10.99% (10)		10.64% (5)		11.36% (5)	
High	89.01% (81)		89.36% (42)		88.64% (39)	
Maternal cortisol						
Cortisol 1	8.80 (2.84)	2.20 – 19.60	8.94 (2.50)	2.20 – 16.40	8.67 (3.17)	3.70 – 19.60
Cortisol 2	8.16 (2.69)	2.50 – 18.20	8.55 (2.86)	2.50 – 14.40	7.77 (2.49)	3.40 – 18.20
Cortisol 3	8.71 (3.75)	2.30 – 24.00	9.98 (4.53)	2.30 – 24.00	7.42 (2.08)	3.10 – 14.30
Cortisol 4	8.87 (4.39)	2.40 – 27.00	11.10 (5.35)	2.40 – 27.00	6.97 (1.91)	3.10 – 12.30
Cortisol 5	6.12 (2.50)	1.60 – 13.80	7.01 (2.85)	1.80 – 13.80	5.20 (1.67)	1.60 – 8.10
Maternal negative affect reactivity						
VAS 1	39.63 (13.42)	16.50 – 78.50	42.06 (15.08)	16.50 – 78.50	37.03 (10.97)	17.50 – 69.00
VAS 2	51.87 (20.74)	16.50 – 93.75	64.68 (17.94)	17.75 – 93.75	38.69 (13.60)	16.50 – 74.63
VAS 3	57.21 (25.31)	17.00 – 118.75	73.60 (23.46)	20.50 – 118.75	40.07 (12.71)	17.00 – 81.25
VAS 4	37.13 (12.76)	16.50 – 68.50	40.13 (12.33)	16.50 – 68.50	48.38 (12.80)	16.75 – 65.13
Infant cortisol						
Cortisol 1	10.43 (5.83)	4.30 – 34.00	10.51 (5.28)	4.50 – 25.00	10.36 (6.37)	4.30 – 34.00
Cortisol 2	10.78 (5.02)	2.60 – 24.00	11.28 (4.91)	2.60 – 22.00	10.30 (5.15)	4.10 – 24.00

Table 2. Continued

	Total (*N* = 91)*.		Stressor (n = 47)		Control (n = 44)	
	M (SD) / % (N)	**Range**	**M (SD) / % (N)**	**Range**	**M (SD) / % (N)**	**Range**
Cortisol 3	10.49 (5.05)	3.90 – 28.00	10.73 (4.93)	4.00 – 26.00	10.23 (5.22)	3.90 – 28.00
Cortisol 4	12.99 (8.05)	5.50 – 46.00	13.50 (7.91)	6.20 – 45.00	12.38 (8.33)	5.50 – 46.00
Cortisol 5	10.17 (4.27)	4.30 – 23.00	10.60 (4.22)	5.50 – 21.00	9.71 (4.35)	4.30 – 23.00
Infant crying**	19.08%	0% – 100%	21.13%	0 – 86.27%	16.97%	0% - 100%
Maternal caregiving						
Sensitivity	5.72 (2.16)	1 – 9	5.55 (2.11)	2 – 9	5.91 (2.22)	1 – 9
Cooperation	5.48 (2.25)	1 – 9	5.36 (2.28)	1 – 9	5.60 (2.24)	1 – 9
Positive regard	4.99 (1.26)	2 – 7	4.86 (1.13)	3 – 7	5.12 (1.38)	2 – 7
Negative regard	1.34 (.55)	1 – 3	1.39 (.58)	1 – 3	1.30 (.51)	1 – 3
General postpartum distress						
EPDS	5.16 (3.61)		5.99 (4.13)		4.27 (2.73)	
2 weeks postpartum	5.27 (4.30)	0 - 22	6.26 (5.02)	0 – 22	4.23 (3.10)	0 – 11
6 weeks postpartum	5.04 (3.93)	0 - 16	5.72 (4.30)	0 – 16	4.32 (3.39)	0 – 14
STAI State	30.66 (6.35)		31.79 (7.30)		29.47 (4.94)	
2 weeks postpartum	30.76 (7.66)	20 – 54	32.11 (8.69)	20 – 54	29.32 (6.19)	20 – 46
6 weeks postpartum	30.57 (7.07)	20 – 58	31.47 (7.42)	20 – 55	29.64 (6.62)	21 – 58
PSS-10 6 weeks postpartum	10.96 (5.89)	0 – 28	11.96 (6.47)	0 – 28	9.89 (5.05)	2 – 24
Babysitter (%research assistant)	61.54% (56)		61.70% (29)		61.36% (27)	
Breastfeeding before manipulation (%yes)	36.67% (33)		36.17 %(17)		37.21% (16)	

Note. Raw values before winsorizing, before log or square-root transformation. Education = low/medium: secondary education or vocational education; high: bachelor or master's degree or higher. Cortisol values in nmol/L. *N* = total sample, *M* = mean, *SD* = standard deviation. * 92 lab visits were performed, but 1 participant was excluded from the analyses (see Methods). **percentage of 5-second intervals when the infant was crying during the mother-infant interaction, ranging from none (0%) to the whole interaction (100%).

Table 3. Pearson Correlation Matrix among all variables in the study ($N = 91$)

	1.	2.	3.	4.	5.	6.	7.	8.
1. Maternal condition	-	.39***	.68***	-.11	.11	.14	.10	.22*
2. Maternal cortisol reactivity		-	.26*	.02	.18	.20	.12	-.02
3. Maternal negative affect reactivity			-	.02	.02	.08	-.05	.14
4. Maternal total caregiving				-	-.11	-.22	-.32**	-.10
5. Infant cortisol at reunion (I4)					-	.86***	.54***	-.03
6. Infant cortisol at the end of the visit (I5)						-	.36**	.02
7. Infant crying							-	.21*
8. General postpartum distress								-

Note. *** <0.001 ** < 0.01 * <0.05. Correlations after winsorizing and log or square-root transformations. Maternal caregiving behavior is a sum score of sensitivity, cooperation, positive regard and negative regard. *Condition is dummy coded with 0 = control condition and 1 = stressor condition. The correlations with maternal condition were calculated with Point-Biserial correlations.

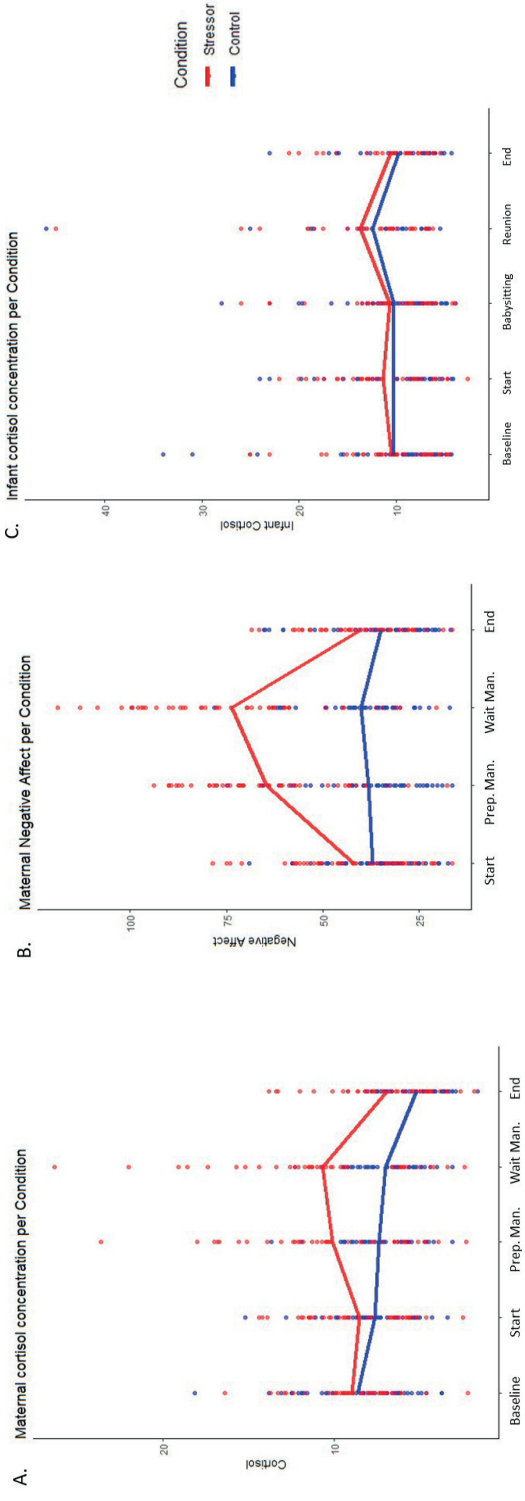

Figure 3. Maternal cortisol concentrations, maternal negative affect and infant cortisol during the lab visit.

Note. Start = start of the lab visit, Prep. Man = preparation manipulation, Wait Man. = waiting after manipulation. Exact timings of assessment can be found in Table 1.

Main analyses

Maternal acute stressor on caregiving behavior quality and infant cortisol and crying

To test our first hypothesis of the effect of the maternal stressor on infant response (i.e. infant cortisol at reunion, infant cortisol at the end of the visit and infant crying) via maternal caregiving behavior quality, we ran three SEMs (see Table 4). Our results showed no direct effect of maternal exposure to the acute stressor on infant cortisol or crying, nor did maternal caregiving quality mediate the association between the acute stressor and infant response. However, higher maternal caregiving behavior quality was associated with lower levels of infant cortisol at the end of the visit and less infant crying, but not infant cortisol levels at reunion. The fit of the models can be found in Supplementary Table 1.

Table 4. Results from three mediation models (hypothesis 1 and 2) of condition (stressor/control) on infant response to maternal stress divided into 1) infant cortisol at reunion 2) infant cortisol at the end of the visit 3) infant crying, mediated by caregiving behavior quality

Model description	Estimate	Standardized Estimate (β)	SE	p-value	CI	
					LL	UL
Model 1. Infant cortisol at reunion (I4)						
Condition → Infant Cortisol I4	.04	.11	.05	.43	-.06	.14
Condition → Caregiving	- .39	-.09	.46	.40	-1.30	.52
Caregiving → Infant cortisol I4	-.02	-.20	.01	.13	-.04	.01
Condition → Caregiving → Infant cortisol	-.04	.13	.05	.37	-.06	.15
Model 2. Infant cortisol at the end of the visit (I5)						
Condition → Infant Cortisol I5	.03	.10	.04	.43	-0.05	0.05
Condition → Caregiving	-.41	-.10	.46	.37	-1.32	.50
Caregiving → Infant cortisol I5	-.02	-.27	.01	.03	-.04	-.00
Condition → Caregiving → Infant cortisol	-.04	.13	.04	.33	-.04	.13
Model 3. Infant crying						
Condition → Infant crying	-.03	.06	.04	.54	-.06	.11
Condition → Caregiving	.37	-.09	.46	.42	-1.28	.53
Caregiving → Infant crying	-.05	-.46	.01	.00	-.07	-.03
Condition → Caregiving → Infant crying	.04	.10	.05	.36	-.05	.14

Note. SE: Standard Effect, CI: Confidence interval, LL: Lower limit, UL: Upper limit. Condition is dummy coded with 0 = control condition and 1 = stressor condition.

Moderating role of maternal general postpartum distress

To assess the moderating role of maternal general postpartum distress, we tested four regression interaction models. All four models were not significant. Maternal general postpartum distress did not moderate the effect of maternal stressor on maternal caregiving behavior quality (b = -.11, SE = .19, p = .57) nor on infant response to maternal stress: cortisol at reunion (b = -.05, SE = .05, p = .35), cortisol at the end of the visit (b = -.04, SE = .04, p = .36), or crying (b = -.07, SE = .06, p = .23).

Maternal reactivity to stressor on caregiving behavior and infant cortisol and crying

To examine the associations between maternal reactivity to the acute stressor (i.e., cortisol and negative affect reactivity) and maternal caregiving behavior and infant response (i.e. cortisol at reunion, cortisol at the end of the visit, crying) regardless of condition, we ran six SEMs (see supplementary Table 2). The models revealed no significant associations between maternal reactivity to the stressor on caregiving behavior and infant cortisol and crying. Again, we found that higher maternal caregiving behavior quality was associated with lower infant cortisol at the end of the visit and less crying (not cortisol at reunion). Furthermore, we found some covariates to be significantly related to the outcome measures (see Supplementary material Table 2). Breastfeeding the infant before the manipulation was significantly related to lower infant cortisol at reunion, less infant crying and lower maternal cortisol reactivity. The fit of the models can be found in Supplementary Table 1.

Sensitivity analyses

We also conducted sensitivity analyses for cortisol reactivity by testing the peaks M3 and M4 separately, subtracting each from the lowest baseline (M1 and M2). Notably, we found that reactivity, defined as the peak cortisol level at the end of the stressor (M4) was significantly related to higher infant cortisol levels at the end of the visit (I5; β = .28, p = .03). No significant associations were found with infant cortisol at reunion (I4; β = .21, p = .10) or infant crying (β = .15, p = .12). These findings were not replicated when reactivity was calculated using the peak from the start of the stressor (M3): infant cortisol at reunion (I4; β = .10, p = .38), the end of the visit (I5; β = .13, p = .26), and infant crying (β = .06, p = .53).

The sensitivity analysis assessing the association between maternal negative affect reactivity by either assessing negative affect at the start (VAS2) or the end of the manipulation (VAS3) on infant outcomes, did not yield different results, as all analyses remained insignificant (all p's > .09).

Discussion

No evidence was found for mother-infant stress contagion, with no main effects of maternal stressor exposure on infant cortisol or crying (hypothesis 1), and no mediation of this relation by caregiving behavior (hypothesis 2). Moreover, no evidence was found for a moderating effect of maternal postpartum distress on the effect of maternal stressor exposure on infant cortisol and crying (hypothesis 3), nor an association between maternal cortisol or negative affect reactivity (irrespective of condition) on infant cortisol, crying and caregiving behavior (hypothesis 4). Secondary findings revealed that higher maternal caregiving behavior quality was associated with lower infant cortisol levels at the end of the visit and less crying. Moreover, sensitivity analysis revealed that when maternal cortisol reactivity was operationalized as the difference between cortisol levels at the end of the manipulation and the lowest baseline, higher maternal cortisol reactivity was associated with elevated infant cortisol levels at the end of the visit.

These results of no association between the acute maternal stressor on infant outcomes contrast those of the two previous studies showing that maternal stressor exposure increased infant sympathetic nervous system activation and avoidance behavior (Waters et al., 2014; 2017). As we utilized infant cortisol concentrations and crying behavior as outcome measures, it is possible that stress contagion happened, but was reflected in different infant biomarkers (e.g., heart rate, skin conductance), or infant behaviors (e.g., motor or looking behavior). Our infants were also much younger compared to the infants from previous mother-infant stress contagion studies by Waters and colleagues (2014, 2017, i.e., 8 weeks instead of 13 months). Infants might have been (too) young to pick up maternal stress, which is congruent with indications of infant's inability to process complex social information or feel other's emotions at this age (Grossmann & Johnson, 2007; Ruffman et al., 2017). However, sensitivity analyses revealed that when maternal cortisol reactivity was operationalized as the difference between cortisol levels at the end of the manipulation and the lowest baseline, higher maternal cortisol reactivity was associated with elevated infant cortisol levels at the end of the visit. This may suggest that contagion is more likely to occur when mothers have higher cortisol levels towards the end of the stressor and approaching the reunion with their infant, compared to the start of the stressor. However, this hypothesis requires further testing. Future research on mother-infant stress contagion is advised to critically consider the timing of cortisol assessments and, more specifically, to measure maternal cortisol levels at least directly before the reunion with their infant.

Furthermore, no evidence was found that maternal stressor exposure caused changes in maternal caregiving quality. Although observational studies often show associations between maternal distress and caregiving quality (Booth et al., 2018), our study is one of the first to experimentally manipulate maternal stress. However, these non-significant findings are consistent with prior research on natural disasters as stressors, which demonstrated that prenatal exposure to objective hardship during the Queensland flood was not associated with maternal caregiving quality (Austin et al., 2017; McLean et al., 2021). While maternal caregiving quality as measured by the Ainsworth scales is found to predict a range of child outcomes (Ainsworth & Bell, 1974), it is also moderately stable over time (Else-Quest et al., 2011; Huang et al., 2009; Madigan et al., 2016). Hence, acute stressors may impact this relatively stable behavior less. Changes due to a stressor might be reflected in other, more subtle caregiving behaviors such as touch and gaze (Beebe et al., 2008, 2011; Granat et al., 2017; Lotzin et al., 2015). Indeed, it has been found that mother and infant exposure to an experimental stressor caused no differences in maternal caregiving quality, but maternal vocalizations had a higher frequency and less prosody tone (Tronick et al., 2021). Future research should investigate more fine-grained maternal behaviors after acute stressors.

Secondary findings indicated that infants of mothers with higher caregiving quality displayed less crying after reunion and had lower cortisol concentrations at the end of the visit. As such, this study replicated earlier findings that higher maternal caregiving quality was related to quicker cortisol recovery in 3-month-old infants (Albers et al., 2008), and not the lack of an association found in 5-week-old infants (Jansen et al., 2010). Mothers might also have been more gentle with the infant, resulting in lower cortisol levels in the infant (Brandes-Aitken et al., 2022). Together, these findings indicate that while maternal behavior may be less effective in buffering cortisol recovery in 1-month-olds, this may change around 2-3 months of age, when caregiving behavior appears to facilitate infant cortisol recovery. Alternatively, heritability of HPA-axis functioning may also play a role in this association (Zänkert et al., 2019).

An additional finding from this study is that infants of mothers with higher levels of general postpartum distress were observed to cry more during the mother-infant interaction, replicating other studies who relied on maternal reports of infant crying (Lux et al., 2023; Mohr et al., 2019). This finding may be attributed to the reduced energy levels of mothers experiencing heightened distress, which could impair their ability to comfort their infants and subsequently lead to increased episodes of infant crying (Kurth, Kennedy, Spichiger, Hösli & Stutz, 2011). Notably, we did not

observe a correlation between maternal caregiving and maternal postpartum distress levels (i.e., symptoms of depression, anxiety, and stress). In contrast to other studies on postpartum distress and caregiving, our sample consisted predominantly of low-risk, healthy participants with self-reported distress, which tends to yield more mixed results compared to studies assessing clinical samples or individuals with diagnosed mental health conditions (Booth et al., 2018). Lastly, we replicated previous studies (Altemus et al., 2001; Thanh Tu et al., 2005) by inducing acute stress in postpartum women despite hormonal fluctuations that typically dampen cortisol reactivity (Bell et al., 2014; Prevost et al., 2014; Uvnas-Moberg et al., 2020).

Next to several strengths, including the randomized controlled design and the observational measures of maternal caregiving behavior and infant crying, limitations should also be mentioned. The participants were mostly Dutch and had higher educational levels than average in the Netherlands, which limits our study's generalizability. Moreover, our sample size was smaller than expected due to the COVID-19 pandemic. While the analyses showed acceptable model fits, simulations of our models showed that the models with maternal stressor condition as a predictor had just enough power. Finally, missingness of infant cortisol samples was high. While our model's imputation techniques avoided loss of power, future research should take care to collect more infant saliva for cortisol analyses. Moreover, as we found no effect of an acute stressor on maternal caregiving behavior, it would be interesting for future research to investigate if maternal caregiving behavior can act as a moderator within the association between maternal stress and infant outcomes, for example by buffering infant cortisol and behavioral responses, which has been found in observational studies (e.g., Grant et al., 2009; Nazzari, Fearon, Rice, Molteni, & Frigerio, 2022).

Conclusions

To our knowledge, this study is the first to examine the impact of an acute maternal stressor on infant cortisol and crying and maternal caregiving quality. While no evidence was found for stress contagion, secondary findings revealed that infants of mothers with higher caregiving quality cried less and had lower cortisol concentrations at the end of the visit. Experimental designs manipulating maternal stress contribute to crucial insights into mechanisms through which maternal postpartum stress affects infant development. This is particularly relevant given the high prevalence of maternal mental health problems during the postpartum period.

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Supplementary Material Chapter 4

Table S1. Fit indices for SEM of caregiving behavior quality mediating maternal stressor on infant cortisol and crying (hypothesis 1 and 2) and caregiving behavior quality mediating maternal cortisol or negative affect reactivity on infant cortisol and crying (hypothesis 4).

	Chi-square	CFI	RSMEA	SRMR
Hypothesis 1 & 2				
Model 1	.25	.98	.08	.06
Model 2	.18	.97	.09	.06
Model 3	.06	.97	.10	.06
Hypothesis 4				
Model 1	.31	.95	.07	.07
Model 2	.27	.95	.07	.07
Model 3	.29	.98	.04	.06
Model 4	.51	.98	.04	.06
Model 5	.28	.94	.07	.07
Model 6	.36	.99	.04	.06

Note. CFI = Robust Comparative Fit Index, RMSEA = Robust Root Mean Square Error Of Approximation, SRMR = Standardized Root Mean Square Residual. The fit of all models was adequate, but not perfect, which is reasonable due to the predictor of experimental condition not adding to any of the three models.

Table S2. Results from the six mediation models (hypothesis 4) of maternal stress reactivity divided into 1) cortisol reactivity and 2) negative affect reactivity on infant stress response divided into 1) infant cortisol at reunion 2) infant cortisol at recovery 3) infant crying, mediated by caregiving behavior quality

Model description	Estimate	Standardized Estimate (β)	SE	p-value	CI	
					LL	UL
Models with maternal cortisol reactivity as predictor						
Model 1 Infant cortisol at reunion (I4)						
1. Maternal cortisol reactivity → Infant Cortisol I4	.01	.15	.01	.24	-.01	.02
Covariates within model						
Infant cortisol baseline I3 → Infant Cortisol I4	.09	.09	.13	.48	-.17	.35
Infant age → Infant Cortisol I4	-.04	-.22	.03	.16	-.09	.01
Breastfeeding → Infant Cortisol I4	-.13	-.33	.05	.01	-.23	-.04
Babysitter → Infant Cortisol I4	-.07	-.16	.05	.20	-.17	.04
Breastfeeding → Maternal cortisol reactivity	-1.49	-.22	.71	.04	-2.88	-.10
Food-intake → Maternal cortisol reactivity	-1.26	-.14	.99	.20	-3.20	.68
Infant age → Caregiving	-.17	-.10	.20	.40	-.56	.22
2. Maternal cortisol reactivity → Caregiving	.01	.02	.07	.84	-.13	.16
3. Caregiving → Infant cortisol I4	-.02	-.21	.01	.07	-.04	.00
4. Maternal cortisol reactivity → Caregiving → Infant cortisol I4	.01	.14	.01	.26	-.01	.02
Model 2. Infant cortisol at recovery (I5)						
Maternal cortisol reactivity → Infant Cortisol at recovery I5	.01	.18	.01	.14	-.00	.02
Covariates within model						
Infant cortisol baseline I3@ Infant Cortisol I5	.07	.08	.13	.59	-.18	.32
Infant age → Infant Cortisol I5	-.03	-.18	.02	.14	-.06	.01
Breastfeeding → Infant Cortisol I5	-.05	-.14	.04	.25	-.60	.18
Babysitter → Infant Cortisol I5	-.09	-.25	.04	.04	-.17	-.00
Breastfeeding → Maternal cortisol reactivity	-1.49	-.22	.71	.04	-2.88	-.10
Food-intake → Maternal cortisol reactivity	-1.26	-.14	.99	.20	-3.20	.67
Infant age → Caregiving	-.21	-.12	.20	.30	-.60	.18

Table S2. Continued

Model description	Estimate	Standardized Estimate (β)	SE	p-value	CI	
					LL	UL
2. Maternal cortisol reactivity → Caregiving	.01	.02	.07	.87	-.13	.15
3. Caregiving → Infant cortisol I5	-.02	-.27	.01	.02	-.04	-.00
4. Maternal cortisol reactivity → Caregiving → Infant cortisol I5	.01	.17	.01	.16	-.00	.02
Model 3 Infant crying						
Maternal cortisol reactivity → Infant crying	.01	.13	.01	.14	-.00	.02
Covariates within model						
Infant age → Infant crying	.04	.19	.02	.03	.00	.07
Breastfeeding → Infant crying	-.12	-.26	.04	.00	-.21	-.04
Babysitter → Infant crying	-.09	-.19	.04	.02	-.17	-.01
Breastfeeding → Maternal cortisol reactivity	-1.50	-.22	.71	.04	-2.89	-.11
Food-intake → Maternal cortisol reactivity	-1.27	-.14	.99	.20	-3.20	.67
Infant age → Caregiving	-.17	-.10	.20	.40	-.56	.22
2. Maternal cortisol reactivity → Caregiving	.01	.02	.07	.88	-.13	.15
3. Caregiving → Infant crying	-.05	-.46	.02	.00	-.07	-.03
4. Maternal cortisol reactivity → Caregiving → Infant crying	.01	.13	.01	.22	-.01	.02
Models with Maternal Negative Affect reactivity as predictor						
Model description	**Estimate**	**Standardized Estimate (β)**	**SE**	**p-value**	**CI**	
					LL	**UL**
Model 4 Infant cortisol at reunion (I4)						
1. Maternal Negative Affect reactivity → Infant Cortisol I4	.02	.08	.03	.59	-.04	.07
Covariates within model						
Infant cortisol baseline I3@ Infant Cortisol I4	.06	.06	.14	.65	-.21	.33
Infant age → Infant Cortisol I4	-.04	-.23	.03	.14	-.09	.01
Breastfeeding → Infant Cortisol I4	-.14	-.35	.05	.01	-.24	-.04
Babysitter → Infant Cortisol I4	-.06	-.14	.05	.27	-.16	.04
Breastfeeding → Maternal Negative affect reactivity	.18	.09	.22	.42	-.25	.59

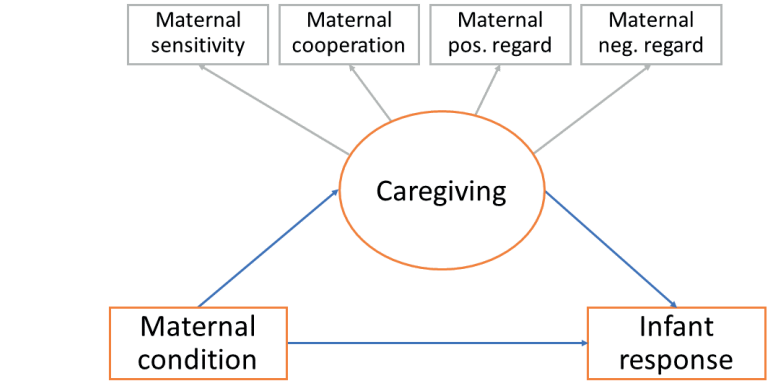
Table S2. Continued

Model description	Estimate	Standardized Estimate (β)	SE	p-value	CI	
					LL	UL
Food-intake → Maternal Negative affect reactivity	.03	.01	.30	.92	-.56	.62
Infant age → Caregiving	-.18	-.10	.19	.36	-.56	.20
2. Maternal Negative affect reactivity → Caregiving	.03	.01	.24	.91	-.44	.49
3. Caregiving → Infant cortisol I4	-.02	-.21	.01	.07	-.04	.00
4. Maternal Negative affect reactivity → Caregiving → Infant cortisol I4	.01	.07	.03	.61	-.04	.07
Model 5 Infant Cortisol at Recovery (I5)						
1. Maternal Negative affect reactivity → Infant Cortisol I5	.02	.14	.02	.24	-.02	.06
Covariates within model						
Infant cortisol baseline I3@ Infant Cortisol I5	.03	.04	.13	.81	-.22	.29
Infant age → Infant Cortisol I5	-.03	-.22	.02	.07	-.06	.00
Breastfeeding → Infant Cortisol I5	-.67	-.19	.04	.12	-.15	.02
Babysitter → Infant Cortisol I5	-.08	-.23	.04	.06	-.16	.00
Breastfeeding → Maternal Negative affect reactivity	.17	.08	.22	.43	-.25	.60
Food-intake → Maternal Negative affect reactivity	.03	.01	.30	.92	-.56	.61
Infant age → Caregiving	-.21	-.12	.20	.28	-.59	.17
2. Maternal Negative affect reactivity → Caregiving	-.01	-.00	.24	.99	-.47	.46
3. Caregiving → Infant cortisol I5	-.02	-.12	.20	.03	-.04	-.00
4. Maternal Negative affect reactivity → Caregiving → Infant cortisol I5	.02	.14	.02	.25	-.02	.06
Model 6 Infant crying						
Maternal Negative affect reactivity → Infant crying	-.01	-.03	.02	.71	-.05	.03
Covariates within model						
Infant age → Infant crying	.03	.17	.02	.06	-.00	.07
Breastfeeding → Infant crying	-.13	-.28	.04	.00	-.22	-.05
Babysitter → Infant crying	-.09	-.19	.04	.03	-.17	-.01
Breastfeeding → Maternal Negative affect reactivity	.18	.09	.22	.42	-.25	.60
Food-intake → Maternal Negative affect reactivity	.03	.01	.30	.91	-.55	.62

Table S2. Continued

Model description	Estimate	Standardized Estimate (β)	SE	p-value	CI	
					LL	UL
Infant age → Caregiving	-.17	-.10	.19	.37	-.55	.21
2. Maternal Negative affect reactivity → Caregiving	.03	.02	.24	.89	-.43	.50
3. Caregiving → Infant crying	-.05	-.46	.01	.00	-.07	-.03
4. Maternal Negative affect reactivity → Caregiving → Infant crying	-.01	-.04	.02	.69	-.06	.04

Note. SE: Standard Effect, CI: Confidence interval, LL: Lower limit, UL: Upper limit. Breastfeeding before manipulation is dummy coded as 0= not breastfed, 1 = breastfed. Babysitter is dummy coded as 0 = brought by the mother, 1= research assistant



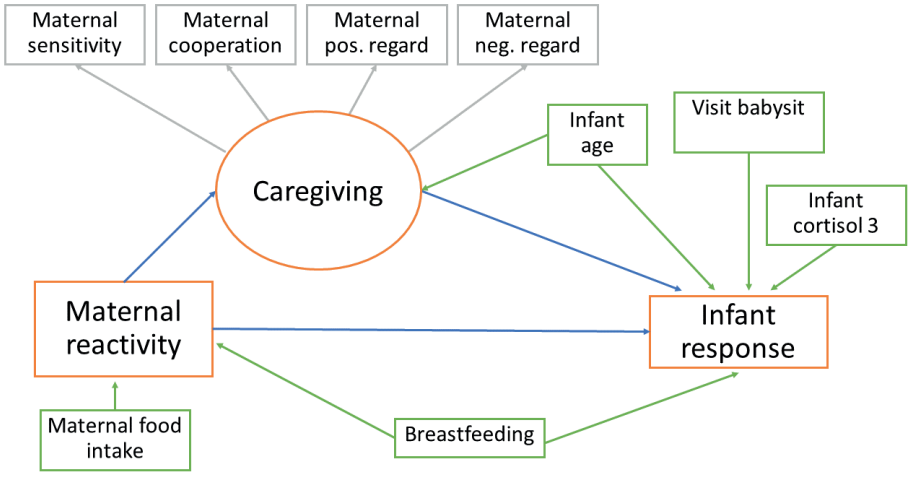


Figure S1 Structural Equation models for hypothesis 1 and 2 (model A) and hypothesis 4 (model B) including all covariates

Note. Infant response reflects infant cortisol 4 (I4), infant cortisol 5 (I5) and infant crying. Boxes in orange reflect the study variables, with the blues lines reflecting the mediation model. Caregiving was a latent score consisting of the latent factors in grey. Boxes in green reflect the covariates. Maternal reactivity reflects cortisol and negative affect reactivity. For model B infant cortisol 3 (reflecting baseline cortisol) was not added for the model with infant crying, only for the models testing I4 and I5.

