Roseriet Beijers

The early maternal and caregiving environment: Longitudinal links with infant regulation and health

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The early maternal and caregiving environment: longitudinal links with infant regulation and health

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Promotor:

Prof. dr. J.M.A. Riksen-Walraven

Copromotor:

Dr. C. de Weerth

Manuscript commissie:

Prof. dr. S.A.E. Geurts (voorzitter)

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Prof. dr. H. Tiemeier (Erasmus University Rotterdam)

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Chapter 1

Introduction

1.1 General introduction

Environmental factors in utero and during early life are predictive of cognitive, behavioural and socio-emotional development and physical health in children and adults (Loman & Gunnar, 2010; Shonkoff, Boyce & McEwen, 2009). Studies on the development of children raised in institutional care illustrate this point well. Conditions within these institutions are often marked by high levels of deprivation, including deprivation of adequate nutrition, medical care, cognitive stimulation, and supportive caregiving (Smyke, Koga, Johnson, Fox, Marshall, Nelson, et al., 2007). When institutionalized children are placed in families, marked improvements in growth and functioning can be observed. However, many of the children maintain developmental difficulties, including problems in cognitive, language and behavioural functioning, even after several years living in the adoptive family (Loman, Wiik, Frenn, Pollak & Gunnar, 2009; Pollak, Nelson, Schlaak, Roeber, Wewerka, Wiik, et al., 2010). These findings underline the long-term impact of early adverse experiences on children's development and physical health. However, questions remain about the underlying mechanisms linking these early experiences to child outcomes later in life (Loman & Gunnar, 2010; Taylor, Way & Seeman, 2010).

The aim of the studies presented in this thesis is to investigate the effects of the early maternal and caregiving environment on regulation and health of infants and young children. Regulation can be measured on multiple levels and includes the regulation of behaviour and emotions and the regulation of physiological states (Haley & Stansbury, 2003; Davis, Bruce & Gunnar, 2002). The concept of regulation received increased attention in the past decades, as regulation problems are acknowledged to play a key role in the development of psychopathology (Yap, Allen & Sheeber, 2007). Unveiling the development of regulation, in conjunction with determining the role of the early maternal and caregiving environment, will increase our understanding of the development of normal behaviour. Moreover, these studies might help to reveal the possible mechanisms underlying the link between early adverse experiences and later psychopathology (Posner & Rothbart, 2000). This is in line with the 'Early Life Stress model', which suggests that early life stressors compromise the regulation of the physiological systems that are responsible

for later behavior and emotion regulatory capacities (Loman & Gunnar; 2010). In turn, difficulties in behaviour regulation might impact the likelihood of psychopathology later in life (Heim & Nemeroff, 2001, Nemeroff, 2004).

Next to infant regulation, this thesis also studied infants' physical health in relation to the early environment. Empirical studies show that children that experience forms of early adversity, such as poverty, abuse and neglect, are at risk for immune system dysfunctioning and disease in adulthood, including cardiovascular disease (Galobardes, Smith & Lynch, 2006; Miller, Chen & Cole, 2009; Slopen, Koenen, & Kubzansky, 2012). However, less is known about the relationship between early adversity and physical health within younger populations, including infants. It remains unclear whether adverse experiences already lead to illnesses early in life, or whether the negative effects of early adversity are stored in a latent form and emerge later in life (Ben-Shlomo & Kuh, 2002).

Fortunately, the role of the early environment is not limited to adverse effects. Beneficial environments, including maternal and caregiving environments of high quality, have been related to positive outcomes. Animal research, for instance, showed that higher quality of maternal care in rats, as quantified by more maternal licking and grooming, is related to lower reactivity to stressors in the offspring (Francis & Meaney, 1999; Weaver, Cervoni, Champagne, D'Alessio, Sharma, Seckl, et al., 2004). In humans, there are also indications that experiencing high quality of maternal care fosters infants' development, e.g., their ability to cope with stressful situations at a behavioural and physiological level (Albers, Riksen-Walraven, Sweep & de Weerth, 2008; Schore, 2001; DeWolff & van IJzendoorn, 1997). Apparently, the dynamics of the maternal and caregiving environment, as experienced by an infant on a daily basis, has an impact on the infant's development. This is in line with Hofer's (1994) concept of hidden regulators. Hofer argued that a number of components of typical parent-infant interactions have long-term regulatory effects on specific components of infant behaviour and physiology. Therefore, basal aspects of the maternal and caregiving environment associated with both adversity or benefits are topic of this thesis.

In conclusion, the aim of this thesis is to study the role of the early maternal and caregiving environment in infant regulation and health. As such, this thesis will shed light upon a research area that has been understudied in humans up till now. The following aspects of the early maternal and caregiving environment are included in this thesis: maternal prenatal stress and anxiety, breastfeeding, parent-infant co-sleeping, mother-infant attachment-in-the-making, and non-parental care. These aspects will be described in more detail in the next paragraphs, followed by a description of the child outcomes measures used: infant regulation and physical health. The studies in this thesis were all part of the BIBO study (BIBO: Basale Invloeden op de Baby Ontwikkeling; Basal Influences on Infant Development): a prospective longitudinal project that followed 193 mothers and their children (91 girls; 80 firstborns) from pregnancy through their first years of life. In paragraph 1.4, the BIBO study will be outlined in more detail, followed by the thesis outline.

1.2 The early maternal and caregiving environment

The earliest environment an infant becomes acquainted with is the womb of the mother. It is generally accepted that the physical condition and psychological well-being of a woman during her pregnancy can influence her developing child. The fact that intrauterine experiences can profoundly affect the fetus and subsequently influence postnatal development is referred to as prenatal programming (Van den Bergh, Mulder, Mennes & Glover, 2005; de Weerth & Buitelaar, 2005). In humans, **maternal prenatal stress and anxiety** have been related to adverse pregnancy outcomes and various developmental problems in childhood and adulthood, including cognitive, behavioural, and emotional problems and mental disorders (for reviews see Beydoun & Saftlas, 2008; Charil, Laplante, Vaillancourt & King, 2010). Furthermore, there are also indications that maternal prenatal stress and anxiety predispose human offspring for diseases and immune system dysfunctioning, although this research is still in a very early stage (Entringer, Buss & Wadhwa, 2010; Merlot, Couret & Otten, 2008).

Immediately after birth, the environment of an infant principally consists of the caregiving behaviour of the primary caregiver, usually the

mother. The emotional bond between the infant and the caregiver, also referred to as the attachment relationship, develops gradually over the first year of life; this process is referred to as **attachment-in-the-making**. The attachment bond is assumed to be fully developed at around 12 months of age, and shows important links to later developmental outcomes, including socio-emotional adjustment and regulation (Panfile & Laible, 2012; Crugnola, Tambelli, Spinelli, Gazzotti, Caprin, & Albizzati, 2011). However, less is known about how infant regulatory capacities develop in the context of the emerging attachment relationship during the first year of life.

Two other important basal aspects of the early caregiving environment are the type of feeding an infant receives and the place where an infant sleeps during the night. The benefits of **breastfeeding** for infant health and growth are highly acknowledged and the practice of breastfeeding has been universally encouraged. Contrarily, the topic of **co-sleeping** has been debated for decades (Duijts, Ramadhani & Moll, 2009; Sobralske & Gruber, 2009). While co-sleeping has been previously discouraged because of the claims that co-sleeping increases risks, including the risk for sudden infant death syndrome (SIDS), other evidence supports the benefits of co-sleeping, e.g. the promotion of breastfeeding and enhanced monitoring of the infant during the night (Sobralske & Gruber, 2009). Although early experiences during feeding and during the night might have important consequences for the development of an infant, both breastfeeding and co-sleeping have been hardly related to the development of regulation, including the regulation of physiological states.

When the infant reaches the age of 3 months, many mothers start working again. Therefore, in the Netherlands, as in many other countries, it is very common for infants to attend **non-parental care** from an early age on. Non-parental care is characterized by separation from the parents, and might therefore be seen as a possible source of stress for children. In addition, non-parental care, and especially centre-based care, is characterized by different routines, unfamiliar settings, different caregivers, larger group sizes and higher levels of noise than parental care (Albers, Riksen-Walraven & de Weerth, 2007). Therefore, the large interest in the possible impact of non-parental care on children's health and socio-emotional development is

not surprising. Over the past decades, scores of studies have been published investigating the effects of non-parental care on child development. Spending large amounts of time in non-parental care, and in particular center-based care, has been found to be a robust predictor of illnesses and behaviour problems (Belsky, Booth-LaForce, Bradley, Brownell, Burchinal, Campbell, et al., 2006; Bradley & Vandell, 2007). However, less is known about how other aspects of non-parental care, such as the number of concurrent arrangements, affect health and socio-emotional development, including regulation. Moreover, studies examining the mechanisms underlying the link between non-parental care and behaviour problems are greatly lacking.

Summarizing the above, with increasing age an infant becomes exposed to more and different environmental factors. Whereas the environment before birth is limited to the womb, postnatally the environment of an infant is expanded by more maternal and caregiving factors. Figure 1 presents a graphical overview of the basal environmental aspects that will be studied in relation to infant development in the current thesis.

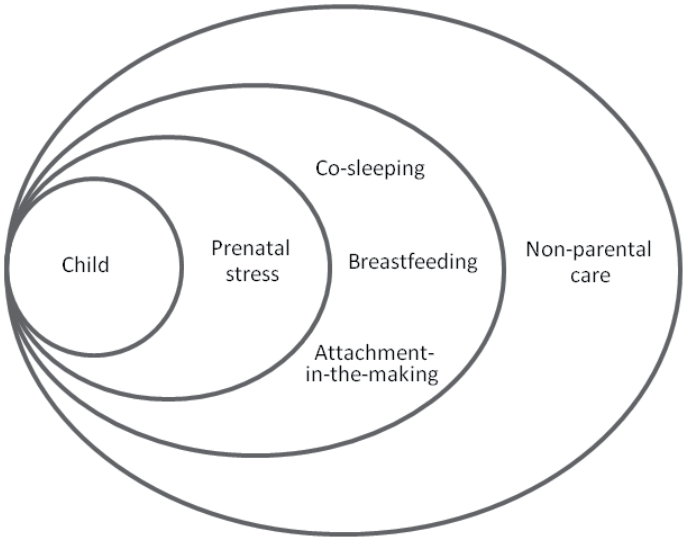

Figure 1: The maternal and caregiving environmental factors studied in the present thesis. The ovals on the right side represent the expanding environment by increasing infant age.

1.3 Infant regulation and health

The child outcome measures investigated in this thesis are infant physiological and behavioural regulation, and physical health. Regulation can best be measured when infants are exposed to challenges and (mild) stressful situations. In response to stressful situations, the human body is activated to mobilize endocrine, autonomic, and behaviour functions that help the body to react efficiently to the stressful situation. Without the ability to regulate these functions, stress responses would be elevated and prolonged, subsequently leading to mental and physical health problems later in life (Sapolsky, Romero & Munck, 2000).

Physiological regulation: In response to challenges and stressful situations, the hypothalamic pituitary adrenal axis (HPA-axis) is activated, resulting in the release of a cascade of hormones. The hormone cortisol, secreted by the adrenal cortex, is the end product of this axis (Sapolsky et al., 2000). Cortisol is not only needed to react efficiently to a stressful situation, but it also regulates many body processes including memory and the immune system. However, repeated or chronic activation of the HPA-axis early in life can eventually result in HPA-axis dysfunctioning. In turn, abnormalities in cortisol physiology have been related to physical health problems and to major psychopathologies, including depression, anxiety disorders, schizophrenia and post-traumatic stress disorders (Petrowski, Herold, Joraschky, Wittchen & Kirschbaum, 2010).

In humans, infants are born with a functional HPA-axis reacting with the secretion of cortisol when confronted with stressors (Jansen, Beijers, Riksen-Walraven & de Weerth, 2010). During development and in response to experience, the HPA-axis' functioning is further fine-tuned and adapted to the early environment. While stressful early life experiences, like childhood abuse and maternal prenatal anxiety, have been related to dysregulated HPA-axis responses to stress later in life (Elzinga, Roelofs, Tollenaar, Bakvis, Van Pelt & Spinhoven, 2008; Laurent, Ablow & Measelle, 2011; Tollenaar, Beijers, Jansen, Riksen-Walraven & de Weerth, 2010), there are also indications that positive early life experiences contribute to more optimal HPA-axis functioning. For example, higher quality of maternal care has been related to a quicker cortisol

recovery from a mild stressor in three- and five-month-old infants (Albers et al., 2008; Haley & Stansbury, 2003). However, influences on infant cortisol regulation of other important aspects of the early caregiving environment, including the type of feeding an infant receives and the place where an infant sleeps during the night, are less well documented. This thesis will shed light upon these aspects by investigating the relations between co-sleeping and breastfeeding early in life and infant cortisol regulation to three different stressors during the first year of life, namely at 5 weeks of age (bathing session), at 2 months of age (vaccination) and at 12 months of age (maternal separation).

Behavioural and emotional regulation: As deficits in behaviour and emotion regulation are known to contribute to psychopathology (Yap et al., 2007), it is imperative to indentify factors that influence their development. Children are viewed as progressing from simple modulation of arousal to more complex forms of behavioral regulation (Eisenberg, Spinrad & Eggum, 2010). Moreover, Kopp and Neufeld (2003) suggested a shift over time in the first years of life from external sources to internal sources of regulation. Starting with almost complete reliance on the caregivers, children gradually become able to regulate their behavior without external monitoring. As such, parenting plays a fundamental role in the development of regulation. Sensitive caregivers are assumed to co-regulate infants' behaviour and emotions and hereby foster the children's own emergent capacities for regulation (Hofer, 1994; Loman & Gunnar, 2010; Schore, 2001).

In this thesis, we investigated behaviour and emotion regulation during the first six months of life and again at 30 months of age. First, we examined the development of infant night waking patterns in the first six months of life. Infants wake up on average three times during the night; the differences between infants stem more from the infant's response, i.e. signaling the waking or self-soothing back to sleep (Touchette, Petit, Tremblay & Montplaisir, 2009). One psychosocial factor that is hypothesized to be linked to infant night waking behaviour is attachment. The attachment relationship between the caregiver, usually the mother, and the infant enables the infant to deal with stressful situations. During stressful situations, securely attached infants turn to the caregiver for comfort and protection, and, when comforted, they return to

exploring the environment. Night wakings do not only occur frequently during infancy, but they can also cause distress in infants because of the threat of being alone and in the dark. As a consequence, the attachment system would be activated at night, and infants would signal their needs according to the attachment strategies they are adopting (Scher & Asher, 2004; Scher, 2008). Therefore, we expect infants who are later classified as securely or insecurely (avoidantly, resistantly, or disorganized) attached, to develop different patterns of night waking signaling in the first six months of life.

At 30 months of age, we examined another aspect of behaviour regulation, i.e. inhibitory control. Inhibitory control can be defined as the ability to inhibit and override dominant responses and behaviours in favour of more appropriate or subdominant responses (Rothbart, Ellis, Rueda & Posner, 2003). As such it is a fundamental aspect of behavioural regulation. According to the 'Early Life Stress' model, stressful and challenging environments early in life can compromise the development of behavioural regulation, possibly impacting the likelihood of psychopathology later in life (Loman & Gunnar; 2010). An environmental factor that can be seen as challenging is early non-parental care. Non-parental care, and especially centre-based care, is often characterized by challenges (i.e. separation from the parents, different caregivers and routines, and large group sizes). Therefore, it might be affecting the budding behavioural regulation capacities of the young infant, and more specifically the infant's inhibitory control capacities. In turn, less inhibitory control would be related to more behavior problems at an older age. In this study we set out to test this hypothesis, by investigating inhibitory control as a mediator of the link between early non-parental care and toddler behavior problems.

Physical health: The concept of the developmental origins of physical health and disease susceptibility is rapidly attracting interest and gaining prominence as a complementary approach for understanding the causation of many common diseases in adulthood (Entringer et al., 2010). The developing child is suggested to react upon conditions in the prenatal and postnatal environment with structural and functional changes in cells, tissues and organ systems. These changes may, in turn, have short-term and long-term consequences for health and disease susceptibility (Gluckman & Hanson,

2004). One of the mechanisms through which the early environment might be related to disease is through stress. In response to stress, the body reacts with a release of hormones, including cortisol. Among others, the hormone cortisol binds to white blood cells and has diverse regulatory effects on their distribution and function within the immune system (Ader, Felten & Cohen, 2001). Although studies convincingly established that stressful experiences alter features of the immune response as well as confer risk factors for disease in adulthood (Segerstrom & Miller, 2004), much less is known about the relationship between early environmental factors and actual illnesses within younger populations, including infants. This thesis focuses on the links between infant illnesses and health complaints within the first year of life and two types of early life experiences, namely prenatal maternal stress and anxiety and multiple aspects of non-parental care.

1.4 The BIBO study

The studies presented in this thesis were carried out as a part of the BIBO study: an ongoing prospective longitudinal study that followed 193 mothers and their children from pregnancy through their first years of life. At this moment, the BIBO study has delivered a wealth of information on the psychobiological development of children till age 5 and the assessment at 6 years of age is being carried out. The study is unique because of its start in pregnancy, and the intensive multi-disciplinary assessments in the first years of life: from temperament, maternal sensitivity, and mother-infant attachment to infant night waking, physical health, and cortisol regulation.

The innovative value of this study lies in the fact that it is the first to longitudinally follow the development of infant regulation and health, in conjunction with the role of the early maternal and caregiving environment, during the crucial period from birth till the age of 12 months and with follow-up studies carried out at 30 months, 4 and 5 years of age. Not only will the findings from this study increase our understanding of the development of normal behaviour, but they will also help reveal the possible mechanisms underlying the link between early experiences and later (psycho)pathology (Posner & Rothbart, 2000).

The BIBO study concentrates on everyday, basal environmental factors. This is reflected in the name of the study; BIBO stands for 'Basale Invloeden op de Baby Ontwikkeling' or, in English, 'Basal Influences on Baby Development'. The monitoring of early basal and everyday environmental factors (e.g. basic caregiving decisions on feeding and sleeping arrangements and non-parental care arrangements), might also provide empirical information relevant for clinical practice to develop optimal childcare advices and policies for parents and professionals in the field.

For the present thesis, the data from the assessment during pregnancy, from the multiple assessments during the infant's first year of life, and from the 30-months assessment were used. For assessing the maternal and caregiving environmental factors, the mothers filled out third-trimester questionnaires and provided saliva samples for circadian cortisol in order to measure prenatal stress and anxiety. Starting at birth, information on breastfeeding and co-sleeping was collected using weekly and daily diaries, respectively, for the first 6 months of life. Furthermore, information on non-parental care arrangements was obtained through monthly maternal interviews across the first year of life. Finally, infant-mother attachment was assessed using the Strange Situation Procedure (Ainsworth, Blehar, Waters & Wall, 1978) when the infants were 12 months of age. An overview of the measurements of the environmental variables used in this thesis can be found in Figure 2.

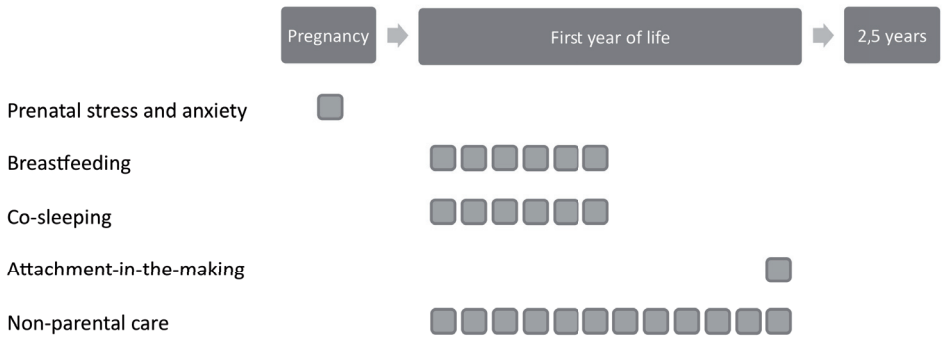

Figure 2: Overview of the measurements of the environmental variables used in the present thesis.

With respect to the child outcome measures, information on infant physical health and illnesses was obtained through monthly maternal interviews across the first year of life. In order to measure cortisol regulation, the infants were subjected to three stressors in the first year of life: at 5 weeks to a bathing session, at 8 weeks to a vaccination, and at 12 months to a maternal separation (Strange Situation Procedure, Ainsworth et al., 1978). Behavioural and emotional regulation was measured in two ways: by examining infant night waking patterns during the first six months of life, and by observing inhibitory control during a home visit when the child was 30 months of age. During this visit we measured the ability to inhibit behaviour and override dominant responses by subjecting the children to three inhibition tasks. An overview of the child outcome measures can be found in Figure 3.

1.5 Thesis outline

The present thesis includes six empirical studies, which are described in six separate chapters. Because the studies are based on the data obtained from the same sample of 193 infants, there is considerable overlap in the Methods sections of the empirical studies. For a more detailed description of the sample, procedures and measures, the reader is referred to the separate chapters.

After the present introductory chapter, the thesis continues in **Chapter 2** with the first empirical study, which aimed to examine whether maternal prenatal anxiety and stress, measured both by self-report and by cortisol

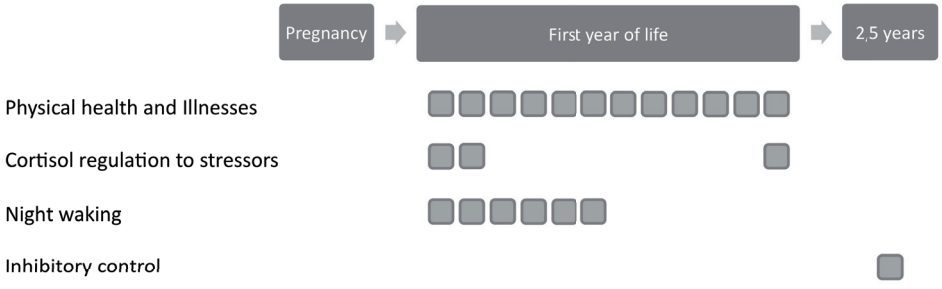

Figure 3: Overview of the infant regulation and physical health outcome measures used in the present thesis.

physiology, were related to more infant illnesses and antibiotic use during the first year of life. The next study in **Chapter 3** investigated the relation between sleeping arrangements in the first two postnatal months and infant cortisol reactivity to a bathing session and a vaccination. This study is followed by the study described in **Chapter 4**, investigating the associations between feeding and sleeping arrangements in the first six months of life and cortisol regulation to a maternal separation at 12 months of age. **Chapter 5** describes the study examining the early history of night waking in infants who were later classified as securely or insecurely (avoidantly, resistantly, or disorganized) attached. The goal of the study presented in **Chapter 6** is to determine whether infant health in the first year of life is related to the amount of time spent in non-parental care and the number of concurrent non-parental care arrangements. The last empirical study, described in **Chapter 7** investigated inhibitory control as a mediator of the link between multiple aspects of early non-parental care and toddlers' behaviour problems. This study also aimed to explore temperamental negative affectivity as a moderator of the link between non-parental care and behaviour problems. Finally, **Chapter 8** presents a summary of the results of the six studies followed by the main conclusion and general discussion.

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Chapter 2

Maternal Prenatal Anxiety and Stress Predict Infant Illnesses and Health Complaints

Roseriet Beijers, Jarno Jansen, Marianne Riksen-Walraven & Carolina de Weerth

Beijers, R., Jansen, J., Riksen-Walraven, J.M.A. & de Weerth, C. (2010). Maternal prenatal anxiety and stress predict infant illnesses and health complaints. *Pediatrics 126*(2): E401-e409.

Abstract

Objective: Evidence from both animals and humans suggests that maternal prenatal anxiety and stress can have adverse consequences on the offspring's development. Animal models also show that prenatal stress has programming effects on the physical health of the offspring, such as immune functioning. In human studies, however, physical health outcomes are often restricted to birth complications, while studies on the effects of acquiring illnesses are scarce. Therefore, this study aims to examine whether maternal prenatal anxiety and stress, measured both by self-report and by cortisol physiology, are related to more infant illnesses and antibiotic use during the first year of life.

Methods: Participants in the study were 174 mothers with normal pregnancies and term deliveries (71 firstborns; 91 boys). The mothers filled in third trimester questionnaires on general and pregnancy-specific anxiety and stress, and provided saliva samples for circadian cortisol. Information on infant illnesses and antibiotic use was obtained through monthly maternal interviews across the infant's first year of life.

Results: Hierarchical multiple regressions showed that, even after controlling for many relevant confounders, prenatal anxiety and stress predicted a considerable amount of variance in infant illnesses and antibiotic use: 9.3% for respiratory, 10.7% for general, 8.9% for skin, and 7.6% for antibiotic use. Digestive illnesses were not related to prenatal anxiety and stress.

Conclusions: Although replication is warranted, to our knowledge this is the first evidence linking maternal prenatal anxiety and stress to infant illnesses and antibiotic use early in life.

1. Introduction

More and more evidence is appearing in both the animal and human literature that maternal anxiety and stress during pregnancy can have adverse consequences on the development of the offspring (Fowden, Giussani & Forhead, 2006; Huizink, Mulder & Buitelaar, 2004; Van den Bergh, Mulder, Mennes & Glover, 2005). The fact that intrauterine experiences can profoundly affect the fetus and subsequently influence postnatal development, is referred to as *prenatal programming* (Van den Bergh et al., 2005; de Weerth &

Buitelaar, 2005). There is evidence from different animal models that prenatal stress also has programming effects on the physical health of the offspring, such as growth and immune functioning (Beydoun & Saftlas, 2008; Merlot, Couret & Otten, 2008). However, in human studies, physical health outcomes are often restricted to birth complications, such as preterm delivery and low birth weight. Only a few studies examined the effects of prenatal anxiety and stress on the risk of illnesses in the offspring (Beydoun & Saftlas, 2008). Stott and colleagues were first in showing that a wide range of prenatal stresses were associated with child morbidity (Stott, 1957; Stott, 1973; Stott & Latchford, 1976). More recent studies showed that prenatal anxiety was positively associated with asthma during childhood (Cookson, Granell, Joinson, Ben-Shlomo & Henderson, 2009), while stress-related maternal factors during pregnancy were associated with an increased risk of eczema during the first 2 years of life (Sausenthaler, Rzehak, Chen, Arck, Bockelbrink, Schafer et al., 2009). Stressful life events, lack of social support and lack of confidence during pregnancy, however, were not linked to celiac disease in the infant offspring (Ludvigsson & Ludvigsson, 2003).

To summarize, human research is scarce regarding the effects of prenatal anxiety and stress on offspring susceptibility for various illnesses. Moreover, none of these studies included physiological measures of stress. Therefore, the goal of the present study was to determine whether maternal anxiety and stress during late pregnancy are related to more infant illnesses and antibiotic use during the first year of life. Prenatal stress was measured by both psychological self-report measures and physiological basal cortisol measures. The reasons for including both measures are that reported prenatal stress has been found to be weakly associated with cortisol diurnal rhythm markers (Harville, Savitz, Dole, Herring & Thorp, 2009; O'Donnell, O'Connor & Glover, 2009), and that there is some evidence that prenatal cortisol predicts infant outcomes independently of prenatal mood (Davis, Glynn, Schetter, Hobel, Chicz-Demet & Sandman, 2007). Infant illnesses were measured with monthly maternal interviews, as maternal report is shown to be a reliable way of assessing the health status of an infant (McCormick, Brooks-Gunn, Shorter, Holems & Heagarty, 1989; Monette, Séguin, Gauvin & Nikiéma, 2007). Our

hypothesis was that pregnant mothers who report more anxiety and stress, and/or who show signs of abnormal cortisol circadian activity, will have infants with more illnesses and antibiotic use during the first year of life.

2. Methods

2.1 Participants

The participants were pregnant women recruited through midwife practices in the cities of Nijmegen, Arnhem and surrounding areas (Netherlands). The study was approved by the Faculty Ethical Committee and informed consent was obtained from each participant. Inclusion criteria were: an uncomplicated and singleton pregnancy, a clear understanding of the Dutch language, no drug use during pregnancy, no current physical health problems (e.g. diabetes and heart diseases) and mental health problems (e.g. major depression), a term delivery (≥37 weeks) and a normal 5-min infant Apgar score (≥7). Of the 220 women that enrolled in the study, 8 were excluded because of medical reasons, such as early birth and major birth complications. Of an additional 20 mothers no prenatal data was available, as they began the study after their infant's birth. Of the remaining 192 mothers, a further 18 discontinued the study during the first 3 postpartum months of life, due to lack of time or other personal circumstances. This resulted in a final sample of 174 mothers and their infants (participation rate = 90.6%). Demographic characteristics of the mothers and infants are provided in Table 1. No differences in these demographic characteristics were found between participating mothers and those that dropped out (*n*=18).

2.2 Procedure

In the last trimester of pregnancy, the mothers filled in questionnaires on general and pregnancy-related anxiety and stress, as also a questionnaire on demographic information. Additionally, they collected circadian samples of saliva. To control for maternal postnatal anxiety and stress, the general anxiety and stress questionnaires were again filled in postnatally at 3, 6 and 12 months.

During the first 12 months of life, information on the frequency of infants' illnesses and health complaints was obtained through monthly

Table 1: Descriptive statistics of the health variables, stress predictors and confounders.

	Mean *(SD)*	Range
Demographics characteristics		
Maternal age (years)	32.6 *(3.80)*	21.9 – 42.9
Maternal educational level (%)		
Primary education	3.6%	
Secondary education	20.5%	
College or university	75.9%	
Maternal marital status (wedlock or unmarried)	98.3%	
Pregnancy smoking (%)	4.0%	
Alcohol ingestion during pregnancy (%)	16.7%	
Birth weight (grams)	3630.32 *(464.32)*	2645.0 – 4730.0
Apgar scores	9.7 *(.60)*	7 – 10
Infant sex (%)		
Girl	47.7%	
Birth order (%)		
First	40.8%	
Second or more	58.2%	
Additional confounders		
Duration of breastfeeding in months	5.42 *(4.28)*	0 – 12
Attendance centre-based daycare (%)[1]	57.5%	
Postnatal state anxiety (STAI)	28.52 *(6.44)*	20.0 – 60.7
Postnatal daily hassles (APL)	1.13 *(0.37)*	0.0 – 2.3
Prenatal psychological anxiety and stress		
State anxiety (STAI)[2]	32.20 *(8.88)*	20.0 – 64.0
Daily hassles (APL)[3]	1.14 *(0.46)*	0.0 – 2.5
Fear of giving birth (PRAQ-R)[4]	5.36 *(2.48)*	3.0 – 15.0
Fear of bearing a handicapped child (PRAQ-R)[5]	8.53 *(2.80)*	4.0 – 18.0
Pregnancy-specific hassles (PES)[6]	0.33 *(0.23)*	0.0 – 1.4
Prenatal cortisol levels (nmol/l)		
Decline	6.74 *(4.47)*	-2.8 – 24.0
Evening	9.44 *(2.75)*	1.0 – 20.0
Infant health		
Respiratory[7]	26.66 *(8.93)*	0 – 53.5
Digestive[7]	4.86 *(3.76)*	0 – 19.0
General[7]	6.83 *(3.81)*	0 – 18.0
Skin[7]	5.92 *(4.07)*	0 – 23.5
Prescribed antibiotic use[7]	.67 *(1.05)*	0 – 6.0

(SD) = standard deviation. [1]Mean (*SD*) age in months at entering daycare = 3.34 (*1.76*), [2] Similar to De Bruijn et al.[18] at 36 weeks gestational age; mean (*SD*) = 31.65 (*9.36*), [3]Compared to Vingerhoets et al.[19] with a group of non-pregnant women; Mean (*SD*) = 1.38 (*0.58*), [4]Similar to Huizink et al.[20] at 37-38 weeks gestational age (Mean (*SD*) = 6.0 (*2.7*), [5] Similar to Huizink et al.[20] at 37-38 weeks gestational age (Mean (*SD*) = 8.5 (*3.2*), [6]Slightly lower than DiPietro et al.[21] at 36 weeks gestational age; mean (*SD*) = 0.72 (0.17). [7]Infant health variables are mean numbers of illness occurrences, summed up over the 12 months.

maternal interviews. These interviews also provided information about breastfeeding and attending centre-based daycare. Finally, information about the delivery and the infant was obtained with a questionnaire filled in immediately after birth.

2.3 Infant health

Mothers reported on their infant's illnesses and health complaints in semi-structured interviews conducted at monthly intervals (3 in person, 9 by phone). With the aim of aiding their memory and of increasing the objectivity of the scoring, the interview also contained a checklist consisting of 24 yes-or-no items listing common infant illnesses and health complaints. Additionally, mothers were asked if their child had received prescribed antibiotics. Subsequently, the health data were coded with the International Classification of Primary Care (ICPC, Lamberts & Wood, 1987). The ICPC is an ordering principle labeling illnesses and health complaints in classes according to established criteria, and is being widely used both by clinicians and scientists (Soler, Okkes, Wood & Lamberts, 2008). Because of low incidence of ear- and eye-related illnesses and complaints, these were added to general illnesses and complaints. Finally, the occurrence of illnesses and complaints were summed up over the 12 months. The following variables were used as dependent variables: respiratory, digestive, general, and skin illnesses and complaints, and antibiotic use. Table 2 shows all illnesses and health complaints reported in our sample, divided per class.

2.4 Prenatal psychological stress

At 37 weeks of pregnancy (*M* = 37 weeks and 1.9 days, *SD* = 7.1 days), mothers reported on their general and their pregnancy-specific anxiety and stress. Daily hassles were used as measure of stress.

2.4.1 General anxiety and stress

Anxiety was assessed by means of the State-Trait Anxiety Inventory (STAI, Spielberger, 1983; Van der Ploeg, Defares & Spielberger, 1981). We used the

state anxiety subscale, which measures anxiety at the moment of scoring. This subscale consists of 20 items, scored on 4-point scales.

Daily hassles were assessed by means of a 49-item Dutch questionnaire, which measures the rate of occurrence and intensity of daily hassles (APL, Vingerhoets, Jeninga & Menges, 1989). Respondents had to check whether a situation had occurred and rated how much each situation bothered them on a 4-point scale. Scoring was based on the mean intensity rating: the sum of how much daily hassles bothered the respondent divided by the frequency of daily hassles. Higher values indicate more experienced negativity as a result of daily hassles.

Table 2: Illnesses and health complaints reported in our sample, divided per class

Respiratory	Digestive	General	Skin
R02 Shortness of breath/ dyspnoea	D01 Abdominal pain/ cramps general	A03 Fever	S01 Pain/tenderness of skin
R04 Breathing problem, other	D03 Heartburn	A12 Allergy	S02 Pruritus
R05 Cough	D10 Vomiting	A72 Chickenpox	S06 Rash localized
R07 Sneezing/nasal congestion	D11 Diarrhoea	A76 Viral exanthem other	S09 Infected finger/toe
R25 Sputum/pleghm abnormal	D12 Constipation	A77 Viral disease other/ NOS	S10 Boil/carbuncle
R71 Whooping cough	D13 Jaundice	A78 Infectious disease other/NOS	S22 Nail symptom/complaint
R72 Strep throat	D16 Rectal bleeding	A94 Perinatal morbidity other	S29 Skin symptom/complaint others
R74 Upper respiratory infection acute	D20 Mouth/tongue/lip symptom/complt.	F03 Eye discharge	S74 Dermatophytosis
R77 Laryngitis/tracheitis acute	D22 Wurms/other parasites	F29 Eye symptom/complaint other	S84 Impetigo
R78 Acute bronchitis/ bronchiolitis	D73 Gastroenteritis presumed infection	F73 Eye infection/inflammation other	S85 Pilonidal cyst/fistula
R80 Influenza	D99 Disease digestive system, other	F80 Blocked lacrimal duct of infant	S86 Dermatitis seborrhoeic
R81 Pneumonia		H01 Ear pain/earache	S87 Dermatitis/atopic eczema
R93 Pleura fluid, other		H04 Ear discharge	S88 Dermatitis contact/ allergic
R96 Asthma		H29 Ear symptom/complaint other	S89 Diaper rash
R97 Allergic rhinitis		H71 Otitis media acute/ myringitis	

2.4.2 Pregnancy-specific anxiety and stress

Pregnancy-specific anxiety was assessed by means of the Pregnancy-specific Anxieties Questionnaire – Revised (PRAQ-R, Huizink, Mulder, Robles de Medina, Visser & Buitelaar, 2004). Similar to previous research in the field of prenatal anxiety and stress (Buitelaar, Huizink, Mulder, Robles de Medina & Visser, 2003; Huizink, Robles de Medina, Mulder, Visser & Buitelaar, 2003) we used two subscales, namely fear of giving birth (3 items) and fear of bearing a handicapped child (4 items). Items were scored on 5-point scales.

Pregnancy-specific daily hassles were measured by means of the Pregnancy Experience Scale (PES) which measures maternal appraisal of 43 pregnancy-specific experiences (DiPietro, Ghera, Costigan & Hawkins, 2004). Mothers were asked to rate the degree to which each experience constituted both a hassle and an uplift on 5-point scales. Scoring was based on the ratio of hassles to uplifts (sum of intensities of hassles divided by sum of intensities of uplifts), where higher values indicate greater negative emotional valence towards pregnancy (DiPietro et al., 2004).

2.5 Prenatal cortisol

At 37 weeks of pregnancy (*M* = 37 weeks and 0.8 days, *SD* = 9.4 days), the mothers provided salivary samples in small 25 ml containers with screw caps, in order to determine cortisol levels. Sampling took place on two consecutive days at awakening, 30 minutes after waking, 1200h, 1600h and 2100h. The subjects were instructed to refrain from eating or brushing their teeth 30 minutes prior to each sample, and to fill in a form indicating exact sampling and eating times. Subsequently the samples were kept at –25°C until further analysis (Laboratory of Endocrinology, University Medical Center Utrecht). Cortisol in saliva was measured using an in house competitive radio-immunoassay (RIA) employing a polyclonal anticortisol-antibody (K7348). [1,2-^{3}H(N)]-Hydrocortisone (Amersham TRK407) was used as a tracer. The lower limit of detection was 1.0 nmol/l and inter-assay and intra-assay variation were below 10%. Because of strong inter-correlations (*r*'s ranging from .53 to .68, all *p*-values <0.01), mean cortisol levels over the two days were calculated for each sampling time. We used evening cortisol and cortisol decline over the

day (waking minus evening) as independent variables, as these variables were not intercorrelated, but were both highly correlated to other cortisol measures (i.e. area under the curve to the ground (Pruessner, Kirschbaum, Meinlschmid & Hellhammer, 2003), morning cortisol, and cortisol awakening response). Moreover, higher evening cortisol levels and flattened diurnal cortisol rhythms have been related to diverse psychopathologies (Goodyer, Park, Netherton & Herbert, 2001).

2.6 Confounders

The following potential confounders were included: maternal educational level, pregnancy smoking, alcohol ingestion during pregnancy, birth weight, 5-min Apgar score, sex, number of siblings, duration of breastfeeding in months, and attending centre-based daycare. To control for maternal postnatal anxiety and stress, state anxiety and daily hassles were assessed postnatally at 3, 6, and 12 months. Because of strong inter-correlations (*r*'s ranging from .49 -.68, and from .51-.61 respectively, all *p*-values <0.01), mean anxiety and daily hassles scores were calculated.

2.7 Statistical analyses

2.7.1 Missing data

The 12 months of infant health data were aggregated per trimester. If one month was missing, the trimester score was calculated by averaging the remaining months. If two months were missing, the trimester score was considered missing (*N* = 4). Additionally, if more than one trimester was missing, the infant was excluded from the study (*N* = 5). Of the 169 infants, 115 infants had no missing months, 44 infants missed 1 month, 7 infants missed 2 months and 3 infants missed 3 months.

From the 174 mothers, 15 mothers were unable to provide pregnancy saliva samples. For the available saliva samples, we used the following time ranges for accepting samples: waking (6:00-10:00h, within 15min after waking), 30 minutes after waking (25-40min), 1200h (11.30-13.30h), 1600h (15.30-17.30h) and 2100h (20.00-23.00h). As a consequence, the following samples

were considered missing: 4 for waking, 12 for 30 minutes after waking, 4 for 1200h, 4 for 1600h and 8 for 2100h.

2.7.2 Statistical analyses

Square root or logarithm transformations were applied to skewed data. To test whether prenatal anxiety and stress uniquely predicted infants' illnesses and antibiotic use, two standard multiple hierarchical regression models (Tabachnik & Fidell, 2007) were computed for every health variable. The first model contained all confounders and prenatal predictors. To eliminate irrelevant confounders and predictors, and to increase power, the second model only contained the variables which individually explained at least 1 percent of the variance in the first model. The explained variance was calculated as (part correlation)^2*100. These final models, with all maintained confounders in hierarchical step 1 and all maintained prenatal predictors in hierarchical step 2, are presented in the results and in Table 5.

3. Results

Descriptive statistics are presented in Table 1 (untransformed data).
Because of skewed data, infant digestive, general, and skin illnesses, as also prenatal anxiety and pregnancy-specific daily hassles were transformed with square root. Table 3 presents the correlations for the prenatal predictors.

Table 3: Pearson correlations for cortisol and psychological stress variables

	Cortisol decline	Evening cortisol	STAI	APL	PRAQ-R $_{birth}$	PRAQ-R $_{handicapped}$	PES
Cortisol decline	-						
Evening cortisol	-.13	-					
STAI	.00	-.01	-				
APL	-.02	-.05	.31**	-			
PRAQ-R $_{birth}$	-.04	.07	.27**	.04	-		
PRAQ-R $_{handicapped}$	-.10	-.05	.21**	.11	.35**	-	
PES	.16	-.19*	.41**	.20**	.25**	.27**	-

Note: STAI = state anxiety, APL = daily hassles, PRAQ-R $_{birth}$ = fear of giving birth , PRAQ-R $_{handicapped,}$ = fear of bearing a handicapped child, PES = pregnancy-specific daily hassles. * = $p < .05$, ** = $p < .01$

Table 4: Pearson correlations for infant health variables, stress predictors and confounders

	Stress predictors							Infant health variables				
	Decline	Evening	STAI	APL	PES	PRAQ-R$_{birth}$	PRAQ-R$_{handicapped}$	Resp.	Digest.	Gen.	Skin	Antibio.
Confounders												
Education	-.01	-.05	.04	-.05	.08	.10	.01	-.06	-.03	.11	-.04	-.04
Prenatal smoking	-.03	-.22**	.16*	.18*	.06	-.03	.15*	.15	.06	.09	-.01	-.02
Prenatal alcohol	.11	.02	-.04	-.04	.01	-.05	-.07	-.07	.10	-.01	-.00	-.01
Birth weight	.01	-.02	-.05	.03	-.01	-.10	-.02	-.07	.01	-.02	.03	-.03
5-min Apgar	.03	-.12	-.11	.09	-.04	-.24**	-.08	.08	.11	.05	-.01	.01
Sex	.07	-.07	-.03	.19*	.10	.01	.06	-.02	.06	-.01	-.08	-.03
Siblings	-.00	-.17*	.03	.05	.04	-.25**	-.21**	.09	.02	.22**	-.08	.15*
Breastfeeding	.06	.11	.13	-.05	.03	-.07	-.10	-.12	-.05	.08	.19*	.07
Attending daycare	.04	.03	.05	.02	.04	.01	-.09	.37**	.18*	.31**	.07	.14
Post anxiety	-.00	.04	.51**	.22**	.37**	.37**	.18*	.12	.10	.19*	.10	-.07
Post daily hassles	.02	-.10	.27**	.56**	.42**	.16*	.17*	.10	.18*	.24**	.15*	.03

Note: Resp. = Respiratory, Digest. = Digestive, Gen. = General, Antibio. = Antibiotics, STAI = state anxiety, APL = daily hassles, PRAQ-R$_{birth}$ = fear of giving birth, PRAQ-R$_{handicapped,}$ = fear of bearing a handicapped child, PES = pregnancy-specific daily hassles.

* = $p < .05$, ** = $p < .01$

The psychological anxiety and stress predictors were not or moderately intercorrelated (*r*'s ranging from .04 to .41). Furthermore, the cortisol and psychological variables were uncorrelated, except for pregnancy-specific hassles and evening cortisol ($r = -.19$, $p<.05$). The correlations between the confounders and the prenatal predictors and health variables are presented in Table 4. Most confounders were not or mildly related to the anxiety and stress predictors, as well as to the health variables.

The final regression models are summarized in Table 5. The results showed that more infant *respiratory* illnesses are predicted by a smaller cortisol decline, which is associated with a flattened diurnal cortisol rhythm. Furthermore, pregnancy-specific hassles tended to predict more infant respiratory illnesses. With respect to *digestive* illnesses, no relations were found. More *general* illnesses were predicted by more pregnancy-specific daily hassles. In addition, higher levels of evening cortisol tended to predict more general illnesses. For *skin* illnesses the analyses showed two effects. Higher levels of evening cortisol were related to more *skin* illnesses, while more daily hassles predicted fewer infant skin illnesses. Finally, higher fear of giving birth was related to more *antibiotic use.* In sum, prenatal anxiety and stress explained a considerable amount of variance in infant illnesses and antibiotic use: 9.3% for respiratory, 10.7% for general, 8.9% for skin, and 7.6% for antibiotic use.

4. Discussion

Our study showed that maternal prenatal anxiety and stress were related to more infant respiratory, general and skin illnesses and antibiotic use in the first year of life. Prenatal stress and anxiety were not related to infant digestive illnesses. Our results are in line with earlier studies showing that prenatal predictors are associated with an increased risk of asthma during childhood (Stott & Latchford, 1976), but not with celiac disease in the infant offspring (Sausenthaler et al., 2009). One specific result, however, did not point in the same direction. While higher evening cortisol was indeed related to more skin illnesses, more daily hassles were related to fewer skin illnesses. Although prenatal stress has been proposed to have implications for the development

Table 5: Final multiple hierarchical regression models of prenatal anxiety and stress on infant illnesses and antibiotic use

		B	β	R^2_{model}	F_{change}	R^2_{change}
Respiratory illnesses and complaints						
Step 1	Duration of breastfeeding in months	-.355	-.175*	.139		
	Attendance daycare	6.127	.349***			
Step 2	State anxiety during pregnancy	1.465	.132	.232	5.614	.093***
	Pregnancy-specific hassles	6.830	.151†			
	Cortisol decline	-.419	-.217**			
Excluded variables:	*Maternal educational level, pregnancy smoking, alcohol ingestion during pregnancy, birth weight, 5-min Apgar score, sex, number of siblings, postnatal anxiety, postnatal daily hassles, daily hassles, fear of giving birth, fear of bearing a handicapped child, and evening cortisol.*					
Digestive illnesses and complaints						
Step 1	5-min. Apgar score	.225	.154*	.099		
	Duration of breastfeeding in months	-.027	-.122			
	Daily hassles postpartum	.368	.142†			
	Attendance daycare	.322	.169*			
Step 2	Pregnancy-specific hassles	.608	.124	.112	2.306	.013
Excluded variables:	*Maternal educational level, pregnancy smoking, alcohol ingestion during pregnancy, birth weight, sex, number of siblings, postnatal anxiety, state anxiety, daily hassles, fear of giving birth, fear of bearing a handicapped child, cortisol decline, and evening cortisol.*					
General illnesses and complaints						
Step 1	Number of siblings	.267	.245***	.149		
	Attendance daycare	.479	.305***			
Step 2	Pregnancy-specific hassles	1.309	.323***	.256	10.473	.107***
	Evening cortisol	.040	.140†			
Excluded variables:	*Maternal educational level, pregnancy smoking, alcohol ingestion during pregnancy, birth weight, 5-min Apgar score, sex, duration of breastfeeding in months, postnatal anxiety, postnatal daily hassles, state anxiety, daily hassles, fear of giving birth, fear of bearing a handicapped child, and cortisol decline.*					
Skin illnesses and complaints						
Step 1	Duration of breastfeeding in months	.036	.169*	.068		
	Daily hassles postpartum	.756	.303***			
Step 2	Daily hassles during pregnancy	-.452	-.228*	.157	7.684	.089***
	Evening cortisol	.079	.235**			
Excluded variables:	*Maternal educational level, pregnancy smoking, alcohol ingestion during pregnancy, birth weight, 5-min Apgar score, sex, number of siblings, attendance daycare, postnatal anxiety, state anxiety, fear of giving birth, fear of bearing a handicapped child, pregnancy-specific hassles, and cortisol decline.*					
Antibiotic use						
Step 1	Number of siblings	.336	.229**	.049		
	State anxiety postpartum	-1.818	-.156†			
	Attendance daycare	.308	.146†			
Step 2	Fear of giving birth	.091	.218*	.125	3.985	.076**
	Cortisol decline	-.030	-.128			
	Evening cortisol	.046	.120			
Excluded variables:	*Maternal educational level, pregnancy smoking, alcohol ingestion during pregnancy, birth weight, 5-min Apgar score, sex, duration of breastfeeding in months, postnatal stress, state anxiety, daily hassles, fear of bearing a handicapped child, and pregnancy-specific hassles.*					

B = regression coefficient, β = standardized regression coefficient, R^2_{model} = total explained variance by the model, F_{change} = F statistic corresponding R^2_{change}, R^2_{change} = partial explained variance by added predictors (Step 2). †= $p < .10$, * = $p < .05$, ** = $p < .01$, *** = $p < .001$

Note: excluded variables explained individually less than 1% variance in infant illnesses and health complaints and antibiotic use in preliminary regression analyses.

of atopic diseases such as eczema (von Hertzen, 2002; Knackstedt, Hamelman & Arck, 2005), only one study actually found a link between stress-related prenatal factors and an increased risk of eczema early in life (Cookson et al., 2009). Taken together, our results indicate that the effects of prenatal anxiety and stress are different depending on the specific health outcome one is looking at. This is consistent with animal literature where the effects of prenatal stress also seem to depend on the specific immune markers measured (Merlot et al., 2008). However, the picture is far from complete, and more research is needed to replicate these findings and to investigate the possible mechanisms involved.

Furthermore, the effects of prenatal anxiety and stress also seem to depend on the nature of the stressor (Merlot et al., 2008). Our results indicate that pregnancy-specific predictors apparently have more detrimental effects on infant health outcomes than general predictors. This is consistent with findings of others (DiPietro et al., 2002; DiPietro et al., 2004; Huizink et al., 2004; Lobel, Cannella, Graham, DeVincent, Schneider & Meyer, 2008). Pregnancy-specific anxiety and stress evolve from a variety of pregnancy-specific issues, including physical symptoms and anxiety about delivery (Huizink et al., 2004). Therefore, these measures may be a more valid means of assessing what women are experiencing during pregnancy, and may have more proximal impact on the outcome of pregnancy (Lobel et al., 2008).