4

Chapter 5.

Moment-to-moment Bidirectional Associations Between Human Milk Glucocorticoids and Infant Behavior

Based on: Bruinhof, N., Beijers, R., and de Weerth, C. (2026). Moment-to-moment Bidirectional Associations Between Human Milk Glucocorticoids and Infant Behavior. *Psychoneuroendocrinology* , 187, 107790. https://doi.org/10.1016/j.psyneuen.2026.107790

Abstract

Background. Human milk composition varies between and within mothers and is impacted by both maternal and infant factors. Glucocorticoids in milk may potentially influence infant development. For example, elevated cortisol concentrations in milk have been associated with more infant negative affect. However, evidence supporting the role of human milk glucocorticoids on infant behavior is inconsistent. The current preregistered study investigated bidirectional associations between diurnal milk glucocorticoids and infant crying and sleep. We hypothesized that 1) higher cortisol and cortisone concentrations would be related to more infant crying and less infant sleep, and 2) more infant crying and less infant sleep would be related to higher cortisol and cortisone concentrations.

Methods. At 6 weeks postpartum, healthy mothers (N=109) collected three milk samples: in the morning, afternoon, and evening. During this same day, mothers kept a logbook on infant crying and sleep. We calculated the duration of crying and sleeping over three time intervals: 1) the complete interval between each milk sample, 2) the 1.5 hours after each breast milk sample, and 3) the 1.5 hours before each milk sample. Next, we performed multilevel models to assess the bidirectional associations between cortisol and cortisone milk concentrations and infant crying and sleep.

Results. Against our hypotheses, we found that higher cortisol and cortisone milk concentrations were related to more infant sleep 1.5 hours after a milk sample. Moreover, more infant sleep 1.5 hours before a milk sample was related to higher cortisol and cortisone milk concentrations. No associations were found between milk glucocorticoids and infant crying.

Conclusion. This study is the first to assess moment-to-moment associations between milk glucocorticoids and infant behavior and broadens our understanding of the role of human milk composition on infant behavior and vice versa.

Introduction

Human milk is a highly complex and changing fluid, containing many nutritive and non-nutritive components, including hormones, such as glucocorticoids (i.e., cortisol and cortisone; Perrella et al., 2021). Understanding whether glucocorticoids in milk are related to infant behavior is important for elucidating the potential relevance of milk as a mechanism through which maternal stress can impact child behavior and development. The current study will unravel this issue further by assessing real-time associations between human milk glucocorticoid concentrations and infant crying and sleep.

Milk glucocorticoids are non-nutritive components that may affect infant development in a long-term manner, a process termed Lactocrine Programming (Hinde, 2013; de Weerth et al., 2023). When confronted with a stressor, the hypothalamic-pituitary-adrenal (HPA) axis becomes activated, producing glucocorticoids (Sapolsky et al., 2000). Glucocorticoids can pass through the mammary epithelium via simple diffusion and enter milk (Hollanders et al., 2017), with milk glucocorticoid concentrations showing a strong correlation with cortisol concentrations in maternal plasma (van der Voorn et al., 2016). Studies have found that, similar to cortisol measured in maternal saliva or serum, cortisol in milk also varies due to maternal factors such as acute stress (Lustermans et al., 2025; Ziomkiewicz et al., 2021), general psychological distress (Aparicio et al., 2020) and maternal adverse childhood experiences (Vacaru et al., 2023).

Animal studies have shown that glucocorticoids in milk can cross the intestinal epithelial barrier, enter the offspring's circulation, and pass the blood-brain barrier (Pácha, 2000). Cortisol in milk could reach the infant brain and subsequently affect infant brain development and functioning, and hence, infant behavior (de Weerth et al., 2023). While some studies have linked milk cortisol to infant behavioral outcomes (Grey et al., 2013; Nolvi et al., 2018), others have found no significant associations between milk cortisol and infant behavior (Hechler et al., 2018; Toorop et al., 2020; Zielinska-Pukos et al., 2022). These inconsistencies may stem from a combination of differences in study designs and the inherent characteristics of both milk glucocorticoids and infant behavior. Previous studies have primarily focused on how a single cortisol sample from human milk predicted infant behavior. However, as milk cortisol follows a 24-hour circadian rhythm (Aparicio et al., 2020; Toorop et al., 2020; van der Voorn et al., 2016), linking a single cortisol sample to infant behavior is difficult. Only one study so far collected multiple milk samples per day and found no link between cortisol and cortisone measurements

at 1 month postpartum and infant sleep 3 months postpartum, as measured with a questionnaire (Toorop et al., 2020). The interpretation of these findings is further complicated by the temporal variability of infant behavior (De Weerth & Van Geert, 2001; Wolke et al., 2017). Daily to weekly fluctuations in infant crying and sleep limit the accuracy of characterizing infant behavior based on a single questionnaire.

Conversely, infant behavior may also influence maternal well-being and, in turn, milk glucocorticoid concentrations. While increased infant crying and reduced infant sleep have been associated with decreased maternal mental well-being (de Barbaro et al., 2023; Brand et al., 2014; Hughes et al., 2015; Joronen & Kaunonen, 2019; Petzoldt, 2018), it remains unclear whether these factors may also lead to higher maternal stress and higher milk glucocorticoid concentrations. In turn, elevated milk glucocorticoids could further contribute to increased infant crying and reduced sleep, perpetuating a self-sustaining cycle of maternal and infant distress.

A moment-to-moment diurnal study design, which captures short-term fluctuations in both milk glucocorticoids and infant behavior, could provide a more accurate approach to studying their potential bidirectional associations. Conducting such a study in a laboratory setting may result in increased stress in the mothers and infants. Contrarily, carrying out such a study with natural observations in the home setting would increase ecological validity and further uncover daily dynamics in mother-infant well-being. In the current study, conducted in a low-risk community sample, we investigated these bidirectional associations by analyzing three milk samples collected at home over a single day alongside measures of infant behavior during the same day. We hypothesize that (1) higher milk glucocorticoid concentrations will be associated with more infant crying and less infant sleep, and (2) more infant crying and less infant sleep will, in turn, be associated with elevated milk glucocorticoid concentrations. Although most studies to date have assessed milk cortisol (with the exception of Toorop et al., 2020), infants are hypothesized to be able to convert cortisone into cortisol due to the presence of 11β-reductase activity (Catalani et al., 2011; Murphy, 1981; Watterberg, 2004). In addition, some bacteria in the gut have been suggested to have steroid-converting properties (Ridlon et al., 2013), which may potentially contribute to the cortisol pool in infants. Consequently, we will examine both milk cortisol and cortisone. While we anticipate similar findings for cortisone as for cortisol, the nature of our analyses on cortisone is exploratory due to the limited research on its associations with infant outcomes.

Methods

Participants

The data from this study is part of the longitudinal SMILEY study (Study of Microbiota and Lifestyle in the Early Years), a longitudinal study following a community sample of 160 healthy women and their infants from 18 weeks of pregnancy to 2 years postpartum. Participants were recruited during pregnancy through our Baby and Child participant database (https://babyandchild.nl/en/), midwifery practices, and social media. Maternal inclusion criteria were: singleton pregnancy, mastery of the Dutch language, ≥ 18 years of age, no severe physical/mental health issues, < 21 weeks of gestation at enrollment, and pre-pregnancy BMI <30. Infant inclusion criteria were: born at ≥37 weeks of pregnancy, birth weight ≥2500g, 5-min Apgar score ≥ 7 and absence of serious malformations, diseases, or developmental conditions. An overview of the participants at each assessment round is presented in a flowchart (see Figure 1). A total of 142 mother-infant dyads were still participating at 6 weeks postpartum, of which 110 were exclusively breastfeeding. We did not include mothers who exclusively pumped their milk, as we could not confirm whether the collected milk was the same milk their infant consumed at each time point throughout the data collection day. One mother was excluded for this reason, resulting in a final sample of 109 mothers and infants. From inclusion until 12 weeks postpartum, participants received three small gifts and 100 euros. Participants gave informed consent and the study procedures were approved by the Ethics Committee of the Faculty of Social Sciences of the Radboud University under the blanket research line 'Pregnancy-4years Developmental Psychobiology Lab' (SW2017–1303–497), including three amendments (ECSW-2019–051, ECSW-2019-106, ECSW-2020–021). The data were collected between July 2020 and December 2021 and this study's specific hypotheses and statistical analyses were preregistered on the Open Science Framework (OSF).

Procedure

Mothers collected milk samples and behavioral data on a normal day at home around 6 weeks postpartum (M= 6.51, SD= 0.48). Milk samples were collected prior to feeding during three predetermined time windows: between 7:00 AM-10:00 AM (i.e. morning), between 11:00 AM-2:00 PM (i.e. afternoon) and between 7:00 PM-11:00 PM (i.e. evening). Mothers were instructed to write down the time of the milk sample collection. On the same day, mothers completed a standardized paper diary (Baby Day Diary; Barr et al., 1988). Mothers were instructed to record their infant's behavior as frequently as possible, with a minimum of every 2–3 hours. The infant's behavior was registered in 5-minute intervals with predefined categories: sleeping,

awake and quiet, fussy, crying, feeding, or unknown. This behavioral diary was maintained in a 24-hour logbook spanning from 7:00 AM on the day of the milk sampling to 7:00 AM the following day.

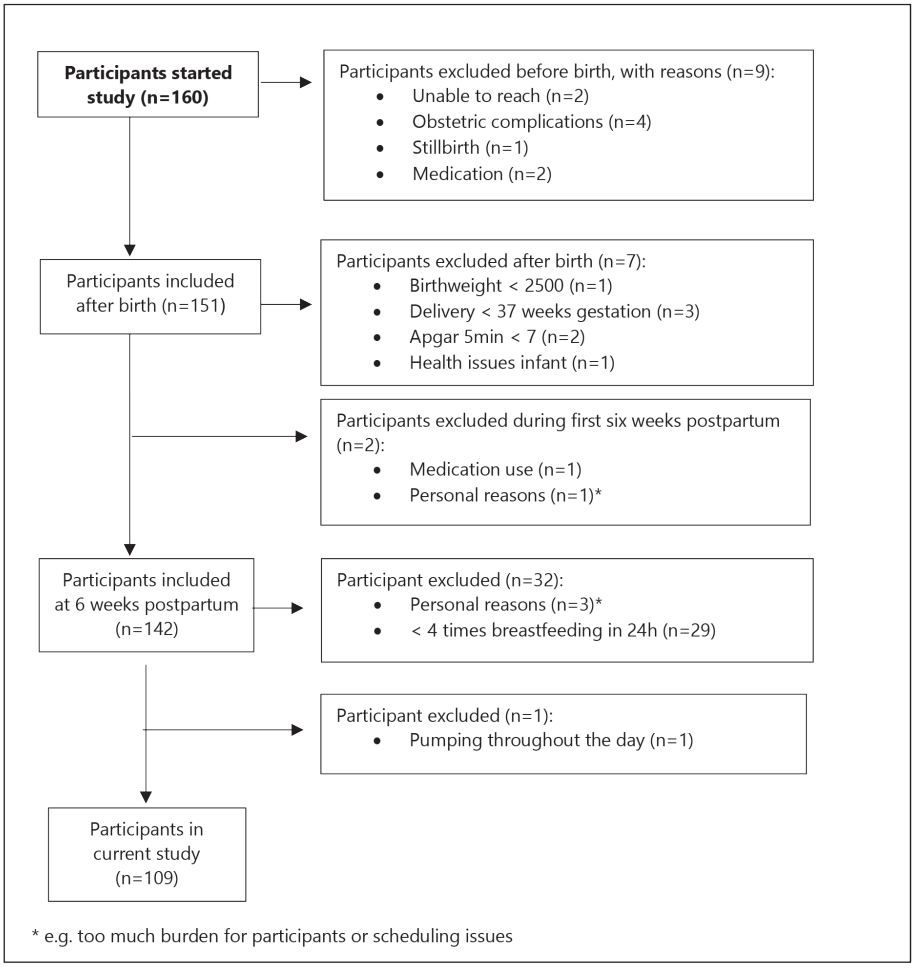


Figure 1. Flowchart of study participants.

Measures

Milk glucocorticoids

To measure glucocorticoids in human milk, participating mothers were asked to collect approximately 4 mL of milk per sample. Mothers were instructed to collect the sample prior to breastfeeding their infants within the specified timeslots to ensure all samples represented foremilk. Due to additional research aims, the

morning milk sample was collected exclusively via hand expression. For the afternoon and evening samples, mothers could select their preferred method of milk collection, such as using a sterilized breast pump or hand expression. After collection participants were instructed to store collected milk samples in a household freezer until a scheduled lab visit. Samples were transported in a cooler to the lab and subsequently stored at −20°C in our lab until data analysis.

After data collection had been completed, all samples were transported by a researcher in temperature-controlled containers to Utrecht University Medical Centre. There, milk was centrifuged for 15 minutes 14000 RCF at 4°C. The fatty layer was removed and the liquid layer was transferred into a second tube and centrifuged for 5 minutes 3000 RCF at room temperature. From this liquid layer, 0.5 mL was mixed with internal standards Cortisol-9,11,12,12-D4 and Cortison-D8. After 30 min incubation cortisol, cortisone and its internal standards were extracted with MTBE and quantified by LC–MS/MS. Calibrator solutions were prepared from Sigma-Aldrich hydrocortisone and cortisone preparations. The UHPLC-MS/MS system consisted of an Accela UHPLC system coupled with a TSQ Vantage mass spectrometer (ThermoElectron Corp, West Palm Beach, FL). A Hypersil Gold C18 50 × 2.1 mm 1.9 µm (Thermo Scientific) column was used with a methanol/water elution solution containing formic acid and ammoniumformate for UHPLC separation cortisone. Day-to-day imprecision was 19 % and 5.2 % at 1.4 and 315 nmol/L cortisol, and 5.9 % and 5.5 % at 4.7 and 45 nmol/L cortisone.

Infant behavior

Paper diary data was entered by 3 trained research assistants. To ensure accuracy, 30% of the entries were double-scored, yielding near-perfect agreement. Any discrepancies were resolved through verification against the raw data. Given the high correlations between fussing and crying, these variables were subsequently merged into one composite measure, hereafter termed 'crying'. For infant crying and sleep three different types of variables were created, each spanning a different time interval surrounding the milk sampling moment. First, a complete time interval was used per participant based on the times they collected their milk sample: a) from 7:00 AM to the participant's morning sample collection time, b) from the participant's morning to afternoon collection time, c) from the participant's afternoon to evening collection time, and d) from the participant's evening collection time to 7:00 AM the next morning. Second, time intervals based on the 1.5 hours right after feeding were used, with 1.5 hours after a) morning collection time, b) afternoon collection time, and c) evening collection time. Third, time intervals based on the 1.5 hours before the feeding were used,

with 1.5 hours before a) morning collection time, b) afternoon collection time, and c) evening collection time. When milk samples and thus sample collection times were missing, we calculated the intervals based on the middle of the instructed interval times, thus 8:30 AM for the morning, 12:30 PM for the afternoon and 9:00 PM for the evening. Exploratorily, we also studied half hour intervals right after and before feeding.

After selecting the intervals, behavioral percentage scores per participant were created for each interval. This was done by dividing the number of intervals consisting of crying or sleeping by the total number of 5-minute intervals (i.e., amount of 5 minutes between sampling times – missing intervals) * 100.

Covariates

We used a directed acyclic graph (DAG; Cinelli et al., 2024) to assess the covariate structure. The covariates that were included in all our models were: parity (primiparous or multiparous), infant age (in weeks) and maternal education. We corrected the variables for milk collection time by regressing cortisol and cortisone on sampling time and using these residuals for analysis (see Statistical Analyses).

Missing data

For hormone data, the following number of milk samples were missing: 7 for morning, 3 for afternoon and 13 for evening. Furthermore, values were checked for implausibly high cortisol and cortisone concentrations (e.g. due to the use of corticosteroid cream; Miller et al., 2013). One participant had extreme values (cortisol >200 nmol/L in the morning, cortisol >50 nmol/L in the evening) and thus these cortisol and cortisone concentrations were recoded as missing. All missing hormone data was imputed.

For infant behavioral data, there were two sources of missing data, all of which were later imputed. The first source was incomplete diary entries by mothers. For infant crying and sleep intervals, we applied a criterion requiring at least 66.66% data availability for each interval (i.e., complete, after, and before feeding for both crying and sleep). Based on this rule, the following intervals were scored as missing: for crying and sleep complete intervals: 4 afternoon, 2 evening and 5 night intervals; for crying and sleep after feeding: 1 morning, 1 afternoon and 3 evening intervals; and for crying and sleep before feeding: 4 afternoon and 2 evening intervals.

The second source of missingness occurred in morning samples due to insufficient observation time before the first milk sample. Since infant behavior data collection

began at 7:00 AM, this resulted in missing data when early morning milk samples were collected. Applying the same criterion as before (i.e., requiring at least 66.66% of data for each interval) meant that for the 1.5-hour intervals, milk samples collected before 08:00 AM had insufficient behavioral data (≥1 hour required). This resulted in 24 missing data points. For the 0.5-hour intervals, milk samples collected before 07:20 AM lacked sufficient behavioral data (≥20 minutes required). This yielded 7 missing intervals. For morning complete intervals, which varied in length depending on the timing of the first milk sample, we also applied a minimum 20-minute behavioral observation threshold, resulting in 7 missing intervals. Sensitivity analyses using a stricter 1-hour minimum for morning complete intervals produced 24 missing data points but did not alter the main findings.

Outliers

Before analyzing the data, all study variables were examined for outliers, defined as values greater or smaller than 3 standard deviations (SD) from the mean. Outliers were then winsorized, meaning they were adjusted to values 3 SD from the mean. Per variable there were between 0 to 4 outliers (see Supplementary table S1 for a list of variables with outliers).

Statistical Analysis

All analyses were performed in R. The milk glucocorticoids variables were log transformed, and the infant crying and sleep intervals were square-root transformed, to conform better to a normal distribution. These transformations were generally effective, though less so for the crying variables. Therefore, Spearman correlations were used, as they handle non-normal distribution in the data better than Pearson correlations (de Winter et al., 2016).

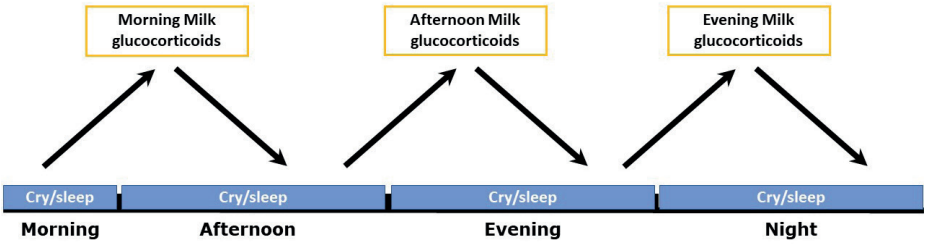
To investigate our hypotheses, we planned on running Random-Intercept Cross-Lagged Panel Models (RI-CLPM; see our pre-registration). However, our models did not converge, even after removing the random intercept, thus we continued our analyses with multilevel models with package lme4 (Bates et al., 2015). This approach allowed us to assess the overall relationships between milk glucocorticoids and infant crying and sleep throughout the day, while being able to account for the nested structure of the data. Before running our multilevel models, we performed linear regression models of sampling time on cortisol and cortisone concentrations and extracted the residuals for inclusion in the multilevel models. This way, we were able to account for differences in sampling times. The intercept, the study variables and the covariates (infant age, maternal parity and education) were added as fixed effects in all multilevel models. We also added a random intercept for participant

ID to account for individual differences in the measured variables. The models were fitted using bobyqa optimizer as a robust optimizer. To account for missing data, we used multiple imputation implemented in the mice package (van Buuren & Groothuis-Oudshoorn, 2011) to impute 20 data sets and pool the estimates using Rubin's rule (Rubin, 1987). This approach was chosen over listwise deletion because it preserves statistical power and reduces bias associated with excluding cases with missing values (Enders, 2017). Inspection of the imputed data showed comparable means and few differences between the original and imputed data distributions.

For each of the three calculated infant behavior intervals, we performed multilevel analyses for each milk glucocorticoid: 1) milk cortisol and 2) milk cortisone, and for each infant behavior: a) infant crying and b) infant sleep (see Figure 2). For the complete intervals we assessed if both milk cortisol and cortisone predicted later infant crying and sleep (hypothesis 1; see figure 2A), in which we used the complete afternoon, evening and night intervals. To assess if infant crying and sleep predicted later milk cortisol and cortisone (hypothesis 2; see Figure 2A), we used the complete morning, afternoon and evening intervals. This resulted in eight multilevel models. For the intervals right after feeding, we tested if milk cortisol and cortisone were related to infant crying and sleep 1.5 hours after feeding (hypothesis 1, see Figure 2B), resulting in four models. For the intervals before feeding, we examined the reverse direction, assessing whether infant crying and sleep in the 1.5 hours before feeding were associated with subsequent milk cortisol and cortisone concentrations (hypothesis 2, see Figure 2B), resulting in four models. In exploratory analyses we also repeated the analyses before and after feeding, but with infant behavior intervals of half an hour.

Because some of our models showed deviations from normality, homoscedasticity, or linearity in the residuals, we also estimated robust parameters and standard errors using the robustlmm package (Koller, 2016), to accommodate these violations.

A. Complete intervals



B. 1.5h before and after feeding intervals

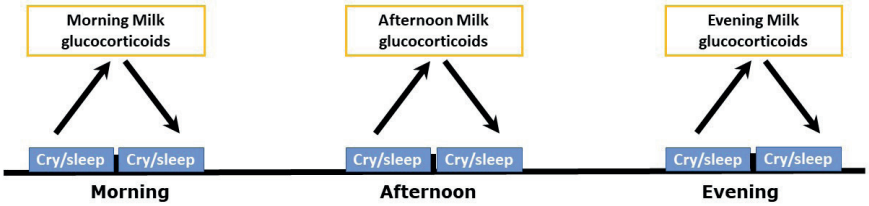


Figure 2. Illustration of different intervals of infant crying and sleep and the association with milk glucocorticoids (cortisol and cortisone).

Note. The length of the complete intervals was heavily dependent on individual mother's sample time.

Table 1. Demographic characteristics and study variables (N = 109)

	M (SD) / %	Range
Maternal age (in years)	32.50 (3.44)	22.92 – 39.63
Infant age (in weeks)	6.51 (0.48)	5.43 – 8.86
Infant sex (% boys)	49.07%	
Parity (% nulliparous)	45.87%	
Dutch background	92.66%	
Education		
Low/medium	11.93%	
High	88.07%	
Sampling time (hh:mm)		
Morning	08:39 (0:59)	06:55 – 11:00
Afternoon	12:33 (1:01)	10:00 – 15:45
Evening	20:37 (1:20)	17:55 – 23:00
Milk cortisol (in nmol/L)		
Morning Cortisol	12.28 (9.56)	0.32 – 45.43
Afternoon Cortisol	3.46 (3.06)	0.19 – 21.13

Table 1. Continued

	M (SD) / %	Range
Evening Cortisol	0.59 (0.67)	0.20 – 4.59
Milk cortisone (in nmol/L)		
Morning Cortisone	25.58 (6.88)	4.12 – 39.61
Afternoon Cortisone	17.09 (6.53)	6.08 – 42.31
Evening Cortisone	5.83 (4.04)	1.31 – 28.95

	Complete intervals		1.5 hour before feeding intervals		1.5 hour after feeding intervals	
	M (SD)	Range	M (SD)	Range	M (SD)	Range
Infant crying %						
Morning crying	11.08 (16.18)	0.00 - 85.71	11.07 (14.05)	0.00 - 66.67	8.78 (9.50)	0.00 - 36.84
Afternoon crying	8.99 (6.86)	0.00 - 34.29	9.88 (10.36)	0.00 - 42.11	6.85 (8.08)	0.00 - 36.84
Evening crying	9.72 (6.97)	0.00 - 35.21	15.17 (17.12)	0.00 - 89.47	10.58 (15.75)	0.00 - 84.21
Night crying	4.36 (5.86)	0.00 - 26.57	x	x	x	x
Infant sleeping %						
Morning sleeping	62.42 (29.13)	0.00 - 100.00	59.31 (29.65)	0.00 -100.00	32.16 (25.83)	0.00 - 100.00
Afternoon sleeping	48.57 (19.53)	0.00 - 93.94	61.63 (28.74)	0.00 - 100.00	36.67 (25.84)	0.00 - 100.00
Evening sleeping	44.48 (13.23)	4.08 - 72.82	41.17 (33.12)	0.00 - 100.00	42.41 (31.74)	0.00 - 100.00
Night sleeping	76.81 (9.45)	44.06 - 95.37	x	x	x	x

Note. N = total sample, M = mean, SD= standard deviation, x = not applicable; only the complete intervals incorporated infant behavior at night. Education = low/medium: secondary education or vocational education; high: bachelor or master's degree or higher. Sampling time is reported as hh:mm (24-hour clock). Values are before winsorization and transformation but after filtering out impossible cortisol and cortisone values and insufficient infant cry and sleep intervals (<66.66% interval available).

Results

Descriptive findings

Table 1 presents descriptive information and Figure 3 shows the mean concentrations of milk cortisol and cortisone across the day. As anticipated, we found that both hormones display a circadian rhythm with high concentrations in the morning and low concentrations in the evening. The figure also illustrates limited cortisol variation in the afternoon and especially in the evening, as compared to morning cortisol and all three cortisone values. Supplementary materials Tables S2 to S6 show the Spearman correlations between our study variables. We found that

cortisol and cortisone concentrations were highly correlated, and that infant crying and sleep were negatively correlated with each other. Cortisol and cortisone were also strongly correlated with time of sampling in the morning and evening, with later times indicating lower cortisol and cortisone values. Cortisone in the evening was positively correlated to sleep 1.5 hours after sampling, with higher cortisone being related to more infant sleep. Crying in the afternoon 1.5 hour before sampling was positively correlated to later afternoon cortisone, thus more infant crying being related to higher cortisone in the following milk sample. We did not find any significant correlations between cortisol and cortisone and the complete intervals of infant crying and sleep.

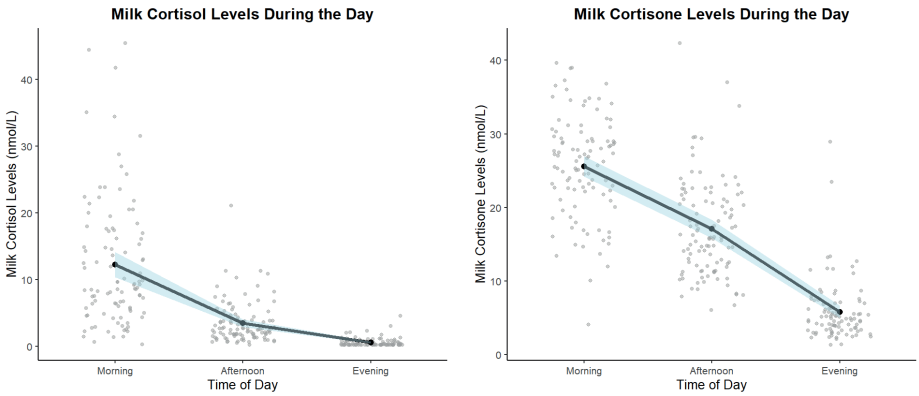


Figure 3. Mean milk glucocorticoids concentrations during the day

Note. Glucocorticoid concentrations are raw values after removing implausible values, and before log transformation and winsorization.