Moreover, higher evening cortisol levels and flattened diurnal cortisol rhythms during late pregnancy were both related to more infant illnesses. Previous studies linked these markers to psychopathology (Goodyer et al., 2001), and this study shows the merit of these diurnal cortisol rhythm markers as well. However, these markers were mostly unrelated to the maternal psychological functioning during late pregnancy. This is in line with recent research, which has either failed to identify a link or observed only small correlations (Harville et al., 2009; Kivlighan, DiPietro, Costigan & Laudenslager, 2008; O'Donnell et al., 2009). One factor that may confound the links between maternal emotional state and cortisol is a dampening of the hypothalamic-pituitary-adrenal (HPA) axis responsiveness during pregnancy (de Weerth & Buitelaar, 2005). As a consequence, the HPA-axis may have a

reduced capacity to respond to psychosocial stress (O'Donnell et al., 2009). Nevertheless, in the present study both psychological predictors and cortisol measures independently predicted infant illnesses, which indicates that measuring prenatal stress on the psychological and physiological level can be complementary and advantageous.

How can the relations between infant illnesses and prenatal anxiety and stress be explained? First, increased/abnormal maternal cortisol activity may lead to increased cortisol in the fetus (Huizink et al., 2004; Wadhwa, 2005). Cortisol plays a key role in the immune system, where it regulates the magnitude and duration of the inflammatory responses and the maturation of lymphocytes (Sapolsky, Romero & Munck, 2000). Exposure to abnormal cortisol levels in utero may program immune function of the fetus (Sapolsky et al., 2000) resulting in an increased infant susceptibility for illnesses. Second, animal models show that prenatal stress can affect placental function, including regulation of the placental barrier enzyme 11β-hydroxy steroid dehydrogenase Type II (11β-HSD2) which converts cortisol to the inactive cortisone (Glover, Bergman, Sarkar & O'Connor, 2009). Recent results in humans also suggest that maternal anxiety may increase the permeability of the placenta to cortisol by causing a downregulation of placental 11β-HSD2 activity, which may allow more cortisol to cross from the maternal to fetal blood (Entringer, Kumsta, Nelson, Hellhammer, Wadhwa & Wüst, 2008). In turn, exposure to abnormal cortisol levels in utero may program immune functioning of the fetus (Benediktsson, Calder, Edwards & Seckl, 1997). Third, an additional mechanism by which both psychological and physiological stress could have effects on infant illnesses, is by affecting how maternal immune factors are passed on to the fetus. In animal models, there are indications that maternal prenatal stress has an impact on the transplacental transfer of passive immunity to the offspring, but only if the stress period covers the end of gestation (Merlot et al., 2008). If this were also the case in humans, late gestation disruptions in the transfer of passive immunity to the fetus could be a mechanism by which prenatal stress are related to more illnesses in the infant.

A common element of these above-mentioned mechanisms is that prenatal anxiety and stress affect the infant's immune system. This hypothesis

is supported by extended animal research on immunity (Merlot et al., 2008), and a few human studies showing heightened levels of specific immune parameters in cord blood (Lin, Wen, Lee & Guo, 2004; Mattes, McCarthy, Gong, van Eekelen, Dunstan, Foster, et al., 2009; Sternthal, Enlow, Cohen, Canner, Staudenmayer, Tsang, et al., 2009; Wright, 2007), and changes in cytokine production in adult offspring (Benediktsson et al., 1997) as a result of prenatal maternal mood. However, there is no direct evidence that the infants in this study were indeed immune-compromised. It is also possible that the effects were mediated by other factors. For instance, Baker et al. showed that restraint stress during mid-pregnancy was related to reduced postpartum maternal care in mother rats (Baker, Chebli, Rees, LeMarec, Godbout & Bielajew, 2008). In the human literature, there are also indications that stress in postpartum women is negatively related to maternal care and health-promotion behaviors (Fowles & Walker, 2009). Although our study controlled for postnatal anxiety and stress, prenatal anxiety and stress could still be related to less maternal care and health-promotion behaviors, subsequently leading to infant health problems. Finally, genetic vulnerability is another possible explanation for the association between infant health and maternal prenatal anxiety and stress (Harding, 2007). Underlying genetic factors could be responsible for more illnesses and health complaints in both the mother and the infant, and subsequently result in heightened maternal psychological stress and abnormal cortisol activity.

Our study has several strengths, including the use of both psychological and physiological measures of stress, the control for many relevant confounders, and the focus on, and robust measuring of infant illnesses. However, some limitations should also be noted. The fact that almost all mothers were highly educated and lived together with their partner, compromises the generalizability of the study. In addition, all the women had healthy pregnancies and reported relatively mild or moderate prenatal stress (albeit comparable to other studies; see Table 1). More severe stress during pregnancy might show stronger or different associations with infant illnesses. Furthermore, our study examined prenatal anxiety and stress during late gestation. In animals, the effects of prenatal stress on immune parameters have been shown to be different depending on the gestational timing of the

stress (Coe, Lubach & Karaszewski, 1999). The question of timing thus remains to be investigated in humans. And lastly, the infant health data were based on maternal report. Although mothers generally report their child's health status accurately (McCormick et al., 1989; Monette et al., 2007), stressed mothers might be more likely to over- or misreport their infant's health, reflecting a reporter bias (O'Connor, Heron, Golding, Beveridge & Glover, 2002). However, two important precautions were taken in this study to reduce a possible reporter bias to the minimum: the data were obtained by using detailed semi-structered interviews conducted at short intervals, and adjustments for postnatal measures of maternal anxiety and daily hassles were made. Other sources of infant health data, such as medical records, were considered less adequate for the present study. First, medical records are not always more objective, in the sense that child health care use also depends on maternal factors, such as attitudes toward health care (Janicke & Finney, 2000). And second and most important, maternal report not only provides us information about common infant illnesses, but also about more subtle complaints, like coughing or a runny nose. This information is less likely to be reported to a family doctor and therefore cannot be obtained from medical records.

5. Conclusion

This study is one of the first linking maternal prenatal anxiety and stress to infant illnesses and antibiotic use early in life. As such it provides a starting point for future research in larger and clinical samples. Follow-up studies are necessary to determine whether the effects of prenatal anxiety and stress on infant susceptibility to illnesses are transient, persistent or even progressive.

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Chapter 3

Solitary Sleeping in Young Infants is Associated with Heightened Cortisol Reactivity to a Bathing Session but not to a Vaccination

Marieke Tollenaar, Roseriet Beijers, Jarno Jansen, Marianne Riksen-Walraven & Carolina de Weerth

Tollenaar, M.S., Beijers, R., Jansen, J., Riksen-Walraven, J.M.A. & de Weerth, C. (2012). Solitary sleeping in young infants is associated with heightened cortisol reactivity to a bathing session but not to a vaccination. *Psychoneuroendocrinology 37*(2): 167-177.

Abstract

Background: In this prospective longitudinal study, we investigated the relation between sleeping arrangements and infant cortisol reactivity to stressors in the first two post-natal months. Co-sleeping, as compared to solitary sleeping, is hypothesized to provide more parental external stress regulation by night, thus reducing general stress sensitivity. We therefore expected lower cortisol reactivity to stress in infants who co-slept more regularly.

Methods: Participants were 163 mothers and infants from uncomplicated, singleton pregnancies. Mothers completed daily diaries on sleeping arrangements in the first 7 weeks of life. Co-sleeping was defined as sleeping in the parents' bedroom (i.e. own or parents' bed). Cortisol reactivity was measured twice: to a mild physical stressor (bathing session) at 5 weeks of age and to a mild pain stressor (vaccination) at 2 months of age.

Results: Infants with a solitary sleeping arrangement in their first month of life showed a heightened cortisol response to the bathing session at 5 weeks compared to infants that co-slept regularly. This effect was not explained by breastfeeding practices, maternal caregiving behavior, or infants' night waking and sleep duration. No effects were found of co-sleeping on the cortisol response to the vaccination at 2 months.

Conclusions: The results suggest that solitary sleeping in the first month of life is associated with heightened sensitivity of the HPA-axis to a mild stressor, possibly due to less nocturnal parental availability as external stress regulator. Whether this effect continues in later life, remains to be investigated.

1. Introduction

In the present study we focus on the relation between parent-infant nocturnal sleeping arrangements in early infancy and infant cortisol reactivity to acute stressors. In Western countries infants' sleeping arrangements during the first months of life show large inter-individual variation: while some infants sleep in their own room from the beginning, others sleep in a crib in the parents' room, and yet others sleep in bed with the parents. These last two options are commonly referred to as 'co-sleeping' (Goldberg & Keller, 2007a; McKenna, Ball & Gettler, 2007).

An area that has been largely unexplored is that of the relation between early sleeping arrangements and infant stress reactivity. In response to stressors the human body reacts with the release of glucocorticoids, mainly cortisol, by the hypothalamic pituitary adrenal (HPA-) axis. The HPA-axis starts to develop prenatally, but fully matures after birth (Lupien, McEwen, Gunnar & Heim, 2009). That is, in the first year of life, basal cortisol levels slowly decrease (Tollenaar, Jansen, Beijers, Riksen-Walraven & de Weerth, 2010), and a circadian rhythm starts to develop within a few months after birth (de Weerth, Zijl & Buitelaar, 2003). Cortisol reactivity to stressors is found early after birth, but seems to diminish after about 6 months (Gunnar, Talge & Herrera, 2009b; Jansen, Beijers, Riksen-Walraven & de Weerth, 2010a). The HPA-axis is found to be shaped by early environmental factors like parental care, parental separations, or early life stress (e.g. neglect or abuse; Elzinga, Roelofs, Tollenaar, Bakvis, van Pelt & Spinhoven, 2008; Gunnar & Donzella, 2002; Gunnar, Frenn & Wewerka & Van Ryzin, 2009a). Sleeping arrangements may constitute an early environmental factor that can influence the HPA-axis, as it relates to the proximity of parents at night. Given that dysregulation of the HPA-axis can be a risk factor for the development of (psycho)pathology (Gunnar & Vazquez, 2001; Heim, Ehlert & Hellhammer, 2000; Lupien et al., 2009), it is important to investigate whether and how early sleeping arrangements are associated to infant HPA-axis functioning.

In the first postnatal months an infant's self-regulation capacities are developing quickly but are as yet limited. Therefore, parents have an important role as external regulators of distress levels of the child, e.g. by sensitively responding to the infant's signals and needs (Albers, Riksen-Walraven, Sweep & de Weerth, 2008; Haley & Stansbury, 2003; Hofer, 2006). Co-sleeping implies more physical closeness to the parents during the night compared to solitary sleeping, making parents more physically available, and more quickly available, to help the infant regulate distress. The nocturnal parental separation for solitary sleepers probably means that infants' subtle signals of discomfort are less, or more slowly, responded to by parental vocalizations and/or touch than for co-sleeping infants. Solitary sleeping may thus be related to more frequent experiences of higher levels of distress (and presumably higher cortisol levels)

during the night, as the infant will probably have to reach higher levels of negative vocalizations in order to alert the parents. This may sensitize the HPA-axis, leading to heightened cortisol reactivity to stressors during the day as well (Heim & Nemeroff, 2001). Support for this hypothesis comes from animal research showing that early maternal separations are a large contributor to the development of the HPA-axis. Daily dam-rat separations of 3hr or more have been associated with a hyper-responsive HPA-axis (including higher corticosterone levels after stress: Aisa, Tordera, Lasheras, Del Rio & Ramirez, 2007; 2008; Meaney, Diorio, Francis, Widdowson, LaPlante, Caldji, et al., 1996), while increased maternal caregiving behaviors are related to lowered corticosterone responses to stress (Liu, Diorio, Tannenbaum, Caldjij, Francis, Freedman, et al., 1997). The current paper will thus investigate whether solitary sleeping is related to higher cortisol reactivity to stress compared with co-sleeping.

Sleeping arrangements may, however, be associated with certain parental and infant factors that can influence infant cortisol reactivity to stress as well. Hence, these factors may provide alternative explanations for a relation between co-sleeping and cortisol reactivity. Several factors may be of special interest for the current paper and will shortly be discussed. Breastfeeding, for example, is a major reason to co-sleep (Ball, 2003; McKenna, Mosko & Richard, 1997), and may by itself also influence cortisol levels (Cao, Rao, Phillips, Umback, Bernbaum, Archer, et al., 2009; Waynforth, 2007). The quality of maternal caregiving behavior is another factor that may be associated with both the choice to co-sleep (Taylor, Donovan & Leavitt, 2008), and the mother's abilities to help the infant regulate stress. For example, Albers et al. (2008), and Haley and Stansbury (2003) found a relation between mothers' sensitivity during caregiving and cortisol regulation in their 3-month-old infants (note however that Jansen, Beijers, Riksen-Walraven and de Weerth (2010b) found no such relations at 5 weeks after birth). Another factor related to co-sleeping may be infant sleep characteristics in the form of night waking and sleep duration (Cortesi, Giannotti, Sebastiani & Vagnoni, 2004; Gaylor & Anders, 2004; Hunsley & Thoman, 2002; Mao, Burnham, Goodlin-Jones). These sleep characteristics may also influence cortisol reactivity or levels during the day

(Lucas-Thompson, Goldberg, Germo, Keller, Davis & Sandman, 2009; Scher, Hall, Zaidman-Zait & Weinberg, 2010) and were therefore included in the study. In sum, in the current study we controlled for the effects of breastfeeding, maternal caregiving behavior, and infant sleep characteristics, by testing whether they were related to co-sleeping and if so, whether co-sleeping explained any additional variance in cortisol reactivity after adjusting for these factors. We also controlled for several other infant and maternal factors (e.g. maternal age and education, number of siblings, infant birth weight).

To our knowledge, only two human studies investigated the relation between parent-infant sleeping arrangements and HPA-axis functioning. One study looked at basal cortisol levels (Waynforth, 2007), and the other at cortisol reactivity (Lucas-Thompson et al., 2009). Waynforth found that fewer years of co-sleeping was related to higher basal cortisol levels in British 3- to 8-year-old children, which is in line with the hypothesis that solitary sleeping may lead to heightened HPA-axis activity. However, Waynforth's study sample constituted a fairly heterogeneous age group with retrospectively collected co-sleeping data, and no cortisol reactivity data. Lucas-Thompson et al. (2009) did collect cortisol reactivity data to vaccinations at 6 and 12 months of age. They found that current co-sleeping, as reported in a maternal questionnaire over the previous month, was associated with *increased* cortisol reactivity. Summarizing, the very few studies on the relation between co-sleeping and cortisol reactivity yielded conflicting results, did not use very detailed measures of co-sleeping (i.e. retrospectively or with a questionnaire), and did not focus on cortisol reactivity in the first months after birth.

The present study is the first to investigate the relation between parent-infant sleeping arrangements and cortisol reactivity during the first two post-natal months, a period in which availability of the parents as external stress regulators may play an important role in the development of the HPA-axis. Moreover, the study used co-sleeping data that were based on 7 weeks of daily diary data on sleeping arrangements, and assessed cortisol reactivity to two stressors. These two stressors were a home bathing session at 5 weeks of age (i.e. a mild physical stressor) and the routine Well Baby clinic vaccinations at 8 weeks of age (i.e. a mild pain stressor). Both stressors are

known to elicit reliable cortisol responses in the first months of life (Albers et al., 2008; Gunnar et al., 2009b; Jansen et al., 2010a).

In sum, in this paper we examined nightly co-sleeping in the first months after birth in relation to infants' cortisol reactivity to stress. Co-sleeping, as compared to solitary sleeping, is hypothesized to provide more parental external stress regulation by night, thus reducing the infant's general stress sensitivity. We therefore expected lower cortisol reactivity to stress in infants who co-slept more regularly.

2. Methods

2.1 Participants

This study is part of an ongoing prospective longitudinal project on the role of early caregiving factors in infant development. Participants were healthy women living in the Netherlands, who were recruited during pregnancy through midwife practices. The study was approved by the university ethical committee for behavioral sciences and written informed consent was obtained from each participant at enrollment.

Inclusion criteria were: uncomplicated, singleton pregnancy, clear understanding of the Dutch language, no drug use, and no current physical or mental health problems. Of the 220 women that originally enrolled, 20 were excluded because of medical reasons such as preterm birth, major birth complications and drug-use during pregnancy. Of the remaining 200 mothers, information on parent-infant sleeping arrangements during the first 2 months of life was collected by 173 mothers. Main reasons for not participating were lack of time, lack of interest, or other private circumstances. For 163 of these 173 mothers, at least one valid cortisol sample was collected during the bathing session (N = 137) or the vaccination (N = 142). Missing cortisol data were due to time and scheduling problems, technical problems (e.g. not enough saliva, or sample timing problems), or outliers (see statistical analyses). These 163 mothers and their infants constituted the study population for the present study. All infants (90 boys, 73 girls) included in the project were healthy, born at full term (≥ 37 weeks) and had a 5 min APGAR score ≥ 7. Demographic characteristics and study variables of the mothers and infants are

provided in Table 1. The mothers in the study population were slightly older than the other 37 women from the overall group of 200 mothers (32.7 yrs and 31.3 yrs, respectively, F (1, 198) = 4.58, p <.04). They did not differ on any other infant or maternal factors (all p's > .05).

Table 1: Overview of demographic characteristics of the mothers and infants (N = 163).

	Mean (*SD*)	Range
Birth weight (gram)	3599 (468)	2645 - 4730
Number of siblings	0.75 (0.7)	0 - 2
Number of breast feedings per day in the first month	5.7 (3.0)	0 - 12
Number of breast feedings per day in the second month	4.6 (3.1)	0 - 12
Maternal age at birth (years)	32.7 (3.7)	21.1 - 42.9
Percentage highly educated mothers (College or University)	78 %	
Percentage that reported smoking during pregnancy	3.1 %	
Percentage that reported alcohol use during pregnancy	14.1 %	
Maternal caregiving behavior (sensitivity and cooperation) at 5 weeks	5.3 (2.0)	1 - 9
Time of day of bathing session	1220 h (0200 h)	0920 h – 1700 h
Time of day of vaccination	1150 h (0215 h)	0855 h – 2055 h

2.2 Procedure

In the last trimester of pregnancy (M = 37.7 weeks, SD = 1.84) the mothers filled in questionnaires on demographics. They also received instructions and materials for the sleeping arrangement diary that would start directly after birth. The sleep diary is explained below.

After we received notice that the mothers had delivered, they were contacted by phone to schedule a home visit when the infants were approximately five weeks of age (M = 33.5 days, SD = 4.9). During the home visit, we asked the mothers to bathe their infant as they would normally do (undressing, bathing, and dressing). The bathing sessions lasted on average 11.2 min (range: 6 – 20 min). We collected infant saliva to measure infant cortisol reactivity to the bathing session. The bathing sessions were also videotaped and later rated for quality of maternal caregiving behavior.

At around 2 months of age (*M* = 62.7 days, *SD* = 6.9) the infants received their first routine vaccinations at the Well Baby clinic. The vaccination included two injections; the first was a combined vaccination for diphtheria, whooping cough, tetanus, poliomyelitis and haemophilus influenzae type b, the second for pneumococcus. Before the vaccinations the babies also received a physical exam. Cortisol responses to this vaccination procedure were measured. Mothers collected the saliva samples themselves.

2.3 Instruments and Measures

Sleeping Arrangements and Sleep Characteristics

Information on parent-infant sleeping arrangements was collected with the use of daily sleep diaries in the first two months of life. Every morning the mothers filled in a diary on how long and where the child had slept during the previous night. They could mark this with lines in a table that consisted of 30-minute time blocks spanning between 0000h and 0800h. They could indicate for every time block whether the child slept in its own room, in the parents' room (in a separate bed), in the parents' bed, or somewhere else. When the child was awake, no line was drawn for that 30-minute block. In the same diary, mothers marked every time the infant woke during the night and required comforting to settle back to sleep. The percentage of time spent in each sleeping arrangement per night was calculated by adding up the number of sleeping blocks for each sleeping arrangement separately, and dividing by the total number of sleeping blocks for that night, multiplied by 100. Weekly averages were calculated for the total amount of hours slept per night, the percentage of time spent in each sleeping arrangement, and the number of night wakings, when at least 4 out of 7 days were filled in.

In the first two months about half of the infants slept at least half the night in their own room or in a separate bed in their parents' room, and only 5% of the infants slept more than half the night in their parents' bed. As we considered this last group too small to analyze separately, we classified co-sleeping as sleeping in the parents' room, including both sleeping in a separate bed and in the parents' bed.[1]

1 Analyses without the bed-sharers gave similar results.

The bathing sessions were scheduled in week 5, so we calculated average co-sleeping in the first 4 weeks from the weekly averages. The vaccinations were scheduled at 2 months of age and therefore average co-sleeping arrangements of the first 7 weeks were used to calculate co-sleeping for those analyses. For analyses on cortisol reactivity to the bathing session participants were only selected if at least 3 out of 4 weeks of co-sleep data were available, and for the vaccination analyses when at least 4 out of the 7 weeks of co-sleep data were available (*N* = 155 and 159 out of 163, respectively).

The distributions of the average percentages co-sleeping per night in the first 4 and 7 weeks are shown in Figure 1a and b. It is clear from this figure that co-sleeping is not normally distributed. For the present study we divided the infants into 3 groups: solitary sleepers (co-sleeping 0 – 10 % of the time), full co-sleepers (91 – 100% of the time), and a middle group of 'partial' co-sleepers (11 – 90 % of the time). For the 4-week analyses the groups were divided as follows; solitary sleep: *N* = 38, partial co-sleep: *N* = 45, full co-sleep: *N* = 72, and for the 7-week analyses; solitary sleep: *N* = 44, partial co-sleep: *N* = 54, full co-sleep: *N* = 61. For the analyses, 2 dummy variables were created to compare the 3 groups. The first dummy variable indicates the contrast of partial and full co-sleepers versus solitary sleepers (the *Solitary sleep dummy*: solitary sleeping = 0, partial and full co-sleeping = 1), and the second dummy variable indicates the contrast of full co-sleepers versus solitary and partial co-sleepers (the *Full co-sleep dummy*: solitary sleeping and partial co-sleeping = 0, full co-sleeping = 1).

Cortisol

Infant saliva samples were collected using Sorbette eye sponges. Samples were taken at arrival of the researcher to the home or of the parents to the Well Baby clinic (T1), and at 25 min (T2) and 40 min (T3) post-stressor (being taken out of the bath and receiving the vaccinations, respectively). After the stress sessions, samples were kept in the freezer (-18 – -25°C) until further analysis. The saliva samples were analyzed with radioimmunoassay at the Laboratory of Endocrinology of the University Medical Center of Utrecht University. The

lower limit of detection was 1 nmol/l, and inter-assay and intra-assay variations were below 10% (de Weerth, Jansen, Vos, Maitimu & Lentjes, 2007).

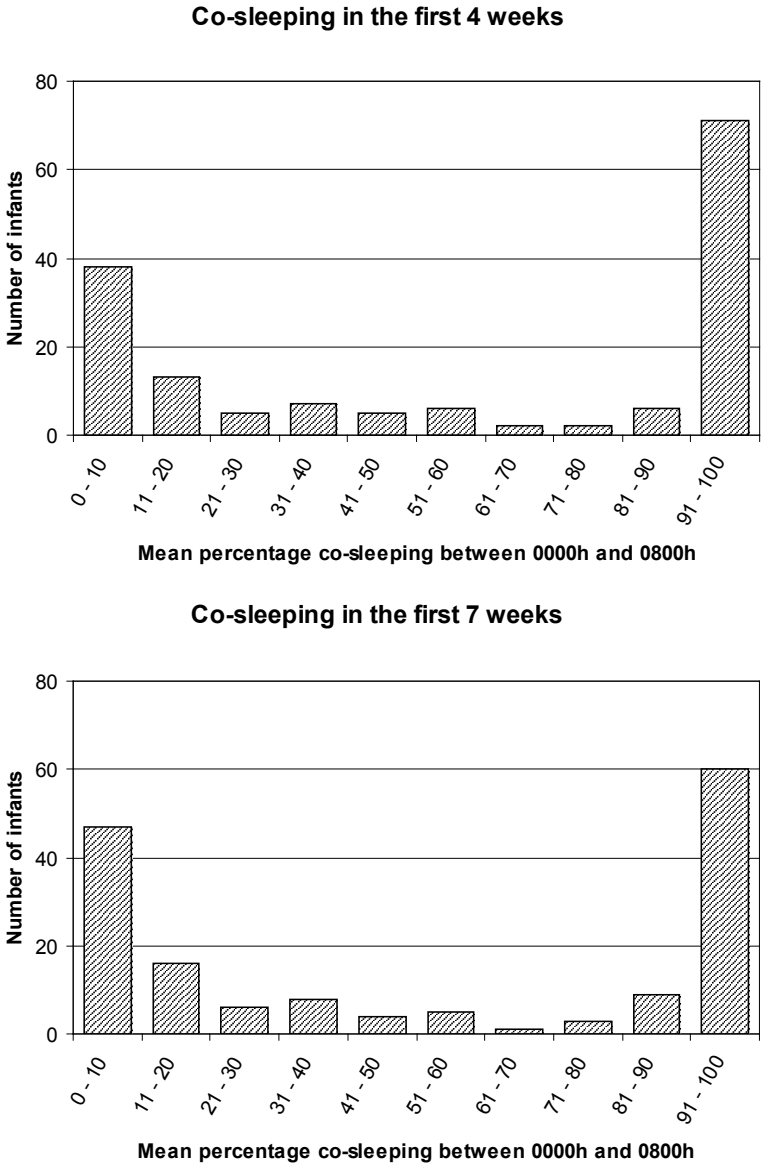

Figure 1: Distribution of parent-infant co-sleeping (a) in the first 4 weeks of life, and (b) in the first 7 weeks of life

Confounders

Potential confounders that were measured in this study were maternal quality of caregiving behavior, breastfeeding, infant night wakings and sleep duration. Similar to Albers et al. (2008), the videotaped bathing routines were rated for maternal quality of caregiving behavior, including measures of *sensitivity* and *cooperation* (Ainsworth, Blehar, Waters & Wall, 1978). *Sensitivity* refers to the extent to which caregivers timely and appropriately respond to the infant's needs and signals, and *cooperation* refers to the extent to which caregivers adjust their behavior to the infant's ongoing activity rather than interfering with the infant's actions. *Sensitivity* and *cooperation* were scored using two 9-point rating scales, with higher scores reflecting more sensitivity and cooperation. Interactions were rated separately by at least two trained students, who did not know the mothers and infants they were observing, and were blind with regard to the other data. Inter-observer reliability was good (Cohen's Kappa 0.90 for both sensitivity and cooperation).

Each week the mothers noted the average number of breast or bottle feedings per day in the sleep diary. The weekly average number of wakings and sleep duration per night were determined from the daily diaries, as described above. From these weekly means, we calculated the average number of daily breastfeedings, night wakings and average sleep duration for the first and second month separately.

In addition, we also included the following child and maternal variables as potential confounders, as these might all be related to co-sleeping and/or influence HPA-axis functioning: gender, birth weight, parity, maternal educational level, maternal age, pregnancy smoking (yes or no), pregnancy alcohol intake (yes or no), and time of day of the stressor.

2.4 Statistical Analyses

Cortisol values higher than 3 SD from the group mean per sample moment were regarded as outliers and treated as missing values (2.5 %). As cortisol scores were not normally distributed, a square root and logarithm transformation were performed on the bath and vaccination session data,

respectively, and these transformed variables were used for the analyses. In the results section untransformed data are presented.

First, paired sampled t-tests were performed to test cortisol reactivity to the stressors. Next, ANOVAs and t-tests were used to examine differences between the groups on each of the potential confounders. Then, to study the relations between co-sleeping and cortisol reactivity to the bathing session and the vaccination, we performed longitudinal regression analyses using mixed-model (multi-level) designs in SPSS 15.0. A major advantage of multilevel modeling over repeated measures analyses is the potential to include infants with missing data at one or two of the time points. With this technique, all valid data points could be included in the model. Time (sample moments T1, T2 and T3) was introduced at level 1 and nested within the individuals (level 2). Time was considered a random factor. Besides time as a linear factor, time squared was entered as a fixed factor to account for the increase and decrease in cortisol over time.

This base model was compared to multiple additional models. First, we entered the potential confounding variables for which the co-sleep groups differed as fixed factors, as these could explain possible effects of co-sleeping on cortisol reactivity.[2] We also included the interactions between the time variables and these variables to test whether they affected cortisol levels over time. In subsequent models, we only included those confounders that were significantly related to co-sleeping, to examine whether co-sleeping explained any additional variance in cortisol reactivity. We added the 2 dummy variables to code for the 3 co-sleep groups. We also included the interactions between the time variables and the dummy variables to examine the effect of co-sleeping on cortisol reactivity over time. The final models were compared on the basis of their deviance on the -2 log likelihood ratio scale after Maximum Likelihood estimation. Finally, to disentangle possible effects of the co-sleep (or other) variables on cortisol reactivity, we used post-hoc one-way ANOVAs and t-tests to study group differences at each cortisol sampling time.

2 In the interest of parsimony and to reduce type I errors, we only included confounders in the regression analyses that differed between the groups.

3. Results

3.1 Preliminary Results

Cortisol Reactivity

Being taken out of the bath resulted in a significant increase in cortisol concentrations from sample moment T1 to T2: 11.5 nmol/L to 14.5 nmol/L ($t(99) = 3.4$, $p < .001$). Cortisol significantly decreased again from T2 to T3: 14.5 nmol/L to 12.3 nmol/L ($t(90) = 4.7$, $p < .001$). Cortisol levels at T3 no longer differed from levels at T1 ($t(89) = 1.1$, $p = .29$).

The vaccination resulted in a significant increase in cortisol concentrations from sample moment T1 to T2: 10.8 nmol/L to 17.1 nmol/L ($t(102) = 5.6$, $p < .001$). Cortisol significantly decreased again from T2 to T3: 17.1 nmol/L to 15.6 nmol/L ($t(92) = 4.0$, $p < .001$). Cortisol levels at T3 were still higher than at T1 ($t(95) = 4.1$, $p < .001$). See Figure 2 for the average cortisol levels at the three sample moments, for each stressor and in each of the three co-sleep groups. The cortisol responses to the two stressors (T2 minus T1) did not correlate ($r = .016$, $p = .89$).

Confounders

Because maternal sensitivity and cooperation were highly correlated ($r = .83$, $p<0.001$), an overall quality of maternal caregiving behavior score was computed by averaging the scores on both scales. As breastfeeding in the first and second months were highly correlated ($r = .88$, $p <.001$), we averaged the number of daily breastfeedings in the first and second month for the mixed model analyses on the vaccination. See Table 2 for the averages of all confounders per co-sleep group. For the bathing session we compared the solitary, partial and full co-sleepers in the first 4 weeks on all potential confounders. The groups differed significantly on maternal education ($\chi^2(1) = 4.2$, $p < .05$) and breastfeeding in the first month ($F(2,148) = 6.8$, $p < .001$), with full co-sleepers having higher educated mothers than solitary sleepers. Partial and full co-sleepers received more breastfeeding. No differences between the co-sleep groups were found on maternal caregiving quality, the sleep variables (duration and waking), or any of the other potential confounders. Hence, only

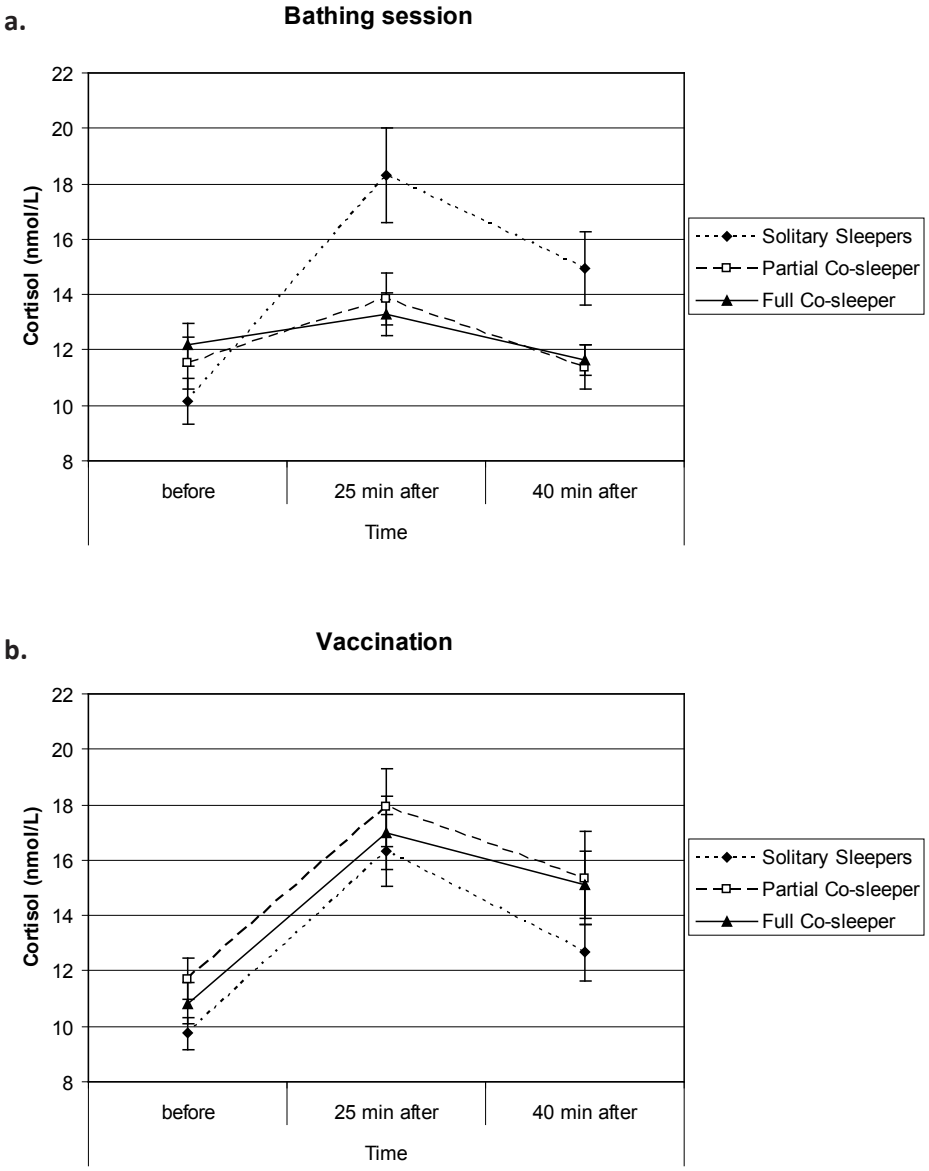

Figure 2: Cortisol levels (mean ± SEM) in the solitary sleep, partial and full co-sleep groups (a) before, 25 min after and 40 min after the bathing session, and (b) before, 25 min after the vaccination.

* <.05

education and breastfeeding were added to the bathing session multilevel models as control variables.

For the vaccination we compared the solitary, partial and full co-sleepers in the first 7 weeks on all potential confounders. The groups differed significantly on maternal education ($\chi^2(1) = 9.0$, $p < .001$), maternal age ($F(2, 136) = 3.1$, $p < .05$), and on breastfeeding in the first two months

Table 2: Means and standard deviations of the confounders for each co-sleep group.

	Co-sleeping in the first 4 weeks			**Co-sleeping in the first 7 weeks**		
	non	partial	full	non	partial	full
Percentage boys	55 (50)	51 (50)	54 (50)	55 (50)	56 (50)	54 (50)
Birth weight (gram)	3654 (392)	3527 (518)	3603 (453)	3608 (400)	3527 (521)	3648 (458)
Number of siblings	0.61 (.68)	0.71 (0.76)	0.88 (0.71)	0.55 (0.66)	0.81 (0.78)	0.84 (0.69)
Number of daily breast feedings in the first month	4.4 (2.8)	5.7 (3.1)	6.5 (2.7)*	4.7 (2.9)	5.7 (3.1)	6.5 (2.7)*
Number of daily breast feedings in the first 2 months	-	-	-	3.9 (2.8)	5.11 (3.1)	6.1 (2.6)*
Maternal age at birth (years)	31.9 (3.8)	32.3 (3.6)	33.6 (3.7)	31.6 (3.9)	32.6 (3.1)	33.7 (3.9)*
Percentage highly educated mothers	71 (46)	72 (45)	88 (33)*	67 (47)	70 (46)	92 (28)*
Percentage that reported smoking during pregnancy	3 (16)	2 (15)	4 (20)	2 (15)	6 (23)	2 (13)
Percentage that reported alcohol use during pregnancy	13 (34)	18 (39)	12 (33)	14 (35)	19 (39)	11 (32)
Quality of maternal caregiving behavior	4.8 (2.1)	5.6 (2.0)	5.4 (2.0)	5.0 (2.1)	5.3 (2.1)	5.6 (2.0)
Mean number of night wakings in the first 4/7 weeks	1.8 (.69)	1.8 (.91)	2.0 (.87)	1.6 (.64)	1.8 (.86)	1.9 (.81)
Mean total sleep duration per night in the first 4/7 weeks (hours)	7.2 (.75)	7.2 (.82)	7.1 (.87)	7.3 (.72)	7.2 (.82)	7.0 (.85)
Time of day of bathing session	1220 h (0215)	1210 h (0155)	1230 h (0210	1210 h (0255)	1230 h (0110)	1215 h (0210)
Time of day of vaccination	1220 h (0225)	1150 h (0215)	1135 h (0205)	1235 h (0215)	1145 h (0210)	1120 h (0200)

Note. * Significant difference between the co-sleep groups in the first 4 or 7 weeks ($p < .05$)

($F(2, 151) = 4.62$, $p < .05$, $F(2, 153) = 9.3$, $p < .001$), with full co-sleepers having the highest educated and oldest mothers, and partial and full co-sleepers receiving more breastfeeding than the solitary sleepers. No differences between the co-sleep groups were found on maternal caregiving quality, the sleep variables or any of the other potential confounders. Hence, only education, maternal age, and breastfeeding were added to the vaccination multilevel models as control variables.

3.2 The Effects of Co-sleeping on Cortisol Reactivity

The Bathing Session

We performed multilevel regression analyses on the cortisol reactivity to the bathing session. The confounding variables that differed between the co-sleep groups (i.e., maternal education and breastfeeding) were added to the model, as well as their interactions with time and time squared (see Table 3, model with confounders). Breastfeeding had no significant effect on cortisol levels over time (all *p*'s > .50). The interactions of education with the time variables (linear and squared) were significant ($B = -0.27$, $p = .02$ and $B = .12$, $p = .03$, respectively). Post-hoc test revealed that infants from higher educated mothers showed lower cortisol levels at 25 min after the bath (T2: $F(1, 109) = 5.28$, $p = .02$), but not before or 40 min after the bath (*p*'s > .19). We entered the education variables into the next model.

Then we added the two dummy variables that classified the three co-sleep groups, as well as the interactions between time, time squared and the two dummy variables. The interactions between the time variables (linear and squared) and the Solitary sleep dummy (contrasting the solitary sleep group with the partial and full co-sleep group) showed significant effects ($B = -1.25$, $p < .005$ and $B = .46$, $p < .05$, respectively), indicating a larger cortisol reaction in the solitary sleep group compared to the partial and full co-sleep group. This model led to a significant better fit (-2 log likelihood = 721.5) compared to the model with only the time variables included (-2 log likelihood = 738.2), χ^2 (3) = 16.7, $p < .001$). Inclusion of the education variables (main effect and interactions with the time variables) did not lead to a better fit of the model (difference in -2 log likelihood: χ^2 (3) = 4.5, $p = .20$), and did not

influence the effect of co-sleeping on cortisol reactivity. They were therefore not included in the final model. The regression results for the final model with the best fit are shown in Table 3.

These analyses indicate that the solitary sleep group has a different reactivity pattern over time than the partial and full co-sleep group. Figure 2a shows the cortisol responses to the bathing session for the 3 groups. Post-hoc one-way ANOVAs with co-sleeping as a between-subject factor showed that there was a difference in cortisol levels at sample moments T2 ($F(2, 109) = 4.79$, $p = .01$) and T3 ($F(2, 98) = 3.75$, $p < .05$). There were no group differences at T1 ($F(2, 112) = 1.19$, $p = .31$). Post-hoc t-tests on the contrast

Table 3: Regression results from the longitudinal analyses for the bathing session and the vaccination.

	Bathing Session				**Vaccination session**			
Factors	Model with confounders		Final model		Model with confounders		Final model	
	B	*p*	*B*	*p*	*B*	*p*	*B*	*p*
Intercept	3.24	<.001	3.14	<.001	0.91	<.001	1.00	<.001
Time (linear)	2.40	<.005	1.71	<.001	0.23	.55	0.30	<.001
Time^2	-1.11	<.01	-.70	<.001	-0.04	.81	-.12	<.001
Breastfeeding	-0.13	.56	-	-	0.01	<.05	-	-
Time * Breastfeeding	0.28	.50	-	-	-0.03	<.05	-	-
Time^2 * Breastfeeding	-0.01	.73	-	-	0.02	<.05	-	-
Education	0.02	.66	-	-	0.05	.24	-	-
Time * Education	-0.27	<.05	-	-	-0.03	.77	-	-
Time^2 * Education	0.12	<.05	-	-	0.02	.72	-	-
Maternal age	-	-	-	-	0.00	.99	-	-
Time * Maternal age	-	-	-	-	0.01	.47	-	-
Time^2 * Maternal age	-	-	-	-	-0.01	.33	-	-
Solitary sleep dummy	-	-	.23	.14	-	-	-	-
Time * Solitary sleep dummy	-	-	-1.25	<.005	-	-	-	-
Time^2 * Solitary sleep dummy	-	-	.46	<.05	-	-	-	-

Note. Solitary sleep dummy = contrast of solitary sleepers (0) versus partial and full co-sleepers (1).

between the solitary sleep group versus the partial and full co-sleep groups (solitary sleep dummy) showed that at moments T2 and T3 solitary sleepers showed significantly higher cortisol levels than partial and full co-sleepers ($t(31) = 3.0$, $p < .05$ and $t(99) = 2.7$, $p < .01$, respectively). Cortisol levels at moments T2 and T3 did not differ between the partial and full co-sleepers (p's > .56).

The Vaccination

Next, we performed multilevel regression analyses on the cortisol reactivity to the vaccination. The confounding variables that differed between the co-sleep groups (i.e., maternal education and age, and breastfeeding) were added to the model, as well as their interactions with time and time squared (see Table 3, model with confounders). Maternal education and age had no significant effects on cortisol levels over time (all p's > .24). Breastfeeding in the first 2 months, and the interactions of breastfeeding with the time variables (linear and squared) were significant ($B = 0.01$, $p = .05$ $B = -0.034$, $p = .02$ and $B = .016$, $p = .02$, respectively, see Table 3), indicating a smaller cortisol reaction in breastfed infants compared to bottle-fed infants. Post-hoc tests showed that infants that were breastfed more than others (based on a median split), showed higher cortisol levels before the vaccination ($F(1, 122) = 4.36$, $p = .04$), but not 25 or 40 min after the vaccination (p's > .19), indicating relatively lower cortisol reactivity in relation to breastfeeding. We entered the breastfeeding variables into the next model.

We then added the two dummy variables that classified the three co-sleep groups, as well as the interactions between time, time squared and the two dummy variables. However, none of the co-sleep variables predicted cortisol reactivity (all p's > .18). Figure 2b shows the cortisol responses to the vaccination for the 3 groups. Post-hoc one-way ANOVAs with co-sleeping as a between-subject factor showed that there was indeed no significant difference between the three co-sleep groups at any of the time points, all p's > .18. Inclusion of the breastfeeding variables (main effect and interactions with the time variables) only led to a marginally better fit compared to the model with only the time variables (χ^2 (3) = 7.75, p =.051), and was therefore not included

in the final regression model. The final regression model with the best fit is shown in Table 3, including only the time variables.

To predict cortisol reactivity to the vaccination in the previous analyses, co-sleeping was averaged over the first 7 weeks. To disentangle possible differential effects of co-sleeping in the first and second month on cortisol reactivity, we also entered co-sleeping in the first month (week 1 – 4) and second month (week 5 – 7) as separate predictors of cortisol reactivity in a mixed model. No different results were found. That is, neither co-sleeping in the first, nor second month independently predicted cortisol reactivity to the vaccination.

4. Discussion

This is the first study to prospectively investigate relations between parent-infant sleeping arrangements and cortisol reactivity in early infancy. We found that solitary sleepers, i.e. infants that slept 90% of the night (between 0000h and 0800h) or more in their own bedroom, respond with a higher cortisol reactivity to a mild (bathing) stressor than partial or full co-sleepers. Several possible confounders, including breastfeeding, a measure of maternal caregiving quality, infant night waking, and sleep duration, were taken into account in this study. Solitary sleepers received less breastfeeding and had mothers with lower education than co-sleepers, and hence these variables were controlled for in the analyses. Breastfeeding did not significantly predict cortisol reactivity to either the bath or the vaccination. Lower education was associated with slightly higher cortisol levels after the bath, but solitary sleeping continued to predict unique variance in cortisol reactivity to the bathing session, next to maternal education.

No associations were found between co-sleeping in the first months and the cortisol response to a vaccination procedure. However, co-sleeping was associated with more breastfeeding in the first two months, and higher maternal age and education. Of these factors, breastfeeding was marginally associated with the cortisol response to the vaccination. Thus, solitary sleepers in the first months of life seem to respond with higher cortisol reactivity to a

mild physical stressor, but their cortisol responses to a vaccination are similar to those of young infants that sleep in close proximity to their parents.

The results of the bathing session are in line with animal studies that report heightened reactivity to stressors after high levels of early parental separation (Aisa et al., 2007; 2008). As maternal caregiving, night waking and sleep duration were not related to co-sleeping, and as breastfeeding was not associated with cortisol reactivity to the bathing session, it may be that lower cortisol reactivity to this mild stressor in the co-sleep groups is related to more availability of the parents at night. More proximity to, and hence more or faster physical and vocal contact with the parents during the night may increase external stress regulation, leading to less frequent high distress levels and hence to lowered stress sensitivity in other domains of life as well. Also, although we controlled for quality of maternal caregiving, as measured through sensitive and cooperative behavior during the bathing session, it is possible that the simple presence of the mother during a mild stressor, irrespective of caregiving quality, was enough to help regulate partial and full co-sleeper's cortisol reactivity to the bathing session. These explanations do not exclude each other and could both partly explain the findings.

We found no relations between solitary sleeping and the cortisol response to a vaccination procedure, 3 weeks after the bathing session. As the vaccination is known to elicit relatively strong cortisol responses in general, it may be a less optimal stressor to show 'hyper-responsivity' in the HPA-axis in comparison to the bathing session (Keenan, Gunthorpe & Grace, 2007).

In the present study co-sleepers received more breastfeeding than solitary sleepers and more breastfeeding was marginally associated with higher basal cortisol levels at the time of the vaccination. Higher cortisol levels in breastfed infants have been reported before by Cao et al. (2009). In the post-vaccination cortisol no effects of breastfeeding were found, indicating relatively lower cortisol reactivity in relation to breastfeeding. Higher basal levels may lead to less reactivity by itself (Law of Initial Value: Lacey, 1956; Wilder, 1957), although previous studies have indicated a link between breastfeeding and lower stress reactivity to painful stressors (Shah, Aliwalas & Shah, 2007).

Interestingly, no differences were found between the co-sleepers and solitary sleepers on our measure of maternal caregiving quality, or on the infants' sleep characteristics. We measured maternal caregiving quality with video observations of sensitivity and cooperation behaviors. These measures have been well validated before (e.g., Albers et al., 2008; Van Bakel & Riksen-Walraven, 2002), predict later mother-infant attachment relationships and infant functioning (e.g. van den Boom, 1994; van Doesum, Riksen-Walraven, Hosman & Hoefnagels, 2008; Egeland, Pianta & O'Brian, 1993), and are stable in the first two years (Kemppinen, Kumppalinen, Raita-Hasu, Moilanen & Ebeling, 2006). However, we measured maternal caregiving quality during a relatively short period, in only one specific care situation. Possibly, extending the observation time and observing the dyad also in other caregiving situations might yield a more robust measure of maternal behavior that could be related to co-sleeping and / or cortisol reactivity.

With regard to the lack of differences in infants' sleep characteristics, while co-sleeping has before been associated with more night wakings (Cortesi et al., 2004; Mao et al., 2004), and has been found to affect sleep duration and quality (Hunsley & Thomas, 2002), Mao and colleagues found co-sleepers to have shorter wakings and hence similar sleep duration. This is in line with our findings of similar sleep durations in the different groups. The fact that we also found no differences in the number of night wakings could be due to our co-sleeping group consisting mainly of room-sharers, while the earlier findings on frequent night waking are based on bed-sharers. Sharing a bed with a caregiver could be linked to the infant waking more often, while sharing the room might not.

Finally, maternal age and education were higher in the co-sleeping groups as compared to the solitary sleepers. While co-sleeping has in the past been related to lower social economic status (Blair & Ball, 2004; Weimer, Dise, Evers, Ortiz, Welldaregay & Steinmann, 2002), this apparent contradiction may be due to culture. In the Netherlands, early independence of children is traditionally valued, and includes solitary sleeping arrangements from the start. It may be that older or more highly educated mothers are more willing to break traditions in the pursuit of their personal caregiving beliefs.

If the apparent heightened reactivity of the HPA-axis of solitary sleepers to (mild) stressors generalizes to later ages, it may explain the findings by Waynforth (2007) in 3- to 8-year-old children. In Waynforth's study, children who had experienced *fewer* years of co-sleeping showed higher basal cortisol levels. This could hypothetically be due to sustained higher cortisol reactivity to mild stressors during infancy, leading to chronically elevated cortisol levels in childhood (Miller, Chen & Zhou, 2007). Future studies will have to show whether early co-sleeping can also influence HPA-axis reactivity at later ages. In this regard, Lucas-Thompson et al. (2009) found solitary sleepers to show lowered cortisol reactivity to vaccinations at 6 and 12 months of age, while in the present study no differences in reactivity to a vaccination at 2 months were found between the groups. One possible explanation for this discrepancy in results is the difference in sleep data collection methodology: questionnaires covering the behavior of the last month versus daily diaries, and different quantifications of co-sleeping. Another is that co-sleeping at 6 and 12 months of age may have a different origin than co-sleeping in the first two months (i.e. reactive versus planned; Keller & Goldberg, 2004), or that co-sleeping effects on cortisol reactivity are age-dependent (e.g. due to developing self-regulating abilities, which may change the need for parental proximity during the night). Clearly, the relations between early and later sleeping arrangements and HPA-axis regulatory mechanisms throughout the first year(s) of life are a relevant topic for future research.