Main analysis

Most models met multilevel assumptions adequately (see Supplementary analysis) and when they did not, additional models with robust estimates and standard errors were run and yielded similar results (Supplementary Table S7).

Milk glucocorticoids as predictors of infant crying and sleep

To assess the association between milk glucocorticoids and infant crying and sleep we ran eight multilevel models, per cortisol or cortisone with crying or sleep, once for complete intervals and once for the first 1.5 hours after milk sampling (see Table 2). For the complete intervals, we did not find a significant association between milk cortisol or cortisone and crying or sleep. For the first 1.5 hours after milk sampling, results showed that higher cortisol concentrations ($p = < 0.001$) and higher cortisone concentrations ($p = 0.005$) were associated with more infant sleep

in the 1.5 hours after feeding. We found no significant associations between milk glucocorticoids and crying 1.5 hours after feeding.

Infant crying and sleep as predictors of milk glucocorticoids

To assess the association between infant crying and sleep and milk glucocorticoids we ran eight multilevel models, per crying or sleep with cortisol or cortisone, once for complete intervals and once for the 1.5 hours before milk sampling (see Table 2). There was no significant association between the complete intervals of crying or sleep and subsequent milk cortisol or cortisone. The models with intervals of 1.5 hours before feeding showed that more infant sleep during the 1.5 hours before feeding was associated with higher milk cortisol concentrations (p = 0.018) and higher cortisone concentrations (p = 0.045). In the robust model the association between more infant sleep and higher milk cortisone became marginally significant (p = 0.076, see Supplementary Table S7). Infant crying in the 1.5 hours before feeding was not related to later milk glucocorticoids.

Exploratory analyses

To evaluate if similar effects were present in shorter intervals surrounding the milk sampling moments, we also performed analyses with crying and sleep intervals of half an hour. We ran multilevel models similar to those described above (see Supplementary Table S8). In line with the 1.5h interval findings, higher milk cortisol (p= 0.007) and cortisone (p= 0.032) concentrations were associated with more infant sleep in the half hour after feeding. Moreover, higher milk cortisone, but not cortisol, was marginally associated with less infant crying (p= 0.088). Half hour intervals of crying and sleep before the milk sampling were not found to predict milk cortisol nor cortisone.

Discussion

Higher concentrations of cortisol and cortisone in milk were related to more infant sleep during the first 1.5 hours after breastfeeding. This finding was in line with the results of our exploratory analyses indicating that these links could already be observed in the first half hour after sampling. In addition, more infant sleep during the 1.5 hours before feeding was related to higher subsequent milk cortisol and cortisone concentrations. Milk cortisol and cortisone concentrations were not associated with infant crying in the 1.5 hours after or before feeding. We also found no associations between milk cortisol and cortisone concentrations and infant crying or sleep when examining the complete time intervals between milk samples.

Table 2. Multilevel Model Results: Associations between Milk Glucocorticoids and Infant Crying and Sleep.

Predictor	Outcome	Measure	Beta(β)	SE	CI_Lower	CI_Upper	*p*-value	ICC
Crying	Milk Cortisol	Complete	0.02	0.03	-0.04	0.07	0.549	0.00
Crying	Milk Cortisone	Complete	0.01	0.01	-0.02	0.04	0.457	0.11
Sleep	Milk Cortisol	Complete	0.03	0.03	-0.02	0.08	0.277	0.00
Sleep	Milk Cortisone	Complete	0.02	0.01	-0.01	0.04	0.142	0.10
Milk Cortisol	Crying	Complete	0.06	0.10	-0.13	0.24	0.557	0.14
Milk Cortisol	Sleep	Complete	-0.09	0.11	-0.31	0.13	0.413	0.00
Milk Cortisone	Crying	Complete	-0.02	0.20	-0.42	0.38	0.924	0.14
Milk Cortisone	Sleep	Complete	-0.05	0.23	-0.50	0.41	0.84	0.00
Crying	Milk Cortisol	1.5h Before	0.00	0.02	-0.04	0.04	0.905	0.00
Crying	Milk Cortisone	1.5h Before	0.01	0.01	-0.01	0.03	0.358	0.10
Sleep	Milk Cortisol	1.5h Before	0.04	0.02	0.01	0.07	0.018	0.00
Sleep	Milk Cortisone	1.5h Before	0.02	0.01	0.00	0.03	0.045	0.11
Milk Cortisol	Crying	1.5h After	-0.09	0.15	-0.38	0.20	0.555	0.13
Milk Cortisol	Sleep	1.5h After	0.74	0.22	0.32	1.16	< 0.001	0.07
Milk Cortisone	Crying	1.5h After	-0.47	0.32	-1.11	0.16	0.145	0.13
Milk Cortisone	Sleep	1.5h After	1.29	0.46	0.39	2.19	0.005	0.05

Note. All glucocorticoid values were residualized against collection time. Models included infant age, maternal parity, and maternal education as covariates. Results were pooled across 20 multiply-imputed datasets. β = standardized, regression coefficient; SE = standard error, CI = confidence interval, ICC = Intraclass Correlation Coefficient, 1.5h, Before = 1.5 hour behavioral interval before feeding, 1.5h After = 1.5 hour interval after feeding.

Our findings of a positive association between elevated milk glucocorticoid concentrations and increased infant sleep duration contradict our initial hypotheses and diverge from previous research showing that higher milk cortisol levels are associated with more difficult infant temperament (Grey et al., 2013; Nolvi et al., 2018), a characteristic typically linked to compromised sleep quality in infants (Morales-Muñoz et al., 2020). Moreover, it contrasts the studies that found no association between milk glucocorticoids and infant sleep (Toorop et al., 2020). However, our study assessed moment-to-moment associations between milk glucocorticoids and infant sleep, while the others investigated relations over longer periods of time. Our findings on sleep are in line with those of a study that investigated sleeping behavior of young infants directly after a stressful procedure. Following circumcision, 2- to 3-day-old infants exhibited both an increase in cortisol and more quiet sleep (Gunnar et al., 1985). According to the authors, quiet sleep may facilitate cortisol recovery from a stressful situation in young infants. In our study, higher maternal glucocorticoid levels could have similarly increased infant systemic glucocorticoid concentrations through milk transfer. While these mechanisms have not been established in humans, animal studies have demonstrated that pups nursing from corticosterone-supplemented dams show elevated circulating corticosterone levels (Catalani et al., 2011; Macrì, 2017; Macrì et al., 2009). Hence, ingesting heightened milk glucocorticoids may have resulted in higher systemic glucocorticoids, that in turn led to more infant sleep. In addition, higher milk glucocorticoids may hypothetically signal the presence of (dangerous) stressors in the maternal environment to the infant. An adaptive response for an infant when being carried is to become quiet, immobile and fall asleep (Ohmura et al., 2022), hence facilitating maternal coping with emergencies and enhancing offspring survival chances (Esposito et al., 2015). This hypothesis could be tested by keeping track of maternal activities and emotions during the sampling day.

We also found that if an infant slept more in the 1.5 hours prior to feeding, higher cortisol and cortisone concentrations were observed in the subsequent milk sample, although this link became marginally significant in further robustness analyses. That more infant sleep was related to higher cortisol and cortisone concentrations was contrary to our expectations, as we had hypothesized that longer infant sleep would decrease maternal stress, thereby reducing maternal systemic glucocorticoids and, consequently, milk glucocorticoid concentrations (Brand et al., 2014; Hughes et al., 2015). A possible explanation is that infant sleep periods may enable mothers to perform physically demanding household (e.g. cleaning) or self-care tasks (e.g., eating), which could elevate maternal cortisol and thereby increase milk glucocorticoid concentrations. Physical activity for at least

10 minutes, and eating in the afternoon, have been linked to small rises in cortisol (Adam & Kumari, 2009; Basso & Suzuki, 2016). Since we did not record maternal behavior, these interpretations remain speculative. Moreover, it is important to add that the association found is not necessarily causal and that other third factors, such as stressful home situations (e.g., quarreling) could have independently affected both milk glucocorticoid concentrations and infant sleeping behavior.

We found associations between higher milk glucocorticoids and more infant sleep for 1.5-hour intervals and vice versa, but these findings were not replicated for the complete intervals. Given that cortisol release within a person is observed to exert relatively direct effects on an individual (i.e., within 20-30 minutes; Foley & Kirschbaum, 2010; Helminen et al., 2019), and that oral glucocorticoid absorption generally occurs within one hour (Oprea et al., 2019), closer temporal intervals between maternal and infant sampling might be more appropriate than more distal ones. The complete intervals in this study had a mean duration of 10 h 10 min, with some extending to 13 h 5 min, and we lacked milk samples of any additional feedings that occurred between the three sampling points. Although a more intensive data collection is challenging in a vulnerable population such as women and their infants in the early postpartum period, future research should consider, if feasible, to include the collection of milk samples at all the diurnal feedings of the sampling day.

No associations were observed between milk glucocorticoid concentrations and infant crying behavior, consistent with prior findings by Hechler et al. (2018) reporting no relationship between morning milk cortisol samples collected at 2, 6, and 12 weeks postpartum and infant crying. These results further align with a previous report from our cohort showing that experimentally elevating maternal salivary cortisol concentrations and psychological stress with a laboratory stressor did not directly influence infant crying (Bruinhof et al., 2025). Another explanation for the lack of associations may be that the crying variable showed less variation and was more clustered around zero than the sleep variable. Possibly, our infants did not cry long enough to stress mothers. Indeed, a recent real-time study found that infant crying had to be at least 8 hours above average to increase maternal distress, while 10 minutes or 1 hour of above average infant crying was unrelated to maternal distress (de Barbaro et al., 2023). Moreover, we assessed infant crying with diaries, and not mothers' subjective interpretation of infant crying or whether this crying was unsoothable. From previous research we know that the nature of an infant's crying and the mother's interpretation of crying (e.g., blaming oneself, helplessness) can affect how stressed mothers actually become from their infant's crying (Muller

et al., 2023). Finally, while crying diaries have been shown to be a robust instrument for observing crying behavior (Barr et al., 1988), newer methods using recording devices show promising results (de Barbaro et al., 2023) and may be considered for future research aiming to confirm and extend our findings.

A secondary finding revealed that cortisone concentrations exhibited large variability at each time point, whereas cortisol concentrations were generally low, with particularly limited variation during the afternoon and evening. Notably, 38.5% of evening milk samples contained cortisol at the lower assay detection limit of 0.20 nmol/L, resulting in a clustering around this value. In contrast, cortisone levels were higher overall, consistent with previous findings (van der Voorn et al., 2016). This greater variability may have contributed to greater robustness of our multilevel models with cortisone compared to cortisol. Though we would continue to assess both cortisol and cortisone in milk in the light of our interesting milk cortisol concentrations and infant sleep findings, we recommend collecting evening milk samples earlier in the evening to reduce the likelihood of very low cortisol concentrations.

Our study has several strengths, including the relatively large sample size and the unique moment-to-moment study design, which incorporated 24 hours of infant behavioral assessments and multiple milk samples in which both cortisol and cortisone were measured. However, some limitations should be noted. First, our sample was low-risk and highly educated, limiting the generalizability of our study. Second, as mothers were requested to start registering infant behavior from 7.00AM onwards, this led to more than 20 % missingness for the morning milk glucocorticoid samples. Third, mothers were instructed to choose a day themselves to collect the milk samples and keep the infant behavior diary, with the aim of reducing participant burden and enhancing data quality. This may have resulted in mothers choosing a day that was more restful, with lower maternal stress, improved infant sleep, and less crying than usual, potentially affecting our results. Fourth, although milk cortisol and cortisone values were comparable to previous pre-COVID-19 studies (e.g., Hechler et al., 2018; Voorn et al., 2016), potential pandemic-related influences (e.g., heightened maternal stress) cannot be fully excluded. Additionally, we suggest that future studies explore how other components in human milk, such as macronutrients, interact with cortisol on a daily level. This field is mainly unexplored, with some indications that milk lipids interact with milk cortisol (Linderborg et al., 2020). Future research can extend our study by also including infant salivary cortisol and cortisone into the design, as well as objective measures of milk intake volume, e.g., by weighing the infant before and after. Lastly,

because data were collected on a single day, future research could replicate these findings across multiple days to better account for day-to-day variation.

As far as we are aware, this study is the first to assess milk glucocorticoid concentrations in direct association with infant behavior during a normal day in the lives of mothers and their young infants. Contrary to our hypotheses, we found that heightened milk glucocorticoids were related to more infant sleep and more infant sleep was associated with subsequently heightened milk glucocorticoids. Future research is needed to confirm these positive associations between milk glucocorticoids and infant sleep and to better understand the underlying mechanisms. Moment-to-moment studies on the complex interplay between human milk components and infant behavior can broaden our understanding of the role of milk composition on infant behavior and vice versa. Finally, our results suggest that in healthy mothers cortisol and cortisone are naturally occurring components of milk that vary throughout the day and from moment-to-moment without any apparent adverse effects on infant behavior.

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Supplementary materials Chapter 5

Table S1. Study variables with outliers

Variable	Number of outliers
Cortisol morning	3
Cortisol afternoon	1
Cortisol evening	2
Cortisone morning	1
Cortisone afternoon	2
Cortisone evening	2
Complete intervals	
Infant crying complete morning	1
Infant crying complete afternoon	2
Infant crying complete evening	2
Infant crying complete night	4
Infant sleep complete evening	1
Infant sleep complete night	1
1.5 hour intervals	
Infant crying before feeding morning	2
Infant crying before feeding afternoon	1
Infant crying before feeding evening	3
Infant crying after feeding afternoon	3
Infant crying after feeding evening	3
Half hour intervals	
Infant crying before feeding morning	3
Infant crying before feeding afternoon	2
Infant crying before feeding evening	2
Infant crying after feeding morning	3
Infant crying after feeding afternoon	2
Infant crying after feeding evening	2

Note. Variables without any outliers are not listed here.

Table S2. Spearman Correlation matrix of glucocorticoid concentrations and infant crying and sleep complete intervals

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. Cortisol morning	—	-0.13	-0.15	0.68***	-0.11	0.00	-0.04	0.05	0.00	-0.02	0.14	-0.14	0.06	-0.06	-0.47***	-0.25*	-0.02	-0.08	0.21*	-0.19
2. Cortisol afternoon		—	0.18	-0.12	0.77***	0.07	-0.07	-0.02	0.12	0.13	-0.07	0.04	0.03	0.02	0.09	0.05	-0.03	0.10	-0.07	0.09
3. Cortisol evening			—	-0.25*	-0.01	0.63***	-0.09	-0.07	0.07	0.10	0.06	-0.01	0.06	-0.11	0.01	-0.09	-0.37***	0.01	0.08	0.06
4. Cortisone morning				—	0.18	0.07	-0.05	0.14	0.13	-0.05	0.08	-0.11	0.01	0.00	-0.30**	-0.28**	0.03	-0.07	0.19	-0.06
5. Cortisone afternoon					—	0.18	-0.06	0.11	0.18	0.16	-0.12	0.00	-0.01	-0.02	0.04	-0.02	-0.08	0.01	0.04	0.16
6. Cortisone evening						—	-0.10	0.02	0.01	0.07	0.03	-0.17	0.15	-0.19	-0.11	-0.21*	-0.49***	0.01	0.26*	0.14
7. Crying morning complete							—	0.13	0.06	0.16	-0.56***	0.11	-0.06	-0.04	0.10	0.11	-0.17	0.06	0.00	0.14
8. Crying afternoon complete								—	0.21*	0.25*	-0.06	-0.49***	-0.03	-0.26*	0.21*	0.21*	0.12	-0.25**	0.14	0.08
9. Crying evening complete									—	0.24*	0.00	0.06	-0.17	-0.01	0.03	0.04	0.19	-0.15	0.10	0.07
10. Crying night complete										—	-0.18	-0.01	0.03	-0.51***	0.03	0.08	-0.23*	-0.14	0.28**	-0.02
11. Sleep morning complete											—	-0.08	0.10	-0.06	-0.15	-0.19	0.20	-0.14	-0.05	-0.21*
12. Sleep afternoon complete												—	0.10	0.08	-0.08	-0.06	0.12	0.18	-0.19	0.12
13. Sleep evening complete													—	-0.22*	-0.24*	-0.21*	0.00	0.07	0.03	-0.14
14. Sleep night complete														—	-0.04	-0.05	0.41***	-0.03	-0.27**	0.06
15. Time morning															—	0.54***	0.12	-0.04	0.01	0.20*
16. Time afternoon																—	0.20*	0.01	-0.14	0.12
17. Time evening																	—	-0.15	-0.23*	0.04
18. Infant age																		—	-0.16	0.10
19. Maternal parity																			—	0.01
20. Maternal education																				—

Note. Upper triangle shows Spearman correlation coefficients. *** p < .001, ** p < .01, * p < .05

Table S3. Spearman Correlations matrix of glucocorticoid and infant crying and sleep intervals 1.5 hours before feeding

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Cortisol morning	—	-0.13	-0.15	0.68***	-0.11	0.00	0.04	0.11	0.02	0.07	-0.18	0.10	-0.47***	-0.25*	-0.02	-0.08	0.21*	-0.19
2. Cortisol afternoon		—	0.18	-0.12	0.77***	0.07	0.06	0.05	0.04	-0.04	0.09	0.04	0.09	0.05	-0.03	0.10	-0.07	0.09
3. Cortisol evening			—	-0.25*	-0.01	0.63***	-0.08	-0.11	0.00	0.02	0.11	0.14	0.01	-0.09	-0.37***	0.01	0.08	0.06
4. Cortisone morning				—	0.18	0.07	-0.06	0.23*	0.13	0.02	-0.20*	-0.03	-0.30**	-0.28**	0.03	-0.07	0.19	-0.06
5. Cortisone afternoon					—	0.18	0.04	0.20*	0.22*	-0.10	-0.04	-0.08	0.04	-0.02	-0.08	0.01	0.04	0.16
6. Cortisone evening						—	-0.01	-0.07	0.12	-0.08	-0.05	0.05	-0.11	-0.21*	-0.49***	0.01	0.26*	0.14
7. Crying morning before							—	0.11	0.10	-0.47***	0.03	-0.01	-0.07	0.10	-0.16	0.01	0.01	0.08
8. Crying afternoon before								—	0.17	0.01	-0.50***	-0.03	0.07	0.04	0.10	-0.28**	0.06	0.03
9. Crying evening before									—	0.05	-0.01	-0.39***	0.05	-0.01	-0.02	-0.07	0.03	0.10
10. Sleep morning before										—	0.05	0.04	-0.09	-0.24*	0.18	-0.14	-0.05	-0.13
11. Sleep afternoon before											—	0.15	-0.10	-0.11	0.07	0.11	-0.03	0.05
12. Sleep evening before												—	-0.24*	-0.13	0.24*	0.12	0.11	-0.12
13. Time morning													—	0.54***	0.12	-0.04	0.01	0.20*
14. Time afternoon														—	0.20*	0.01	-0.14	0.12
15. Time evening															—	-0.15	-0.23*	0.04
16. Baby age																—	-0.16	0.10
17. Maternal parity																	—	0.01
18. Maternal education																		—

Note. Upper triangle shows Spearman correlation coefficients. *** $p < .001$, ** $p < .01$, * $p < .05$

Table S4. Spearman Correlations matrix of glucocorticoid and infant crying and sleep intervals 1.5 hours after feeding

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Cortisol morning	—	-0.13	-0.15	0.68***	-0.11	0.00	-0.05	0.02	0.02	0.06	-0.10	-0.12	-0.47***	-0.25*	-0.02	-0.08	0.21*	-0.19
2. Cortisol afternoon		—	0.18	-0.12	0.77***	0.07	0.08	0.02	0.16	-0.02	0.18	-0.04	0.09	0.05	-0.03	0.10	-0.07	0.09
3. Cortisol evening			—	-0.25*	-0.01	0.63***	0.11	0.11	0.10	-0.07	0.09	0.02	0.01	-0.09	-0.37***	0.01	0.08	0.06
4. Cortisone morning				—	0.18	0.07	-0.05	0.01	-0.06	0.07	0.00	-0.05	-0.30**	-0.28**	0.03	-0.07	0.19	-0.06
5. Cortisone afternoon					—	0.18	0.08	-0.01	0.14	0.04	0.22*	0.01	0.04	-0.02	-0.08	0.01	0.04	0.16
6. Cortisone evening						—	0.08	-0.04	0.05	-0.15	0.28**	-0.07	-0.11	-0.21*	-0.49***	0.01	0.26*	0.14
7. Crying morning after							—	0.13	0.11	-0.31**	0.12	-0.13	0.18	0.22*	-0.01	0.01	0.06	0.00
8. Crying afternoon after								—	0.16	0.05	-0.26**	-0.09	0.00	0.01	-0.05	-0.23*	0.14	0.00
9. Crying evening after									—	0.15	0.06	-0.42***	0.13	0.08	-0.20*	-0.20*	0.08	-0.02
10. Sleep morning after										—	0.03	0.05	0.08	-0.02	0.04	0.07	-0.12	0.07
11. Sleep afternoon after											—	0.08	-0.05	-0.13	-0.11	0.00	-0.12	-0.13
12. Sleep evening after												—	-0.02	0.05	0.27**	0.02	-0.12	0.01
13. Time morning													—	0.54***	0.12	-0.04	0.01	0.20*
14. Time afternoon														—	0.20*	0.01	-0.14	0.12
15. Time evening															—	-0.15	-0.23*	0.04
16. Baby age																—	-0.16	0.10
17. Maternal parity																	—	0.01
18. Maternal education																		—

Table S5. Spearman Correlations matrix of glucocorticoid and half hour of infant crying and sleep after feeding

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Cortisol morning	—	-0.13	-0.15	0.68***	-0.11	0.00	-0.15	0.00	-0.06	0.09	-0.14	-0.10	-0.47***	-0.25*	-0.02	-0.08	0.21*	-0.19†
2. Cortisol afternoon		—	0.18	-0.12	0.77***	0.07	0.03	0.07	0.15	-0.09	0.17	-0.03	0.09	0.05	-0.03	0.10	-0.07	0.09
3. Cortisol evening			—	-0.25*	-0.01	0.63***	0.02	0.14	0.07	0.02	0.21*	0.02	0.01	-0.09	-0.37***	0.01	0.08	0.06
4. Cortisone morning				—	0.18	0.07	-0.19	0.10	-0.15	0.19	-0.07	-0.07	-0.30**	-0.28**	0.03	-0.07	0.19	-0.06
5. Cortisone afternoon					—	0.18	0.02	0.10	0.10	0.02	0.12	0.03	0.04	-0.02	-0.08	0.01	0.04	0.16
6. Cortisone evening						—	0.03	0.06	0.06	0.11	0.25*	-0.05	-0.11	-0.21*	-0.49***	0.01	0.26*	0.14
7. Crying morning after							—	0.09	-0.04	-0.19*	-0.05	-0.03	0.20	0.18	0.01	-0.07	0.05	0.06
8. Crying afternoon after								—	0.09	0.03	0.04	0.03	0.06	-0.05	-0.20*	-0.30**	0.15	-0.07
9. Crying evening after									—	0.07	0.14	-0.20*	0.11	-0.07	-0.15	-0.01	0.14	-0.06
10. Sleep morning after										—	0.09	-0.11	0.11	0.08	0.05	0.05	-0.03	0.15
11. Sleep afternoon after											—	0.07	0.03	-0.04	-0.13	-0.02	-0.01	-0.02
12. Sleep evening after												—	-0.03	0.02	0.10	-0.09	-0.12	-0.09
13. Time morning													—	0.54***	0.12	-0.04	0.01	0.20*
14. Time afternoon														—	0.20*	0.01	-0.14	0.12
15. Time evening															—	-0.15	-0.23*	0.04
16. Baby age																—	-0.16	0.10
17. Maternal parity																	—	0.01
18. Maternal education																		—

Note. Upper triangle shows Spearman correlation coefficients. *** p < .001, ** p < .01, * p < .05

Table S6. Spearman Correlations matrix of glucocorticoid and half hour of infant crying and sleep before feeding

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Cortisol morning	—	-0.13	-0.15	0.68***	-0.11	0.00	-0.08	0.12	0.18	0.04	-0.20*	0.03	-0.47***	-0.25*	-0.02	-0.08	0.21*	-0.19
2. Cortisol afternoon		—	0.18	-0.12	0.77***	0.07	-0.06	0.00	-0.01	0.03	0.17	0.12	0.09	0.05	-0.03	0.10	-0.07	0.09
3. Cortisol evening			—	-0.25*	-0.01	0.63***	-0.17	-0.06	0.03	-0.01	0.08	-0.01	0.01	-0.09	-0.37***	0.01	0.08	0.06
4. Cortisone morning				—	0.18	0.07	-0.10	0.23*	0.12	0.05	-0.18	-0.04	-0.30**	-0.28**	0.03	-0.07	0.19	-0.06
5. Cortisone afternoon					—	0.18	-0.05	0.10	0.10	0.00	0.05	0.00	0.04	-0.02	-0.08	0.01	0.04	0.16
6. Cortisone evening						—	-0.14	0.00	0.13	0.11	-0.02	-0.11	-0.11	-0.21*	-0.49***	0.01	0.26*	0.14
7. Crying morning before							—	0.09	0.12	-0.41***	-0.07	0.03	0.12	0.20*	-0.07	-0.01	-0.04	0.11
8. Crying afternoon before								—	0.08	-0.01	-0.48***	-0.08	-0.01	-0.08	0.22*	-0.28**	-0.06	0.06
9. Crying evening before									—	-0.15	-0.09	-0.37***	0.02	0.05	-0.12	-0.01	0.01	0.07
10. Sleep morning before										—	0.11	0.05	-0.02	-0.09	0.17	-0.05	-0.14	-0.06
11. Sleep afternoon before											—	0.23*	-0.05	0.01	0.00	0.16	0.00	-0.01
12. Sleep evening before												—	-0.19	-0.01	0.25*	0.07	0.02	-0.04
13. Time morning													—	0.54***	0.12	-0.04	0.01	0.20*
14. Time afternoon														—	0.20*	0.01	-0.14	0.12
15. Time evening															—	-0.15	-0.23*	0.04
16. Baby age																—	-0.16	0.10
17. Maternal parity																	—	0.01
18. Maternal education																		—

Note. Upper triangle shows Spearman correlation coefficients. *** p < .001, ** p < .01, * p < .05

Supplementary analysis details

Diagnostic evaluation revealed that most models met multilevel assumptions adequately. However, some of the models with the glucocorticoids as a predictor showed deviations from normality, homoscedasticity, or linearity in the residuals. Moreover, 6 out of 16 models, the models with milk cortisol or complete sleep interval as outcomes, showed singular fits for random effects, reflecting near zero intraclass coefficient (ICC; see Table 2). Indeed, visual inspection of individual cortisol, cortisone, crying and sleep trajectories (Supplementary Figure S1) confirmed that the cortisol and complete sleep variables had low between-person differences, especially in the evening. Because we based our models on theory, we continued the analyses with a random intercept of ID for these participants. As a check, we ran the models without random intercept, which replicated our significant results. Due to the mentioned violation of model assumptions (i.e., normality, homoscedasticity, linearity), we ran additional models with robust estimates and standard errors, which yielded similar results, with one exception discussed in the Results. These robust results can be found in Supplementary Table S7.

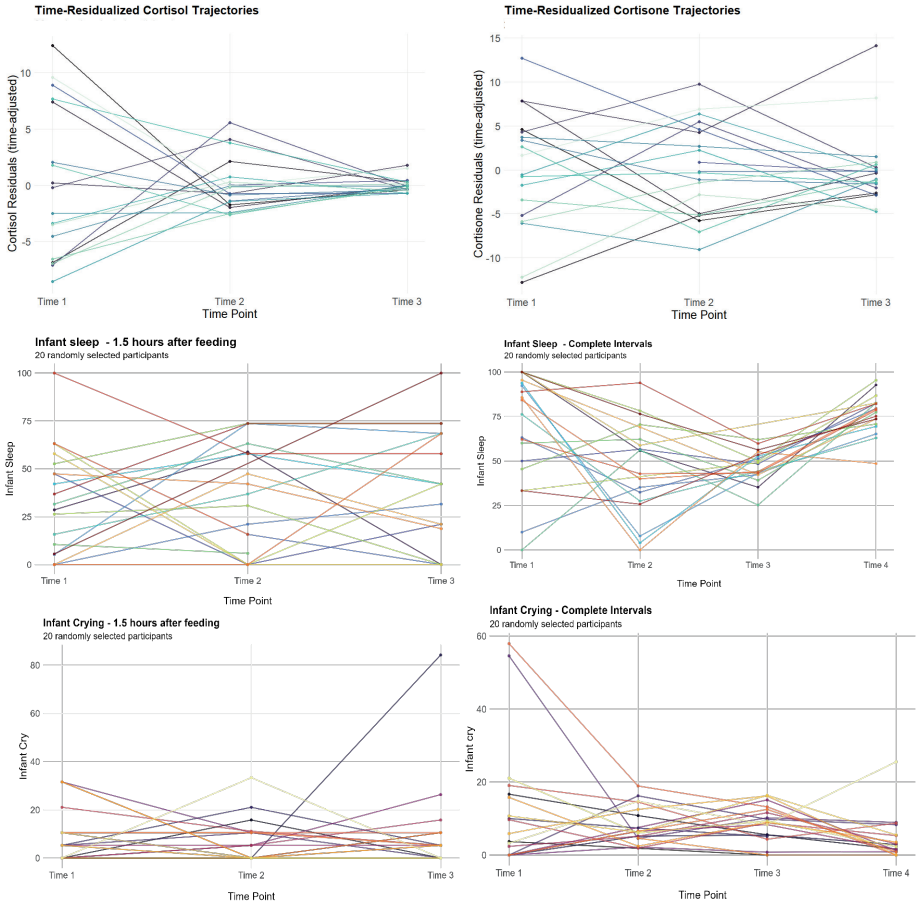

Figure S1. Randomly plotted participants for all outcome variables.