Strong points of this study are that we collected daily sleep arrangements data for an extensive period in a relatively large group of young infants, and that cortisol reactivity to two different, effective early life stressors was assessed. Limitations are that although we measured many relevant confounders in the present study, we did not control for infant health, recent feedings, naps, or for recent stressors while these can all influence cortisol levels. Individual differences in maternal behavior during the vaccination were also not accounted for. In addition, because of the small group of bed sharers in our study, we defined co-sleeping as sleeping in the parents' bedroom without distinguishing between bed sharers and room sharers (Ball, 2003), while both groups may naturally differ in cortisol reactivity. Generalizability of these

findings to other stressors and ages, and long term health outcomes remain open questions for future research. And as reasons to co-sleep differ per culture (Keller & Goldberg, 2004), these findings may not generalize outside of the Netherlands.

As we studied associations between sleeping arrangements and cortisol reactivity, we cannot draw any conclusions on causality. There may be underlying causes explaining both sleeping arrangements and cortisol reactivity, e.g., infant temperament or (distress) behavior. Also, as parents chose the sleeping arrangement for their infant, there may be differences between the nurturing elements of the environment for co-sleepers and solitary sleepers that we didn't measure but may influence infants' stress reactivity. While we investigated several important candidates, including breastfeeding, maternal caregiving quality, and sleep characteristics (i.e. infant night wakings and sleep duration), future studies should investigate other potential confounders or underlying causes.

Based on these findings, we would suggest that co-sleeping in the first month of life may be beneficial, as it is associated with lowered infant cortisol responding to a mild daily physical stressor. The fact that in our study only a small part of the co-sleepers were actual bed-sharers suggests that mere proximity to the parents at night (by sleeping in a separate bed in the parent's room) may be sufficient. This is important given that bed-sharing has been associated with an increased risk of Sudden Infant Death Syndrome (SIDS, for discussions see Ball, 2009; Goldberg & Keller, 2007a). When considering the potential beneficial effects of co-sleeping (or bed-sharing), multiple health effects should be taken into account (e.g. SIDS or heightened arousal during infant sleep: Ball, 2009; Goldberg & Keller, 2007b; Hunsley & Thoman, 2002), as well as relations to later behavioral and emotional outcomes (e.g. social independence: Keller & Goldberg, 2004).

In sum, solitary sleeping in early infancy is associated with heightened cortisol responding to a bathing, but not a vaccination session, as compared to co-sleeping. Breastfeeding, maternal caregiving quality, and sleep characteristics of the infant could not explain this association. The underlying mechanisms therefore still have to be unraveled.

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Chapter 4

Cortisol regulation in 12-month-olds: associations with the infants' early history of breastfeeding and co-sleeping

Roseriet Beijers, Marianne Riksen-Walraven & Carolina de Weerth

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Abstract

Background: Early experiences are found to play a role in programming infants' HPA-axis functioning. Where stressful early experiences have been associated with dysregulated cortisol responses, positive early experiences, i.e. maternal caregiving quality, contribute to more optimal cortisol regulation. Although it is suggested that a number of components of typical parent-infant interactions have long-term regulatory effects on infant physiology, influences of other caregiving factors on infant cortisol regulation are less well documented. The goal of this study was to examine whether a history of breastfeeding and co-sleeping during the first six months of life was associated with cortisol regulation, i.e. cortisol reactivity and recovery, to a stressor at 12 months of age.

Methods: Participants were 193 infants and their mothers. Information on breastfeeding and co-sleeping was collected using weekly and daily sleep diaries, respectively, for the first 6 months of life. At 12 months of age, infants were subjected to a psychological stressor (Strange Situation Procedure, Ainsworth et al., 1978). Salivary cortisol was obtained pre-stressor, and at 25 min, 40 min and 60 min post-stressor, to measure cortisol reactivity and recovery.

Results: Regression analyses showed that after controlling for maternal sensitivity and many other confounders, more weeks of co-sleeping predicted lower infant cortisol reactivity to the Strange Situation Procedure (SSP), but only for children who also received breastfeeding for a relatively long period (six months). Also, more weeks of breastfeeding predicted quicker cortisol recovery.

Conclusion: These results suggest that early breastfeeding and co-sleeping contribute positively to cortisol regulation in 12-month-old infants.

1. Introduction

Stress responses involve activity in the central nervous system to mobilize endocrine, autonomic, and behavior functions that help the body to react efficiently to the stressful situation. A major part of the neuroendocrine system is the hypothalamic-pituitary-adrenal (HPA)-axis that not only controls

reactions to stress, but also regulates many body processes including the immune system and energy expenditure. Infants are born with a functional HPA-axis, reacting with the secretion of cortisol when confronted with stressors (Jansen, Beijers, Riksen-Walraven & de Weerth, 2010). Where small to moderate increases of cortisol are adaptive and needed to react efficiently to stressful situations, repeated or chronic exposure to high levels of cortisol early in life has been shown to have negative effects on brain development, socio-emotional adjustment, and immune functioning (Loman & Gunnar, 2011; Petrowski, Herold, Joraschky, Wittchen & Kirschbaum, 2010).

There is evidence that early experiences have a role in programming infants' HPA-axis functioning. Stressful early life experiences, like childhood abuse and maternal prenatal anxiety, are related to dysregulated cortisol responses to psychosocial stress later in life (Bouma, Riese, Ormel, Verhulst & Oldehinkel, 2011; Carpenter, Shattuck, Tyrka, Geracioti & Price, 2011; Elzinga, Roelofs, Tollenaar, Bakvis, Van Pelt & Spinhoven, 2008; Laurent, Ablow & Measelle, 2011; Tollenaar, Beijers, Jansen, Riksen-Walraven & de Weerth, 2010). Moreover, positive early experiences contribute to more optimal infant HPA-axis functioning, i.e. a less strong reaction to a stressor and a quicker recovery afterwards. For example, higher quality maternal care in rats, as quantified by more maternal licking and grooming, is related to lower offspring HPA-axis reactivity to stressors (Francis & Meaney, 1999; Szyf, Weaver, Champagne, Diorio & Meaney, 2005; Weaver et al., 2004). In humans, higher quality of maternal care has been related to a quicker cortisol recovery from a mild stressor in three- and five-month-old infants (Albers, Riksen-Walraven, Sweep & de Weerth, 2008; Haley & Stansbury, 2003).

These results are in line with Hofer's (1994) concept of hidden regulators embedded in parent-infant relationships. Hofer argued that a number of components of typical parent-infant interactions have long-term regulatory effects on specific components of infant behavior and physiology. Loss of these hidden regulators results in dysregulation of physiological and behavioral responses to stress (for a recent review, see also Loman & Gunnar, 2011). Given that HPA-axis functioning appears to be shaped by early experiences and that dysregulation of the HPA-axis can be a risk factor for the

development of (psycho)pathology (Gunnar & Vazquez, 2001; Heim, Newport, Heit, Graham, Wilcox, Bonsall, Miller & Nemeroff, 2000; Lupien, McEwen, Gunnar & Heim, 2009), it is important to obtain a better picture of which early influences are associated with infant HPA-axis functioning. While previous studies concentrated on stressful life experiences and quality of maternal care, influences of other caregiving factors on infant HPA-axis functioning are less well documented. During the first months of life two important aspects of the early caregiving environment are the type of feeding an infant receives and the place where the infant sleeps during the night. The aim of this study is to investigate whether a history of breastfeeding and co-sleeping within the first six months of life are associated with cortisol regulation, i.e. reactivity and recovery, to a psychological stressor in 12-month-old infants.

1.1 Breastfeeding

Young infants spend a great portion of their waking time feeding. Therefore, early experiences during feeding, for example as a result of differences in type of feeding (breastfeeding versus bottle) and duration of feeding, could have important consequences for the development of the HPA-axis. With regard to type of feeding: both short-term and long-term positive effects of breastfeeding over bottle-feeding have been found in many domains of development. The chemistry of human milk is such that it has an important role in supporting infant growth and development (Duijts, Ramadhani & Moll, 2009; Kramer, Aboud, Mironova, Vanilovich, Platt, Matush, et al., 2008; Savino, Fissore, Liguori & Oggero, 2009). However, the role of breastfeeding in the development of the HPA-axis remains largely unexplored.

Different mechanisms could link breastfeeding to the development of infant HPA-axis functioning. First, it has been hypothesized that components of the mother-infant interaction unique to the breastfeeding context, e.g. the body contact and the increased frequency and longer duration of feeds, might have stimulatory effects on HPA-axis development (Kramer et al., 2008; Sievers, Oldigs, Santer & Schaub, 2002). This hypothesis is in line with Hofer's (1994) concept of hidden regulators, as he argued that a number of sensorimotor, thermal, and nutrient-based events that are components of typical

parent-offspring interactions have long-term regulatory effects on infant behavior and physiology. Second, in contexts other than the feeding context, breastfeeding mothers have been shown to engage in more additional interactions with their infant, including touching and gazing. In addition, breastfeeding mothers show increased parasympathetic nervous system modulation, lower perceived stress levels, and fewer anxiety and depressive symptoms (Jansen, de Weerth & Riksen-Walraven, 2008; Kim, Feldman, Mayes, Eicher, Thompson, Leckman & Swain, 2011; Oberlander et al., 2008). Moreover, it has also been suggested that breastfeeding may strengthen the maternal bond with the infant, although evidence for this is as yet greatly lacking (Jansen et al., 2010). In turn, improved quality of the mother-infant interaction and better maternal mood may support maternal co-regulation. Maternal co-regulation protects the infant from excessive levels of behavioral distress, e.g. signs of discomfort and crying, and hence promotes the infant's emergent capacity to regulate emotional and physiological arousal (Schore, 2001).

Up till now only a few studies have examined the relation between feeding practices and cortisol physiology. Breastfeeding status was found unrelated to infants' cortisol reactivity to physical stressors, such as vaccinations and physical examinations (Davis & Granger, 2009; Larson, White, Cochran, Donzella & Gunnar, 1998; de Weerth & Buitelaar, 2007). However, in response to a habituation task, Oberlander et al., (2008) found a cortisol reaction in breastfed 3-month-old infants, but not in non-breastfed infants. In sum, breastfeeding appears to be generally unrelated to cortisol reactivity to physical stressors, and one study found it related to higher cortisol reactivity to a testing session. However, much remains unclear regarding breastfeeding in relation to psychological stressors and cortisol regulation later in life, and will therefore be topic of this study.

1.2 Co-sleeping

The topic of co-sleeping has been debated for decades. Where it has been previously discouraged because of the claims that co-sleeping increases risks, including the risk for sudden infant death syndrome (SIDS), other evidence

supports the benefits of co-sleeping, e.g. the promotion of breastfeeding (for a review, see Sobralske & Gruber, 2009). Nowadays, co-sleeping is an upcoming phenomenon in Western cultures. Nevertheless, infants' sleeping arrangements during the first months of life show large inter-individual variation. While some infants sleep in their own room from the beginning, others sleep in a crib in the parents' room or in bed with the parents. These last two options are commonly referred to as 'co-sleeping' (Goldberg & Keller, 2007; McKenna, Ball & Gettler, 2007).

Co-sleeping implies more physical closeness to the parents, making the parents more available during the night. When compared to co-sleeping, the separation from parents during the night for solitary sleepers probably means that infants' subtle signals of behavioral distress, e.g. vocal signs of discomfort, are less frequently or less quickly responded to by parents. As a consequence, these infants will probably have to reach higher levels of negative vocalizations in order to alert the parents. Where solitary sleeping may thus be related to more frequent experiences of higher levels of distress, co-sleeping might be related to more frequent experiences of parental co-regulation during the night. In turn, these experiences may influence HPA-axis functioning (Heim & Nemeroff, 2001; Schore, 2001). Furthermore, co-sleeping is seen as a healthy bonding experience and thought to provide warmth, protection and a general feeling of well-being (Sobralske & Gruber, 2009). These aspects of parental behavior have been related to infants' HPA-axis functioning in response to stressors (Albers et al., 2008).

Only a few studies have investigated the relation between co-sleeping and HPA-axis functioning. In a retrospective study, Waynforth (2007) found that more years of co-sleeping were related to lower basal cortisol levels in 3- to 8-year-old children. Lucas-Thompson, Goldberg, Germo, Keller, Davis and Sandman (2009) found that current co-sleeping, as reported by mothers in a questionnaire over the previous month, was associated with increased cortisol reactivity to vaccination at 6 and 12 months of age. Furthermore, in our lab we showed that solitary sleeping in very young infants is associated with heightened cortisol reactivity to a bathing session at 5 weeks of age, but not to a vaccination at 8 weeks of age (Tollenaar, Beijers, Jansen, Riksen-Walraven

& de Weerth, 2011). In sum, the results are mixed. While Waynforth (2007) and Tollenaar et al., (2011) found lower basal cortisol levels and lower cortisol reactivity, Lucas-Thompson et al., (2009) found that co-sleeping was related to higher cortisol reactivity. Moreover, these previous infant studies are relatively limited in scope as they concentrated on basal cortisol or on cortisol reactivity to physical stressors (vaccinations and bathing sessions). Therefore, this study examines the relation between an early history of co-sleeping in relation to a psychological stressor (maternal separation) in 12- month-old infants.

1.3 The association between breastfeeding and co-sleeping

Breastfeeding women are nearly twice as likely to sleep with their infants in the first month after birth as mothers who bottle-feed (McCoy et al., 2004). In addition, multiple longitudinal studies have demonstrated that co-sleeping is positively associated with a longer duration of breastfeeding, particularly in the first six months of life (McCoy et al., 2004; Ball, 2007).

Remarkably, these apparently closely associated aspects of the early caregiving environment have never been examined simultaneously in relation to infant HPA-axis functioning, so their unique contributions remain to be explored. In addition, interactive effects might also be present, as (additional) effects of breastfeeding and co-sleeping might be seen when experienced in combination. As longer durations of breastfeeding and co-sleeping would increase parental opportunities of serving as external stress regulators, the combination of high levels of breastfeeding and co-sleeping would provide maximal opportunities for parental co-regulation. Moreover, the breastfeeding and co-sleeping combination has been shown to hold unique characteristics specific to this combination. For instance, Gettlert and McKenna (2011) showed that breastfeeding and bed-sharing mother-infant pairs engaged in a greater number of feeds per night compared to breastfeeding mother-infants pairs that sleep in separate rooms. Also, the combination of breastfeeding and co-sleeping leads to more contact and proximity between mother-infant pairs in contexts and situations other than the feeding and sleeping context (Kim et al., 2011). In light of the above, it can be theorized that the breastfeeding and co-sleeping combination is also positively related to infant cortisol regulation.

1.4 Summary and present study

To summarize, human research regarding the effects of early breastfeeding and co-sleeping on HPA-axis functioning in the first year of life is scarce. Moreover, earlier research has focused mostly on cortisol reactivity to physical (instead of psychological) stressors, and on current co-sleeping or breastfeeding status, while neglecting a history of breastfeeding and/or co-sleeping. As stated before, breastfeeding and co-sleeping may support parental co-regulation of the infant, which is most important early in life when the infant's own regulating capacities are limited. Because parental co-regulation would protect the infant from high levels of distress, breastfeeding and/or co-sleeping even in the very first weeks may affect cortisol regulation later in life. Furthermore, none of the previous studies have examined breastfeeding and co-sleeping simultaneously in relation to infant HPA-axis functioning, so their unique, shared and/or interactive contributions remain unexplored. Also, none of the studies examined breastfeeding or co-sleeping in relation to cortisol recovery. Prior research already underlined the importance of considering not just reactivity, but also recovery, to characterize HPA-axis functioning (Albers et al., 2008; Laurent et al., 2011; de Weerth & Buitelaar, 2005). The general inability to recover following stress termination might be as important as or even more important for future development than reactivity to a stressor. High and prolonged cortisol elevations as a response to stressors are known to further influence infant HPA-axis functioning and to have pathological effects (Sapolsky, Romero & Munck, 2000).

The goal of this study was to examine whether the history of breastfeeding and co-sleeping during the first months of life was associated with cortisol regulation, i.e. cortisol reactivity and recovery, to a psychological stressor at 12 months of age. We chose the Strange Situation Procedure (SSP - Ainsworth, Blehar, Waters & Wall, 1978) as an age-appropriate psychological stressor (Jansen et al., 2010). We hypothesized that both co-sleeping during the night and breastfeeding for a longer period would predict better cortisol regulation in the infant, i.e. a less strong reaction and a quicker recovery afterwards.

2. Methods

2.1 Participants

This study is part of an ongoing prospective longitudinal project that investigates the influences of early caregiving factors on the development of children during their first years of life. The subjects were healthy infants living in the Netherlands, whose mothers were recruited during late pregnancy. Mothers responded to flyers that were spread among midwife practices in the cities of Nijmegen, Arnhem, and surrounding areas. The study was approved by the faculty ethical committee, and informed consent was obtained from each mother before starting. Inclusion criteria were an uncomplicated, singleton pregnancy, no drug use, and no current physical or mental health problems. Of the 220 women who enrolled in the study, 8 were excluded because of medical reasons, such as preterm birth. Of the remaining 212 mothers, a further 19 discontinued the study during the first 3 postpartum months due to personal circumstances. This resulted in a final sample of 193 mothers and their infants, from which the demographic characteristics are provided in Table 1. No differences in these demographic characteristics were found between participating mothers and those that dropped out (N=19).

2.2 Procedure

Information on breastfeeding (exclusive breastfeeding or breastfeeding in combination with bottle-feeding) was collected with the use of a weekly diary for the first 6 months of life (wk 1-27), and information on type of sleeping arrangements (in the infant's own room or in the parent's room/bed) was collected using a daily diary for the first 6 months of life. The mothers received the diaries and accompanying instructions at the end of pregnancy, so they could start immediately after birth. When the infants were 12 months of age (M=53 weeks and 6 days, SD=19 days), the mothers were asked to bring their infant to the laboratory of the Behavioural Science Institute, Radboud University, Nijmegen. Upon arrival, the SSP (Ainsworth et al., 1978) was carried out and cortisol regulation was measured.

Table 1. Descriptive statistics of the study variables (*N* =193).

	Mean *(SD)*	Range
Demographics characteristics		
Maternal age (years)	32.46 *(3.79)*	21.10–42.90
Maternal educational level (%)		
Primary education	3.80%	
Secondary education	20.40%	
College or university	75.80%	
Maternal marital status (living with partner[1])	97.90%	
Birth weight (grams)	3616.97 *(465.32)*	2645.00–4730.00
Infant sex (%)		
Girl	47.20%	
Birth order (%)		
First	41.60%	
Second	43.70%	
Third or fourth born	14.70%	
Confounders		
Maternal quality of caregiving	5.50 (*2.06*)	1.0-9.0
Maternal depression	5.01 (*2.93*)	0.0-14.33
Infant temperament (negative affectivity)	2.60 (*0.46*)	1.5-3.93
Infant attachment security (%)	68.80%	
Night waking 0-6 months	0.59 (*0.38*)	0.00-1.70
Night waking at 12 months of age	0.21 (*0.32*)	0.00-1.36
Breastfeeding at 12 months of age (%)	16.00%	
Co-sleeping at 12 months of age (%)	10.00%	
Attendance childcare centre during the first year (%)	57.10%	
Time of day Strange Situation (hours)	11:18 (*2:02*)	8:38-17:31
Hours since last feeding before Strange Situation	1:14 (*0:45*)	0:00-3:21
Hours since last sleep before Strange Situation	1:35 (*1:05*)	0:00-4:48
Predictors		
Number of weeks breastfeeding 0-6 months	16.77 (*11.07*)	0.00-27.00
Number of weeks co-sleeping 0-6 months	11.24 (*10.06*)	0.00-27.00
Infant cortisol regulation		
Reactivity (highest value of T2/T3)	8.79 (*4.26*)	3.10-24.00
Recovery (T4-T1)	-0.24 (*4.15*)	-15.10-10.50

[1]In wedlock or unmarried

To control for the quality of maternal caregiving behavior, two key characteristics of maternal behavior were rated, i.e., maternal sensitivity and maternal cooperation versus intrusiveness, during a videotaped mother-infant bathing session when the infants were 5 weeks of age. The previously mentioned daily diary to measure sleeping arrangements also provided information about infant night wakings for the first 6 months of life. To control for type of feeding, type of sleeping arrangements and number of night wakings at 12 months of age, a similar diary was kept again for 2 consecutive weeks (wk 51-52). The SSP also provided information about the quality of the infant-mother attachment (secure/insecure). Furthermore, we controlled for maternal depression and infant temperament, which were assessed using questionnaires that were filled in by the mother postnatally at 3, 6, and 12 months. Finally, additional information on mother and infant was obtained with questionnaires filled in by the mother during the last trimester of pregnancy and immediately after birth.

2.3 Breastfeeding

Information on feeding (exclusive breastfeeding, breastfeeding in combination with bottle-feeding, or exclusive bottle-feeding) was collected using weekly diaries for the first 6 months of life (wk 1–27), and again for 2 consecutive weeks at 12 months of age. Each week the mothers noted the average number of breast and/or bottle-feedings per day. The number of weeks during the first six months of life that an infant received exclusive breastfeeding or breastfeeding in combination with bottle-feeding, was used as predictor.

2.4 Sleeping arrangements

Information on infant sleeping arrangements was collected using daily diaries for the first 6 months of life (wk 1–27), and again for 2 consecutive weeks at 12 months of age (Beijers, Jansen, Riksen-Walraven, & de Weerth, 2011; Tollenaar et al., 2011). Mothers were instructed to complete the diary every morning by indicating whether the infant had slept in his or her own room, in the parents' room in a separate bed, in the parents' bed, or somewhere else. The mothers could mark this information with lines in a table that consisted

of 30-minute time blocks spanning between 2000h and 0800h. As only about 5% of the infants had the parents' bed as the standard arrangement, we classified co-sleeping as sleeping in the parents' bed and/or sleeping in the parents' room during the nighttime (Tollenaar et al., 2011). Similar to Anders and Keener (1985), we defined nighttime as 0000h to 0500h, the usual time of the parents' sleep. Per nighttime period, the number of hours co-sleeping was divided by the total hours of infant sleep and multiplied by 100 to obtain the percentage of co-sleeping for each night. The mean weekly percentage of co-sleeping was calculated by averaging the daily percentages. We classified the weeks in which 10-100% of co-sleeping had taken place as co-sleeping weeks. The 10% cut-off score was chosen to avoid including small amounts of incidental co-sleeping, and is also in line with Tollenaar et al., (2011). The total number of co-sleeping weeks during the first six months was used as predictor.[3]

2.5 Strange Situation Procedure (SSP)

During the laboratory visit, and upon arrival, the SSP was administered (Ainsworth et al., 1978). This procedure comprises a sequence of eight 3-min episodes that introduce an unfamiliar environment, the arrival of a stranger, and two brief separations from the mother. The SSP is considered a psychological stressor and has been found to elicit cortisol responses in one-year-olds (Van Bakel & Riksen-Walraven, 2004).

2.6 Infant cortisol concentrations

Infant saliva samples were collected using Sorbette eye sponges (De Weerth, Jansen, Vos, Maitimu & Lentjes, 2007). The samples were taken directly upon arrival of the parents at the laboratory (T1), to reflect the pre-stressor state of the infants. The next samples were taken at 25 min (T2), 40 min (T3), and 60 min (T4) post-stressor (i.e. after the second infant-mother separation in the SSP). Samples were stored at -25°C until further analysis at the Laboratory of

3 The variable concerning the total number of hours co-sleeping during the first six months of life (*M*=344.97, *SD*=349.23, min=0.00, max=945.00) correlated very highly with the variable number of co-sleeping weeks (*r*=.99, *p*<.000), and the results with this variable did not differ from the analyses reported in the paper.

Endocrinology of the University Medical Center of Utrecht University. Cortisol in saliva was measured using an in-house competitive radioimmunoassay by employing a polyclonal anticortisol antibody (K7348). [1,2-3H(N)]-Hydrocortisone (Amersham TRK407) was used as a tracer (de Weerth et al., 2007). The lower limit of detection was 1 nmol/L, and inter-assay and intra-assay variations were below 10%. We used the following time ranges for accepting the post-stressor samples: T2, 20–30 min post-stressor; T3, 35–50 min post-stressor; and T4, 55-75 min post-stressor. Cortisol samples containing enough saliva and taken at the right time were available for 176 infants at T1, 171 infants at T2, 173 infants at T3, and 148 infants at T4.

To measure cortisol reactivity, we used the highest post-stressor concentration (T2 or T3), and corrected for pre-stressor cortisol concentrations (T1) to adjust for baseline values (Llabre et al., 1990). Half of the infants showed peak values at T2 (51.1%), and the other half at T3. An independent-samples t-test showed no difference in the magnitude of the peak between the infants that responded at T2 (M=9.23, SD=4.45) versus T3 (M=8.43, SD=4.09, t=1.24, p>.05).

Furthermore, peaking at T2 versus T3 was not significantly related to the number of weeks breastfeeding or co-sleeping (Pearson's r=-.07, p>.10 and r=-.02, p>.10 respectively). Cortisol recovery was measured by obtaining the difference between the last post-stressor sample and the pre-stressor cortisol sample (T4-T1). This measure reflects infants cortisol recovery from the cortisol reaction, with higher scores reflecting slower recovery, and as such is dependent on the height of the peak. Therefore, we corrected for the highest post-stressor concentration (T2/T3) in the analyses on recovery. Peaking at T2 versus T3 was not significantly related to cortisol recovery (Pearson's r=.12, p>.10).