Note. Only outcome variables are shown. For each outcome 20 participants were randomly selected for illustrative purposes. The measures reflect the raw values after quality control for the intervals (i.e., ≥66.66% of intervals available). The comparison of these figures illustrates how milk cortisol (Figure S1.A) displays less between-subject variation, especially at time points 2 and 3, contributing to singular multilevel model warnings.

Table S7. Robust Multilevel Model Results: Associations between Milk Glucocorticoid and Infant Sleep and Crying

Predictor	Outcome	Measure	Robust Beta	Robust SE	Robust CI Lower	Robust CI Upper	Robust p value
Crying	Milk Cortisol	Complete	0.02	0.03	-0.04	0.07	0.551
Crying	Milk Cortisone	Complete	0.01	0.01	-0.02	0.03	0.535
Sleep	Milk Cortisol	Complete	0.03	0.03	-0.02	0.08	0.193
Sleep	Milk Cortisone	Complete	0.02	0.01	-0.01	0.04	0.189
Milk Cortisol	Crying	Complete	0.06	0.11	-0.14	0.27	0.556
Milk Cortisol	Sleep	Complete	-0.06	0.11	-0.27	0.16	0.612
Milk Cortisone	Crying	Complete	0.03	0.22	-0.40	0.46	0.894
Milk Cortisone	Sleep	Complete	-0.09	0.23	-0.53	0.35	0.700
Crying	Milk Cortisol	1.5h Before	0.00	0.02	-0.04	0.03	0.818
Crying	Milk Cortisone	1.5h Before	0.01	0.01	-0.01	0.03	0.183
Sleep	Milk Cortisol	1.5h Before	0.04	0.02	0.01	0.07	0.006
Sleep	Milk Cortisone	1.5h Before	0.01	0.01	0.00	0.03	0.076
Milk Cortisol	Crying	1.5h After	-0.06	0.17	-0.39	0.27	0.721
Milk Cortisol	Sleep	1.5h After	0.81	0.23	0.36	1.27	< 0.001
Milk Cortisone	Crying	1.5h After	-0.43	0.35	-1.12	0.27	0.23
Milk Cortisone	Sleep	1.5h After	1.51	0.49	0.56	2.47	0.002

Note. All glucocorticoid values were residualized against collection time. Models included infant age, maternal parity, and maternal education as covariates. Results were pooled across 20 multiply-imputed datasets. ICC is the same for robust and non-robust multilevel analyses and thus not represented in this table. β = standardized, regression coefficient; SE = standard error, CI = confidence interval, Before = 1.5 hour behavioral interval before feeding, 1.5h After = 1.5 hour interval after feeding.

Table S8. Multilevel Model Results: Associations between Milk Glucocorticoids and Half Hour of Infant Crying and Sleep

Predictor	Outcome	Measure	Beta(β)	SE	CI_Lower	CI_Upper	*p*-value	ICC
Crying	Milk Cortisol	0.5h Before	-0.01	0.02	-0.04	0.02	0.651	0.00
Crying	Milk Cortisone	0.5h Before	0.00	0.01	-0.01	0.02	0.932	0.09
Sleep	Milk Cortisol	0.5h Before	0.02	0.01	0.00	0.04	0.112	0.00
Sleep	Milk Cortisone	0.5h Before	0.01	0.01	-0.01	0.02	0.352	0.09
Milk Cortisol	Crying	0.5h After	-0.20	0.20	-0.59	0.18	0.294	0.02
Milk Cortisol	Sleep	0.5h After	0.72	0.27	0.20	1.24	0.007	0.06
Milk Cortisone	Crying	0.5h After	-0.70	0.41	-1.50	0.10	0.088	0.01
Milk Cortisone	Sleep	0.5h After	1.20	0.56	0.11	2.29	0.032	0.03

Note. All glucocorticoid values were residualized against collection time. Models included infant age, maternal parity, and maternal education as covariates. Results were pooled across 20 multiply-imputed datasets. β = standardized regression coefficient; SE = standard error, CI = confidence interval, ICC = Intraclass Correlation Coefficient, 0.5h Before = 0.5 hour behavioral interval before feeding, 0.5h After = 0.5 hour interval after feeding.

Chapter 6.

Infant Colic and Behavioral Problems from Toddlerhood to Adolescence – Moderation by Maternal Adverse Childhood Experiences and Attachment Style

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Based on: Bruinhof, N.*, Latini, A.*, Beijers, R., and de Weerth, C. Infant Colic and Behavioral Problems from Toddlerhood to Adolescence – Moderation by Maternal Adverse Childhood Experiences and Attachment Style. *Submitted.*
*Shared first author

Abstract

Background. While infant colic has been linked to early childhood behavioral difficulties, its association with behavioral problems through childhood and adolescence remains understudied. Additionally, colic may particularly impact children's behavioral development when mothers have vulnerabilities affecting caregiving toward their infants. By modelling children's internalizing, externalizing, and total behavioral problems trajectories, this study aimed to investigate whether infant colic predicts more negative problem behavior trajectories and whether maternal adverse childhood experiences and insecure attachment style, as vulnerability factors, moderates this association.

Methods. Mothers (N=166) from a Dutch low-risk birth cohort reported infant colic with a 4-day diary at 6 weeks postpartum and filled out questionnaires at eight time points between 2.5 and 16 years to measure children's behavioral problems. Maternal adverse childhood experiences and attachment style were assessed using self-report.

Results. We obtained three trajectories (low, moderate, high) for each behavioral problem type. Infant colic was not related to behavioral problem trajectories. However, at high levels of maternal anxious attachment, infant colic was more predictive of the moderate externalizing problems trajectory compared to the high trajectory.

Conclusion. Given high prevalences of infant colic, it is important to elucidate whether, through what mechanisms, and under which contextual factors infant colic shapes later behavioral development.

Introduction

When infant crying becomes prolonged and inconsolable in an otherwise healthy baby for at least three hours a day, three days per week, for three weeks, it is defined as infant colic (Wessel et al., 1954). More recently, the Rome IV criteria have adapted this definition to periods of infant crying, fussing or irritability, that occur in infants under 5 months, are recurrent and prolonged, have no obvious cause and cannot be prevented or resolved by caregivers (Zeevenhooven et al., 2017). Both definitions indicate that infant colic, also called excessive crying, is a symptom-based phenomenon rather than a disease.

Infant colic usually emerges at two weeks postpartum, peaks at six weeks, and resolves by three months for most children (Savino, 2007; Wessel et al., 1954; Zeevenhooven et al., 2017). Despite its temporary nature and apparent lack of underlying pathology, colic is highly prevalent (up to 25% of infants; de Weerth et al., 2013; Muhardi et al., 2022; Wolke et al., 2017). Moreover, colic often produces high levels of stress in caregivers and families (Kurth et al., 2011; Stifter & Bono, 1998), potentially negatively affecting parent-infant interactions and family dynamics. However, long-term effects of infant colic are largely unknown. While some literature suggests an association between infant colic and psychopathology in early childhood (Zeevenhooven et al., 2018), it is currently unclear whether and how infant colic is associated with behavioral and mental health outcomes later in childhood and adolescence. Furthermore, while infant colic can be stressful for parents, it may be especially challenging for parents with vulnerabilities. The present study will investigate associations between infant colic and behavioral problems from toddlerhood to adolescence and whether maternal psychological vulnerabilities modify the strength of these associations.

Many children suffer from behavioral problems, with 18.4 % of European children aged 6 to 11 years reporting high internalizing problems (e.g., mood disturbances, anxiety) and 7.8% high externalizing problems (e.g., hyperactivity, aggression; Husky et al., 2018). Moreover, behavioral problems are associated with other negative outcomes, including lower academic achievement and suicide ideation and attempts (Orri et al., 2020; Veldman et al., 2014). In previous studies, infant colic was associated with a higher risk for behavioral problems in early childhood. Children with a history of infant colic were found to be at increased risk of elevated emotional reactivity, internalizing problems, and total behavioral problems between the ages of 3 and 5 years (Valla et al., 2021; Zeevenhooven et al., 2022). Moreover, infant colic was also related to a twofold increased risk of overall child

problem behavior, conduct problems, hyperactivity, and mood problems between the ages of 5 and 6 years (Smarius et al., 2017).

These associations between infant colic and behavioral problems in early childhood may indicate that, at least for some children, infant colic may be a sign of more persistent regulatory issues. If this were the case, one would expect to find relations between colic and behavior problems also in later childhood and adolescence. Recent studies suggest that adolescents with a history of colic show higher rates of gastrointestinal and sleeping issues, heightened HPA axis functioning, and lower quality of life (Brett et al., 2024; Rheinheimer & de Weerth, 2025; Zeevenhooven et al., 2024). A single study investigated associations between early crying and behavior problems in adolescence. Sammallahti et al. (2023) found that excessive crying at 3 months was linked to increased behavioral problems up to 14 years. Note, however, that excessive crying assessed at 3 months, the age in which colic has resolved in most infants, might actually be reflecting more substantial regulatory problems or distinct underlying mechanisms (Von Kries et al., 2006). The extent to which colic, determined at its peak of around 6 weeks of age, is associated with subsequent behavioral problems up to adolescence remains unknown and will be addressed in the current study.

Certain infants with colic might be at higher risk of developing behavioral problems than others, for example, because their parents struggle more with managing the infant's colic. In general, mothers of a colicky infant report having low levels of self-efficacy (Stifter & Bono, 1998) and high levels of tiredness and fatigue (Kurth et al., 2011). This negative impact of infant colic on mothers' wellbeing could vary based on maternal vulnerabilities, including a mother's own adverse childhood experiences (ACEs) and adult attachment style. ACEs are defined as experiences of emotional, physical, or sexual abuse, or emotional and physical neglect, that take place before the age of 18 years (Bernstein et al., 2011; Felitti et al., 1998). Maternal ACEs are associated with adverse outcomes in their offspring, suggesting intergenerational transmission. For example, children of ACE-exposed mothers show higher levels of behavioral problems (Cooke et al., 2021; Loheide-Niesmann et al., 2024; Zhang et al., 2023). Importantly, higher maternal ACEs are associated with a negative and more aggressive reaction of the mother to her infant's crying (Buisman et al., 2018). In line with this, maternal ACEs have been associated with more negative maternal attributions towards infant crying and lower sensitivity to infant distress (Girod et al., 2023), which may exacerbate the association between infant colic and later behavioral problems. However, whether maternal ACEs can moderate the association between infant colic and later behavioral problems is as yet unknown.

Similarly, maternal adult attachment style might also be a vulnerability factor that increases the risk of infants with colic developing later behavioral problems. Adult attachment styles develop from early childhood experiences through repeated interactions with multiple caregivers, which together shape internal working models of relationships and emotional regulation (Cassidy, 2000; Hazan & Shaver, 1987). An insecure attachment style reflects having negative models of the self and of others and is often divided into avoidant attachment (i.e., distrusting of the intentions of others and dismissing of the importance of close relationships) and anxious attachment (i.e., high dependency on others and fear of rejection and abandonment, Ainsworth et al., 1978; Bartholomew & Horowitz, 1991). Literature shows that parents with more insecure attachment styles tend to have children with more behavioral problems in childhood (Jing & Michiyo, 2023; Yoo et al., 2006). Changes in parenting behavior might play an underlying role in this association as neuroimaging and behavioral studies indicate that maternal attachment insecurity is related to overall negative parenting and lower sensitivity, reduced tolerance, and more aversion to offspring crying (Ablow et al., 2013; Jones et al., 2014; Strathearn et al., 2009). These findings raise the question of whether infants of mothers with insecure attachment styles, like those of mothers with ACEs, may be more prone to developing behavioral problems after experiencing colic.

Our study will be carried out on data from a low-risk, community-based birth cohort in which colic was determined at 6 weeks and behavioral problems were assessed 8 times by means of maternal report between 2.5 and 16 years. Note that substantial individual heterogeneity exists in developmental trajectories of behavioral problems from childhood to adolescence: approximately 20% of children exhibit high internalizing and externalizing problems, while 50-80% follow low or moderate trajectories. However, whether behavioral problems increase, decrease or remain stable across children's development vary considerably across studies (Korhonen et al., 2014; Nivard et al., 2017; Papachristou & Flouri, 2020). This underscores the importance of examining individual differences in developmental patterns of problem behavior. Therefore, the first aim of this study is to replicate previous research to model internalizing, externalizing and total behavioral problems from toddlerhood to adolescence into trajectories, using Latent Class Growth Analysis (Jung & Wickrama, 2008; Muthén & Muthén, 2000). Based on the heterogeneity of findings in the literature, we do not have a specific first hypothesis regarding the number or slope of the internalizing, externalizing, and total problem behavior trajectories. Our second aim is to assess if a history of colic is associated with specific behavioral trajectories. We hypothesize that infants with a history of colic are more likely to follow behavioral trajectories characterized by more behavioral problems.

Our third and final aim will be to assess the potential moderating role of maternal vulnerabilities (ACEs, avoidant and anxious attachment styles) on the association between infant colic and behavioral trajectories. We hypothesize that higher levels of maternal ACEs, anxious and avoidant attachment will strengthen the association between infant colic and more adverse internalizing, externalizing and total behavioral problems trajectories. Together, our results will aid in uncovering whether infant colic is related to a higher risk of longer-term behavioral problems in community samples, and whether maternal ACEs and insecure attachment styles increase this risk. The results may serve as a basis for future interventions designed to provide support to families with infants at risk of developing childhood and adolescent psychopathology.

Methods

Participants

This preregistered study (https://osf.io/4nvhm) used data from the ongoing prospective longitudinal BIBO cohort (stands for Basal Influences on Child Development in Dutch). The study follows the Helsinki declaration and was approved by the Ethical Committee of Radboud University (ECG300107/SW2017-1303-497/SW2017-1303-498). Pregnant women (N = 220) from a community sample were recruited from midwife practices in the Nijmegen and Arnhem region between 2005 and 2007. Inclusion criteria were an uncomplicated, singleton pregnancy with a term delivery, Dutch language fluency, no drug or alcohol use during pregnancy, no serious physical or mental health problems, and a 5-minute infant Apgar score of 7 or higher. The final sample consisted of N = 193 mother-child dyads, after exclusion due to medical issues (n = 8) and withdrawal from the study due to personal circumstances (n = 19, see also: Beijers et al., 2010, 2011). In the current study we included participants who had at least two problem behavioral measures, which resulted in a final sample size of 166 participants. These 166 mothers did not differ significantly from the 27 excluded mothers on measures of maternal parity ($p = .897$), education ($p = .349$), or age at birth ($p = .530$).

Procedure

After giving informed consent at 37 weeks of pregnancy, mothers filled in questionnaires. One of these questionnaires was aimed at determining their attachment style. When the child was 6 weeks of age, mothers completed a validated daily dairy with information about infant crying. At child ages 2.5, 4, 6, 7, 10, 12.5, 14, and 16 years, mothers reported on their children's problem behavior.

When the child was 8 years old, mothers completed a questionnaire on their own childhood adversities. Until the child was 4 years old, questionnaires were completed on paper, while in later waves they were administered online.

Measures

Infant Colic

At 6 weeks postpartum (41 ± 5 days) mothers filled in a validated daily diary (Barr et al., 1988), in which they recorded infant behavior (e.g., sleeping, crying, eating) over a 96-hour period divided into 5-minute intervals. Mothers were instructed to retrospectively fill in this diary every 2-3 hours or, upon awakening, for the whole night period. Infant crying was defined as the total time spent fussing, crying, and unsoothably crying each day. Daily reports were summed over these three behaviors and averaged over the four days to create a score of average time spent crying per day. Infants who cried for an average of at least 180 minutes a day over the 4 days were classified as having colic, while the rest as not having colic, also in accordance with our previous calculations of colic in this cohort (Brett et al., 2024; de Weerth et al., 2013; Rheinheimer & de Weerth, 2025). This binary variable was used in the analyses as a predictor (effect coding; non-colic=-0.5, colic = 0.5).

Children's Behavioral Problems

At approximate child ages 2.5, 4, 6, 7, 10, 12.5, 14, and 16 years mothers filled in the Dutch version of the Child Behavior Checklist (CBCL; Achenbach & Rescorla, 1991; 2000). This questionnaire comprises 100 items (in the 1,5-5 years old version) or 113 items (in the 4-18 years old version) on a 3-point Likert scale, with 0 = "Not at all", 1 = "A little bit or sometimes", 2 = "True or often". The scores obtained at each time point were standardized as T-scores (i.e., transformed to a score based on age and sex at birth of the child) and used to model the internalizing, externalizing and total problems trajectories of the children. To calculate the behavioral problems scores, we followed CBCL guidelines, which differ per version. At 2.5 and 4 years old, the internalizing problems score results from the sum of the Emotionally Reactive, Anxious/Depressed, Somatic complaints, and Withdrawn subscales, while the externalizing problems score from the sum of the Attention Problems and Aggressive Behaviors subscales. The total problems score is calculated from the sum of all items. Between 6 and 16 years old, the internalizing problems score is calculated from the sum of the Anxious/Depressed, Somatic Complaints, and Withdrawn subscales. The externalizing problems score is obtained from the sum of the Rule-Breaking Behavior and Aggressive Behaviors subscales. The total problems score is given by the sum of all items, except item 2 and item 4. All

Cronbach's alpha and McDonald's omega coefficients were good. Cronbach's alpha coefficients related to total problems ranged from 0.89 to 0.97 and McDonald's omega coefficients from 0.90 to 0.97 for each time point. Internalizing problems' Cronbach's alpha coefficients were between 0.79 and 0.94, while the McDonald's omega coefficients ranged from 0.81 and 0.90. Cronbach's alpha coefficients for externalizing problems were between 0.80 and 0.92 and McDonald's omega coefficients between 0.84 and 0.93.

Maternal ACEs

When children were 8 years old, mothers completed the Childhood Trauma Questionnaire (CTQ; Bernstein et al., 1998) as a retrospective measure of their own ACEs. Participants rated 25 items on 5-point Likert scales ranging from 1 (never true) to 5 (very often true). The CTQ yields five subscale scores: Emotional Abuse, Physical Abuse, Sexual Abuse, Emotional Neglect, and Physical Neglect, each consisting of five items. A total ACEs score was calculated by summing the five subscale scores. Total scores can range from 25 to 125, with higher scores indicating more ACEs. Cronbach's alpha coefficient and McDonald's omega coefficient for the CTQ total score were 0.87 and 0.91, respectively.

Maternal Attachment Style

At 37 weeks of pregnancy, mothers completed the Relationship Questionnaire (RQ; Bartholomew & Horowitz, 1991) to assess adult attachment style. Mothers read 4 brief paragraphs describing 4 attachment styles (Secure, Dismissing, Preoccupied, and Fearful) and rated on a 1 to 7 scale how much they identified with each statement. Based on previous literature (Griffin & Bartholomew, 1994; Jones et al., 2020), anxious attachment style scores were calculated as the sum of the scores to the Preoccupied and Fearful paragraphs, minus the scores to the Secure and Dismissing paragraphs, while avoidant attachment style scores were obtained from the sum of the scores to the Dismissing and Fearful paragraphs, minus the scores to the Secure and Preoccupied paragraphs. Higher scores indicate more anxious and more avoidant attachment styles, respectively, with a possible range of -12 to 12. The score for secure attachment style is obtained from the Secure paragraph, with higher scores indicating higher levels of secure attachment.

Covariates

To account for potential confounding, we selected covariates based on a directed acyclic graph (DAG; Cinelli et al., 2024). The DAG identified the following variables as relevant covariates: maternal education level, maternal age at childbirth, child's pubertal onset age, child age, and child sex assigned at birth. We controlled for

child age and sex assigned at birth by converting CBCL behavioral problems scores into T-scores which are standardized based on child age and sex (Achenbach & Rescorla, 1991, 2000). Consequently, we included the following covariates in our statistical model: maternal education level (effect coding: -0.5 = lower education, 0.5 = higher education), maternal age at childbirth, and child's pubertal onset age. Pubertal onset was measured with the Tanner Questionnaire (Marshall & Tanner, 1969, 1970) and defined as ≥ Tanner stage 2 (also see Bolhuis et al., 2022). The Tanner Questionnaire was filled in by the child at ages 10, 11, 12.5, and 14 years (data at 16 were available but all participants had passed stage 2 by then).

Missing Data

Due to the longitudinal nature of our data, we had missing data on different levels. Missing data percentages for all variables were below 20%. First, on an item level, due to being unable to pool estimates, the missingness was handled with simple imputation using missForest (v1.5; Stekhoven et al., 2025). For the CBCL, a total of 31 items were missing across all participants at 2.5 years, whereas 22 items were missing at 4 years. After imputation, problem behavioral scores were summed. However, if participants had more than eight items missing, their total score was not computed for that specific time point and considered as missing for that data collection wave (n = 1 at the 2.5-year wave and n = 4 at the 4-year wave). Due to inconsistencies in how participants responded, item 100 in the CBCL 1,5-5 years version (administered at waves 2,5 years and 4 years) and item 113 in the CBCL 4-18 years version (administered from wave 6 years to 16 years) were not included in the scoring. Additionally, due to a mistake, two items were missing from the data at 6 years (items 56d and 56h) and one item at 7 years (item 56h). Therefore, total scores at these time points were calculated without these items.

Second, at a participant level, infant colic, maternal ACEs, attachment style, and covariates (maternal age at childbirth and child's pubertal onset age) showed some missingness (see Table 1), which we addressed through missForest imputation to generate complete data. The missingness of our outcome, the complete CBCL questionnaire, was handled within the Latent Class Growth Analysis models by the lcmm package (v2.2.1; Proust-Lima et al., 2017) using Full Information Maximum Likelihood (FIML).

Statistical Analyses

All analyses were conducted in R Statistical Software (v4.4.1; R Core Team, 2024). First, outliers were identified (i.e., values 3SD above or below the mean) and winsorized to 3SD above or below the mean (n = 2 internalizing problems at

4 years, n = 1 total problems at 4 years, n = 1 externalizing problems at 5 years, n = 3 CTQ, and n = 1 anxious attachment style). We ran Spearman correlation after winsorization and imputation of missing data. Latent Class Growth Analysis (LCGA; Jung & Wickrama, 2008; Muthén & Muthén, 2000), was implemented using the lcmm package (v2.2.1; Proust-Lima et al., 2017) to model the internalizing, externalizing and total behavioral problems trajectories separately. LCGA is a person-centered statistical technique used to identify latent classes within a population. It groups individuals who exhibit similar patterns of change over time (e.g., trajectories) on a given variable assessed at multiple time points and each class represents a distinct trajectory. To pick the best model, Bayesian Information Criterion (BIC), sample size adjusted BIC (aBIC), and Integrated Completed Likelihood (ICL) were considered as indices of model fit, with lower values indicating better fit (Biernacki et al., 2000; Nylund et al., 2007). Additionally, entropy values (i.e., closer to 1), posterior classification probabilities (i.e., mean posterior probabilities at least 70%, (Antoniussen et al., 2024), class sizes (i.e., 12% of sample size; Ehrler et al., 2023), and interpretability were considered to evaluate the best models (van de Schoot et al., 2017).

For aim 1, an unconditional (i.e., without predictors included in the model formula) 1-class model was fit for each behavioral problem domain (i.e., internalizing, externalizing and total) to model trajectories from 2.5 years to 16 years. The number of classes was increased until model fit indices, posterior classification, and class sizes showed no meaningful improvements. Latent classes were characterized by their intercept and linear slope parameters, with slopes tested for significance $p < .05$ to determine whether trajectories showed a significant increase or decrease over time. For aim 2, infant colic (i.e. effect coded binary variable) was added to the 2-class model together with the identified covariates (maternal age, education level, and child's puberty onset age) as predictors of class membership (van de Schoot et al., 2017). Continuous variables (maternal age, child puberty onset age) were centered prior to the analyses. Similarly to aim 1, the number of classes was increased until model fit indices, posterior classification, and class sizes displayed no meaningful improvements. As an additional check regarding the LCGA model estimation, the class enumeration steps were repeated with the conditional LCGA models. For all behavioral problems, a 2-class model was initially fit and classes were added until fit showed no significant improvements. Within the conditional models, a multinomial logistic regression was performed, comparing the high trajectory to the moderate and the low trajectories. Using the permut() function from the lcmm package, the low trajectory was also re-labeled as the reference class, allowing the comparison of the low with the moderate trajectory.

For aim 3, the best conditional model was picked and maternal ACEs or adult attachment style and its interaction term with infant colic were added as predictors of class membership. These analytical steps were repeated three times, for 1) internalizing, 2) externalizing and 3) total behavioral problems.

The best unconditional and conditional (i.e., with predictors included in the formula) LCGA models were also tested with Growth Mixture Modeling (GMM; Jung & Wickrama, 2008; Muthén & Muthén, 2000). While LCGA assumes that all individuals within a specific class follow the same trajectory, GMM is a more flexible approach that allows for within-class variability (Jung & Wickrama, 2008; Muthén & Muthén, 2000). However, due to its complexity, it might require a larger sample size. For this reason, we included these analyses as an exploration of the data.

As a sensitivity check, all analyses were repeated with multilevel analysis using the lme4 package (v1.1.37; Bates et al., 2015), with random intercepts and random slopes of time point per participant. First, a multilevel model including only random intercepts per participant was performed to model internalizing, externalizing and total behavioral problems. Then, a second model was fit that additionally included random slopes of timepoint per participant. An ANOVA test indicated that the models including both random slopes and intercepts fit significantly better than the model including random intercepts only. To investigate whether infant colic is predictive of internalizing, externalizing and total behavioral problems, and whether maternal ACEs and insecure attachment style moderate this association, a model with infant colic and the three covariates (maternal education level and age, and child's puberty onset age) was fit first. Then, for the third aim we added the interaction between infant colic and maternal vulnerabilities (ACEs, avoidant attachment style, and anxious attachment style; centered), and centered time point. All these analytical steps were repeated three times, for 1) internalizing, 2) externalizing and 3) total behavioral problems. As additional sensitivity checks, the main analytic models were repeated with the continuous variable of average minutes crying per day and with the inclusion of outliers. Lastly, as an exploratory analysis, we investigated the role of secure attachment as a moderator between infant colic and behavioral problem trajectories with LCGA.

Table 1. Descriptive Statistics of All Variables.

Variable		N	Mean (SD)	Range	Percentage
Maternal variables					
Age*		153	32.57 (3.66)	21.1 – 42.9	
Educational level **	High	132			79.52%
	Low	34			20.48%
Country of birth	Netherlands	155			94.5%
	Other	9			5.5%
Relationship status	Partner	165			99.4%
	Single	1			0.60%
ACEs		160	32.78 (8.69)	25 – 67	
Avoidant attachment		165	-1.50 (3.28)	-9 – 8	
Anxious attachment		165	-2.85 (3.14)	-8 – 9	
Child variables					
Sex assigned at birth	Girls	75			45.18%
	Boys	91			54.82%
Firstborn	Yes	68			40.96%
	No	98			59.04%
Puberty onset age		150	10.88 (1.03)	9.61 – 13.50	
Colic					
	Yes				
No	36				
114			24%		
76%					
CBCL total T-scores	2.5 years	162	49.22 (8.63)	29 – 68	
	4 years	155	46.93 (9.56)	28 – 99	
	6 years	159	48.16 (9.87)	20 – 72	
	7 years	164	48.06 (10.28)	22 – 77	
	10 years	149	48.82 (10.59)	20 – 80	
	12.5 years	150	48.85 (9.78)	26 – 78	
	14 years	147	52.57 (10.25)	26 – 79	
	16 years	138	51.66 (9.20)	28 - 72	
CBCL internalizing					
T-scores	2.5 years	162	47.95 (9.10)	29 – 66	
	4 years	155	46.68 (10.48)	29 – 100	
	6 years	159	47.91 (10.31)	32 – 73	
	7 years	164	48.64 (10.44)	32 – 75	
	10 years	149	50.84 (10.58)	32 – 82	

Table 1. Continued

Variable		N	Mean (SD)	Range	Percentage
	12.5 years	150	50.09 (9.78)	33 – 80	
	14 years	147	54.35 (10.66)	33 – 82	
	16 years	138	54.17 (9.65)	34 – 78	
CBCL externalizing					
T-scores	2.5 years	162	50.99 (8.93)	28 – 71	
	4 years	155	48.28 (9.75)	28 – 100	
	6 years	159	49.17 (8.87)	32 – 72	
	7 years	164	48.30 (9.83)	32 – 74	
	10 years	149	48.35 (9.84)	32 – 76	
	12.5 years	150	47.19 (9.36)	33 – 74	
	14 years	147	50.18 (10.38)	33 – 77	
	16 years	138	49.40 (9.27)	33 – 70	
Crying per day ***		150	143.11 (55.80)	17.50 – 301.25	

Note. * Maternal age at delivery. **High = college or university; low = between primary school and high school diploma. ***Crying per day was calculated in minutes, as the mean over four days.

Results

Descriptives

All descriptive statistics can be found in Table 1 and correlations in Supplementary Table S1. Spearman correlations revealed no significant associations between infant colic and behavioral problems except for colic being related to more internalizing problems at 10 years. Higher maternal ACEs scores were associated with more internalizing and total behavioral problems at 6 years. Higher maternal anxious attachment was correlated with more total and externalizing problems at ages 6 and 7 years, and with more total problems at ages 12.5 and 14 years. Higher maternal avoidant attachment was correlated with more total problems at age 12.5 years and more internalizing problems at age 16 years. Neither maternal ACEs, anxious attachment, nor avoidant attachment were correlated with infant colic. Behavioral problem subscales showed significant intercorrelations both within and across time points.

Main Analyses

Aim 1: behavioral problem trajectories

For all behavioral outcomes, a three-class solution was identified as the optimal model across the 1- to 5-class comparisons (see Figure 1). This decision for three trajectories was supported by favorable model fit indices, mean posterior probabilities exceeding 70%, and sufficiently large class sizes (see Table 2 for fit indices, see Supplementary Text 1 for details on the estimation of unconditional LCGA models). Trajectory classes are described in Table 3 and were labeled based on two parameters: 1) the slope coefficient and its statistical significance (p-value) to determine temporal direction (stable, increasing, or decreasing), and 2) the intercept value to characterize baseline level (low, moderate, or high). As sensitivity analyses, all LCGA unconditional models were also tested with GMM. However, none of the GMMs were meaningful according to our model estimation criteria and thus unable to interpret. Model fit indices of the unconditional GMMs can be found in the Supplementary Table S2 and more information in Supplementary Text 2.

Table 2. Model Fit Indices of the Unconditional LCGA Models.

	BIC	aBIC	ICL	E	%class1	%class2	%class3	%class4	%class5
Internalizing									
INT 1-class	9146.08	9136.58	9146.08	1.00	100.00				
INT 2-class	8874.82	8855.82	8898.59	0.79	57.83	42.17			
INT 3-class	**8796.54**	**8768.04**	**8829.55**	**0.82**	**38.55**	**48.80**	**12.65**		
INT 4-class	8788.23	8750.24	8851.52	0.72	23.49	30.72	33.73	12.05	
INT 5-class	8791.44	8743.95	8869.06	0.71	24.70	25.90	8.43	30.12	10.84
Externalizing									
EXT 1-class	8997.97	8988.47	8997.97	1.00	100.00				
EXT 2-class	8598.40	8579.40	8611.14	0.89	60.84	39.16			
EXT 3-class	**8525.27**	**8496.78**	**8557.51**	**0.82**	**46.39**	**35.54**	**18.07**		
EXT 4-class	8494.44	8456.44	8533.60	0.83	11.45	44.58	27.11	16.87	
EXT 5-class	8495.09	8447.60	8558.83	0.76	11.45	32.53	13.86	25.30	16.87
Total									
TOT 1-class	9062.62	9053.12	9062.62	1.00	100.00				
TOT 2-class	8686.36	8667.36	8702.70	0.86	60.84	39.16			
TOT 3-class	**8565.91**	**8537.41**	**8588.53**	**0.88**	**16.27**	**51.20**	**32.53**		
TOT 4-class	8550.62	8512.63	8592.32	0.82	15.66	47.59	24.10	12.65	
TOT 5-class	8544.08	8496.59	8592.84	0.82	3.61	15.06	45.18	12.65	23.49

Note. BIC = Bayesian Information Criterion, aBIC = sample size adjusted BIC, ICL = Integrated Completed Likelihood, E = entropy, TOT = total behavioral problems; INT = internalizing behavioral problems; EXT = externalizing behavioral problems. Classes are ordered based on trajectory intercept, from lowest to highest. In bold are the chosen models.

Table 3. Internalizing, externalizing and total behavioral problem trajectories

	N	Intercept	Slope	SE	p-value
Internalizing					
Increasing low	64	39.67	0.51	0.09	< .001
Increasing moderate	81	49.33	0.44	0.08	< .001
Increasing high	21	55.95	1.01	0.18	< .001
Externalizing					
Stable low	77	43.57	-0.12,	0.07	.079
Stable moderate	59	51.39	-0.04	0.09	.606
Increasing high	30	58.86	0.22	0.11	.037
Total					
Stable low	27	37.27	0.05	0.12	.688
Increasing moderate	85	45.27	0.32	0.07	< .001
Increasing high	54	55.31	0.37	0.08	< .001

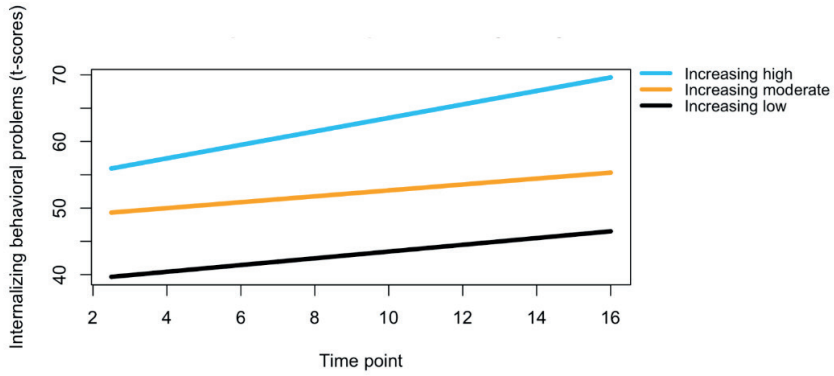
Note. N = number of participants per group, SE = standard error, p values reflect tests of the slope parameter (i.e., rate of change over time).

Aim 2: Infant colic as predictor of behavior problems

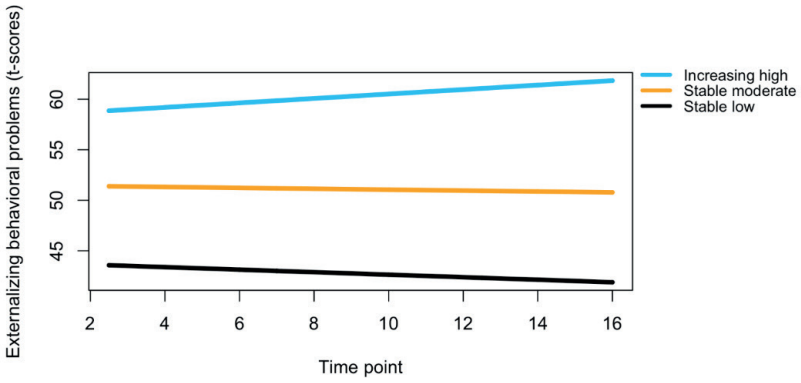
Similar to the unconditional models, for the conditional models with infant colic and the covariates (i.e., maternal education level, age at childbirth and child's pubertal onset age), a 3-class solution appeared as best model for all three problem behavior measures. Trajectory classes were not affected for internalizing, externalizing and total behavioral problems but the addition of predictors caused minimal changes, with some participants being reassigned to different classes (see Supplementary Table S3 for fit indices and Supplementary Text 3 for more information). These results strengthen the validity of the 3-class structure identified with the unconditional models from aim 1. The 3-class GMMs did not converge and could thus not be interpreted.

For internalizing problems, the multinomial logistic regression showed that infant colic was not predictive of the increasing high internalizing behavioral problems trajectory compared to the increasing low internalizing behavioral problems trajectory (B = -0.70, SE = 0.72, p = .334) nor compared to the increasing moderate internalizing behavioral problems trajectory (B = 0.53, SE = 0.65, p = .411). There was a trend-level association between colic and increased likelihood of membership to the increasing moderate internalizing behavioral problems trajectory compared to the increasing low trajectory (B = 1.23, SE = 0.64, p = .053). Among the covariates, only maternal educational level was significant, showing that high maternal educational level significantly increased the likelihood of belonging to the increasing moderate class compared to the increasing high class (B = 1.75, SE = 0.62, p = .005).

A. Internalizing Behavioral Problems Trajectories



B. Externalizing Behavioral Problems Trajectories



C. Total Behavioral Problems Trajectories

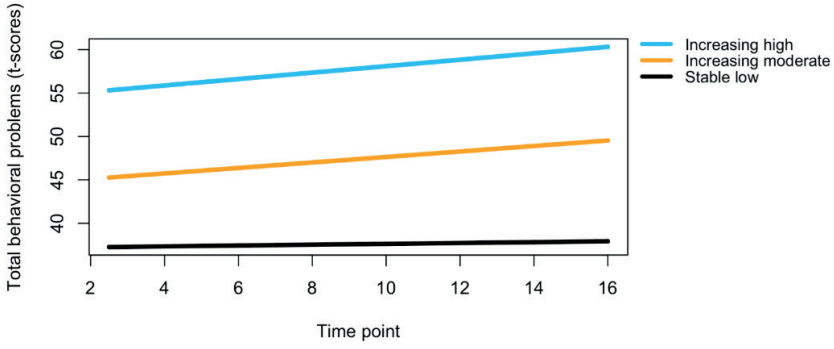


Figure 1. Behavioral problem trajectories as identified by the Unconditional LCGA 3-class Model.

Similarly, for externalizing problems, results showed no association between infant colic and increasing high externalizing behavioral problems trajectory compared to the stable low externalizing behavioral problems trajectory (B = 0.70, SE = 0.69, p = .310), but a trend-level association appeared between colic and increased likelihood of membership to increasing high compared to the stable moderate externalizing behavioral problems trajectory (B = 1.23, SE = 0.73, p = .091). Colic was not predictive of the stable moderate trajectory compared to the stable low externalizing problems trajectory (B = 0.53, SE = 0.46, p = .249). None of the covariates in this model reached significance at the $p < .05$ level.

Lastly, for total behavioral problems, the results of the multinomial logistic regression showed that infant colic was not predictive of membership to the increasing high total behavioral problems trajectory compared to the stable low total behavioral problems trajectory (B = -0.65, SE = 0.70, p = .353) nor compared to the increasing moderate total behavioral problems trajectory (B = -0.00, SE = 0.25, p = .988) and neither to the increasing moderate total behavioral problem trajectory compared to the stable low (B = 0.65, SE = 0.73, p = .375). None of the covariates reached significance.

Aim 3: moderating role of maternal ACE's and adult attachment style

For internalizing, externalizing and total behavioral problems, the interaction between infant colic and maternal ACEs was not predictive of the high behavioral problems trajectories compared to the low behavioral problems trajectories and the moderate behavioral problems trajectories, nor of the moderate behavioral problems trajectories compared to the low (see Table 4). There were no direct effects of maternal ACEs on internalizing, externalizing and total behavioral problems (see Table 4). A significant direct effect of infant colic was detected for internalizing problem behaviors: having a history of infant colic being associated with increased risk of belonging to the increasing moderate compared to the increasing low internalizing problems trajectory (B = 1.73, SE = 0.82, p = .034), which was in line with above-mentioned trend finding in the infant colic and internalizing behavior problems model.

For internalizing, externalizing and total behavioral problems, the interaction between infant colic and maternal avoidant attachment style was not predictive of the high behavioral problem trajectories compared to the low or the moderate behavioral problems trajectories, nor of the moderate trajectories compared to the low. No significant direct effects of maternal avoidant attachment style on internalizing, externalizing and total behavioral problems were identified (see Table 4).

Table 4. Results from moderation analyses on the association between infant colic on internalizing, externalizing and total behavioral problems as moderated by maternal ACEs, avoidant and anxious attachment style (Aim 3).

Variable	Internalizing			Externalizing			Total		
	B	SE	p-value	B	SE	p-value	B	SE	p-value
Class of reference: high									
ACEs (low)	-0.13	0.07	.086	-0.01	0.06	.816	-0.12	0.10	.215
ACEs (moderate)	-0.01	0.04	.752	0.04	0.05	.493	0.00	0.03	.949
ACEs * Colic (low)	-0.11	0.15	.431	0.01	0.12	.956	-0.17	0.19	.373
ACEs * Colic (moderate)	0.13	0.09	.138	0.05	0.11	.633	0.04	0.06	.532
Avoidant attachment style (low)	-0.26	0.13	.056	-0.01	0.09	.899	-0.19	0.13	.135
Avoidant attachment style (moderate)	-0.04	0.09	.662	0.06	0.10	.538	-0.06	0.07	.358
Avoidant attachment style * Colic (low)	-0.24	0.28	.392	-0.06	0.19	.759	-0.24	0.25	.333
Avoidant attachment style * Colic (moderate)	0.09	0.18	.642	-0.09	0.19	.636	0.03	0.13	.836
Anxious attachment style (low)	-0.19	0.14	.164	-0.02	0.13	.881	-0.24	0.15	.099
Anxious attachment style (moderate)	-0.01	0.11	.920	0.04	0.14	.796	-0.07	0.09	.395
Anxious attachment style * Colic (low)	-0.26	0.28	.358	0.36	0.27	.185	-0.03	0.30	.913
Anxious attachment style * Colic (moderate)	-0.21	0.24	.385	0.60	0.29	.038*	0.04	0.18	.805
Class of reference: low									
ACEs (moderate)	0.11	0.07	.111	0.05	0.05	.286	0.12	0.10	.209
ACEs*Colic (moderate)	0.24	0.14	.079	0.05	0.09	.616	0.21	0.19	.283
Avoidant attachment style (moderate)	0.22	0.12	.069	0.07	0.08	.337	0.13	0.14	.349
Avoidant attachment style*Colic (moderate)	0.33	0.25	.185	-0.03	0.15	.821	0.27	0.26	.308
Anxious attachment style (moderate)	0.18	0.12	.132	0.06	0.10	.550	0.17	0.15	.270
Anxious attachment style * Colic (moderate)	0.05	0.24	.832	-0.36	0.27	.183	0.08	0.31	.803

Note. Results from the multinomial logistic regression comparing the high trajectory to the moderate and the low trajectories are shown first. Results from comparing the low trajectory to the moderate trajectory are shown after. Trajectories are identified based on the 3 classes (low, moderate, high) as this was consistent for all behavioral problem variables. B= Unstandardized Beta, SE=Standard Error, * = significant at $p < .05$ level

For externalizing behavioral problems, infant colic and higher maternal anxious attachment style predicted greater odds of belonging to the stable moderate externalizing problems trajectory compared to the increasing high externalizing problems trajectory (see Table 4 and Figure 2). The interaction between infant colic and maternal anxious attachment was not predictive of membership to the increasing high externalizing behavioral problems compared to the stable low externalizing behavioral problems, nor to the stable moderate compared to the stable low. For internalizing and total behavioral problems, the interaction between infant colic and maternal anxious attachment style was not significantly predictive of the high behavioral problems trajectories compared to the low or the moderate trajectories, nor of the moderate compared to the low (see Table 4). Again, a significant direct effect of infant colic was detected for internalizing problem behaviors: having a history of infant colic was associated with increased risk of belonging to the increasing moderate compared to the increasing low internalizing problems trajectory (B = 1.24, SE = 0.63, p = .048). No significant direct effects of maternal anxious attachment style were found on children's internalizing, externalizing, or total behavioral problems (see Table 4).

Sensitivity and Exploratory Analyses

For internalizing and total behavioral problems, the multilevel models showed an increase in problem behavior over time. For internalizing behavioral problems, time was positively associated with internalizing behavioral problems, showing internalizing behavioral problems increased by 0.53 units for each additional timepoint (see Supplementary Figure S1 and Table S4). For externalizing behavioral problems, the time point was not significant (see Supplementary Figure S1 and Table S4), suggesting that externalizing problems remained stable over time. Total behavioral problems increased by 0.30 units for each additional timepoint (see the Supplementary Figure S1 and Table S4). For all multilevel analyses, infant colic was not predictive of internalizing, externalizing or total behavioral problems. Moderation analyses by maternal ACEs, avoidant attachment style, and anxious attachment style did not reach significance at the $p < .05$ level (see Supplementary Table S4).

The LCGA analyses with average minutes crying per day, yielded similar results, with crying not being associated with internalizing, externalizing and total behavioral problems trajectories, and showing a lack of significant moderation effects of maternal ACEs, avoidant and anxious attachment (see Supplementary Table S5). Moreover, the repetition of the analysis without the outliers did not differ from the main results. Lastly, the interaction of secure attachment and infant colic on behavioral problem trajectories was not significant.

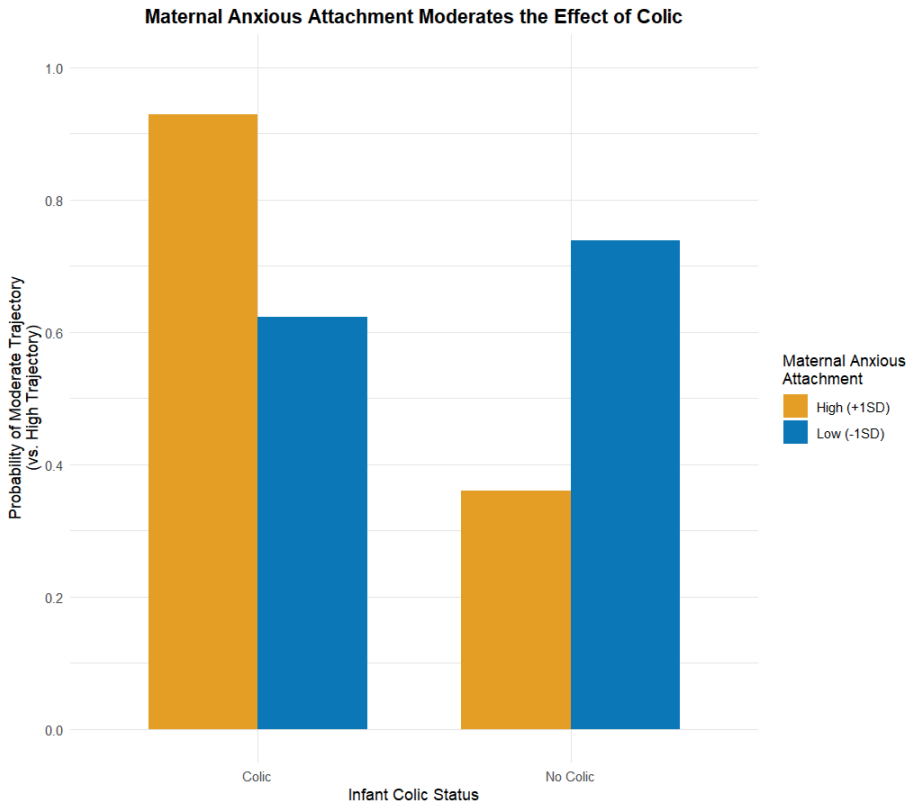


Figure 2. Predicted probability of membership to the stable moderate externalizing problems trajectory (vs. increasing high trajectory) as a function of infant colic and maternal anxious attachment style.

Note. High and low maternal anxious attachment represent values at +1 SD and -1 SD from the sample mean, respectively. Among children with colic, higher maternal anxious attachment was associated with greater probability of moderate (vs. high) trajectory membership (probability = .93 vs. .62 for high vs. low anxious attachment). Among children without colic, lower maternal anxious attachment was associated with greater probability of moderate (vs. high) trajectory membership (probability = .36 vs. .74 for high vs. low anxious attachment).

Discussion

In the current study, we identified three trajectories for internalizing, externalizing and total behavioral problems. Having colic as an infant did not predict more adverse behavioral problem trajectories in the main models. However, introducing maternal ACEs and anxious attachment to the model did result in infant colic being associated with moderate compared to low levels of internalizing problems. With respect to maternal vulnerabilities, maternal anxious attachment style moderated the

association between infant colic and externalizing behavioral problem trajectories. Among mothers with high levels of anxious attachment, infants with colic had higher odds of following the stable moderate trajectory rather than the increasing high trajectory. These moderating effects of maternal anxious attachment were not observed in internalizing or total behavioral problem models. Moreover, maternal adverse childhood experiences (ACEs) and avoidant attachment style failed to moderate the association between infant colic and any behavioral problem trajectory.

We modeled trajectories of children's internalizing, externalizing and total problems from ages 2.5 to 16 years and identified three patterns in each case: high, moderate, and low. Our trajectories show both convergence and divergence with previous research. Similar to prior studies (Korhonen et al., 2014; Nivard et al., 2017; Papachristou & Flouri, 2020), most of the children in the current study (67.5-87.5%) follow low or moderate trajectories, with 12.5-32.5% in the highest group. However, our low, moderate and high internalizing trajectories all increased over time, whereas previous studies found mixed increasing, stable, and decreasing patterns. Additionally, our externalizing problems remained stable for low and moderate groups rather than decreasing as previously reported (Korhonen et al., 2014; Papachristou & Flouri, 2020). The discrepancies with these studies, likely reflect differences in our analytical approach, including our longer follow-up into adolescence, employing LCGA rather than GMM and our smaller sample size as compared to previous studies.

Despite previous literature suggesting an association between infant colic and later behavioral problems (Sammallahti et al., 2023; Smarius et al., 2017; Valla et al., 2021; Wolke et al., 2002; Zeevenhooven et al., 2022), this finding was only partially replicated in the present study. Colic was predictive of the increasing moderate internalizing problems trajectory, compared to the increasing low trajectory, but only in the models in which maternal ACEs and anxious attachment style were included as moderators. For this reason, we have to interpret these results with caution. Evidence from the same cohort showed that colic was associated with physiological dysregulation, including altered HPA axis functioning in late childhood (Brett et al., 2024) and sleep difficulties in adolescence (Rheinheimer & de Weerth, 2025). In contrast, we found no association between infant colic and behavioral problems throughout toddlerhood and adolescence. This difference suggests that infant colic may serve as an early marker for subsequent physiological regulatory difficulties including circadian rhythmicity, HPA axis function, sleep architecture, and metabolic regulation, as evidenced by the previously reported association with disordered eating patterns (Von Kries et al., 2006), rather than behavioral psychopathology.

Additionally, the developmental timing of infant colic may be critical in explaining the differences in findings between our study and previous studies. Studies that reported associations between excessive crying and later behavioral problems assessed crying at a later age, namely between 3 and 6 months postpartum (Sammallahti et al., 2023; Smarius et al., 2017; Valla et al., 2021), after the typical crying peak (Von Kries et al., 2006; Wolke et al., 2017). In contrast, our 6-week assessment coincides with the normative crying peak, a developmental period characterized by transient crying in most infants (James-Roberts, 1998; Sloman et al., 1990; Stifter & Braungart, 1992). However, a subgroup of infants may continue to exhibit excessive crying beyond the crying peak. Persistent crying could index more severe and enduring regulatory dysfunction, identifying those infants at elevated risk for behavioral problems. Future longitudinal research with crying data extending beyond 6 weeks, could determine whether indeed only the subset of infants that show persistent excessive crying behavior are at greater risk of developing behavioral problems.

We found that infant colic was predictive of the moderate externalizing problems trajectory at high levels of maternal anxious attachment, compared to the high trajectory, which was contrary to our hypothesis. Anxious attachment style has been linked to increased seeking of external help and support (Vogel & Wei, 2005). Therefore, anxiously attached mothers with an infant that cries excessively, may be more likely to seek help for the colic, than mothers with a secure or avoidant attachment style. This additional support may have served as a protective factor against externalizing problems, particularly given that parents of colicky infants often report barriers to seeking help (Garratt et al., 2019). Another explanation for this unexpected result is related to the possibility that infant colic may differentially elicit caregiving responses based on maternal attachment style. Mothers with anxious attachment are found to exhibit heightened vigilance and protective behaviors toward their infants (Adam et al., 2004), which may be particularly well-suited to the demands of caring for a colicky infant. Mothers with avoidant attachment styles or ACE histories have been found to demonstrate more reduced responsiveness and emotional availability (Doinita & Maria, 2015; Jing & Michiyo, 2023; Rholes et al., 1995; Rowell & Neal-Barnett, 2022; Weistra et al., 2025), and thus may not provide this buffering effect. However, the moderating role of anxious attachment on infant colic and externalizing problems was not replicated in our multilevel models, so caution and future replication studies are needed.

A key strength of this study is its 16-year longitudinal design, which enabled modeling of behavioral problem trajectories and examination of infant colic

as an early risk factor. Additionally, the use of the Baby Day Diary (Barr et al., 1988) over the course of 4 days to measure infant colic provided an ecologically valid assessment that is less likely to be subject to recall bias. However, we also report several limitations. First, LCGA, and especially GMM, are usually performed with larger sample sizes than ours. For this reason, we may have lacked enough statistical power to find associations. Moreover, we sought to preserve model simplicity by not including a quadratic term for time in our models, which could have highlighted potential nonlinear associations between our variables. Secondly, our sample consisted mainly of highly educated mothers, which are not representative of the general population. This could have biased our results and may explain some of the non-significant effects. Future research may consider replicating our study with larger, more representative birth cohorts in order to overcome these limitations. Third, the behavioral problems included in the present study are derived from mother-report questionnaires. While this allowed us to model the same questionnaire filled in from 2.5 to 16 years, it may have introduced maternal bias.

In conclusion, to our knowledge, this is the first study to investigate the longitudinal associations between infant colic and behavioral problems spanning from toddlerhood through adolescence, and to examine how maternal characteristics such as ACEs and insecure attachment styles may moderate these associations. We found no strong evidence for associations between infant colic and subsequent behavioral problems, suggesting that infant colic at around 6 weeks of age may not be a significant risk factor for poor behavioral development in community samples. Additionally, maternal anxious attachment served a protective role, weakening the association between infant colic and later externalizing problems. Our findings underscore the importance of longitudinal designs and person-centered analytic approaches for capturing heterogeneous developmental trajectories within populations.

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Supplementary Material Chapter 6

Supplementary Text 1. LCGA Unconditional Models Estimation
The 3-class solution was the best model in all cases. For internalizing and total behavioral problems, the three-class solution was selected despite not yielding the lowest BIC and aBIC values, as it demonstrated the lowest ICL, the highest entropy, and adequate class sizes. For externalizing problems, the 3-class solution had adequate class sizes, more meaningful trajectories, and higher mean posterior probabilities. The 4-class solution, despite having good model fit indices, also showed lower mean posterior classification probabilities, inconsistencies across multiple model runs, and unusually high estimates. Mean posterior classification probabilities across all 3-class models ranged from 89% to 96% (see Supplementary Table S2).

Supplementary Text 2. Unconditional GMMs Estimation
Unconditional GMMs with either a random intercept or a random intercept and slope were fit. Due to the low entropy values and small class sizes, none of the models were considered meaningful, and no more than 3 classes were tested (see Table S2).

Supplementary Text 3. LCGA Conditional Models Estimation with Infant colic
For internalizing and total behavioral problems, the 3-class solutions showed the lowest ICL and highest entropy. Class sizes were adequate and mean posterior probabilities ranged from 90% to 96% across all 3-class models. For externalizing behavioral problems, the 3-class solution was picked despite the 4-class model showing overall better fit indices, as the 4-class solution contained one small class, below the minimum of 12% (see Table S3).

For trajectory classes there was no difference in the conditional models compared to unconditional models in the significance of the slopes or order of intercept. However, the addition of predictors into the formula caused minor changes of participants being reassigned to different classes. For internalizing problems, in the conditional model 58 individuals (64 in unconditional) were classified into the stable low class, 85 individuals (81 in unconditional) into the increasing moderate class, and 23 individuals (21 in unconditional) into the increasing high class. For externalizing problems, in the conditional model 81 individuals (unconditional 77) were classified into the stable low class, 56 individuals (unconditional 59) into the stable moderate class, and 29 individuals (30 unconditional) into the increasing high class. Lastly, in the conditional model for total behavioral problems, 26 individuals (27 in unconditional) were classified into the stable low class, 87 individuals (85 in unconditional) into the increasing moderate class, and 53 individuals (54 in unconditional) into the increasing high class.