2.7 Potentially confounding variables

Maternal quality of caregiving: sensitivity and cooperation: During a home visit at 5 weeks of age (M=33.5 days, SD=4.9), mothers were asked to bathe their infant as they would normally do (undressing, bathing, and dressing). The videotaped bathing sessions were rated for maternal sensitivity and cooperation (Ainsworth et al., 1978). Sensitivity refers to the extent to which

the mother timely and appropriately responds to the infant's signals and needs, while cooperation refers to the extent to which she adjusts her behavior to the infant's ongoing activity rather than interfering with the infant's actions. Sensitivity and cooperation were scored on 9-point scales, with higher scores reflecting more sensitivity and cooperation. All sessions were rated separately by 2 trained students. In case of non-agreement, the session was also rated by a senior observer. The inter-observer reliability was good, with an intraclass correlation of .94 for sensitivity and .95 for cooperation. Because of the strong correlation between sensitivity and cooperation (Pearson's r=.83, p<.05), the mean score was used.

Maternal depression: Mothers completed the Edinburgh Postnatal Depression Scale (EPDS, Cox, Holden & Sagovsky, 1987) at 3, 6, and 12 months postpartum. This 10-item questionnaire, scored on a 4-point scale, is widely used to measure depression. Because of moderate inter-correlations between the three ages (Pearson's r's ranging from .51 to .63, all p's <.05), mean depression scores across the first year were calculated.

Infant attachment: Tapes of the SSP's were coded at the Institute of Child Development, University of Minnesota. The coders were trained and supervised by Dr. Elizabeth Carlson. The infant's attachment to the mother was categorized as secure or insecure (avoidant, resistant, or disorganized - Ainsworth et al., 1978; Main & Solomon, 1990). Based on a subset of 46 infants, inter-observer reliability was good (Cohen's κ =0.82).

Infant night waking: The daily diaries for sleeping arrangements also provided information about infant night wakings. The mothers were instructed to report the number of night wakings during the previous night by marking this information with crosses in the table. Night waking was defined as an episode of infant arousal during the nighttime to which the parent reacted with physical approach and resettling the child. The mean number of night wakings between 0000h to 0500h for the first six months of life, and again for weeks 51-52, was calculated and used as confounders.

Infant temperament: Mothers completed the Infant Behavior Questionnaire-Revised (IBQ-R, Gartstein & Rothbart, 2003) at 3, 6, and 12 months of age. The IBQ-R consists of 191 items, scored on a 4-point scale. The

191 items constitute 14 scales that have been factor analyzed to yield 3 broad temperament dimensions (Gartstein & Rothbart, 2003). In this study we used the infant's score for the temperamental dimension of negative affectivity. Cronbach's alphas for this scale were sufficient on all three ages, ranging from .71 to .91. For each infant a mean negative affectivity score over the three ages was calculated (Pearson's *r*'s ranging from .40 to .58, all p's <.05), and used as a confounder.

Additional confounders: We also included infant sex, maternal educational level, number of siblings, attending centre-based childcare (yes or no) in the first 12 months of life, the time of day when the SSP took place and the time since the last feeding and sleep as confounders.

2.9 Statistical analyses

Missing data: First, we aggregated the infant sleeping arrangements data per week. Co-sleeping percentages were calculated when at least 3 of 7 days had been completed. Infants were included only if data was available for 17 weeks or more. Of the 193 mothers, 173 adequately collected information on infant sleeping arrangements during the first 6 months of life. No differences in demographic characteristics were found between the 173 mothers and those that did not collect sufficient sleep data (N=20). From the sample of 173 infants, 105 infants had no missing weeks, 44 infants missed 1 to 2 weeks and 24 infants missed 3 to 10 weeks. Of the total number of 4368 weeks, 88.0% of the weeks (3844 weeks) were based on data from 7 nights, 9.5% of the weeks (414 weeks) were based on data from 5 or 6 nights and 2.5% of the weeks (110 weeks) were based on data from 3 or 4 nights. Missing weeks were replaced with the average score of the previous and following weeks.

In addition, weekly feeding data were adequately collected by 173 mothers (data for at least 17 weeks). From this sample, 139 infants had no missing weeks, 21 infants missed 1 to 2 weeks and 13 infants missed 3 to 10 weeks. Similar to sleeping arrangements, missing weeks were replaced with the average score of the previous and following week.

Statistical analyses: Square root or logarithm transformations were applied to skewed data. To analyze if the SSP induced a significant change

in cortisol for the whole group of infants, a repeated measures analysis of variance (ANOVA) was conducted. To explore the interaction between breastfeeding and co-sleeping, these variables were centered prior to computing the interaction term (Aiken & West, 1991). For testing whether breastfeeding, co-sleeping and breastfeeding X co-sleeping predicted infant cortisol regulation, two standard multiple hierarchical regression models were computed for both cortisol reactivity and cortisol recovery. The first model contained all confounders and predictors. For elimination of irrelevant confounders and predictors and for increasing power, the second model contained only the variables that individually explained at least 1% of the variance in the first model. The explained variance was calculated as (part correlation)^2 * 100. These final models, with all maintained confounders in hierarchical step 1 and all maintained predictors in hierarchical step 2, are presented in the Results and in Table 3.

3. Results

3.1 Preliminary analyses

Descriptive statistics of the raw data are presented in Table 1. Infant reactivity scores (highest cortisol value of T2/T3) were log transformed, and the skewness improved importantly. Figure 1 shows the percentage of infants co-sleeping and being breastfed (exclusively breastfed or in combination with bottle-feeding) during the first six months of life. In our sample, 13.29% of the infants were exclusively bottle-fed and 19.08% of the infants slept alone from birth onwards. Furthermore, it can be seen that the percentage of infants co-sleeping with their parents decreased more rapidly during the first six months than the percentage of infants being breastfed. At 8 weeks of age, about half of the infants co-slept and half of the infants slept alone. At 22 weeks of age, about half of the infants received breastfeeding (or breastfeeding in combination with bottle-feeding), and half of the infants received bottle-feeding.

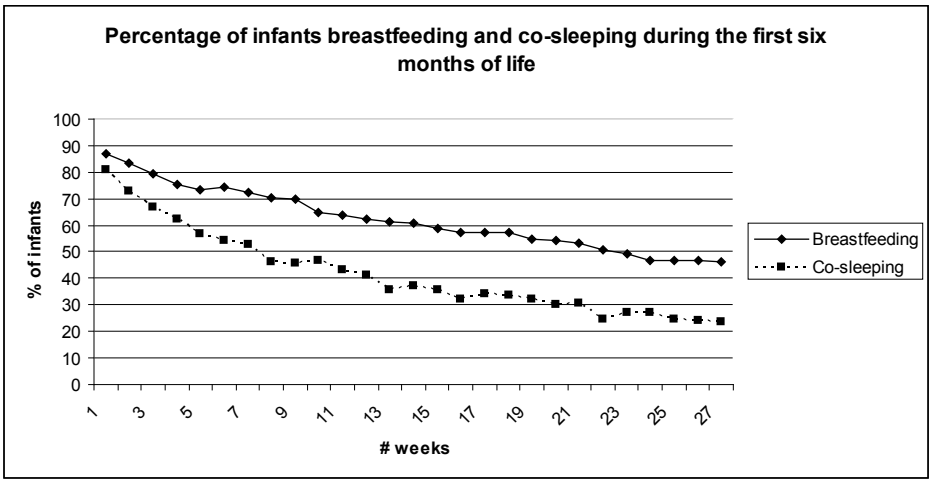

Figure 1: Percentages of breastfeeding and co-sleeping infants

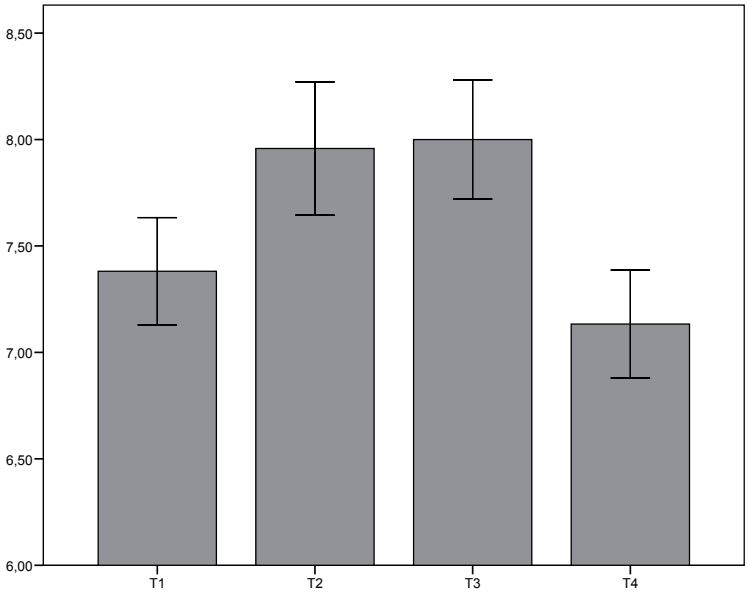

Figure 2. The cortisol response to the Strange Situation Procedure (N's ranging from 148-176).

Note: Error bars are displayed as SE

The cortisol responses to the SSP are presented in Figure 2 (untransformed data). Repeated measures ANOVA revealed a marginally significant quadratic time effect for the repeated measurements of cortisol using all four measurements (T1, T2, T3 and T4; $F(3, 127)=2,96$, $p=.09$, $\eta^2=.02$), and a significant quadratic time effect using only the highest post-stressor concentration of each individual infant (T1, T2 or T3, T4; $F(2, 139)= 17.70$, $p<.0005$, $\eta^2=.11$). Table 2 presents the correlations between breastfeeding, co-sleeping and the cortisol variables. Early breastfeeding and co-sleeping in the first six months of life were moderately intercorrelated ($r=.43$, $p<.05$), just as infant cortisol reactivity and recovery to the SSP ($r=.39$, $p<.05$). Furthermore, breastfeeding and co-sleeping were not related to the cortisol variables, except for a marginally significant correlation between breastfeeding and recovery ($r=.14$, $p<.10$). Table 3 shows the correlations between the confounders and the independent and dependent variables.

Table 2. Pearson correlations for breastfeeding, co-sleeping and infant cortisol variables.

	Breastfeeding	Co-sleeping	Reactivity	Recovery
Breastfeeding	-			
Co-sleeping	.43**	-		
Reactivity	.09	-.01	-	
Recovery	-.14†	-.11	.39**	-

Notes: Breastfeeding and co-sleeping are measured as number of weeks in the first six months of life (wk 1-27). Lower recovery scores indicate quicker recovery. $^{\dagger}= p < .10$, * = $p < .05$, ** = $p < .01$

3.2 Main analyses

The final regression models are summarized in Table 4. The results show that cortisol reactivity to the SSP was predicted by the interaction between breastfeeding and co-sleeping. The interaction is plotted in Figure 3. Subsequently, the single slopes of the regression lines reflecting the relation between co-sleeping and reactivity for infants with different durations of breastfeeding (low, mean, or high) were tested for significance, using the method of Hayes and Matthes (2009). Only the slope for infants with high levels of breastfeeding (mean+1SD) was significant, indicating that more

co-sleeping predicted lower cortisol reactivity in infants who were breastfed for a relatively long period (i.e. six months; t=-2.61, p<.05). For infants who received less breastfeeding, co-sleeping was not significantly related to cortisol reactivity (t=1.50, p=.14 for low duration of breastfeeding, and t=.38, p=.71 for mean duration of breastfeeding). In addition to these findings, higher infant cortisol reactivity was predicted by having more siblings, less negative affectivity, more night wakings during the first six months of life, longer time since last sleep, and higher pre-stressor cortisol. Furthermore, secure attachment[4] showed a trend to be related to lower infant cortisol reactivity. Infant cortisol recovery after the SSP, with cortisol reactivity controlled for, was only predicted by duration of breastfeeding. More weeks of breastfeeding predicted quicker recovery from the SSP.

Table 3. Pearson correlations for the dependent and independent variables and confounders

	Breastfeeding	Co-sleeping	Reactivity	Recovery
Maternal educational level	.19*	.26**	-.10	-.11
Infant sex	.02	-.04	.03	.06
Number of siblings	-.02	.18*	.17*	.06
Maternal quality of caregiving	.01	.09	.03	.03
Maternal depression	.02	.09	-.08	-.03
Temperament (negative affectivity)	.18*	.01	-.05	-.13
Attachment status (0=secure, 1=insecure)	.00	.07	.18*	-.01
Night waking 0-6 months	.29**	.39**	.09	.02
Night waking at 12 months	.17*	.15	.02	.07
Breastfeeding at 12 months	.36**	.26**	.03	-.07
Co-sleeping at 12 months	.05	.31**	.05	-.05
Attendance childcare centre	.10	.05	-.08	-.17*
Time of day	.09	.22**	-.14	-.10
Hours since last feeding	-.02	.02	.09	-.01
Hours since last sleep	-.07	-.11	.16*	.07

Notes: Breastfeeding and co-sleeping are measured as number of weeks in the first six months of life (wk 1-27). Lower recovery scores indicate quicker recovery. * = p < .05, ** = p < .01

4 In contrast to the findings by Luijk et al., (2010), the variable 'resistant attachment versus non-resistant attachment', instead of 'attachment security', was not significantly related to cortisol regulation in our sample.

Table 4. Final multiple hierarchical regression models for the prediction of infant cortisol regulation from breastfeeding and co-sleeping.

		B	β	R^2_{model}	F_{change}	R^2_{change}
Reactivity						
Step 1	Number of siblings	.06	.22**			
	Temperament (negative affectivity)	-.07	-.17*			
	Night waking 0-6 months	.09	.18*			
	Attachment status (0=secure, 1=insecure)	.06	.14†			
	Time since last sleep	.76	.18*			
	Pre-stressor cortisol	.01	.17*	.14	3.78	.14**
Step 2	Breastfeeding 0-6 months	.00	.07			
	Co-sleeping 0-6 months	-.00	-.08			
	Breastfeeding X co-sleeping	.00	-.22*	.20	3.41	.06*
Recovery						
Step 1	Night waking at 12 months	1.51	.12			
	Attendance childcare centre	-.97	-.12			
	Reactivity cortisol	.43	.44**	.22	10.74	.22**
Step 2	Breastfeeding 0-6 months	-.07	-.18*	.25	4.86	.03*

Notes: B = regression coefficient, β = standardized regression coefficient, R^2_{model} = total explained variance by the model, F_{change} = F statistic corresponding R^2_{change}, R^2_{change} = partial explained variance by added predictors (Step 2). Excluded variables explained individually less than 1% variance in cortisol reactivity and recovery in preliminary regression analyses. † = $p < .10$, * = $p < .05$, ** = $p < .01$

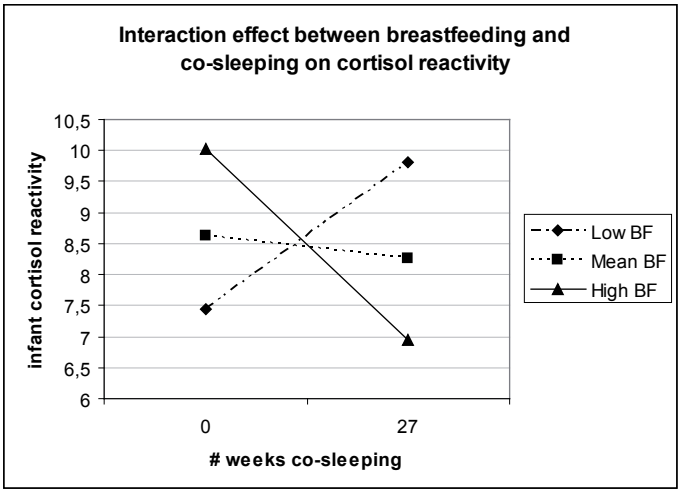

Figure 3. Interaction effect between breastfeeding and co-sleeping on cortisol reactivity

Notes: BF stands for breastfeeding. Mean breastfeeding is 16.8 weeks. Low breastfeeding is mean-1SD and high breastfeeding is mean+1SD. Only the slope for infants with high levels of breastfeeding was significant (t=-2.61, p<.05), while the slopes for infants with low and mean levels of breastfeeding were not significant (t=1.50, p=.14, and t=.38, p=.71 respectively).

4. Discussion

This study examined whether the history of breastfeeding and co-sleeping during the first 6 months of life was associated with infant cortisol regulation, i.e. reactivity and recovery, to a psychological stressor (the Strange Situation Procedure) at 12 months of age. We hypothesized that longer periods of co-sleeping and breastfeeding would predict better cortisol regulation in the infant, i.e. a less strong cortisol reaction to the SSP and a quicker recovery afterwards. The results partly support our hypothesis. A longer period of co-sleeping indeed predicted lower cortisol reactivity to the SSP, but only for infants who also received breastfeeding for a relatively long period (i.e. six months). Longer periods of co-sleeping and breastfeeding by themselves did not predict cortisol reactivity. Also, more weeks of breastfeeding, but not co-sleeping, predicted quicker cortisol recovery. These effects were found after correcting for several mother, infant and environmental characteristics, including maternal sensitivity, infant attachment security, and feeding and co-sleeping practices at 12 months of age.

In line with our hypothesis, we found that, for infants who received breastfeeding for a relatively long period, more weeks of co-sleeping in the first six months of life predicted lower cortisol reactivity at 12 months of age. Unique features specific for the breastfeeding and co-sleeping combination, i.e. the increased number of feedings and/or the increased contact outside the feeding and sleeping context, might be one explanation for lower infant cortisol reactivity. Another explanation might be that the combination of high levels of breastfeeding and co-sleeping provide maximal opportunities for parental co-regulation. By both breastfeeding and co-sleeping, infants would have their parents available to help them regulate and to protect them from high levels of distress during the whole day. Especially when these caregiving practices continue for a long period of time, i.e. six months, a synergy could be developed in the relationship between mothers and infants that in time facilitate the infants' own capacities to regulate physiological stress.

This study not only examined infant cortisol reactivity, but also recovery. Prior research already underlined the importance of considering cortisol recovery, as high and prolonged cortisol elevations as a response to

stressors are known to further influence HPA-axis functioning and to have pathological effects (Sapolsky et al., 2000). Our study found that more weeks of breastfeeding predicted quicker cortisol recovery. There are several possible explanations for this finding. One is that the quicker cortisol recovery to the psychological stressor lies in the breast milk itself. Breast milk is a source of various hormones and growth factors, which are either not found in commercial milk formulas or their presence is still controversial (Savino et al., 2009). As such, breast milk has an important role in the regulation of growth, energy metabolism and development in the neonatal age and infancy (Kramer et al., 2008; Savino et al., 2009), and could influence the development of HPA-axis physiology. Another possible explanation could be based on the fact that breastfeeding mothers might regularly use the breast as a means of soothing the infant during the first months of life. A recent study has shown that during a painful procedure early in life, i.e. a heelstick procedure, the odor of maternal breast milk calms the infant and reduces the infant's HPA-reaction to the stressor (Nishitani et al. 2009). In this line, one could hypothesize that the link between breastfeeding and quicker cortisol recovery from a stressor at 12 months of age is the result of a history of early life maternal soothing and co-regulating by means of putting the infant to the breast.

Alternative explanations can also be raised for the links between a history of breastfeeding and co-sleeping and cortisol regulation in 12-month-old infants. One explanation could be that parents' decisions with respect to where and how their infant sleeps and feeds, reflects different underlying antecedent parental personality characteristics, views on parenting and/or different parenting styles (Britton, Britton & Gronwaldt, 2006). Although our study controlled for maternal sensitivity and cooperation, other parenting and personality characteristics such as ego-resiliency, could still be related to both the choice for specific caregiving arrangements as well as the ability to serve as an external stress regulator. Ego-resilient persons, for example, are known to be able to adapt adequately to stressful situations and are characterised by the dynamic capacity to regulate impulses and emotions (Van Bakel & Riksen-Walraven, 2002). Furthermore, the choice for breastfeeding and/or co-sleeping may not have been based on voluntary and intrinsic parental motivations,

but on circumstances that forced parents to do otherwise. For example, it could be that parents slept or did not sleep together with their infant because of space limitations. In turn, these circumstances might be associated with life events or other environmental stressors that could be related to infant cortisol regulation. Finally, infant cortisol regulation may be affected or programmed by underlying (epi)genetic mechanisms, rather than caregiving practices, as underlying genetic factors could be responsible for both parental HPA-axis functioning and parental motivation for specific caregiving practices.

Our study has several strengths, including the control for many relevant confounders, and the weekly and daily measuring of breastfeeding and co-sleeping, respectively, in the first 6 months of life. Where prior research focused mostly on current co-sleeping or breastfeeding status, we assessed the early feeding and sleeping history of the infant. However, some limitations should also be noted. Almost all mothers were highly educated and lived together with their partner, which compromises the generalizability of the study. Furthermore, due to the relatively intensive longitudinal design, the breastfeeding and sleep data had to be based on maternal report. However, a recent validation study showed good agreement between paper sleep diaries and actigraphy, and concluded that the paper diary is a valid and well-accepted method for the assessment of infant sleep (Müller, Hemmi, Wilhelm, Barr & Schneider, 2011). Nonetheless, future research might consider observational methods, as these would also provide insight in the parent-infant interaction patterns during feeding and sleeping practices. In that vein, it would also be interesting to look at infant sleep and proximity to the parents during the day.

Finally, our study did not differentiate between initial and reactive co-sleeping infants. Reactive co-sleeping refers to infants who start to co-sleep or return to co-sleep at a later age, and following an extended period of solitary sleeping (Hayes, Fukumizu, Troese, Sallinen & Gilles, 2007; Keller & Goldberg, 2004). Ramos and colleagues (2007) also define reactive co-sleeping as children who co-sleep because they have difficulty sleeping alone, even though the parents prefer separate sleeping arrangements. Prior research already suggests that reactive co-sleepers (compared to infants of parents who plan and choose to co-sleep from early infancy onward), might be at risk for sleep-related

problems (Keller & Goldberg, 2004; Ramos, Youngclarke & Anderson, 2003). Also, a study by Taylor, Donovan, and Leavitt (2008) showed that mother-infant dyads who experienced consistency in sleeping arrangements in a typical week at 6 months (i.e., habitual co-sleeping or non co-sleeping) were characterized by more positive behavior and interactions at 9 months as compared to dyads who experienced inconsistency in sleeping arrangements. In this line, cortisol regulation can be hypothesized to develop differently in initial and reactive co-sleeping infants. Unfortunately, our study did not keep track of the parental reasons behind their sleeping practices, or for starting or returning to co-sleeping at a later infant age, which is crucial for disentangling initial and reactive co-sleeping and its possible effects on HPA-axis development.

This study used cortisol as an indicator of infant HPA-axis functioning. Including behavioral measures or measures of the sympathetic nervous system, such as cardiovascular measures and measures of alpha amylase, could render a more complete picture of the influence of early caregiving practices on infants' stress reactions. Lastly, this study found an interaction effect between breastfeeding and co-sleeping during the first six months of life on infant cortisol reactivity at12 months of age. To gain more insight in the mechanisms behind this interaction, follow-up studies examining the dynamics between these early caregiving practices are necessary.

In sum, these findings support the hypothesis that positive early experiences, including breastfeeding and co-sleeping, contribute positively to infant HPA-axis functioning later in life. As such, variations within a normal range of parental care apparently can alter HPA-axis development (Szyf, Weaver, Champagne, Diorio & Meaney, 2005). However, the underlying mechanisms remain unclear and should be topic of future research.

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Chapter 5

Attachment and infant night waking: a longitudinal study from birth through the first year of life

Roseriet Beijers, Jarno Jansen, Marianne Riksen-Walraven & Carolina de Weerth

Beijers, R., Jansen, J., Riksen-Walraven, J.M.A. & de Weerth (2011). Attachment and infant night waking: a longitudinal study from birth through the first year of life. *Journal of Developmental and Behavioural Pediatrics 32*(9): 635-643.

Abstract

Objective: Night wakings are common in infancy. Although a link between infant night wakings and attachment to the primary caregiver has been previously proposed, empirical support is limited so far. The aim of this longitudinal study was to examine the early history of night waking in infants that were later classified as securely or insecurely (avoidantly, resistantly or disorganized) attached.

Methods: Participants in the study were 193 infants and their mothers. Information on infant night wakings was collected with the use of daily sleep diaries for the first 6 months of life and again for two weeks at 12 months of age. Infant-mother attachment was assessed using the Strange Situation (Ainsworth, Blehar, Waters & Wall, 1978) when the infants were 12 months of age.

Results: Longitudinal regression analyses showed that, after controlling for many covariates, infants with an insecure-resistant attachment at 12 months of age awoke more during the night in their first six months of life than the other infants. Furthermore, infants with different attachment classifications developed different patterns of night wakings over the first six months, with the insecure-avoidant infants waking the least towards the end of the six months. Hierarchical multiple regression analyses showed no associations between attachment and night wakings at 12 months of age.

Conclusion: This study is the first in showing that attachment at 12 months of age is related to infant night waking patterns in the first six months of life. Patterns of infant night wakings early in life apparently reflect the emerging attachment relationship.

1. Introduction

During the first year of life, night wakings are a natural phenomenon for infants. Measures obtained by actigraphy, video, and polysomnography reveal that infants wake up on average three times during the night from birth to one year of age (Touchette, Petit, Tremblay & Montplaisir, 2009). The differences between infants stem more from the infant's response, i.e. either signalling the waking or self-soothing back to sleep, than from

the waking itself. At birth, 95% of the infants signal at least one waking at night, while at five months of age 20% of the infants signal at least one waking per night. Most infants are able to self-sooth and resume sleep without alerting their parent by the time they are 12 months of age (Touchette et al., 2009). However, not all infants manage to sleep through the night. Night wakings can cause distress and exhaustion for parents, and are among the most common complaints to paediatricians and other professionals (Sadeh, Tikotzky & Scher, 2010).