Supplementary Table S1. Spearman Correlation Matrix among all study variables

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.
1. INT 2.5y	—	0.57 ***	0.79 ***	0.48 ***	0.28 ***	0.42 ***	0.37 ***	0.28 ***	0.35 ***	0.27 ***	0.17*	0.27 ***	0.41 ***	0.19*	0.33 ***
2 .EXT 2.5y		—	0.90 ***	0.24 **	0.56 ***	0.45 ***	0.28 ***	0.47 ***	0.45 ***	0.26 **	0.41 ***	0.39 ***	0.30 ***	0.37 ***	0.40 ***
3. TOT 2.5y			—	0.39 ***	0.52 ***	0.52 ***	0.40 ***	0.48 ***	0.52 ***	0.34 ***	0.41 ***	0.45 ***	0.43 ***	0.40 ***	0.48 ***
4. INT 4y				—	0.57 ***	0.85 ***	0.40 ***	0.30 ***	0.41 ***	0.39 ***	0.27 ***	0.37 ***	0.36 ***	0.26 **	0.35 ***
5. EXT 4y					—	0.88 ***	0.26 **	0.49 ***	0.46 ***	0.21 **	0.51 ***	0.42 ***	0.27 **	0.46 ***	0.46 ***
6. TOT 4y						—	0.38 ***	0.45 ***	0.50 ***	0.36 ***	0.46 ***	0.48 ***	0.35 ***	0.40 ***	0.46 ***
7. INT 6y							—	0.44 ***	0.75 ***	0.72 ***	0.30 ***	0.54 ***	0.58 ***	0.19*	0.44 ***
8. EXT 6y								—	0.84 ***	0.33 ***	0.72 ***	0.60 ***	0.42 ***	0.67 ***	0.65 ***
9. TOT 6y									—	0.57 ***	0.62 ***	0.72 ***	0.55 ***	0.51 ***	0.67 ***
10. INT 7y										—	0.42 ***	0.76 ***	0.57 ***	0.22 **	0.46 ***
11. EXT 7y											—	0.81 ***	0.38 ***	0.71 ***	0.66 ***
12. TOT 7y												—	0.54 ***	0.53 ***	0.69 ***
13. INT 10y													—	0.42 ***	0.74 ***
14. EXT 10y														—	0.83 ***
15. TOT 10y															—
16. INT 12.5y															
17. EXT 12.5y															
18. TOT 12.5y															
19. INT 14y															
20. EXT 14y															
21. TOT 14y															
22. INT 16y															
23. EXT 16y															
24. TOT 16y															
25. Infant colic															
26. Minutes crying															

16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.
0.30***	0.27***	0.31***	0.33***	0.25**	0.36***	0.21*	0.19*	0.26**	0.10	0.04	0.04	0.10	0.09	-0.05	0.01	-0.02
0.20*	0.39***	0.35***	0.32***	0.41***	0.42***	0.12	0.37***	0.26**	0.08	-0.02	0.11	0.08	0.04	-0.06	-0.03	-0.04
0.32***	0.45***	0.44***	0.41***	0.44***	0.50***	0.24**	0.34***	0.34***	0.08	0.01	0.10	0.12	0.11	-0.08	-0.03	-0.03
0.30***	0.20*	0.31***	0.34***	0.16	0.32***	0.26**	0.14	0.26**	0.01	-0.07	-0.01	0.09	0.09	-0.01	-0.01	0.03
0.16	0.36***	0.34***	0.27**	0.36***	0.37***	0.25**	0.40***	0.37***	0.00	-0.14	-0.01	0.01	0.10	0.00	-0.05	0.02
0.26**	0.33***	0.38***	0.36***	0.29***	0.41***	0.31***	0.32***	0.38***	-0.01	-0.12	0.01	0.04	0.12	-0.02	-0.04	0.04
0.41***	0.25**	0.37***	0.42***	0.17*	0.35***	0.36***	0.10	0.28**	0.11	-0.01	0.19*	0.10	0.15	-0.14	-0.05	0.03
0.23**	0.59***	0.49***	0.34***	0.54***	0.52***	0.31***	0.50***	0.46***	-0.09	-0.16	0.13	0.06	0.20*	-0.09	-0.01	0.02
0.36***	0.48***	0.53***	0.43***	0.43***	0.53***	0.42***	0.38***	0.50***	0.07	-0.02	0.22**	0.08	0.23**	-0.11	-0.01	0.05
0.50***	0.21*	0.42***	0.46***	0.09	0.36***	0.28***	0.09	0.26**	0.14	0.11	0.12	0.05	0.12	-0.01	-0.08	0.15
0.23**	0.56***	0.49***	0.30***	0.50***	0.51***	0.20*	0.47***	0.38***	0.01	-0.03	0.08	-0.02	0.16*	0.00	-0.01	0.10
0.42***	0.44***	0.57***	0.43***	0.35***	0.53***	0.34***	0.34***	0.44***	0.10	0.10	0.10	0.02	0.20*	0.02	-0.06	0.16*
0.56***	0.36***	0.51***	0.54***	0.39***	0.53***	0.46***	0.26**	0.44***	0.16*	0.09	0.10	0.12	0.08	0.03	-0.10	-0.02
0.25**	0.66***	0.55***	0.25**	0.62***	0.51***	0.25**	0.50***	0.41***	-0.07	-0.04	-0.02	0.02	0.12	0.02	-0.03	-0.08
0.47***	0.60***	0.69***	0.44***	0.59***	0.64***	0.42***	0.46***	0.55***	0.06	0.06	0.02	0.07	0.13	0.06	-0.06	-0.04
—	0.45***	0.81***	0.60***	0.21*	0.52***	0.45***	0.09	0.39***	0.15	0.15	0.10	0.17*	0.10	0.05	-0.07	0.01
	—	0.78***	0.42***	0.71***	0.65***	0.32***	0.52***	0.50***	-0.04	-0.07	-0.06	0.11	0.12	-0.10	-0.01	-0.11
		—	0.59***	0.52***	0.72***	0.45***	0.38***	0.58***	0.05	0.08	0.00	0.18*	0.19*	0.01	-0.06	-0.02
			—	0.41***	0.85***	0.64***	0.30***	0.58***	0.05	0.03	0.15	0.10	0.13	0.03	-0.07	0.02
				—	0.75***	0.31***	0.68***	0.53***	0.02	0.02	0.01	-0.10	0.16	-0.09	-0.02	-0.12
					—	0.58***	0.52***	0.69***	0.04	0.07	0.11	0.04	0.21*	-0.01	-0.03	-0.02
						—	0.37***	0.85***	-0.01	-0.04	0.09	0.18*	0.13	0.07	0.11	-0.04
							—	0.72***	-0.07	-0.11	0.08	-0.07	0.08	-0.03	-0.07	-0.09
								—	0.00	-0.02	0.11	0.08	0.14	0.05	0.05	-0.05
									—	0.76***	-0.05	0.02	0.00	0.08	0.01	0.00
										—	-0.01	0.01	0.06	0.12	0.01	0.06

Supplementary Table S1. Continued

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.
27. Maternal ACEs															
28. Avoidant attachment															
29. Anxious attachment															
30.Maternal age															
31.Maternal education															
32.Puberty onset															

Note. Correlations after winsorizing, imputation and log or square-root transformations. Infant colic (-0.5 = no colic, 0.5 = colic) and maternal education (-0.5 =low, 0.5 = high) are effect coded.
***p <.001; ** p <.01; * p <.05.

	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.
												—	0.19*	0.12	0.09	0.02	-0.04
													—	0.04	-0.02	-0.03	0.02
														—	-0.03	-0.04	0.05
															—	0.11	-0.03
																—	0.02
																	—

Supplementary Table S2. Fit Indices of the Unconditional GMMs.

	BIC	aBIC	ICL	E	%class1	%class2	%class3
Total (random intercept)							
TOT 1-class	8510.19	8497.52	8510.19	1.00	100.00		
TOT 2-class	8499.74	8474.41	8571.75	0.37	48.19	51.81	
TOT 3-class	8510.19	8472.20	8580.69	0.61	72.89	22.29	4.82
Total (random intercept and slope)							
TOT 1-class	8484.95	8465.96	8484.95	1.00	100.00		
TOT 2-class	8499.75	8468.09	8512.46	0.89	94.58	5.42	
TOT 3-class	8515.82	8471.50	8600.25	0.54	6.63	80.72	12.65
Internalizing (random intercept)							
INT 1-class	8772.34	8759.68	8772.34	1.00	100.00		
INT 2-class	8759.69	8734.36	8822.97	0.45	30.12	69.88	
INT 3-class	8770.06	8732.07	8838.01	0.63	75.30	21.69	3.01
Internalizing (random intercept and slope)							
INT 1-class	8744.67	8725.67	8744.67	1.00	100.00		
INT 2-class	8757.12	8725.46	8814.74	0.50	46.39	53.61	
INT 3-class	8771.88	8727.55	8828.98	0.69	48.80	0.60	50.60
Externalizing (random intercept)							
EXT 1-class	8482.01	8469.34	8482.01	1.00	100.00		
EXT 2-class	8464.22	8438.89	8522.38	0.49	24.70	75.30	
EXT 3-class	8474.07	8436.07	8539.58	0.64	30.12	62.65	7.23
Externalizing (random intercept and slope)							
EXT 1-class	8452.18	8433.18	8452.18	1.00	100.00		
EXT 2-class	8463.09	8431.43	8502.31	0.66	71.69	28.31	
EXT 3-class	8479.95	8435.63	8535.51	0.70	47.59	47.59	4.82

Note. BIC = Bayesian Information Criterion, aBIC = sample size adjusted BIC, ICL = Integrated Completed Likelihood, E = entropy, TOT = total behavioral problems; INT = internalizing behavioral problems; EXT = externalizing behavioral problems. Classes are ordered from low trajectories to high trajectories.

Supplementary Table S3. Fit Indices of Behavioral Problems LCGA Models (Conditional).

	BIC	aBIC	ICL	E	%class1	%class2	%class3	%class4	%class5
Total									
TOT 2-class	8705.12	8673.46	8721.10	0.86	61.45	38.55			
TOT 3-class	8599.76	8545.93	8621.13	0.88	15.66	52.41	31.93		
TOT 4-class	8599.51	8523.53	8641.88	0.82	15.66	46.99	19.88	17.47	
TOT 5-class	8607.42	8509.28	8656.41	0.82	6.02	13.86	42.17	15.66	22.29
Internalizing									
INT 2-class	8893.18	8861.52	8916.84	0.79	56.63	43.37			
INT 3-class	8821.21	8767.39	8853.20	0.82	34.94	51.20	13.86		
INT 4-class	8828.88	8752.89	8888.68	0.74	24.10	31.33	31.93	12.65	
INT 5-class	8843.67	8745.53	8901.92	0.78	23.49	18.67	13.25	32.53	12.05
Externalizing									
EXT 2-class	8615.77	8584.11	8628.51	0.89	59.04	40.96			
EXT 3-class	8556.54	8502.72	8585.84	0.84	48.80	33.73	17.47		
EXT 4-class	8542.89	8466.91	8575.34	0.86	9.04	44.58	28.92	17.47	
EXT 5-class	8558.59	8460.44	8608.98	0.81	7.23	38.55	25.30	12.05	16.87

Note. BIC = Bayesian Information Criterion, aBIC = sample size adjusted BIC, ICL = Integrated Completed Likelihood, E = entropy, TOT = total behavioral problems; INT = internalizing behavioral problems; EXT = externalizing behavioral problems. Classes are ordered from lowest trajectory intercept to highest. In bold are the chosen models.

Supplementary Table S4. Multilevel analyses on association between infant colic and internalizing, externalizing and total behavioral problems and the moderating role of maternal ACEs and attachment style.

Variable	Estimate	SE	p-value
Total problems			
Intercept	47.97	0.85	.000 ***
Timepoint	0.30	0.06	.000 ***
Colic	1.26	1.43	.379
Moderation models: Total problems			
ACEs	0.11	0.09	.249
Colic * ACEs	-0.01	0.19	.937
Avoidant attachment	0.29	0.20	.157
Colic * Avoidant attachment	0.38	0.41	.365
Anxious attachment	0.42	0.26	.102
Colic * Anxious Attachment	-0.24	0.52	.647
Internalizing problems			
Intercept	47.42	0.85	.000 ***
Timepoint	0.53	0.06	.000 ***
Colic	2.13	1.37	.122
Moderation models: Internalizing problems			
ACEs	0.13	0.09	.131
Colic * ACEs	0.02	0.18	.924
Avoidant attachment	0.39	0.19	.046*
Colic * Avoidant attachment	0.35	0.40	.377
Anxious attachment	0.45	0.25	.076
Colic * Anxious Attachment	0.24	0.50	.635
Externalizing problems			
Intercept	49.36	0.83	.000 ***
Timepoint	-0.03	0.06	.553
Colic	-0.26	1.37	.848
Moderation models: Externalizing problems			
ACEs	0.04	0.09	.617
Colic * ACEs	-0.03	0.18	.858
Avoidant attachment	0.16	0.20	.415
Colic * Avoidant attachment	0.40	0.40	.314
Anxious attachment	0.21	0.25	.400
Colic * Anxious Attachment	-0.46	0.50	.363

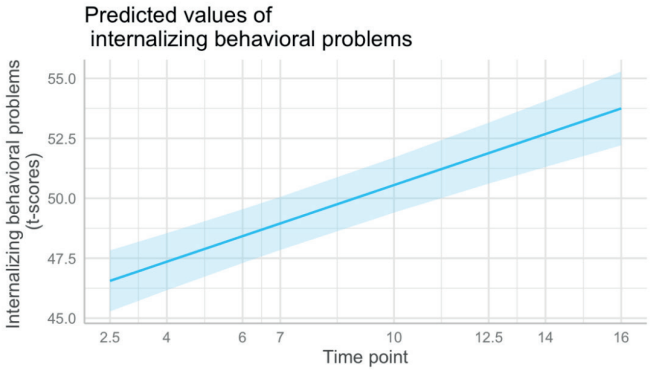
Note. * = p-value significant at the >.05 level. ***= p-value significant at the >.001 level

Supplementary Table S5. Exploratory results from moderations analyses on the Association between Infant mean minutes of crying on Internalizing, Externalizing Total and Behavioral Problems as moderated by maternal ACEs, avoidant and anxious attachment style.

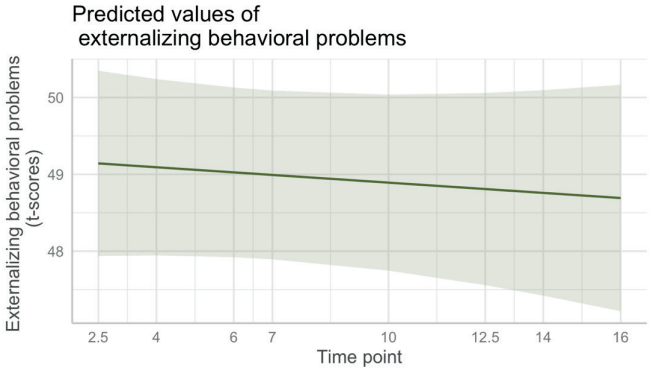
Variable	Internalizing			Externalizing			Total		
	B	SE	p-value	B	SE	p-value	B	SE	p-value
Main effect									
Class of reference: Increasing high									
Crying (low)	0.00	0.01	0.927	0.01	0.00	0.251	-0.01	0.01	0.293
Crying (moderate)	0.01	0.01	0.140	0.01	0.01	0.171	0.00	0.00	0.538
Class of reference: low									
Crying (moderate)	0.01	0.00	0.082	0.00	0.00	0.654	-0.01	0.00	0.122
Moderation models									
Class of reference: high									
ACEs * Crying (low)	0.00	0.00	0.056	0.00	0.00	0.370	0.00	0.00	0.327
ACEs * Crying (moderate)	0.00	0.00	0.067	0.00	0.00	0.271	0.00	0.00	0.433
Avoidant attachment style * Crying (low)	0.00	0.00	0.630	0.00	0.00	0.923	0.00	0.00	0.943
Avoidant attachment style * Crying (moderate)	0.00	0.00	0.826	0.00	0.00	0.390	0.00	0.00	0.774
Anxious attachment style * Crying (low)	0.00	0.00	0.924	0.00	0.00	0.398	0.00	0.00	0.534
Anxious attachment style * Crying (moderate)	0.00	0.00	0.579	0.00	0.00	0.066	0.00	0.00	0.833
Class of reference: low									
ACEs*Crying (moderate)	0.00	0.00	0.996	0.00	0.00	0.745	0.00	0.00	0.636
Avoidant attachment style*Crying (moderate)	0.00	0.00	0.737	0.00	0.00	0.367	0.00	0.00	0.901
Anxious attachment style * Crying (moderate)	0.00	0.00	0.409	0.00	0.00	0.171	0.00	0.00	0.447

Note. Results from the multinomial logistic regression comparing the high trajectory to the moderate and the low trajectories are shown first. Results from comparing the low trajectory to the moderate trajectory are shown after. Trajectories are identified based on the 3 classes (low, moderate, high) as this was consistent for all behavioral problems. B= Unstandardized Beta, SE=Standard Error.

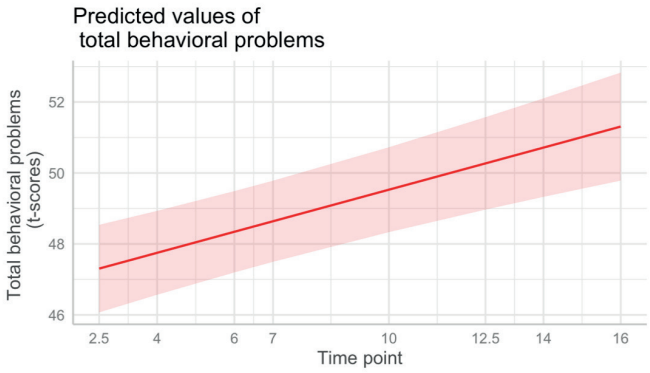
A. Internalizing Behavioral Problems



B. Externalizing Behavioral Problems



C. Total Behavioral Problems



Supplementary Figure S1. Predicted Values of Internalzing, Externalzing and Total Behavioral Problems in Multilevel Analys

Chapter 7.

Summary and General Discussion

This section begins with a concise summary of all five thesis chapters, followed by an overview of the thesis conclusions. Subsequently, a general discussion interprets the findings within a broader context and explores future directions for research on perinatal mental health and infant development.

Summary

The perinatal period is frequently accompanied by substantial challenges that can contribute to or trigger mental health problems (Saxbe et al., 2018). Around 1 in 5 Dutch women experience serious mental health issues during pregnancy and the postpartum period (Browne et al., 2021a; Van Der Zee-Van Den Berg et al., 2021). These mental health issues have been linked to adverse child outcomes including difficult temperament, lower cognitive scores, neurobiological alterations, and later mental health problems (Slomian et al., 2019; Tarabulsy et al., 2014). Despite growing evidence linking maternal mental distress to child development, the mechanisms by which maternal stress impacts child outcomes remain fairly unclear. Understanding these underlying mechanisms is crucial for deepening knowledge of developmental processes and for designing effective interventions. Therefore, this thesis aimed to unravel how prenatal and postnatal distress and maternal childhood experiences affect infant and child behavioral development.

During pregnancy, increased levels of cortisol from maternal circulation can cross the placental barrier and affect the fetal neurobiological development (Seckl & Holmes, 2007). Over time, this can influence the child's development, behavior and wellbeing (Van Den Bergh et al., 2017; Palma-Gudiel et al., 2015). Postnatally, maternal distress can affect child development through multiple pathways. Maternal distress can undermine a mother's ability to provide consistent, sensitive, and responsive caregiving. This can result in emotion regulation problems and insecure attachment in the child, both of which have been associated to later relational and emotional problems (Deans, 2020; Granat et al., 2017). Another postpartum mechanism linking maternal stress to child behavior involves breast milk composition. Lactocrine programming describes how maternal factors, such as stress, can alter non-nutritive bioactive components in breast milk, including glucocorticoids (i.e., cortisol and cortisone). These alterations can affect infant neurodevelopment and behavior (Hinde, 2013). Beyond these direct pathways, maternal vulnerabilities stemming from her own childhood, such as adverse childhood experiences (ACEs) and insecure attachment style, may also play a role in children's development. For example, mothers with ACEs or insecure attachment style have been found to show decreased sensitivity and increased hostility to infant crying (Ablow et al., 2013; Buisman et al., 2018; Girod et al., 2023).

Chapter 2

Prenatal distress encompasses a range of different emotions, worries, and experiences of stress. While specific pregnancy-related distress questionnaires exist,

an instrument assessing pregnancy worries about the postnatal period was missing. To fill this gap, the Baby Preparation and Worry Scale (Baby-PAWS) was recently developed to target these anticipatory worries (Erickson et al., 2020). However, the **Baby-PAWS questionnaire** was only examined in the United States of America, limiting the questionnaire's generalizability to different countries. To address this issue, we performed a **psychometric evaluation** of the questionnaire in a Dutch sample and examined associations between the Baby-PAWS questionnaire and established measures of maternal distress (i.e., depression, anxiety and pregnancy-specific stress) and infant temperament (i.e., IBQ-R). Healthy pregnant women (N = 521) completed online questionnaires during their third trimester, including the Baby-PAWS and distress measures. A subsample of mothers (N = 194) also reported on postnatal distress and infant temperament at 12 weeks postpartum. Exploratory factor analysis suggested a four-factor structure for the 16-item questionnaire in our Dutch sample. This is different from the three-factor structure found in the original psychometric evaluation with the American sample. The Dutch subscales that emerged where: 1) Self and Partner Worry, 2) Baby Caregiving Worry, and 3) Non-parental Childcare Worry, all also found in the American sample, and the Dutch sample-specific factor 4) Separation from Infant Worry. The total Baby-PAWS score was related moderately to pre-and postnatal depression, anxiety, and stress, indicating that Baby-PAWS scores did not substantially overlap with the established constructs. This confirms that the Baby-PAWS addresses distinct worries of pregnant women about the transition to parenthood, and is thus conceptually distinct from general depression, anxiety and stress targeted by existing instruments. Moreover, higher scores of the Baby-PAWS questionnaire were related to infant distress to limitations and sadness. In general, American women had higher worry scores on Baby-PAWS items than Dutch women. In conclusion, the current analyses indicated good validity of the Baby-PAWS in a Dutch sample. Furthermore, our results highlight cross-cultural differences in perinatal mental health and show the importance of examining instrument structure of context-dependent constructs, such as prenatal worries.

Chapter 3

Maternal psychological **stress during pregnancy** has been linked to temperamental difficulties in offspring (Van den Bergh et al., 2020). While heightened cortisol concentrations are often hypothesized as an underlying mechanism, evidence supporting this mechanism is inconsistent (Zijlmans et al., 2015). In this study, we addressed two explanations why these associations might not be found: the nature of cortisol measurements (i.e., mostly saliva) and participant samples (i.e., low-risk pregnant women with low levels of stress). First, we assessed long-term

and retrospective HPA axis activity utilizing prenatal **hair cortisol**, as it would be more suitable biomarker for chronic stress (Kirschbaum et al., 2009, D'Anna-Hernandez et al., 2011). Second, we assessed prenatal stress during the COVID-19 outbreak as a major worldwide psychological stressor that increased stress levels in the population, especially in vulnerable groups such as pregnant women (Lebel et al., 2020, Gustafsson et al., 2021, Perzow et al., 2021, Vacaru et al., 2021). This preregistered study investigated associations between 1) COVID-19-related prenatal psychological stress levels (i.e., social support-, work- and general COVID-19 -related worries) and prenatal hair cortisol, and 2) maternal hair cortisol during the COVID-19 outbreak and later infant temperamental negative affectivity and orienting/regulation. Additionally, we explored whether associations were different for women with lower versus higher socioeconomic status (SES) and at different stages of pregnancy. Pregnant women (N = 100) filled out online questionnaires during the first COVID-19 lockdown. Maternal hair samples were collected during home visits. When infants were six months old, mothers completed a questionnaire on their infant's temperament. While hierarchical regression analyses revealed no associations between prenatal COVID-19 psychological stress and hair cortisol during the COVID-19 outbreak, SES proved to be a moderator in this association. Only pregnant women from higher SES, showed a positive association between work-related and social-related COVID-19 worries and hair cortisol. Prenatal hair cortisol was not associated with later infant temperamental negative affectivity and orienting/regulation. While the COVID-19 outbreak proved to be a major psychological stressor worldwide, the physiological impact of the crisis might be different for pregnant women with higher SES as compared to lower SES.

Chapter 4

Postpartum maternal distress has been associated with adverse infant outcomes (Field, 2018; Kingston et al., 2012). A potential pathway of how maternal distress affects infant outcomes could be alterations in maternal **caregiving** behavior. Observational studies have found that postpartum distress decreases caregiving behavior quality (Booth et al., 2018) and decreases in caregiving behavior mediate associations between maternal distress and infant outcomes (Choe et al., 2013). Interestingly, also on a moment-to-moment basis maternal stress has been found to affect the infant through **mother-infant stress contagion**, whereby negative affective states transfer from mother to infant (Waters et al., 2014, 2017). However, the associations between maternal distress, caregiving behavior, and infant outcomes have never been tested in a controlled experiment. This study utilized an experimental design to investigate the effects of an acute maternal stressor, compared to a control task, on infant cortisol and crying and the possible

mediating role of maternal caregiving behavior. We also assessed if the effects were stronger for mothers with pre-existing high levels of postpartum distress. Finally, associations between maternal cortisol, negative affect, caregiving quality, infant cortisol, and crying were examined irrespective of condition. Mother-infant dyads (N = 91) participated in a lab visit at 8 weeks postpartum. Mothers were separated from their infants and placed in a stress condition (Trier Social Stress Test, TSST; Kirschbaum et al., 1993) or a control condition (non-stressful task). After these tasks, the mothers were reunited with their infant and asked to engage in a semi-structured mother-infant interaction. This interaction was videotaped to assess maternal caregiving behavior and infant crying. Mother and infant salivary cortisol samples were taken throughout the visit. Our structural equation model (SEM) found no differences between conditions (stressor or control) on maternal caregiving behavior and infant response to maternal stress. Furthermore, no evidence was found for a moderating effect of maternal postpartum distress on the effect of maternal condition on infant cortisol and crying. Additionally, maternal cortisol and negative affect reactivity (irrespective of condition) were not associated with infant cortisol, crying, or caregiving behavior. Secondary findings revealed that higher maternal caregiving behavior quality was associated with lower infant cortisol levels at the end of the visit and less crying at reunion. Moreover, sensitivity analysis revealed that when maternal cortisol reactivity was operationalized as cortisol at the end of the manipulation minus the lowest baseline, higher maternal cortisol reactivity was associated with elevated infant cortisol levels at the end of the visit. Thus, although the randomized acute stressor did not induce mother-infant stress contagion, maternal caregiving quality and concurrent infant cortisol and crying levels showed associations. Given the high prevalence of maternal mental health problems and their possible negative association with offspring development, further (experimental) research is needed to understand just how maternal postpartum distress affects infants.

Chapter 5

Human milk is a highly complex and changing fluid, containing many nutritive and non-nutritive components. The latter include hormones, such as glucocorticoids (i.e., cortisol and cortisone; Perrella et al., 2021). These milk glucocorticoids may potentially influence infant development. While some studies have linked milk cortisol concentrations to infant behavioral outcomes (Grey et al., 2013; Nolvi et al., 2018), others have found no significant associations between breast milk cortisol and infant behavior (Hechler et al., 2018; Toorop et al., 2020; Zielinska-Pukos et al., 2022). Understanding whether the stress hormone cortisol in breast milk is related to infant behavior is important for elucidating the potential relevance of breast

milk as a mechanism through which maternal stress can impact child behavior and development. The current preregistered study investigated bidirectional associations between diurnal breast milk glucocorticoids and infant crying and sleep. We hypothesized that 1) higher cortisol and cortisone concentrations would be related to more infant crying and less infant sleep, and 2) more infant crying and less infant sleep would be related to higher cortisol and cortisone concentrations. At 6 weeks postpartum, healthy mothers (N=109) collected three milk samples: in the morning, afternoon, and evening. During this same day, mothers kept a logbook on infant crying and sleep. We calculated the duration of crying and sleeping over three time intervals: 1) the complete interval between each breast milk sample, 2) the 1.5 hours after each breast milk sample, and 3) the 1.5 hours before each breast milk sample. Next, we performed multilevel models to assess the bidirectional associations between cortisol and cortisone milk concentrations and infant crying and sleep. Against our hypotheses, we found that higher cortisol and cortisone milk concentrations were related to more infant sleep 1.5 hours after a milk sample. Moreover, more infant sleep 1.5 hours before a milk sample was related to higher cortisol and cortisone milk concentrations. No associations were found between milk glucocorticoids and infant crying. Milk cortisol and cortisone concentrations were also not associated with infant crying or sleep when examining the complete time intervals between milk samples. This study is the first to assess moment-to-moment associations between breast milk glucocorticoids and infant behavior and broadens our understanding of the role of breast milk composition on infant behavior and vice versa.

Chapter 6

Infant colic is defined as prolonged and inconsolable crying in an otherwise healthy baby under five months of age for at least three hours per day, three days per week, for three weeks (Wessel et al., 1954; Zeevenhooven et al., 2017). Although infant colic has been linked to early childhood behavioral difficulties (Smarius et al., 2017; Valla et al., 2021; Zeevenhooven et al., 2022), its association with behavioral problems from childhood to adolescence remains understudied. Moreover, certain infants with colic may be at higher risk of developing behavioral problems, particularly when parents struggle to manage the infant's crying. **Maternal vulnerabilities**, including adverse childhood experiences (ACEs) and insecure attachment style could amplify this risk, as both have been associated with more negative and aggressive reactions to infant crying and lower parenting sensitivity (Ablow et al., 2013; Buisman et al., 2018; Girod et al., 2023; Jones et al., 2014; Strathearn et al., 2009). This study aimed to investigate whether infant colic predicts more negative problem behavior trajectories by modelling children's internalizing, externalizing,

and total behavioral problems trajectories from toddlerhood to adolescence. Moreover, we assessed whether maternal ACEs and insecure attachment style, as vulnerability factors, moderate this association between infant colic and behavioral problems trajectories. Mothers (N=166) from a Dutch low-risk birth cohort reported infant colic with a 4-day diary at 6 weeks post-partum and filled out questionnaires for children's behavioral problems at eight time points between 2.5 and 16 years. Maternal adverse childhood experiences and attachment style were retrospectively assessed using self-report. With latent class growth analyses (LCGA) we obtained three trajectories (low, moderate, high) for each type of behavioral problems. Infant colic was overall not associated with a higher behavioral problem trajectory. However, at high levels of maternal anxious attachment, infant colic was more predictive of the moderate externalizing problems trajectory compared to the high trajectory. We did not find evidence that maternal ACEs and avoidant attachment style moderate the association between infant colic and behavioral problem trajectories. Given the high prevalence of infant colic, it is essential to elucidate whether, through what mechanisms, and under which contextual factors infant colic shapes later behavioral development.

Conclusions

- Exploratory factor analysis indicated good validity of the Baby Preparation and Worry Scale (Baby-PAWS) in a Dutch sample.
- The factor structure of the Dutch Baby-PAWS differed from the US version, with a fourth factor emerging that represented worries about separation from the infant, indicating that these worries may be more salient for Dutch than US women.
- The Baby-PAWS was moderately, but not strongly, related to existing measures of prenatal depression, anxiety and pregnancy-specific stress, suggesting it assesses distinct prenatal worries beyond these established constructs.
- We did not find that hair cortisol concentrations of Dutch pregnant women during the COVID-19 outbreak, was related to prenatal worries nor infant temperament at 6 months.
- Women with higher socioeconomic status (SES), but not lower SES, showed a positive association between work-related and social support-related COVID-19 worries and hair cortisol.
- Although the COVID-19 outbreak constituted a major psychological stressor worldwide, the physiological impact of the crisis might be different for women differing in SES.
- At 8 weeks postpartum, maternal independent exposure to an acute stressor (versus a non-stressful control task) did not affect infant crying or cortisol concentrations upon reunion.
- Exposure to the acute maternal stressor was also not related to maternal caregiving behavior quality.
- Independent of conditions, when maternal cortisol reactivity was operationalized as change from the lowest baseline value to post-stressor levels, higher maternal cortisol reactivity was associated with elevated infant cortisol levels at the end of the visit.
- Higher quality maternal caregiving was associated with decreased infant crying and cortisol at visit end, but not at reunion.
- Higher milk cortisol and cortisone concentrations were related to more infant sleep during the first 1.5 hours after breastfeeding.
- In addition, more infant sleep during the 1.5 hours preceding a feeding was related to higher subsequent milk cortisol and cortisone concentrations.
- Bidirectional associations were not present for milk glucocorticoids and infant behavior when examining the complete time intervals between milk samples.
- We obtained three trajectories (low, moderate, high) for internalizing, externalizing and total behavioral problems spanning from toddlerhood to adolescence.

- Infant colic was not related to later internalizing, externalizing or total behavioral problems.
- High maternal anxious attachment was protective for infants with colic, increasing the likelihood of stable moderate rather than increasing high externalizing problems.
- Maternal adverse childhood experiences (ACEs) and avoidant attachment style did not play a role in strengthening or weakening the association between infant colic and internalizing, externalizing or total behavioral problem trajectories.

General discussion

This thesis aimed to advance the understanding of the mechanisms through which maternal perinatal distress influences children's behavioral development. The thesis focused on the role of prenatal and postpartum distress on child behavior and how maternal childhood experiences can affect children's behavioral problems. The following discussion addresses three overarching themes that contextualize these findings: first, the value of daily and bidirectional approaches for studying mother-child interactions; second, challenges in operationalizing perinatal distress in contemporary society; and third, how cultural and societal factors can shape maternal distress and child outcomes.

Daily and bidirectional approaches to studying mother-child interactions

Existing literature has provided substantial insight into long-term associations between maternal distress and child outcomes. Traditional longitudinal studies typically assess maternal distress, caregiving quality and infant behavior at widely spaced intervals (e.g., months or years apart) and examine whether trait-level differences, such as general levels of maternal distress, predict caregiving quality and infant outcomes, often measured at one time. Such designs capture important stable between-person differences over time, such as if maternal distress affects child development, as discussed in **Chapter 3**. However, these studies are unable to unravel how mothers and infants dynamically interact throughout the day. Early developmental researchers recognized this importance of studying real-time interactions to understand caregiver-infant dynamics. Pioneering work using experimental paradigms includes the Still-Face Paradigm (Tronick et al., 1978) and detailed observational methods in attachment research (Ainsworth et al., 1978), establishing that infant behavior develops through ongoing exchanges with caregivers. More recent technological advances have enabled researchers to extend this work with more intensive repeated measures that assess maternal and infant behavior continuously or in rapid succession (de Barbaro, 2019; Wass et al., 2024). This approach allows examination of specific within-person processes, such as capturing heterogeneity and inconsistency in caregiving behavior, or how infants learn from and are affected by their caregivers in real time (de Barbaro & Fausey, 2022).

This thesis incorporated moment-to-moment designs in **Chapters 4** and **5** to study direct, real-time associations between maternal distress and infant behavior. Beyond their empirical findings, these studies showcased two unique methodological features that such designs offer: their ability to enable otherwise impossible

experimental manipulation of acute stress and their capacity to capture bidirectional processes as they unfold in real time.

Acute stress manipulation

While experimental stress paradigms for infants, such as the Still-Face Paradigm (Tronick et al., 1978), have been studied intensively, very few studies have induced acute stress in mothers to observe its effects on the infant stress response. These experimental designs serve to establish causal mechanisms between maternal stress, caregiving behaviors and infant outcomes. While researchers cannot induce long-term maternal stress exposure for obvious ethical reasons, short-term manipulations of acute maternal stress are possible. Studies using these experimental designs have documented stress transmission in dyads (Engert et al., 2019; Marheinecke et al., 2025), including older mother-child dyads (Blasberg et al., 2023). More precisely, Blasberg et al. (2023) found that school-aged children (8-12 years) exhibit empathic stress responses when observing their mothers undergo an acute stressor. Children of mothers who underwent the stressor had significant increases in the stress hormone cortisol and decreased parasympathetic activity (i.e., reduced heart-rate variability, indicating stress) that synchronized with their mothers' stress responses. Waters and colleagues (2014; 2017) studied stress contagion in mother-infant dyads. They found that acutely stressing the mother with a laboratory stressor and then reuniting her with her infant led to reactions in the infant. The infant showed increased sympathetic nervous system activity and avoidance behavior, indicating stress transmission from mother to infant. Touch seemed to play a role in this transmission, with infants displaying higher mother-infant sympathetic nervous system covariance when the mothers were instructed to hold the infants (Waters et al., 2017). **Chapter 4** extended this work of Blasberg et al. (2023) and Waters et al. (2014; 2017) to 8-week-old infants by assessing changes in maternal caregiving behavior and infant cortisol. We were the first to test stress contagion in such young infants and did not find the expected mother-infant stress transmission in our sample. This could be due to the single episode of stress or because of the chosen measure of caregiving behavior. Future research could investigate whether repeated exposure to maternal stress could accumulate to affect the infant's development. Additionally, further research is recommended to study how an acute maternal stressor might affect maternal caregiving behaviors that were not part of our observations, such as very subtle changes in gaze or voice. Such work would help explain whether, through frequent repetition in daily life, maternal stress accumulates to produce the long-term negative infant outcomes observed in longitudinal research. Given children's vulnerability to chronic stress and its programming effects on the developing stress system, understanding these transmission mechanisms in mother-child dyads is especially important.

Capturing Bidirectionality

Moment-to-moment designs can also capture bidirectional influences as they unfold. The notion that mother-infant interaction is bidirectional has been acknowledged in developmental research for decades. For example, Bell argued that correlational findings typically interpreted as parent-to-child behavioral effects could be reinterpreted as child-to-parent influences (Bell, 1968). Through this reframing, he established the child's active role in shaping parental behavior. While researchers have acknowledged bidirectional parent-child influences, fewer studies have actually tested these associations from infant to mother. More recent naturalistic, bidirectional studies have found that throughout the day, fluctuations in maternal and infant emotions significantly covary (Hibel et al., 2024). Moreover, when infant crying was above average for the prior eight hours, mothers reported subsequent increases in depressive symptoms (de Barbaro et al., 2023). These findings illustrate how mother-infant associations are bidirectional and dynamic, and that when the states of the partners is mostly negative, this may create feedback loops of negativity that intensify over time.

To empirically examine such bidirectional mother-infant processes, repeated measurements, such as multiple daily assessments, of constructs with high intra-individual variability are particularly well-suited (Paschall & Mastergeorge, 2016). We demonstrated bidirectional effects in **Chapter 5**, finding that higher maternal milk glucocorticoids were associated with more infant sleep and more infant sleep led to higher milk glucocorticoids. Interestingly, both associations were opposite to our predictions. Based on longitudinal findings that higher milk cortisol predicts more difficult infant temperament (Grey et al., 2013; Nolvi et al., 2018) and that infant sleeping problems predict higher maternal distress (Brand et al., 2014; Hughes et al., 2015), we had expected higher glucocorticoids to be associated with less infant sleep and vice versa. These seemingly contradictory findings underscore the need for a better understanding of the daily dynamics of breastfeeding mothers and their infants. To fulfill this need, future research can additionally include measures of maternal behavior. This could enable a better understanding of what mothers do while their infants sleep and how this may affect their mood and physiology. Important for these types of data is assessing both autoregressive (i.e., behavior or milk predicting itself at the next time point) and cross-lagged effects (i.e., bidirectional associations between maternal and infant behavior). Sophisticated analytical approaches, such as dynamic structural equation models (DSEM; Asparouhov et al., 2018) allow researchers to do this. While not yet frequently used in parent-child interaction research, DSEM allows researchers to study various timescales, such as minute-by-minute dynamics within dyads.

The method has shown promising initial applications, for example in examining dynamics of physiological regulation and affect within and between mothers and infants during real-time interactions (Somers et al., 2021, 2022).

Conclusion and future directions

The limited focus on naturalistic moment-to-moment constructs may reflect technological and time constraints in coding naturalistic mother-infant interactions. Fortunately, recent technological advancements enable researchers to capture dynamic changes in maternal and infant behavior within home settings. Wearable devices can assess mother and child vocalizations (Richards et al., 2017; Smith et al., 2022), infant crying (de Barbaro et al., 2023; Yao et al., 2022), heart rate (Wass et al., 2019) and touch (Yao et al., 2019) throughout the day in home settings. These technologies enable examination of cascading events that take place in real-world environments. For example, an initial maternal stressor can result in less sensitive responses towards the infant, the infant may start crying, the mother may become more stressed, triggering interconnected reactions that progressively amplify arousal in both mother and infant.

Future research can use these new technologies to study variability in interactions. At the daily level, interactions between mother and child may differ across times of day, such as morning versus afternoon or evening. Small changes in maternal or infant fatigue or irritability could affect how the dyad interacts. At the developmental level, interaction patterns will change from the first postnatal days through infancy and toddlerhood as children develop social, behavioral, motor and language skills (Else-Quest et al., 2011; Madigan et al., 2016). These developmental changes may alter how moment-to-moment dynamics between mother and infant change over time. For example, while **Chapter 4** found no evidence for mother-infant stress contagion at 8 weeks, other studies have documented such effects at 13 months postpartum (Waters et al., 2014, 2017). Lastly, the same infant behavior (e.g., crying) may affect maternal distress or caregiving differently over time, as mothers develop their caregiving views and skills. Understanding these moment-to-moment bidirectional processes more in depth will help in uncovering the mechanisms through which maternal distress and infant behavior affect one another.

Operationalization of perinatal distress in contemporary society

In the current thesis, maternal prenatal (**Chapters 2-3**) and postnatal distress (**Chapters 4-5**), as well as maternal own childhood experiences (**Chapter 6**), were assessed to obtain a broad perspective on maternal distress. Moreover, in **Chapter 2**,

the Baby-PAWS, a novel prenatal distress questionnaire, was validated as there was not yet a prenatal questionnaire assessing anticipatory stress about the postnatal period. Despite these diverse measures, important gaps remain in current perinatal distress operationalization. First, several emotions remain critically understudied, despite their potential relevance to maternal wellbeing and infant outcomes. While women report negative emotions, such as loneliness, anger, shame and guilt during the perinatal period (Adlington et al., 2023), these types of emotions have rarely been systematically addressed in research. Second, substantial societal changes over recent decades have created new sources of contemporary perinatal distress that existing measurement tools may not adequately capture. Together, these gaps may limit our understanding of maternal experiences and their effects on child development.

Understudied and Stigmatized Perinatal Emotions

Over the years, perinatal maternal stress has been studied using different measures. The Edinburgh Postnatal Depression Scale (EPDS) was the first questionnaire developed to assess perinatal-specific mental health (Cox et al., 1987). This questionnaire recognized the importance of perinatal-specific screening scales to detect and study perinatal mental health complaints. In subsequent decades, additional pregnancy-specific questionnaires were developed to address other prenatal-specific worries, including the Pregnancy-Related Anxiety Questionnaire (PRAQ; Van den Bergh, 1990) and the Pregnancy Experience Scale (PES; DiPietro et al., 2004). In the coming paragraphs four different emotions will be discussed that appear to be relevant in the context of perinatal distress, namely, loneliness, anger, shame and guilt, but received much less research attention.

Loneliness may be particularly relevant during the perinatal period in modern Western societies, relating to feelings of isolation due to shifted social roles or being unable to share the perinatal experience with someone else (Kent-Marvick et al., 2022; Nowland et al., 2024). Current studies on loneliness reveal high prevalence rates, with between 32% and 42% of parents from pregnancy through the first five years of their child's life reporting loneliness. This increases to 70% among parents of children with disabilities or poor health, and approximately 82% of parents report experiencing loneliness at least occasionally (for review see Kent-Marvick et al., 2022). While research on perinatal loneliness is rare, these initial prevalence rates seem higher than loneliness in other age groups (Surkalim et al., 2022). Despite these high prevalence rates, perinatal loneliness receives disproportionately less research attention than loneliness in other life stages. For example, adolescent or elderly loneliness yielded respectively 3610 and 9379 results on Pubmed,

compared to 303 results for pregnancy or perinatal loneliness as of November 2025. Depression and loneliness in the perinatal period seem to show bidirectional relationships, with each potentially serving as both cause and consequence of the other (Kent-Marvick et al., 2022). Beyond maternal outcomes, perinatal loneliness is also associated with adverse child behavioral development, such as heightened internalizing problems and increased respiratory tract infections (Luoma et al., 2019; Nowland et al., 2024). Given the high prevalence of maternal loneliness during the perinatal period and its associations with both maternal and child outcomes, further research on perinatal loneliness is important.

Another understudied maternal reaction to the perinatal period is perinatal anger. Mothers report anger during the postpartum period when they experience violation of their expectations about motherhood and powerlessness when feeling trapped in circumstances such as poverty and intimate partner problems (Ou & Hall, 2018). A qualitative study further showed that mothers experienced anger when they have compromised needs, lack of support and inability to control infants' sleep (Ou et al., 2022a). Given that sleep deprivation is a frequently reported cause of anger (Ou et al., 2022b), this suggests that anger may be particularly salient during the perinatal period. While research is scarce, initial prevalence of intense levels of maternal postpartum anger has been found to be around 31% (Ou et al., 2022c). Anger shows significant associations to maternal perinatal depression and anxiety. However, besides this association, anger was also shown to be a distinct mood disturbance, as only half of the women experiencing intense perinatal anger, also experience depressive symptoms (Ou, et al., 2022c). While the direct role of perinatal anger on child outcomes has not yet been studied, research on parents of older children shows that increased rates of parental anger can lead to harsh parenting and potentially also to child abuse (Rodriguez & Green, 1997; Shay & Knutson, 2008).

Perinatal shame may be particularly pronounced when mothers feel they fall short of idealized "perfect mother" standards or through comparisons with other parents (Constantinou et al., 2021). Shame can negatively impact perinatal mental health and is related to decreased maternal tendency to seek help (Caldwell et al., 2021; Dunford & Granger, 2017). Due to this decreased help-seeking, shame may worsen maternal mental health and potentially affect child development. More research is needed on how shame can sustain or worsen different maternal mental health symptoms and its possible role on child development. Related to shame, but defined as a distinct construct, is perinatal guilt. Unlike the global self-evaluation characteristic of shame, guilt typically arises when mothers feel they have made specific parenting mistakes, such as not responding quickly enough to their infant's

cries or struggling with breastfeeding (Jackson et al., 2022). Given this focus on behavior rather than on the self, guilt shows significant, but weaker associations with perinatal depression than shame, especially when controlling for concurrent shame (Caldwell et al., 2021).

Further research is needed to develop validated psychometric measures specifically designed to assess negative perinatal emotions. Currently these negative emotions are measured using scales for general populations, such as the UCLA Loneliness Scale (Russell, 1996), PROMIS Anger Short Form (Pilkonis et al., 2011) or Guilt Inventory (Jones & Kugler, 1993). However, these questionnaires have not been validated in perinatal samples, with the exception of the UCLA Loneliness Scale, which demonstrated acceptable psychometric properties in Japanese perinatal women (Arimoto & Tadaka, 2019). Beyond the lack of validation, perinatal-specific dimensions of these emotions, such as feelings of isolation related to shifted social roles or shame and anger due to unique caregiving concerns, may not be adequately captured by general population measures, highlighting the need for perinatal-specific assessments.

Contemporary perinatal stressors

Modern society may have created new sources of perinatal distress. Two main interconnected societal shifts in Western societies have potentially altered the perinatal experience: the transition from communal to individualistic societies and the digitalization of society.

Historically, parenting was a communal activity, with caregiving responsibilities shared among extended family and community members, a phenomenon called alloparenting. The industrial era shifted caregiving responsibility to the nuclear family, away from communal support structures (Sear, 2016). This transformation has pressured mothers toward self-reliance and independent caregiving (Bridgers & Fox, 2024), potentially creating a more isolating and stressful perinatal experience. Indeed, a cross-country evaluation found that more individualistic countries compared to more communal countries have higher rates of parental burnout and stress (Roskam et al., 2022). This social isolation may be compounded by a decreased willingness to ask for social support. In the Netherlands, for example, two-thirds of parents do not dare to ask for help from their direct environment, such as family, friends, or neighbors (Gezonde Generatie, 2021). This reluctance to seek support, combined with reduced community structures, leaves many mothers navigating parenthood alone, consistent with the previously documented high prevalence of perinatal loneliness.

Another important change in our current society is the increased rate of digitalization of daily life. While substantial research has examined perinatal distress, most studies were conducted before this digital shift. Digitalization may have altered the nature of distress that pregnant and postpartum mothers experience. Nowadays, many women use social media for information related to pregnancy and their child's wellbeing. Numbers show that more than 52% of pregnant women use social media for medical information and 89% of women for pregnancy or parenting advice (Baker & Yang, 2018; Huddleston et al., 2025). Some women experience positive effects of social media use, such as receiving support from peers and enhancing pregnancy-related knowledge (Chan & Chen, 2019; Mertens et al., 2024). However, there has also been critique of its effects, especially on vulnerable parents (Johnson, 2014). For example, looking up medical advice seems to exacerbate later anxiety and distress during pregnancy (Huddleston et al., 2025) and especially young, first-time mothers show increased levels of anxiety and stress due to information seeking (Hughson et al., 2018). Using digital sources for parental advice could increase distress due to information overload, conflicting advice or misinformation, and difficulty distinguishing reliable from unreliable information. This can be especially challenging in the perinatal period given the inherent heightened uncertainty and vulnerability (Frey et al., 2022; Mertens et al., 2024).

Besides looking for parenting and medical advice, social media platforms can also create opportunities for social comparison and enforce how perfect motherhood should look like (Tate, 2023). This social comparison can drive negative emotions in the perinatal period in mothers, such as stress, depression and guilt (Constantinou et al., 2021; Tate, 2023). Thus, perinatal social media use seems to show both positive and negative associations with maternal wellbeing (Mertens et al., 2024). Given this dual nature, perinatal social media use may represent a particularly important, yet largely unmeasured, contemporary perinatal stressor.

Conclusions and future research on contemporary operationalization of perinatal distress

Given these substantial societal changes over recent decades, including increased individualism, the shift to nuclear families and digitalization, it is important to question whether currently utilized screening instruments remain adequate for contemporary populations. As the most widely used measures were developed over 20 years ago (Cox et al., 1987; DiPietro et al., 2004; Van den Bergh, 1990), it remains unclear whether these instruments still capture all the primary sources of parental distress. The drivers of perinatal distress may have evolved, and some constructs might be less applicable to (expectant) mothers today than when

the instruments were originally developed. Future research should continue developing new questionnaires or updating existing instruments to incorporate previously understudied emotions and contemporary stressors.

Understudied emotions are additionally interesting as innovative approaches in clinical psychology have moved away from focusing merely on diagnostic categories such as depression or anxiety, instead emphasizing symptom-level assessment (Borsboom & Cramer, 2013). This framework could prove valuable for understanding perinatal distress, recognizing that different mothers may experience distinct symptom profiles. For example, one mother may experience stress, guilt and anxiety, while another mother's distress is characterized by depression and loneliness. Recent data-driven analyses of postpartum depression empirically demonstrate this heterogeneity, identifying nine distinct clinical subtypes differentiated by types of symptoms, severity, trauma history and timing of onset (Bauer et al., 2025). Thus, mothers across subtypes could obtain similar scores on general distress measures despite experiencing different symptom profiles and requiring different interventions to decrease the distress. Future research could examine how these specific maternal symptom combinations differentially affect infant developmental outcomes.

Furthermore, as society continues to evolve with new parenting and perinatal challenges each decade, ongoing qualitative work is necessary to capture the full range of maternal experiences and understand why particular emotions emerge in specific contexts. For example, parents nowadays might experience climate anxiety or have concerns about relying on AI for parenting guidance. Further qualitative research is essential to understand these new perinatal worries before next steps, such as the development of new quantitative questionnaires, are taken.

How cultural and societal factors can affect maternal and infant wellbeing

When mothers experience anxiety, depression, or other mental health challenges, they are sometimes implicitly viewed as failing to cope adequately with motherhood. This framing conceals how cultural and societal expectations can undermine maternal wellbeing. Perinatal mental health research and practice predominantly focus on screening, diagnosing and treating individual mothers, with insufficient attention to the systemic contexts that generate distress (see also Luijk & Roseboom, 2025). Following Bates' (2022) argument to "fix the system, not the women", this section shifts focus from individual maternal responsibility for mental health improvement to the institutional contexts in which women

operate. Cultural (ideologies, mental load) and societal factors (parental leave) are examined to understand their role in shaping maternal distress and child behavioral development.

Cultural factors: ideologies and gender roles

Theoretical frameworks developed in the 1990s and 2000s remain influential in understanding how contemporary parenting norms emerged and continue to shape maternal experiences. Prominent among these are the motherhood myth (Douglas & Michaels, 2004) and intensive parenting (Hays, 1996). These theories argue that in modern Western cultures, motherhood is defined as aiming for perfection, self-sacrifice and intensive investment in children's development. In turn, these ideologies can emphasize the idea that mothers are primarily responsible for children's outcomes, both reflecting and reinforcing structural inequalities in parental responsibilities. For example, research shows that the more expectant fathers view men and women as essentially different in their caregiving abilities, the lower their intentions and actual involvement in childcare is (Ross-Plourde et al., 2022).

Around the transition to parenthood, the unequal division of household labor can become more skewed (Hiekel & Ivanova, 2023; Yavorsky et al., 2015). While 50% of parents in the Netherlands want paid and household work divided equally, only 9% of couples actually achieve this equal division (Centraal Bureau voor de Statistiek, 2024). An unequal division of household labor between parents has been associated with increased maternal psychological distress and sleep problems (Ervin et al., 2022; Goldberg & Perry-Jenkins, 2004) and even lower cognitive outcomes in children (Keizer et al., 2020).

Beyond the unequal distribution of care itself, the planning and managing of household and care tasks are also often disproportionately shouldered by mothers. This is termed "maternal mental load" in the literature and is defined as the invisible cognitive and emotional labor of managing household and family life (Offer, 2014; Offer & Schneider, 2011). It encompasses planning, anticipating, organizing, multitasking, and remembering household and caregiving tasks that fall disproportionately to mothers. Feminist scholars already argued in the 1990s that gender inequality includes invisible cognitive and managerial household work (DeVault, 1991; Walzer, 1996). However, empirical research on this concept emerged only recently, with attention accelerating during the COVID-19 pandemic when work-home boundaries were challenged (Callaghan et al., 2024). Importantly, increased maternal mental load has been related to worse maternal mental

health outcomes (Dean et al., 2022). Moreover, an American study showed that mothers reported greater responsibility than partners for cognitive labor in 29 of 30 household tasks and for physical labor in 28 of 30 tasks. Importantly, only the unequal cognitive division (i.e., the mental load) predicted maternal depression, stress, burnout and overall worse mental health, while unequal physical labor showed no such associations (Aviv et al., 2024). This finding suggests that maternal mental load is an important construct for furthering our understanding of perinatal distress.

One possibility to help distribute household and management responsibilities more equitably is through cultural shifts towards egalitarian parenting norms, combined with structural support measures such as extended partner leave. Past research in the field of perinatal mental health and child development has predominantly focused on mothers but would benefit from increased inclusion of fathers in research designs. By not actively studying fathers, research might unintendedly support the notion that they are not main caregivers, reinforcing existing gender roles.

Societal factors: parental leave

Beyond cultural factors, several societal and policy factors can profoundly affect maternal perinatal distress and, consequently, child development outcomes. An important societal factor affecting maternal distress is the length and reimbursement (ranging from fully paid to unpaid) of parental leave. Studies have found that a shorter duration or less reimbursement of parental leave has been associated with worse maternal mental health, including depressive symptoms, psychological distress and burnout (Heshmati et al., 2023). In turn, shorter maternity leave has been related to worse child outcomes, such as child behavioral problems (Van Niel et al., 2020). Part of this evidence comes from quasi experimental studies, comparing parental and child wellbeing before and after policy changes in parental leave duration or payment, providing evidence that controls for socioeconomic and cultural confounds (Houmark et al., 2024).

Differences in parental leave policies may also explain some of the differences in prenatal distress between the US and Netherlands as found in **Chapter 2**. We observed a different factor structure of the Baby-PAWS questionnaire in the US and the Netherlands and higher levels of distress during pregnancy in the US, compared to the Netherlands. One main difference between the countries is maternity leave policies, as the US lacks a national paid maternity leave policy, allowing at most 12 weeks of unpaid leave for eligible employees (FMLA, 1993). Consequently, in

the US women return to work more quickly than women in countries with paid maternity leave (Slopen, 2020). In contrast, the Netherlands mandates a minimum of 16 weeks of fully paid leave, with additional parental leaves available (OECD, 2021). Availability of paid maternity leave has been shown to decrease stress levels in families already in the prenatal period, due to less anticipatory financial and childcare related worries (Van Niel et al., 2020). Additionally, this longer leave in the Netherlands may alter the types of worries women have during pregnancy. In **Chapter 2**, we found that Dutch pregnant mothers were more concerned about being separated from their infants. This could be because Dutch mothers expect to spend more time with their babies initially, which allows them to worry more about expected separation.

Beyond maternal leave, longer, paid partner or paternal leave has also shown initial positive effects. For example, longer partner leave has been shown to decrease father's stress and fatigue (Cardenas et al., 2021), improve relationship quality (Petts & Knoester, 2020), and enable a more equitable caregiving division, allowing fathers to develop close nurturing relationships as primary caregivers (André et al., 2025). These paternal benefits extend to maternal wellbeing, with longer partner leaves being associated with decreased maternal distress (Cardenas et al., 2021). The Netherlands mandates 1 week of fully paid partner leave plus 5 optional weeks at 70% pay, however, other countries such as the US do not mandate any paid partner leave (OECD, 2021; FMLA, 1993). This policy difference may have additionally contributed to our **Chapter 2** finding of lower stress among pregnant Dutch compared to US women.

Conclusions and future research on cultural and societal factors

In conclusion, maternal distress can at least in part be a consequence of cultural and societal factors. These factors can be interdependent as cultural ideologies can affect policy decisions that place caregiving responsibility primarily on individual mothers. Moreover, policy structures, such as short partner leave, can reinforce cultural beliefs about mothers as primary caregivers.

These cultural and societal factors carry implications for perinatal distress research. First, validation of perinatal distress instruments across countries is important, as the perinatal experience can be shaped by specific factors per country. Second, the underlying mechanisms of how maternal distress can affect infant development may vary across mothers depending on social and cultural contexts. Therefore, it is important to replicate findings in diverse samples, such as between countries. Third, as parental leave policies continue to evolve, future research should keep

assessing their effects on parental involvement and wellbeing, maternal mental load and caregiving division. Fourth, as mentioned above, the field should move beyond research designs that focus exclusively on mothers to also include other caregivers. While this thesis focused on mothers who carried and gave birth to their biological children, it is important to acknowledge that not all caregivers experience pregnancy, childbirth, or are biologically related to their children. The current research aims to help and inspire future research to apply these designs also to fathers and non-traditional or nonbiological parents, such as, LGBTQIA+ or adoption parents. Finally, intervention research is recommended to address systemic and policy factors alongside individual-level factors. While screening and treating maternal mental health problems remain important, such approaches might be insufficient if they do not also address the cultural and societal contexts that can generate distress. In conclusion, maternal perinatal distress is shaped by cultural ideologies, gender dynamics, and structural support systems. Research must remain attentive to these factors when trying to understand, measure and address maternal perinatal distress and its effects on child development.

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Appendix

Nederlandse samenvatting

De perinatale periode gaat vaak gepaard met uitdagingen die kunnen leiden tot psychische problemen (Saxbe et al., 2018). Ongeveer 1 op de 5 Nederlandse vrouwen ervaart serieuze psychische klachten tijdens de zwangerschap en de postpartumperiode (Browne et al., 2021; Van Der Zee-Van Den Berg et al., 2021). Deze psychische klachten werden door eerdere studies in verband gebracht met negatieve uitkomsten bij het kind, waaronder een moeilijker temperament, lagere cognitieve scores, neurobiologische veranderingen en latere psychische problemen (Slomian et al., 2019; Tarabulsy et al., 2014). Ondanks dit groeiende bewijs dat maternale psychische problemen een rol kunnen spelen in de ontwikkeling van kinderen, blijven de mechanismen hoe maternale stress het kind kan beïnvloeden onduidelijk. Het begrijpen van deze onderliggende mechanismen is belangrijk om de processen beter begrijpen en effectieve interventies te ontwikkelen. Daarom had dit proefschrift het doel het in kaart brengen van hoe prenatale en postnatale stress en maternale jeugdervaringen de gedragsontwikkeling van baby's en kinderen kunnen beïnvloeden.