Although sleep-wake regulation relies primarily on the maturation of biological networks, it is also modulated by psycho-social factors (Anders, 1994). Night wakings have been previously linked to maternal variables, including depression, contextual variables, including co-sleeping, and infant variables, including temperament (Sadeh et al., 2010). Another psychosocial factor that is hypothesized to be linked to infant night wakings is infant-mother attachment. Attachment refers to the relatively enduring emotional tie of the infant to the caregiver that enables the infant to deal with stressful situations and negative emotions (Bowlby, 1969). During stressful situations, securely attached infants turn to the caregiver for comfort and protection, and, when comforted, they return to exploring the environment. Insecurely attached infants cannot use their caregiver as a secure base. The different types of insecurely attached infants react in a different manner under stress: while *insecure-avoidant* infants minimize the expression of negative emotions and tend to avoid proximity to the caregiver, *insecure-resistant* infants maximize the expression of negative emotions and remain focused on the parent at the expense of exploration. These insecure forms of attachment are considered to be adaptive to the (sub-optimal) child rearing environments that these infants experience (Ainsworth et al., 1978). *Disorganized* attachment is considered to be the most insecure form of attachment; attachment disorganisation is thought to reflect the breakdown of other, "organized" strategies to deal with a stressful situation (Main & Solomon, 1990).

The threat of separation or loss of the attachment figure is a major stressor, especially during infancy and early childhood. As in the

Western world sleep typically involves separating from the caregiver, separation at bedtime and the absence of the attachment figure during night wakings could constitute anxiety-provoking experiences for the child. As a consequence, the attachment system would be activated at night, and infants would signal their needs according to the attachment strategies they have adopted (Scher & Asher, 2004; Scher, 2008).

Although a link between infant night wakings and attachment can be hypothesized, empirical support so far is limited (Sadeh et al., 2010). In a study including 94 one-year-olds, the percentage of infants who were defined by their mothers as night wakers was high in both the secure and resistant infants (Scher, 2001). Also, in a subgroup of 37 infants, actigraphic measures revealed no links between the infant's attachment classification and night waking. Importantly, because disorganized attachment was not rated in this study, and there was a low incidence of avoidant attached infants, night wakings were not examined in these two groups of infants. In another study with 57 one-year-old infants, the degree of security was also not associated with the frequency of reported night wakings or with actigraphic measures of night wakings in a subsample of 44 infants (Scher & Asher, 2004). In conclusion, these two studies failed to find support for the link between attachment and infant night wakings.

Morrell and Steele (2003) compared attachment classifications in 14- to 16-month-old infants with sleep problems (N=40) and without sleep problems (N=60). The frequency and duration of reported night wakings was greater in the resistant group, and a follow-up assessment at two years of age indicated that sleep problems were more likely to persist in this group. Another study examining the link between night waking and attachment was based on the large-scale longitudinal NICHD Study of Early Child Care (McNamara, Belsky & Fearon, 2003). Compared to the avoidant infants, resistant infants were reported to wake more at night when they were 6 months old, but not when they were 15 months old.

To summarize, research provides limited support for the link between infant night wakings and attachment. While some studies related resistant attachment to more night wakings, other studies failed to find support for

the link between attachment and night wakings. Moreover, the few studies that have been done have only marginally controlled for other factors that can affect an infant's sleep or sleep-attachment associations (e.g. co-sleeping and maternal depression), and they mostly concentrated on one-year-olds. The quality of the parent-child attachment relationship can not be reliably assessed before 12 months of age, when a "clear-cut attachment" has emerged (Ainsworth et al., 1978). It is therefore not surprising that previous research examined night waking in relation to attachment in infants beyond the first year of life. However, the parent-child attachment relationship develops gradually over the first year of life. From birth onwards, infants build up expectations of how their needs will be met by their caregivers, and these expectations may be reflected in signalling or other contact-promoting behaviours (Ainsworth et al., 1978). Hence, infants' reaction to their waking up at night could reflect the emerging parent-child attachment relationship. In the present prospective longitudinal study we aimed to examine this by relating infants' night waking during the first six months of life to the quality of the mother-child attachment relationship at 12 months of age, the first age at which mother-child attachment can be reliably assessed and classified as secure or insecure (avoidant, resistant or disorganized). Also, in order to replicate prior research, this study examined the relationship between attachment and night wakings at 12 months of age.

2. Methods

2.1 Participants

This study is part of a prospective longitudinal project that investigates the influences of early caregiving factors on the development of children during their first years of life. The subjects were healthy infants living in the Netherlands, whose mothers were recruited during late pregnancy. Mothers responded on a voluntary basis to flyers that were spread amongst midwife practices in the cities of Nijmegen, Arnhem and surrounding areas. The study was approved by the faculty ethical committee and informed consent was obtained from each mother before starting. Inclusion criteria were: an uncomplicated, singleton pregnancy, no drug use, and no current physical or

mental health problems. Of the 220 women that enrolled in the study, 8 were excluded because of medical reasons, such as preterm birth. Of the remaining 212 mothers, a further 19 discontinued the study during the first 3 postpartum months due to personal circumstances. This resulted in a final sample of 193 mothers and their infants, from which the demographic characteristics are provided in Table 1. No differences in these demographic characteristics were found between participating mothers and those that dropped out (n=19).

2.2 Procedure

Information on infant night wakings was collected with the use of daily sleep diaries for the first six months of life and again for two consecutive weeks at 12 months of age. These sleep diaries also provided information about the type of sleeping arrangements (in the infant's own room or in the parent's room/bed) and the type of feeding (breast or bottle). Furthermore, infant-mother attachment was assessed when the infants were 12 months of age using the

Table 1: Descriptive statistics of the demographic characteristics (n=193).

	Mean *(SD)*	Range
Demographics characteristics		
Maternal age (years)	32.46 *(3.79)*	21.1–42.9
Maternal educational level (%)		
Primary education	3.8%	
Secondary education	20.4%	
College/University	75.8%	
Maternal marital status (in wedlock/living together - %)	97.9%	
Birth weight (grams)	3616.97 *(465.32)*	2645.0–4730.0
Apgar score at 5 minutes	9.66 *(.63)*	7.0–10.0
Infant sex (%)		
Girl	47.2%	
Boys	52.8%	
Birth order (%)		
Firstborn	41.0%	
Second born	43.8%	
Third or fourth born	15.2%	

(SD)=standard deviation.

Strange Situation (Ainsworth et al., 1978). Maternal sensitivity and cooperation were controlled for using ratings of maternal behaviour during a videotaped mother-infant bathing session when the infant was five weeks of age. We also controlled for maternal depression and infant temperament, which were assessed using questionnaires that were filled in postnatally at 3, 6 and 12 months. Finally, additional information on mother and infant was obtained with questionnaires filled in during the last trimester of pregnancy and immediately after birth.

2.3 Sleep diary

Information on infant night wakings was collected using daily sleep diaries for the first 6 months of life (weeks 1-27) and again for two consecutive weeks at 12 months of age (weeks 51-52). The mothers received the sleep diary and accompanying instructions at the end of pregnancy, so they could start immediately after birth. Mothers were instructed to complete the diary every morning by reporting the hours of sleep and the number of night wakings during the previous night. The mothers could mark this information with lines for sleep and crosses for wakings in a table that consisted of 30-minute time blocks spanning between 2000h and 0800h. Night waking was defined as an episode of infant arousal during the nighttime to which the parent reacted with physical approach and resettling the child. Similar to Anders and Keener (1985), we defined nighttime as 0000-0500h, the usual time of the parents' sleep.

2.4 Attachment

During a lab visit at 12 months of age (*M*=53 weeks and 6 days, *SD*= 19 days), the infants were observed during the Strange Situation to assess their attachment pattern. The 20-minute Strange Situation involves a sequence of episodes which progressively activate the attachment system with an unfamiliar environment, the arrival of a stranger and two brief separations from the mother (Ainsworth et al., 1978). Tapes of all the Strange Situations were coded at the Institute of Child Development, University of Minnesota. The coders were trained and supervised by Dr. Elizabeth Carlson. The infant's attachment behavior was categorized as either secure, avoidant, resistant, or

disorganized. Based on a subset of 46 infants, inter-observer reliability was very good (Cohen's kappa of 0.82, Intraclass correlation of 0.86).

2.5 Covariates

Infant temperament. Mothers completed the Infant Behavior Questionnaire-Revised (IBQ-R, Gartstein & Rothbart, 2003) at 3, 6 and 12 months of age. The IBQ-R consists of 191 items, scored on 4-point scales. These 191 items constitute 14 scales that have been factor analyzed to yield three overarching temperament dimensions: surgency, regulatory capacity and negative affectivity. Because irritability and fussiness have been found to be associated with infant sleeping problems, negative affectivity was used in this study. This dimension consists of the scales sadness, distress to limitations, fear, and falling reactivity (loads negatively). Because of moderate inter-correlations (r= .53, p <.001), negative affectivity scores at three and six months were averaged and used as a confounder in the relation between attachment and night wakings in the first six months of life. The infant negative affectivity scores at 12 months of age were used as a confounder in examining the relationship between attachment and night wakings at 12 months of age.

Maternal depression. Mothers completed the Edinburgh Postnatal Depression Scale (EPDS, Cox, Holden & Sagovsky, 1987) at 3, 6, and 12 months postpartum. This 10-item questionnaire, scored on 4-point scales, is widely used to measure depression. Because of moderate inter-correlations (r= .51, p<.001), depression scores at three and six months were averaged and used as a confounder in the analyses regarding the first six months of life. The depression scores at 12 months postpartum were used as a confounder in examining the relationship between attachment and night wakings at 12 months of age.

Maternal sensitivity and cooperation. During a home visit at five weeks of age (M=33.5 days, SD=4.9), mothers were asked to bathe their infant as they would normally do (undressing, bathing, and dressing). The videotaped bathing sessions were rated for maternal sensitivity and cooperation (Ainsworth et al., 1978). Sensitivity refers to the extent to which caregivers timely and

appropriately respond to the infant's signals and needs, while cooperation refers to the extent to which caregivers adjust their behaviour to the infant's ongoing activity rather than interfering with the infant's actions. Sensitivity and cooperation were scored using 9-point rating scales, with higher scores reflecting more sensitivity and cooperation. The sessions were rated separately by at least two trained students, and the inter-observer reliability was good (Cohen's Kappa's of 0.90 for both sensitivity and cooperation).

Co-sleeping. The sleep diaries also provided information about sleeping arrangements. For every time block the parent indicated whether the infant had slept in its own room, in the parents' room in a separate bed, in the parents' bed, or somewhere else. Because less than 5% of the infants had the parents' bed as the standard arrangement, we classified sleeping in the parents' bed and sleeping in the parents' room in a separate bed both as co-sleeping. Per night, the number of hours co-sleeping was divided by the total hours of infant sleep and multiplied by 100 in order to obtain the percentage of co-sleeping. The average percentage of co-sleeping per week was used as a covariate.

Breastfeeding. As part of the sleep diary, each week the mothers noted the average number of breast and/or bottle feedings per day. The average number of daily breast feedings was used as a covariate.

Additional covariates. We also included infant sex, maternal educational level and number of siblings as covariates.

2.6 Statistical analyses

Missing data. First we aggregated the infant sleep data per week. Averages were calculated when at least three out of seven days were completed. Infants were included only if sleep data were available for five weeks or more. Of the 193 mothers, information on infant sleep during the first six months of life was adequately collected by 177 mothers. From this sample, 105 infants had no missing weeks, 44 infants missed 1-2 weeks, 24 infants missed 3-10 weeks and 4 infants missed more than 10 weeks. Out of the total number of 4543 weeks, 88.1% of the weeks (4001 weeks) were based on data from seven nights.

From the 177 infants, 160 mothers also provided sleep data when the infants were 12 months of age. In accordance to the sleep data in the first six months of life, the data were aggregated per week and averages were calculated when at least three out of seven days were completed. For 156 infants, the sleep data on 12 months were based on two weeks. Out of the 316 weeks, 95.3% of the weeks (301 weeks) were based on data from seven nights.

Finally, out of the 177 infants, 3 infants were not able to participate in the Strange Situation because of scheduling problems. Also one infant was excluded because of being ill during the Strange Situation.

Statistical analyses. To test whether attachment was related to night wakings during the first six months of life, longitudinal regression analyses using mixed-model (multilevel) designs in SPSS 15.0 were conducted. Time in weeks was introduced at level 1 and nested within the infants at level 2. First, the intraclass correlation was calculated using a null model, to examine whether the nested structure is needed for the analyses. The intraclass correlation was .39 (.08/(.08+.12)), so 39% of the variability in night wakings were associated with differences between infants, and multilevel analyses were appropriate. Second, a build-up strategy was used, adding predictors into the model one by one at the time and examining their deviance on the -2loglikelihood ratio scale after generalized least square estimation. None of the predictors were centered prior to entering them into the model. Linear time, quadratic time and cubic time were first entered into the model. Linear time was considered a random factor. The time model which improved the model fit the best was retained, and thereafter the covariates were entered one by one at the time. We then added the dummy variables to code for attachment classification (the securely attached infants served as reference group). In the last models we also included the interactions between the dummies and time variables (calculated by multiplying the dummy by the time variable) to examine the effect of attachment on night waking over time. The best fitting models were presented in the results.

To test whether attachment classification was related to infant night wakings at 12 months of age, multiple hierarchical regression analyses were conducted in SPSS 15.0. The mean night waking score, derived from week 51

and 52, was used as dependent variable. Relevant covariates were based on their (marginal) significant Pearson correlation with infant night waking (p<.10). The regression model, with all maintained covariates in hierarchical step 1 and the dummy variables representing the attachment classifications in hierarchical step 2, are presented in the results.

In common with many other researchers in the field, we regard the disorganized classification as orthogonal to the avoidant, secure or resistant classification. Therefore, the analyses were done first using the ABCD classification, and then repeated with the ABC classification whereby the disorganised infants were added to their underlying ABC category.

3. Results

3.1 Descriptive statistics

Of the 173 infants, 120 (68.2%) were classified as securely attached, 7 (4%) as insecure-avoidant, 21 (11.9%) as insecure-resistant, and 24 (13.6%) as disorganized. One infant was unclassifiable. Among the infants with D-classification, the number of infants having an underlying secure, avoidant or resistant attachment classification was 12, 3 and 9, respectively.

Figure 1 shows the course of infant night waking in the first half year of life, divided per attachment classification. During the first few weeks of life, little differences in night wakings between infants of different attachment classifications can be seen, but these differences begin to emerge at around seven to eight weeks. Infants ending up being avoidantly attached show a sharp decrease and then remain stable at around 0.2 wakings per night. This means that, on average, parents of avoidantly attached infants need to resettle their infant one or two times per week from nine weeks onwards. Securely, resistant and disorganized infants show a more gradual decrease in their night wakings. At 12 months of age, avoidantly attached infants rarely need to be resettled by their parent at night (M=.05, SD=.05), while securely, resistant and disorganised attached infants need to be resettled once or twice per week (M=.24, SD=.34; M=.18, SD=.22; and M=.13, SD=.31 respectively).

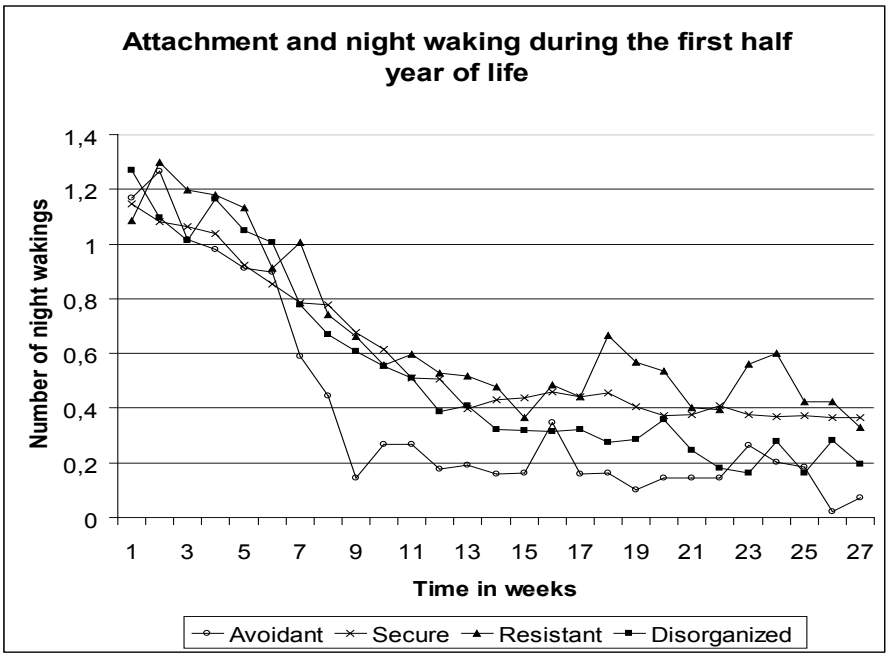

Figure 1: The course of infant night wakings in the first six months of life (N=177).
Note: Night waking was defined as an episode of infant arousal during the nighttime to which the parent reacted with physical approach and resettling the child.

3.2 Main analyses

Attachment and night wakings during the first six months of life. The best fitting multilevel models are presented in Table 2. Besides the three time variables, both breastfeeding and co-sleeping were positively related to more infant night wakings. Furthermore, the multilevel model based on the ABCD-classification showed a marginally significant effect from the dummy resistant attachment. Also, the interactions between the time variables and the attachment dummies showed significant effects, suggesting that the avoidant and disorganised attached infants develop a different pattern of night wakings over time than the securely and resistant attached infants. The model with the attachment dummies and interaction variables between time and attachment led to a significantly better fit (-2 log likelihood = 3357.890), compared to the model with only the time variables and covariates (-2 log likelihood = 3419.003, $\chi^2\,(9) = 61.113$, $p < .001$).

Table 2: Estimates for the best fitting multilevel models for night wakings during the first six months of life.

	ABCD-classification			ABC-classification		
	Estimate	S.E.	*p*	Estimate	S.E.	*p*
Fixed effects						
Intercept	.689	.236	.004**	.680	.235	.004**
Time linear	-.097	.008	.000***	-.098	.008	.000***
Time quadratic	.004	.001	.000***	.004	.001	.000***
Time cubic	.000	.000	.000***	.000	.000	.000***
Infant negative affectivity[1]	.024	.037	.507	.023	.037	.533
Breastfeeding	.023	.004	.000***	.024	.004	.000***
Co-sleeping	.001	.000	.000***	.001	.000	.000***
Maternal educational level	.041	.025	.103	.043	.025	.089†
Avoidant attachment	.112	.179	.532	.006	.151	.966
Resistant attachment	.199	.112	.078†	.252	.100	.012*
Disorganized attachment	.097	.108	.372			
Linear time by avoidant attachment	-.039	.016	.014*	-.026	.014	.055†
Linear time by resistant attachment	-.016	.010	.111	-.021	.008	.015*
Linear time by disorganized attachment	-.023	.010	.015*			
Quadratic time by avoidant attachment	.001	.000	.012*	.001	.000	.015*
Quadratic time by resistant attachment	.000	.000	.134	.001	.000	.015*
Quadratic time by disorganized attachment	.001	.000	.036*			
Random effects						
Intercept	.168	.021	.000***	.168	.021	.000***
Time linear	.000	.000	.000***	.000	.000	.000***
Deviance	3357.890			3345.147		

†=*p*<.10, *=*p*<.05, **=*p* <.01, ***=*p* <.001, [1] Averaged score between 3 and 6 months of age

The multilevel model based on the ABC-classification showed a significant effect of the dummy resistant attachment. This indicates that infants who are resistantly attached at 12 months of age, showed more night wakings in their first six months of life. Also, the interactions between the time variables and the attachment dummies showed significant effects, suggesting that the avoidant and resistant infants develop a different pattern of night wakings over time as compared to the securely attached infants. The resistant infants end up waking the most, while the avoidant infants end up waking the least towards the end of the six months. The model with the attachment dummies and interaction variables led to a better fit (-2 log likelihood = 3345.147), compared to the model with only the time variables and covariates (-2 log likelihood = 3419.003, $\chi^2\,(6) = 73.856$, p <.001).

Attachment and infant night wakings at 12 months of age. Table 3 shows Pearson correlations between infant night wakings and all possible covariates. Four (marginally) significant correlations can be seen. More night wakings were associated with higher infant negative affectivity at 12 months of age (r=.23, p<.01), higher percentages of co-sleeping at 12 months of age (r=.22, p<.01), receiving breastfeeding at 12 months of age (r=.15, p<.10), and higher maternal depression scores at 12 months of age (r=.15, p<.10).

Table 3: Pearson correlations between infant night wakings at 12 months of age and covariates.

		1	2	3	4	5	6	7	8	9
1	Night wakings	-								
2	Negative affectivity	.23**	-							
3	Maternal depression	.15†	.22**	-						
4	Maternal sensitivity	.02	-.04	.11	-					
5	Infant sex	-.13	.01	-.04	.03	-				
6	Maternal education	.11	.13†	.01	.15*	.06	-			
7	Number of siblings	.04	.14†	-.08	-.12†	.03	-.16*	-		
8	Co-sleeping	.22**	.11	.17*	.11	-.01	.06	.03	-	
9	Breastfeeding	.15†	.06	.00	-.08	-.18*	.01	.05	.03	-

Table 4: Multiple hierarchical regression models for infant night wakings at 12 months of age.

	ABCD-classification					ABC-classification				
	B	β	R^2_{model}	F_{change}	R^2_{change}	B	β	R^2_{model}	F_{change}	R^2_{change}
Step 1										
Negative affectivity	.100	.184*				.104	.191*			
Co-sleeping	.002	.173*				.003	.180*			
Breastfeeding	.061	.141†				.064	.148†			
Maternal depression	.006	.076	.119**			.006	.068	.119**		
Step 2										
Avoidant attachment	-.166	-.104				-.172	-.127			
Resistant attachment	-.028	-.029				-.024	-.029			
Disorganized attachment	-.085	-.092	.136	.971	.017			.135	1.376	.016

Note: Repetition of the analyses without the two marginally significantly correlated variables (breastfeeding and maternal depression) produced similar results. †=p<.10, *=p<.05, **=p<.01, ***=p< .001

Table 4 shows the two regression models with negative affectivity, co-sleeping, breastfeeding, and maternal depression as covariates in hierarchical step 1 and the dummy variables in hierarchical step 2. While infant night wakings at 12 months of age were related to significantly more negative affectivity and co-sleeping at 12 months of age, it was unrelated to attachment classification.

4. Discussion

This study is the first in showing that infant attachment at 12 months of age is related to night waking patterns in the first six months of life. To this end, important evidence is added to the field, by indicating that patterns of infant night waking apparently reflect the emerging attachment relationship. Our results showed that infants that were resistantly attached at 12 months of age were reported to show more night wakings in their first six months of life. Furthermore, infants with different attachment classifications develop different patterns of night waking. While there are no apparent differences in levels of waking in the first five to six weeks, and all infants decrease in their night wakings over the first six months of life, the avoidantly attached infants end

up with the lowest frequency of reported night wakings at six months of age. Attachment classification was not related to night wakings at 12 months of age. Together, these results are in line with earlier research, relating resistant attachment to more night wakings and avoidant attachment to fewer night wakings in six-month-olds, but not in one-year-olds (McNamara et al., 2003).

Several plausible explanations exist to explain the link between attachment at 12 months of age and (earlier) night waking patterns. A first possible explanation is that endogenous characteristics of the infant, like temperament, are related to both night waking and the manifestation of attachment type. Former research related infant irritability and negative emotionality to more night waking (Carey, 1974; Schaefer, 1990), and to resistant attachment (Mangelsdorf, McHale, Diener, Goldstein & Lehn, 2000; Susman-Stillman, Kalkoske & Egeland, 1996). However, it is unlikely that infant endogenous characteristics are completely responsible for our findings. The development of infant sleep has been conceptualized in the context of a transactional model that emphasizes the ongoing bi-directional links between endogenous (e.g. medical, temperament) factors and exogenous (e.g. ecology, parent-infant relationship) factors (Sadeh et al., 2010). In addition, our study controlled for negative affectivity, and only healthy infants born out of non-complicated pregnancies participated.

Another possible explanation is that night waking patterns reflect the experiences of the infant with the environment, e.g. the primary caregivers. While the attachment bond with the primary caregiver, usually the mother, is assumed to be fully developed around 12 months of age, it is still emerging in the course of the first year of life (Ainsworth et al., 1978). During the very first weeks after birth, called the Initial Preattachment Phase, infants orient and signal to their mothers in much the same way as they do to any other person. This phase normally continues for a few weeks, and possibly corresponds to our first five to six weeks, that were characterized by a lack of clear differences in waking between the attachment groups. In the second phase of attachment development, called the Phase of Attachment-in-the-making, infants not only more clearly discriminate between persons, but also, based on former experiences, build up expectations of how their needs will be met, and

increasingly orient contact-promoting behaviours to the attachment figure. Hence, attachment-in-the-making could be affecting how infants (learn to) cope with their night wakings. Infants with a secure attachment-in-the-making have had satisfying experiences and would signal night wakings to their mothers because they expect her to be available and comforting when called upon. In contrast, infants with a resistant attachment-in-the-making may have had experiences in which bids for proximity have been inconsistently responded to (Ainsworth et al., 1978). Therefore, they would maximize the expression of negative emotions in order to increase the chances of their attachment needs being met, resulting in more signalling during night wakings. Furthermore, infants with an avoidant attachment-in-the-making may have had experiences in which bids for proximity have been rejected, and would therefore avoid maternal contact and minimize the expression of negative emotions, resulting in less signalling during night wakings.