Tijdens de zwangerschap kunnen verhoogde cortisolniveaus de placentabarrière passeren en de neurobiologische ontwikkeling van de foetus beïnvloeden (Seckl & Holmes, 2007). Op lange termijn kan dit de ontwikkeling, het gedrag en het welzijn van het kind beïnvloeden (Van Den Bergh et al., 2017; Palma-Gudiel et al., 2015). Na de geboorte kan stress van de moeder de ontwikkeling van het kind via meerdere andere routes beïnvloeden. Zo kan stress het vermogen van een moeder om consistente, sensitieve en afgestemde zorg te bieden verminderen. Dit kan leiden tot problemen met emotieregulatie en onveilige hechting bij het kind, die beide in verband zijn gebracht met latere problemen (Deans, 2020; Granat et al., 2017). Een ander postpartum mechanisme waardoor maternale stress het kind kan beïnvloeden, is de samenstelling van moedermelk. Lactocriene programmering verwijst naar hoe maternale factoren, zoals stress, de niet-voedende componenten van moedermelk kunnen beïnvloeden. Dergelijke veranderingen kunnen vervolgens de hersenontwikkeling en het gedrag van baby’s beïnvloeden (Hinde, 2013). Naast deze directe routes kunnen maternale kwetsbaarheden die voortkomen uit haar eigen jeugd, zoals negatieve jeugdervaringen en een onveilige hechtingsstijl, ook een rol spelen in de ontwikkeling van kinderen. Zo is aangetoond dat moeders met negatieve jeugdervaringen of een onveilige hechtingsstijl minder sensitief reageren en meer vijandigheid tonen wanneer hun baby huilt (Ablow et al., 2013; Buisman et al., 2018; Girod et al., 2023).

Hoofdstuk 2

Prenatale stress omvat verschillende emoties, zorgen en stresservaringen. Hoewel er specifieke zwangerschapsgerelateerde stress-vragenlijsten bestaan, ontbrak een instrument dat zwangerschapszorgen over de postnatale periode beoordeelt. Om deze leegte te vullen werd onlangs de Baby Preparation and Worry Scale (Baby-PAWS) ontwikkeld om deze anticiperende zorgen te meten (Erickson et al., 2020). De Baby-PAWS-vragenlijst is echter alleen onderzocht in de Verenigde Staten, wat de generaliseerbaarheid van de vragenlijst naar verschillende landen beperkt. Om dit probleem aan te pakken hebben we een psychometrische evaluatie van de vragenlijst uitgevoerd in een Nederlandse steekproef. Daarnaast hebben we associaties onderzocht tussen de Baby-PAWS-vragenlijst en andere bestaande vragenlijsten van maternale stress (d.w.z. depressie, angst en zwangerschaps-specifieke stress) en het temperament van baby's. Gezonde zwangere vrouwen (N = 521) vulden online vragenlijsten in tijdens hun derde trimester van de zwangerschap, waaronder de Baby-PAWS en andere stressvragenlijsten. Een subgroep van moeders (N = 194) rapporteerde ook over postnatale stress en het temperament van de baby bij 12 weken postpartum. Exploratieve factoranalyse liet een vier-factorenstructuur zien voor de 16-item vragenlijst in onze Nederlandse steekproef. Dit verschilt van de drie-factorenstructuur die gevonden werd in de oorspronkelijke psychometrische evaluatie met de Amerikaanse steekproef. De Nederlandse subschalen die naar voren kwamen waren: 1) Zorgen over Zelf en Partner, 2) Zorgen over Babyverzorging, en 3) Zorgen over Niet-ouderlijke Kinderopvang. Daarnaast vonden we een vierde schaal die specifiek voor de Nederlandse vrouwen was: 4) Zorgen over Scheiding van de Baby. De totale Baby-PAWS-score was gematigd gerelateerd aan pre- en postnatale depressie, angst en stress, wat aangeeft dat Baby-PAWS-scores niet volledig overlapten met de bestaande constructen. Dit bevestigt dat de Baby-PAWS zich richt op specifieke zorgen van zwangere vrouwen over de overgang naar het ouderschap, en dus conceptueel verschilt van algemene depressie, angst en stress die door bestaande instrumenten worden gemeten. Bovendien waren hogere scores op de Baby-PAWS-vragenlijst gerelateerd aan het temperament van het kind, meer specifiek moeders met hogere scores rapporteerde ook meer negatieve emoties in de baby. Over het algemeen hadden Amerikaanse vrouwen hogere zorgscores op Baby-PAWS-items dan Nederlandse vrouwen. Concluderend gaven de huidige analyses een goede validiteit van de Baby-PAWS in een Nederlandse steekproef aan. Verder benadrukken onze resultaten cross-culturele verschillen in perinatale mentale gezondheid. Ook tonen ze het belang van het onderzoeken van de instrumentstructuur van contextafhankelijke constructen, zoals prenatale zorgen.

Hoofdstuk 3

Maternale psychologische stress tijdens de zwangerschap is in verband gebracht met een moeilijker temperament bij kinderen (Van den Bergh et al., 2020). Hoewel verhoogde cortisolconcentraties vaak als onderliggend mechanisme worden voorgesteld, is het bewijs hiervan inconsistent (Zijlmans et al., 2015). In deze studie hebben we twee verklaringen onderzocht waarom deze associaties mogelijk niet worden gevonden: de aard van cortisolmetingen (d.w.z. meestal via speeksel) en de aard van de deelnemerssteekproeven (d.w.z. zwangere vrouwen met een laag risico en lage stressniveaus). Ten eerste hebben we als biomarker voor chronische prenatale stress haarcortisol gebruikt. Dit is een langetermijn en retrospectieve marker van de activiteit van de hypothalamus-hypofyse-bijnier-as (HPA-as; Kirschbaum et al., 2009, D'Anna-Hernandez et al., 2011). Ten tweede hebben we prenatale stress beoordeeld tijdens de COVID-19-uitbraak als een grote wereldwijde psychologische stressor die de stressniveaus in de bevolking verhoogde, met name in kwetsbare groepen zoals zwangere vrouwen (Lebel et al., 2020, Gustafsson et al., 2021, Perzow et al., 2021, Vacaru et al., 2021). Deze vooraf geregistreerde studie onderzocht associaties tussen 1) COVID-19-gerelateerde prenatale psychologische stressniveaus (d.w.z. sociale steun-, werk- en algemene COVID-19-gerelateerde zorgen) en prenataal haarcortisol, en 2) maternaal haarcortisol tijdens de COVID-19-uitbraak en latere temperament van de baby. Daarnaast onderzochten we of de associaties verschillend waren voor vrouwen met een lagere versus hogere sociaaleconomische status (SES) en in verschillende stadia van de zwangerschap. Zwangere vrouwen (N = 100) vulden online vragenlijsten in tijdens de eerste COVID-19-lockdown. Maternale haarmonsters werden verzameld tijdens huisbezoeken. Toen de baby's zes maanden oud waren, vulden de moeders een vragenlijst in over het temperament van hun kind. Hoewel de regressieanalyses geen associaties aantoonden tussen prenatale COVID-19 psychologische stress en haarcortisol tijdens de COVID-19-uitbraak, bleek SES een moderator in deze associatie te zijn. Alleen bij zwangere vrouwen met een hogere SES werd een positieve samenhang gevonden tussen werkgerelateerde en sociaal-gerelateerde COVID-19-zorgen en haarcortisol. Prenataal haarcortisol was niet geassocieerd met het latere temperament van de baby. Hoewel de COVID-19-uitbraak wereldwijd een grote psychologische stressor was, kan de fysiologische impact van de crisis verschillend zijn voor zwangere vrouwen met een hogere SES in vergelijking met een lagere SES.

Hoofdstuk 4

In meerdere studies is postpartum maternale stress geassocieerd met nadelige uitkomsten bij de baby (Field, 2018; Kingston et al., 2012). Een mogelijke route waarlangs maternale stress de uitkomsten van baby's beïnvloedt, kunnen veran-

deringen in maternaal zorggedrag zijn. Observationele studies hebben gevonden dat postpartum stress de kwaliteit van zorggedrag vermindert (Booth et al., 2018) en dat deze afnames in zorggedrag kunnen leiden tot meer negatieve uitkomsten bij de baby (Choe et al., 2013). Interessant genoeg is ook aangetoond dat acute maternale stress de baby kan beïnvloeden via moeder-baby-stressbesmetting, waarbij negatieve gevoelens van de moeder op de baby worden overgedragen (Waters et al., 2014, 2017). De associaties tussen maternale stress, zorggedrag en uitkomsten bij baby's zijn echter nooit getest in een experiment. Deze studie gebruikte een experimenteel design om de effecten van een acute maternale stressor, vergeleken met een controletaak, op het cortisol en huilen van de baby te onderzoeken, evenals de mogelijke mediërende rol van maternaal zorggedrag. We beoordeelden ook of de effecten sterker waren voor moeders met reeds bestaande hoge niveaus van postpartum stress. Ten slotte werden associaties tussen maternaal cortisol, negatief affect, zorgkwaliteit, het cortisol en huilen van de baby onderzocht ongeacht de conditie. Moeders met hun baby's (N = 91) namen deel aan een labbezoek bij 8 weken postpartum. Moeders werden gescheiden van hun baby's en toegewezen aan een stressconditie (Trier Social Stress Test, TSST; Kirschbaum et al., 1993) of een controleconditie (niet-stressvolle taak). Na deze taken werden de moeders herenigd met hun baby en gevraagd om deel te nemen aan een moeder-baby-interactie. Deze interactie werd op video opgenomen om maternaal zorggedrag en het huilen van de baby te beoordelen. Speekselmonsters om cortisol te meten van moeder en baby werden gedurende het bezoek afgenomen. Ons structural equation model (SEM) vond geen verschillen tussen condities (stressor of controle) op maternaal zorggedrag en het cortisol en huilen van de baby. Verder werd er geen bewijs gevonden voor een versterkend effect van maternale postpartum stress op het effect van de maternale conditie op de reactie van de baby. Bovendien waren maternale cortisol- en negatieve affectreactiviteit (ongeacht conditie) niet geassocieerd met het cortisol, huilen of zorggedrag van de baby. Bijkomende bevindingen toonden aan dat een hogere kwaliteit van maternaal zorggedrag geassocieerd was met lagere cortisolniveaus bij de baby aan het einde van het bezoek en minder huilen bij hereniging. Bovendien toonde sensitiviteitsanalyse aan dat wanneer maternale cortisolreactiviteit werd gemeten als cortisol aan het einde van de manipulatie minus de laagste baseline, hogere maternale cortisolreactiviteit geassocieerd was met verhoogde cortisolniveaus bij de baby aan het einde van het bezoek. Dus hoewel de gerandomiseerde acute stressor geen moeder-baby-stressbesmetting induceerde, vonden we voor maternale zorgkwaliteit en gelijktijdige cortisol- en huilniveaus van de baby wel associaties. Gezien de hoge prevalentie van maternale psychische problemen en hun mogelijke negatieve effecten op nakomelingen, is verder (experimenteel) onderzoek nodig om te begrijpen hoe maternale postpartum stress baby's precies beïnvloedt.

Hoofdstuk 5

Moedermelk is een zeer complexe en veranderende vloeistof, die veel voedzame en niet-voedzame componenten bevat. Niet-voedzame componenten omvatten hormonen, zoals glucocorticoïden (d.w.z. cortisol en cortison; Perrella et al., 2021). Deze glucocorticoïden in melk kunnen mogelijk de ontwikkeling van baby's beïnvloeden. Hoewel sommige studies melkcortisol hebben gekoppeld aan gedragsuitkomsten van baby's (Grey et al., 2013; Nolvi et al., 2018), hebben anderen deze significante associaties niet gevonden (Hechler et al., 2018; Toorop et al., 2020; Zielinska-Pukos et al., 2022). Het is van belang om de relatie tussen melkcortisol en het gedrag van baby's beter te doorgronden, omdat dit mogelijk een mechanisme vormt waardoor maternale stress het gedrag van kinderen beïnvloedt. De huidige studie onderzocht wederzijdse associaties tussen dagelijkse melkglucocorticoïden en het huilen en de slaap van baby's. We verwachtten dat 1) hogere cortisol- en cortisonconcentraties gerelateerd zouden zijn aan meer huilen en minder slaap van de baby, en 2) meer huilen en minder slaap van de baby gerelateerd zouden zijn aan hogere cortisol- en cortisonconcentraties. Bij 6 weken postpartum verzamelden gezonde moeders (N=109) drie melkmonsters: 's ochtends, 's middags en 's avonds. Gedurende dezelfde dag hielden moeders een logboek bij over het huilen en de slaap van de baby. We berekenden de duur van huilen en slapen over drie tijdsintervallen: 1) het volledige interval tussen elk moedermelkmonster, 2) de 1,5 uur na elk moedermelkmonster, en 3) de 1,5 uur vóór elk moedermelkmonster. Vervolgens voerden we multilevel-modellen uit om de wederzijdse associaties tussen cortisol- en cortisonmelkconcentraties en het huilen en de slaap van de baby te beoordelen. Tegen onze verwachtingen in vonden we dat hogere cortisol- en cortisolconcentraties gerelateerd waren aan meer slaap van de baby 1,5 uur na een melkmonster. Bovendien was meer slaap van de baby 1,5 uur vóór een melkmonster gerelateerd aan hogere cortisol- en cortisonconcentraties. Er werden geen associaties gevonden tussen melkglucocorticoïden en het huilen van de baby. Melkcortisol- en cortisonconcentraties waren ook niet geassocieerd met het huilen of de slaap van de baby bij het onderzoeken van de volledige tijdsintervallen tussen melkmonsters. Deze studie is de eerste die dagelijkse associaties heeft beoordeeld tussen moedermelkglucocorticoïden en het gedrag van de baby. Dit vergroot ons inzicht in hoe de samenstelling van moedermelk het gedrag van baby's beïnvloedt, en andersom.

Hoofdstuk 6

Koliek bij baby's wordt gedefinieerd als langdurig en ontroostbaar huilen gedurende ten minste drie uur per dag, drie dagen per week, gedurende drie weken, bij een verder gezonde baby onder de vijf maanden oud (Wessel et al., 1954; Zeevenhooven et al., 2017). Hoewel koliek bij baby's in verband is gebracht met gedragsmoeilijkheden

in de vroege kindertijd (Smarius et al., 2017; Valla et al., 2021; Zeevenhooven et al., 2022), blijft de associatie met gedragsproblemen van de peutertijd tot adolescentie onderbelicht. Bovendien kunnen bepaalde baby's met koliek een hoger risico lopen op het ontwikkelen van gedragsproblemen, wanneer ouders moeite hebben om met het huilen van de baby om te gaan. Maternale kwetsbaarheden, waaronder negatieve jeugdervaringen en onveilige hechtingsstijl, kunnen dit risico versterken, aangezien beide geassocieerd zijn met meer negatieve en agressieve reacties op het huilen van baby's en lagere ouderlijke sensitiviteit (Ablow et al., 2013; Buisman et al., 2018; Girod et al., 2023; Jones et al., 2014; Strathearn et al., 2009). Deze studie had tot doel te onderzoeken of koliek bij baby's meer negatieve gedragtrajecten voorspelt. Hiervoor modelleerden we trajecten van internaliserende, externaliserende en totale gedragsproblemen van peutertijd tot adolescentie. Bovendien beoordeelden we of maternale negatieve jeugdervaringen en onveilige hechtingsstijl, als kwetsbaarheidsfactoren, deze associatie tussen koliek bij baby's en gedragsprobleemtrajecten versterken. Moeders (N=166) uit een gezond Nederlands geboortecohort rapporteerden koliek bij baby's met een 4-daags dagboek bij 6 weken postpartum en vulden vragenlijsten in voor gedragsproblemen van kinderen op acht tijdspunten tussen 2,5 en 16 jaar. Maternale negatieve jeugdervaringen en hechtingsstijl werden beoordeeld door middel van retrospectieve zelfrapportage. Met latent class growth analyses (LCGA) vonden we drie trajecten (laag, gemiddeld, hoog) voor elk type gedragsprobleem. Koliek bij baby's was over het algemeen niet geassocieerd met een hoger gedragsprobleemtraject. Bij hoge niveaus van maternale angstige hechtingsstijl voorspelde koliek bij baby's echter meer externaliserende problemen. We vonden geen bewijs dat maternale negatieve jeugdervaringen en vermijdende hechtingsstijl de associatie tussen koliek bij baby's en gedragsprobleemtrajecten versterken. Gezien de hoge prevalentie van koliek, is het belangrijk om beter te begrijpen via welke mechanismen koliek de latere gedragsontwikkeling kan beïnvloeden.

Conclusies

- Exploratieve factoranalyse toonde een goede validiteit aan van de Baby Preparation and Worry Scale (Baby-PAWS) in een Nederlandse steekproef.
- De factorstructuur van de Nederlandse Baby-PAWS verschilde van de Amerikaanse versie, waarbij een vierde factor naar voren kwam die zorgen over scheiding van de baby vertegenwoordigde, wat aangeeft dat deze zorgen mogelijk meer kenmerkend zijn voor Nederlandse dan voor Amerikaanse vrouwen.
- De Baby-PAWS was gematigd, maar niet sterk, gerelateerd aan bestaande maten van prenatale depressie, angst en zwangerschapsspecifieke stress, wat suggereert dat het andere, specifieke prenatale zorgen meet dan deze gevestigde constructen.

- We vonden dat haarcortisolconcentraties van Nederlandse zwangere vrouwen tijdens de COVID-19-uitbraak niet gerelateerd waren aan prenatale zorgen noch aan het temperament van baby's bij 6 maanden.
- Vrouwen met een hogere sociaaleconomische status (SES), maar niet lagere SES, vertoonden een positieve associatie tussen werkgerelateerde en sociale steun-gerelateerde COVID-19-zorgen en haarcortisol.
- Hoewel de COVID-19-uitbraak wereldwijd een grote psychologische stressor vormde, kan de fysiologische impact van de crisis verschillend zijn voor vrouwen die verschillen in SES.
- Bij 8 weken postpartum beïnvloedde maternale blootstelling aan een acute stressor (versus een niet-stressvolle controletaak) het huilen of de cortisolconcentraties van de baby niet.
- Blootstelling aan de acute maternale stressor was ook niet gerelateerd aan verschillen in de kwaliteit van maternaal zorggedrag.
- Als maternale cortisolreactiviteit werd gemeten als verandering van de laagste baselinewaarde naar post-stressor niveaus, was hogere maternale cortisolreactiviteit geassocieerd met verhoogde cortisolconcentraties bij de baby aan het einde van het bezoek.
- Hogere kwaliteit van maternale zorgverlening was geassocieerd met verminderd huilen en cortisol van de baby aan het einde van het bezoek, maar niet bij hereniging.
- Hogere melkcortisol- en cortisonconcentraties waren gerelateerd aan meer slaap van de baby gedurende de eerste 1,5 uur na het geven van borstvoeding.
- Daarnaast was meer slaap van de baby gedurende de 1,5 uur voorafgaand aan een voeding gerelateerd aan hogere daaropvolgende melkcortisol- en cortisonconcentraties.
- Wederzijdse associaties werden niet gevonden voor melkglucocorticoïden en het gedrag van baby's bij het onderzoeken van de volledige tijdsintervallen tussen melkmonsters.
- We verkregen drie trajecten (laag, gemiddeld, hoog) voor internaliserende, externaliserende en totale gedragsproblemen van peutertijd tot adolescentie.
- Koliek bij baby's was niet gerelateerd aan latere internaliserende, externaliserende of totale gedragsproblemen.
- Hoge maternale angstige hechtingstijl was beschermend voor baby's met koliek, waarbij de kans om te behoren tot een stabiele gemiddelde trajectorie in plaats van een toenemende hoge externaliserende gedragtrajectorie werd vergroot.
- Maternale negatieve jeugdervaringen en een vermijdende hechtingsstijl bleken geen rol te spelen in het versterken of verzwakken van de samenhang tussen koliek bij baby's en de trajecten van internaliserende, externaliserende of totale gedragsproblemen.

Research data management statement

Ethics and privacy

Data collection of all studies (SMILEY, COPE, BIBO, SKIPPY and online study) were conducted in accordance with the 1964 Declaration of Helsinki and its amendments, and with relevant national legislation and regulations, guidelines, and codes of conduct.

The ethics committee of the Social Science faculty of the Radboud University, Nijmegen, the Netherlands, approved the study protocols of the SMILEY study (SW2017-1303-497/ECSW-2019-051/ECSW-2019-106/ECSW-2020-021), SKIPPY study (ECSW2015-2311-358), BIBO study (ECG300107/SW2017-1303-497/SW 121 2017-1303-498/ECSW-2023-046) and the Online study (ECSW2022–1303-497). The COPE online study was approved by The Ethics Review Board of Tilburg University (RP2019–143) and the collection of biological materials, such as the hair samples, was separately approved by The Ethics Committee Faculty of Social Sciences of Radboud University (ECSW-2020–056).

Informed consent was obtained from all participants regarding their data usage and storage. Consent was also obtained for sharing and reuse of the pseudonymized data for future research.

Data collection and storage

Online questionnaire data were collected through secure platforms: Castor EDC for the SMILEY and more recent BIBO study waves, Easion for older BIBO waves, LimeSurvey platform for the SKIPPY study, and Qualtrics for the Online and COPE study. Some SMILEY and BIBO data were collected via paper questionnaires, subsequently digitized in Excel.

For all studies, the privacy of participants is ensured through pseudonymization using participant codes. Moreover, identifiable data, such as addresses or dates of birth, are stored separately from other data (e.g., questionnaires, biological sample values) and are password protected. The key file for pseudonymization is password protected and stored on the secure network drive, separate from the research data, and can only be accessed by a few trained individuals, such as the head researcher and the lab manager. All pseudonymized research data are stored on secure network servers of the Donders Institute.

Additionally, some paper questionnaires, informed consent forms and logbooks were collected, which are physically stored in a locked archive at the Donders Institute. Lastly, collected biological samples (i.e., hair, saliva, stool, and milk samples) are physically stored in locked cabinets (hair) or freezers (saliva, stool, and milk). These samples are labeled only with participant codes and do not contain identifiable data. Most of these samples were sent out for analysis and were subsequently destroyed (hair) or returned to the aforementioned freezers (stool).

Data sharing

Chapters 2 to 5 are all published with open access. Chapters 6 has been submitted for publication and is currently under review.

The dataset from chapter 2 to 6 is published with restricted access in Radboud Data Repository (see: https://doi.org/10.34973/0x79-3a21). Requests for access will be checked by prof. Carolina de Weerth, against the conditions for sharing the data as described in the signed Informed Consent. The data of all chapters are archived by Donders Institute. As aligned with the informed consent form, all study data will be stored for a minimum of 15 years from the moment of being collected.

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Curriculum vitae

Nina Bruinhof was born on April 26th, 1997 in Breda, the Netherlands. In 2015, she began her Bachelor's degree in Psychology at Radboud University, where she developed an interest in research through multiple internships at the Behavioral Science Institute (BSI). During her undergraduate studies, she completed a minor in Gender Studies and spent a semester abroad at Australian Catholic University in Melbourne, Australia. She started the Research Master's program in Behavioral Science at Radboud University in 2019, graduating cum laude in 2021. As part of this program, she completed an internship at the Developmental Psychobiology Lab under the supervision of Dr. Roseriet Beijers. In 2021, she began her doctoral research at the same research group under the supervision of prof. Dr. Carolina de Weerth and Dr. Roseriet, investigating perinatal influences on infant behavioral development. During her PhD, she completed the SMILEY data collection as a maternity leave replacement and set up and executed the SMILEY 2-year follow-up assessment. The final phase of her doctoral work involved managing and conducting the BIBO 18-year assessment round, which included a study of mothers, fathers, and children, as well as MRI visits, for which she obtained MRI certification.

List of publications

Bruinhof, **N**., Beijers, R., de Weerth, C. Moment-to-moment Associations Between Breast Milk Glucocorticoids and Infant Crying and Sleep. *Psychoneuroendocrinology*, *187*, 107790. https://doi.org/10.1016/j.psyneuen.2026.107790

Lustermans, H., **Bruinhof**, **N**., Beijers, R., de Weerth, C. (2025) From Pregnancy to Postpartum: The Interplay Between Maternal Mental Health and Diet. *Appetite*, 108253. https://doi.org/10.1016/j.appet.2025.108253

Bruinhof, **N**., Beijers, R., Lustermans, H., & de Weerth, C. (2025). Mother–infant stress contagion? Effects of an acute maternal stressor on maternal caregiving behavior and infant cortisol and crying. *Journal of Child Psychology and Psychiatry*. https://doi.org/10.1111/jcpp.14119

Bruinhof, **N**., Sehic, E., Hancock, G. R., Gartstein, M. A., & de Weerth, C. (2024). Prenatal anticipatory stress: Baby preparation and worry scale-revised in the Dutch context. *Comprehensive Psychiatry,* 128, 152437 . https://doi.org/10.1016/j.comppsych.2023.

Rheinheimer, N., Beijers, R., **Bruinhof**, **N**., Cooijmans, K. H. M., & de Weerth, C. (2023). Effects of daily full-term infant skin-to-skin contact on behavior and cognition at age three–secondary outcomes of a randomized controlled trial. *Journal of Child Psychology and Psychiatry*, *64*(1), 136-144. https://doi.org/10.1111/jcpp.13679

Bruinhof, **N**., Vacaru, S. V., van den Heuvel, M. I., de Weerth, C., & Beijers, R. (2022). Prenatal hair cortisol concentrations during the COVID-19 outbreak: Associations with maternal psychological stress and infant temperament. *Psychoneuroendocrinology*, *144*, 105863. https://doi.org/10.1016/j.psyneuen.2022.105863

Lustermans, H., Missler, M., Peters, L., **Bruinhof**, **N**., de Weerth, C., & Beijers, R. Maternal Stress Upon Returning to Work after Maternity Leave: Associations with room-sharing, breastfeeding and partner support. *Under review.*

Bruinhof, **N**., Latini, A., Beijers, R., de Weerth, C. Infant colic and behavioral problems from childhood to adolescence – moderation by maternal childhood trauma and attachment style. *Submitted.*

Portfolio

Course participation	
2025	The Art of Finishing up
2024	Mindfulness Based Stress Reduction
2024	Getting Ready for your First Grant Application
2023	Science Journalism and Communication
2023	DGS. Graduate School Day 2
2023	Analyzing longitudinal and multilevel data using R
2023	Scientific Integrity Course
2022	Writing Scientific Articles
2022	Achieving your Goals and performing more successfully in your PhD
2022	DGS - Graduate School Day 1
2022	Radboudumc, eBROK course
2022	Kinderen enthousiasmeren voor Wetenschap
2022	Education in a Nutshell
2021	DGS - GS Introduction Day
Supervision	
2024 – 2025	Asia Latini. Master thesis titled: Infant colic and behavioral problems from childhood to adolescence – moderation by maternal childhood trauma and attachment style.
2024	Luna Hoogeveen. Master thesis titled: "The role of prenatal maternal stress on maternal diet and child cognition."
2021 – 2022	Ingrid de Boer. Honours bachelor thesis titled: "Depressive symptoms and dietary quality during pregnancy and in the early postpartum period: an analysis of a community-based sample"
Conferences	
2025	Poster at Vereniging Nederlandse Ontwikkelings Psychologie (VNOP), Nijmegen, NL. Infant colic and behavioral problems from childhood to adolescence – moderation by maternal childhood trauma and attachment style.
2025	Presentation at Society for Reproductive and Infant Psychology (SRIP), Liverpool, UK. Moment-to-moment Associations Between Breast Milk Glucocorticoids and Infant Crying and Sleep. And poster: Infant colic and behavioral problems from childhood to adolescence – moderation by maternal childhood trauma and attachment style.
2025	Symposium presentation at European Society of Child and Adolescent Psychiatry (ESCAP) Strasbourg, FR. Infant colic and behavioral problems from childhood to adolescence – moderation by maternal childhood trauma and attachment style.
2024	Symposium presentation at an international webinar of the General University Hospital of Heraklion (Invited lecturer), online conference. Moment-to-moment Associations Between Breast Milk Glucocorticoids and Infant Crying and Sleep.
2024	Early Career Pitch at Stress-NL meeting, Utrecht, NL. Mother-Infant Stress Contagion? The Effect of Acute Maternal Stress on Maternal Caregiving Behavior and Infant Stress Response

2023	Presentation at Donders Discussion, Nijmegen, NL. Mother-Infant Stress Contagion? The Effect of Acute Maternal Stress on Maternal Caregiving Behavior and Infant Stress Response
2023	Symposium presentation at International Society for Psychoneuroendocrinology (ISPNE), London UK. Mother-Infant Stress Contagion? The Effect of Acute Maternal Stress on Maternal Caregiving Behavior and Infant Stress Response
2023	Poster presentations at International Society of Developmental Psychobiology (ISDP), Utrecht. 1. Mother-Infant Stress Contagion? The Effect of Acute Maternal Stress on Maternal Caregiving Behavior and Infant Stress Response and, 2. Moment-to-moment Associations Between Breast Milk Glucocorticoids and Infant Crying and Sleep.
2023	Symposium presentation at Dutch Neuroscience Meeting (DNM), Tiel, NL. Moment-to-moment Associations Between Breast Milk Glucocorticoids and Infant Crying and Sleep.
2022	Presentation at International Society for Psychoneuroendocrinology (ISPNE), Chicago USA. Prenatal hair cortisol concentrations during the COVID-19 outbreak: Associations with maternal psychological stress and infant temperament
2021	Online poster presentation at International Society of Developmental Psychobiology (ISDP). Prenatal hair cortisol concentrations during the COVID-19 outbreak: Associations with maternal psychological stress and infant temperament.
Other activities	
2025	**Green team committee Medical Neuroscience.**
2022 – 2024	**PR committee Baby & Child Research Center.**

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education. Others continue in a wide range of positions, such as policy advisors, project managers, consultants, data scientists, web- or software developers, business owners, regulatory affairs specialists, engineers, managers, or IT architects. As such, the career paths of Donders PhD graduates span a broad range of sectors and professions, but the common factor is that they almost all have become successful professionals.

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