Another, perhaps complementary, explanation is that how caregivers respond to infant night waking (i.e. nighttime sensitivity) is involved in the shaping of attachment relations (Friedman & Boyle, 2008). Because we defined night waking as an episode of infant arousal to which the caregiver reacted with physical approach and resettling the infant, night waking could also be reflecting the behaviour of the caregiver. According to attachment theory, infants develop secure attachment bonds with caregivers who are sensitive, predictable and available (Ainsworth et al., 1978; Bowlby, 1969). More recent research has revealed that sensitivity to infant distress, but not greater sensitivity to non-distress, is central in promoting attachment security (McElwain & Booth-LaForce, 2006). Night wakings do not only frequently occur during infancy, but can also cause distress in infants because of the threat of being alone and in the dark. Although maternal sensitivity during daytime interactions has been frequently linked to attachment security, only one study examined the association between mother-infant nighttime interactions and attachment security (Highley & Dozier, 2009). This study with 44 one-year-olds showed that mothers of securely attached infants were more consistent, sensitive and responsive during nighttime interactions, than mothers of insecurely attached infants. Specifically, in secure dyads, mothers generally

picked up and soothed their infants when they fussed or cried after waking. Nevertheless, to examine the possibility of parents' responses to infant night waking being involved in the shaping of attachment bonds, developmental studies are clearly needed.

The last explanation is that mothers of infants with different attachment classifications might have different perceptions of infant behaviour because of differences in their own attachment style and personality. Although this study controlled for maternal depression, daytime sensitivity and educational level, other maternal characteristics such as maternal attachment status, could influence their interpretations and expectations of infant behaviour. For example, avoidant infants may actually signal as much night wakings as infants with other classifications, but their mothers may perceive them more so than mothers from other classification groups as capable of settling themselves and "not needing" their mothers to go back to sleep. In addition, the sharp decrease that these infants show in Figure 1 could be the result of the mothers of avoidant infants increasing the threshold at which they respond to arousal during the night due to the enactment of maternal expectations that infants should sleep through the night by 7-8 weeks. In this specific example, these different perceptions of infant nighttime signalling would be followed by less resettling during the night, and therefore by fewer reports of infant waking.

Less clear is why infants from the disorganised attachment classification showed more night wakings than the avoidantly attached infants, but less than the resistantly attached infants. Two explanations for these findings can be raised. First, because attachment disorganisation reflects poorly organised attachment behaviour in the face of stress (Main & Solomon, 1990), it could be that these infants use different attachment strategies from night waking to night waking, so that some of the night wakings will be signalled and others will not. Another explanation could be that the disorganised attached infants during night wakings mainly behave according to their subsidiary avoidant, secure or resistant attachment classification. When adding the disorganised attached infants according to their subsidiary attachment classification to their underlying ABC attachment classification, the effects of

the resistant classification turned out to be stronger. Although this strengthens our last explanation, more research is certainly needed to understand how infants that develop a disorganized attachment to their mother, cope with their night wakings early in life.

At 12 months of age, our results indicated that infant night wakings were not related to attachment. Previous research also provided limited support for the hypothesis that attachment would be related to infant night waking in one-year-olds (Sadeh et al., 2010). A possible explanation for finding an association between attachment and night waking in the first six months, but not at 12 months, is that by the end of the first year sleep is well-consolidated and regulated (Touchette et al., 2009). Therefore, clear differences between attachment classifications can probably not be seen, because one-year-olds show less night wakings than infants in their first months of life. Also, the effects of the emerging attachment bond, as an environmental influence on infant sleep, may be stronger when infant sleep is less consolidated (Teti, Kim & Mayer, 2010).

Our study has several strengths, including the control for many relevant confounders (e.g. co-sleeping and breastfeeding, measured on a daily and weekly basis, respectively), and the daily and longitudinal measuring of infant night wakings in the first six months of life. However, some limitations should also be noted. First, mothers responded on a voluntary basis to flyers and it is unknown what motivated mothers to participate and others to decline. This compromises the generalizability of the study to mothers that did not respond to the flyer. Second, due to the relatively large sample size and the intensive longitudinal design, the night waking data and temperament data had to be based on maternal report. Because maternal report can be influenced by maternal characteristics, precautions were taken in this study to reduce a possible reporter bias to the minimum. First, we controlled for maternal depression, maternal educational level and maternal daytime sensitivity. Second, scores were based on data from multiple time points. The infant night waking data were collected using daily sleep diaries, and infant temperament was measured three times during the first year of life. Nonetheless, future research should consider observational methods for infant night waking and

temperament, assessed at multiple time-points to capture stable or evolving infant characteristics.

Our study focused on the frequency of night wakings during the first six months of life. Future research is needed to look at other measures of infant sleep (e.g. length of night wakings or sleep onset problems), which also could be related to attachment. In addition, future research on infant sleep in the age period between 6 and 12 months of age is needed to see how the relationship between night waking and attachment further develops.

5. Conclusion

This study is the first in showing that infant attachment at 12 months of age was related to night waking patterns in the first six months of life. To this end, important evidence is added to the field, by indicating that patterns of infant night waking early in life apparently reflect the emerging attachment relationship. Because night waking was defined as an episode of infant arousal during the nighttime to which the parent reacted with physical approach and resettling the child, it is undecided if differences by attachment classification are related to differences in infant behaviour, differences in adult behaviour, or differences in perceptions of infant behaviour. Future studies including observational measures of caregiver responses to infant night waking, can help unravel how differences in night waking patterns appear and how attachment bonds are formed.

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Chapter 6

Non-parental care and infant health: Do number of hours and number of concurrent arrangements matter?

Roseriet Beijers, Jarno Jansen, Marianne Riksen-Walraven & Carolina de Weerth

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Abstract

Objectives: Previous research found that centre-based childcare is related to more illnesses early in life. The goal of this longitudinal study is to determine whether infant health in the first year of life is also related to the amount of time spent in non-parental care and the number of concurrent non-parental care arrangements.

Methods: Information on infant health and non-parental care was obtained through monthly maternal interviews across the first year of life. The occurences of respiratory, digestive, general, and skin illnesses and complaints were used as dependent variables, while the number of hours and the number of arrangements per week were used as predictors. Analyses were done separately in infants for whom centre-based childcare is included in their arrangements (*n*=107), and for those which it is not (*n*=61).

Results: Infants spending more hours in non-parental care had more respiratory and general illnesses. Infants who were cared for in more concurrent arrangements had fewer respiratory and general, but more skin illnesses. These results only applied to infants that included centre-based childcare in their arrangements. In the group of infants that did not attend centre-based childcare, health was not related to either the number of hours or the number of arrangements.

Conclusions: Number of hours and number of arrangements do matter in relation to infant health early in life, but only for infants who attend centre-based childcare. While more hours were related to more illnesses, more arrangements were related to both fewer and more illnesses, depending on the type of illnesses.

1. Introduction

Previous research consistently found that children who attend centre-based childcare experience more communicable illnesses, like respiratory illnesses, otitis media and diarrhea (Bradley, 2001; Bradley, 2003; Dales, Cakmak, Brand & Judek, 2004; Lu, Samuels, Shi, Baker, Glover & Sanders, 2004; Kamper-Jorgensen, Wohlfahrt, Simonsen, Gronbaek & Benn, 2006). These differences in morbidity are particularly large in children under two years of age (Dales et al.,

2004; Lu et al., 2004; Kamper-Jorgensen et al., 2006). However, studies about the influence of the amount of time spent in non-parental care on children's illnesses have been few and less consistent. While some studies found that the likelihood of acquiring illnesses increases with the number of hours spent in non-parental childcare (Hagerhed-Engman, Bornehag, Sundell & Berg, 2006; Kotch & Bryant, 1990), the large NICHD study found little evidence that the number of hours of childcare per week resulted in an increased illness rate (Bradley, 2001). However, this finding may have emerged because there was little variability in hours of attendance, as the majority of children in this study spent more than 20 hours per week in childcare (Bradley, 2001).

Furthermore, research looking at whether children's health status is affected by the number of concurrent care arrangements is lacking. Number of concurrent care arrangements, also known as arrangement multiplicity, is the number of separate non-parental care arrangements a child regularly experiences during a single day or week. Prior research already showed that children attending more concurrent arrangements have more behavioral problems (De Schipper, Tavecchio, Van IJzendoorn & Van Zeijl, 2004; De Schipper, Van IJzendoorn, & Tavecchio, 2004; Morrisey, 2009), but it is also important to understand the role of number of non-parental care arrangements in infant health, especially since a considerable number of infants experience these changes in arrangements day by day (Morrisey, 2009). The number of concurrent care arrangements is often confounded with the number of changes in care arrangements over time, also known as long-term stability of care arrangements (Morrisey, 2009). Extensive research related long-term stability to negative developmental outcomes, including behavior and health problems (Morrisey, 2009; NICHD, 1998). For instance, the risk of acute respiratory infections was shown to be the highest within the first period of enrollment into a new childcare arrangement, and increased again with a shift to a new childcare facility (Kamper-Jorgensen et al., 2006).

The present study focuses on the number of regular concurrent care arrangements. Moving among different settings exposes an infant to different environments with varied household products and chemicals, which in turn may lead to respiratory illnesses and allergies (Becher, Hongslo, Jantunen &

Dybing, 1996; Sheriff, Farrow, Golding, The Alspac Study Team & Henderson, 2005). In addition, moving among different settings may be a stressful experience for children. Unpredictable, irregular transitions may be especially stressful, but even if regular daily or weekly transitions between arrangements can quickly become routine for parents and caregivers, moving among care settings could be burdening for infants. Frequent transitions may delay or prevent the adjustment to the different care situations and the formation of dietary and sleep routines, which in turn could contribute to increased health problems (Fiese, Tomcho, Douglas, Josephs, Poltrock & Baker, 2002).

Transitions between different care settings can be particularly burdening if these settings differ in group size, routine and physical setting (Morrisey, 2009). While some non-parental care arrangements resemble the home-setting more (for instance care by grandparents), centre-based childcare is characterized by larger group sizes, higher levels of noise, multiple caregivers, and very different routines and physical settings. Previous research has already shown that infants exhibit higher levels of the stress hormone cortisol on days when they are at centre-based childcare compared with days when they are at home (Dettling, Gunnar & Donzella, 1999; Dettling, Parker, Lane, Sebanc & Gunnar, 2000; Watamura, Donzella, Alwin & Gunnar, 2003; Watamura, Sebanc & Gunnar, 2002). This could be indicating that centre-based childcare constitutes a possible challenge for infants. Therefore, and also because it is well-known that centre-based childcare is related to more illnesses early in life, the number of hours and the number of concurrent care arrangements could potentially have different effects for infants attending centre-based childcare in comparison to infants not attending centre-based childcare. Therefore, the group of infants for whom centre-based childcare is included in their 'package' of care arrangements was examined separately from the group of infants for whom centre-based childcare is not included.

The goal of the present longitudinal study is to determine whether more hours spent in non-parental care, and more concurrent care arrangements, are related to more infant illnesses and health complaints during the first year of life. The infants in the present study were followed monthly, from birth until the age of 12 months. This has the advantage of

making it possible to obtain a full array of arrangements and hours, and to obtain robust measures of infant health during the first months in non-parental care. Moreover, our study focused on a broad spectrum of infant illnesses and health complaints, including respiratory, digestive, general, and skin illnesses and complaints.

2. Methods

2.1 Participants

This study is part of an ongoing prospective longitudinal project that investigates the influences of maternal and caregiving factors on the behavioral development and physical health of children during their first years of life. The subjects were healthy infants living in the Netherlands, whose mothers were recruited during pregnancy through midwife practices in the cities of Nijmegen, Arnhem and surrounding areas. The study was approved by the faculty ethical committee and informed consent was obtained from each mother before starting. Of the 220 women that enrolled in the study, 8 were excluded because of medical reasons, such as major birth complications. Of the remaining 212 mothers, a further 19 discontinued the study during the first 3 postpartum months, due to lack of time or other personal circumstances. This resulted in a final sample of 193 infants. No differences in demographic data were found between participating mothers and those that dropped out (n = 19).

Most women lived with their partner, either in wedlock or unmarried (96.9%), and were born in the Netherlands (95.8%). Furthermore, 60% of the mothers professed the Christian religion while 40% were non-religious. All mothers had normal, uncomplicated pregnancies and term deliveries (>37 weeks). The infants had normal 5-min. Apgar scores (M = 9.6, SD = 0.6, min = 7, max = 10), and none of the infants experienced major birth complications.

2.2 Procedure

In the first year of life, information on the frequency of infant illnesses and health complaints was obtained through monthly maternal interviews (3 in person, 9 by phone). These maternal interviews also provided information about the type of non-parental care used, the amount of time the child spent

in non-parental care, the number of concurrent arrangements, and the type of feeding (bottle or breast). Additional information on mother and infant was obtained with questionnaires filled in during the last trimester of pregnancy, immediately after birth or postnatally at 3, 6 and 9 months.

2.3 Infant health

Mothers reported on their infant's health during the previous month in semi-structured interviews conducted at monthly intervals. With the aim of aiding their memory and of increasing the objectivity of the scoring, the interview also contained a checklist consisting of 24 yes-or-no items listing common infant illnesses and health complaints. Subsequently, the infant health data were coded with the International Classification of Primary Care (ICPC, Lamberts & Wood, 1987). The ICPC is an ordering principle labeling illnesses and health complaints in classes according to established criteria, and is widely used both in daily family practice and in research (Soler, Okkes, Wood & Lamberts, 2008). Finally, the occurrence of illnesses and complaints were summed up per month. Because of low incidence of ear- and eye-related illnesses and complaints, these were added to general illnesses and complaints. The following variables were used as dependent variables: respiratory, digestive, general and skin illnesses and complaints.

2.4 Non-parental care predictors

During the semi-structured interviews, mothers were also asked to list the concurrent non-parental care arrangements and the hours per week the child spent in each arrangement. Non-parental care was defined as any type of care by caregivers other than the parents for at least 4 hr per week, and lasting for at least one month. This criterion was chosen because we aimed to assess the more regular non-parental care arrangements; fewer hours and/or a shorter period of care could be due to incidental care by others. Nine types of non-parental care arrangements were distinguished: centre-based childcare, care in the child's home by a nonrelative, care by a non-relative elsewhere (i.e., a child care home), four types of care by grandparents (care by maternal grandparents in the child's home, care by

maternal grandparents elsewhere, care by paternal grandparents in the child's home, care by paternal grandparents elsewhere), and two types of care by a relative other than the grandparents (care by relative in the child's home, care by relative elsewhere). For each month, the total hours per week, and the number of non-parental care arrangements per week, were summed up.

2.5 Potential covariates

Adjustments were made for the following covariates: maternal educational level, maternal native country, maternal religion, pregnancy smoking (yes or no), alcohol ingestion during pregnancy (yes or no), birth weight, sex, breastfeeding and number of siblings. Furthermore, to control for maternal postnatal depression and maternal daily hassles, the Edinburgh Postnatal Depression Scale (EPDS, Cox, Holden & Sagovsky, 1987) and the Everyday Problem Checklist (EPCL, Vingerhoets, Jeninga & Menges, 1989) were assessed at 3-months, 6-months and 12-months postpartum. Because of strong inter-correlations (*r*'s ranging from .52 to .62, and from .51-.61 respectively, all *p*-values <0.01), mean depression and daily hassles scores were calculated. Finally, a covariate was included to control for the number of trimesters an infant attended centre-based childcare.

2.6 Statistical analyses

First, in order to reduce the amount of data and to increase reliability of the analyses, the infant health data and non-parental care predictors were aggregated per trimester. If one month in a trimester was missing, the mean trimester score was calculated by averaging the remaining two months. If two of the three months were missing, the child's trimester score was considered missing (*N* = 6). Infants that missed six months or more, were excluded from the study (*N* = 5). Of the remaining 188 infants, 125 infants had no missing data, 50 infants missed 1 month, 9 infants missed 2 months, 3 infants missed 3 months, and 1 infant missed 4 months. No differences were found between infants with missing months and infants with no missing months in demographic data, health data or non-parental care predictors, with two exceptions. Infants with missing months had fewer siblings (*M*=.57 versus

M=.81, *p*=.029) and had mothers with higher depression scores (*M*=5.75 versus *M*=4.68, *p*=.028) than infants with no missing months.

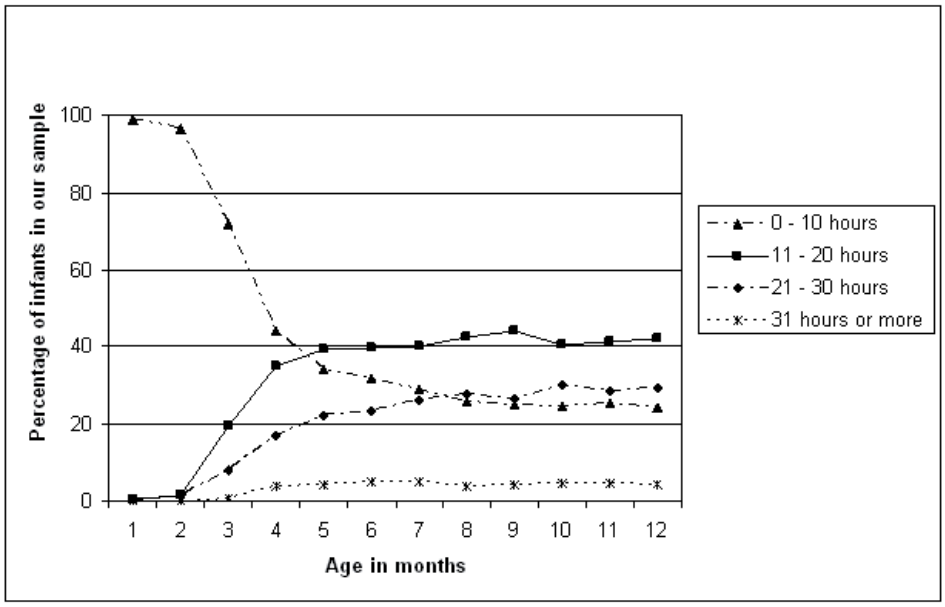
Second, descriptive analyses were carried out to test for differences in health, nonparental care and covariate characteristics between infants that included centre-based childcare in their arrangements and those that did not. Infants that did not receive non-parental care during their first year of life were excluded (*N*=20).

Third, multilevel analyses were conducted to test whether the number of hours and the number of concurrent arrangements uniquely predicted infants' illnesses and health complaints. Respiratory, digestive, general, and skin illnesses and complaints were modeled separately for the group of infants that included centre-based childcare in their arrangements and for those that did not. The trimesters were introduced at level 1 and nested within the infants at level 2. We compared multiple models in which linear time, quadratic time and cubic time were first entered into the empty model as explanatory variables. Linear time was considered a random factor. The time model which improved the model fit the best was retained, and thereafter the predictors number of hours and number of arrangements were added. If the non-parental care predictors improved the model fit, then lastly the covariates were entered into the model. The best fitting models were determined on the basis of their deviance on the -2loglikelihood ratio scale after generalized least square estimation, and presented in the results.

3. Results

3.1 Descriptive analyses

As shown in Figure 1, the percentage of infants in one or more care arrangements, and the percentage of infants spending ten or more hours in non-parental care, increased sharply between two and four months of age. From four months of age onwards, about 35% of the infants attended two arrangements or more.



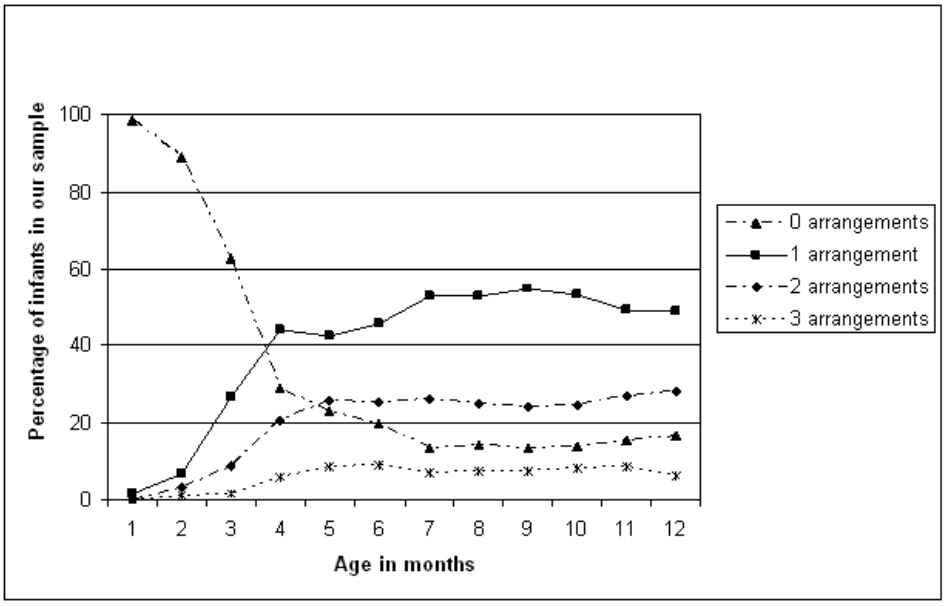


Figure 1: Percentages of infants spending time in non-parental care and attending concurrent arrangements during the first year of life.

Table 1: Correlations between the infant health variables (N=188).

	Respiratory				Digestive				General				Skin			
	1-3 months	4-6 months	7-9 months	10-12 months	1-3 months	4-6 months	7-9 months	10-12 months	1-3 months	4-6 months	7-9 months	10-12 months	1-3 months	4-6 months	7-9 months	10-12 months
Respiratory																
1-3 months	-															
4-6 months	.28**	-														
7-9 months	.12	.46**	-													
10-12 months	.18*	.23**	.37**	-												
Digestive																
1-3 months	.13	.01	.04	.05	-											
4-6 months	.07	.22**	.11	.01	.19*	-										
7-9 months	.02	.15*	.29**	.13	.23**	.36**	-									
10-12 months	.13	.03	.10	.18*	.29**	.20**	.23**	-								
General																
1-3 months	.13	.14	.03	.08	.12	.16*	.25**	.15*	-							
4-6 months	-.07	.34**	.12	.11	.00	.29**	.13	.10	.31**	-						
7-9 months	.13	.12	.32**	.08	.12	-.02	.23**	.08	.16*	.18*	-					
10-12 months	.02	.06	.23**	.36**	.12	.11	.25**	.33**	.15*	.09	.34**	-				
Skin																
1-3 months	.01	-.16*	-.00	.06	.14	.04	.04	.10	.03	.02	.08	.14	-			
4-6 months	.02	-.04	-.05	.12	.06	.29**	.04	-.04	.01	.14	-.15*	-.00	.28**	-		
7-9 months	.04	.15*	.14	.20**	.07	.12	.09	.05	.17*	.24**	.01	.10	.27**	.44**	-	
10-12 months	.02	-.05	.14	.05	.12	.13	.09	.15*	.05	-.04	.11	.17*	.26**	.31**	.48**	-

* = $p < .05$, ** = $p < .01$

Table 1 presents the correlations between the infant health variables. The correlations between the different classes of illnesses and health complaints are small to moderate. Skin illnesses and complaints are the least likely to co-occur with the other health variables. Also, the stability over age within a class of illnesses and health complaints is low to moderate for all classes. Because 20 infants received no non-parental care at all during their first year of life, they were excluded from further analyses. The remaining 168 infants were divided by centre-based childcare; 107 infants included centre-based childcare in their arrangements (referred to as 'centre-based childcare group'), and 61 infants did not (referred to as 'non centre-based childcare group').

Table 2 presents descriptive statistics for the covariates, separately for the centre-based and the non centre-based childcare group. T-tests show no significant differences between the two groups.

Table 2: Descriptive statistics of the covariates, divided per childcare group.

	Non Centre-based	Centre-based
Covariates		
Maternal educational level	6.44 (1.52)[1]	6.84 (1.45)[1]
Alcohol ingestion during pregnancy (%)	11.5 %	15.0 %
Pregnancy smoking (%)	3.3 %	4.7 %
Birth weight	3541.62 (453.39)[1]	3628.70 (497.36)[1]
Sex of neonates (%)		
Boy	55.7 %	56.1 %
Girl	44.3 %	43.9 %
Birth order (%)		
First	44.3 %	43.0 %
Second	39.3 %	43.0 %
Third or more	14.8 %	12.1 %
Postnatal maternal depression (EPDS)	5.58 (2.88)[1]	4.78 (3.31)[1]
Postnatal maternal daily hassles (EPCL)	1.15 (.39)[1]	1.14 (.36)[1]
Duration of breastfeeding in months[2]	4.84 (4.10)[1]	5.87 (4.33)[1]

Note. Centre-based' refers to the infants that included centre-based childcare in their childcare arrangements (N=107), and 'Non centre-based' refers to those infants that did not (N=61). No significant differences between the childcare groups are found. [1]Data are expressed as Mean *(SD)*. [2]Mean over the first year of life.

Table 3: Descriptive statistics of the health variables and childcare predictors at each trimester, divided per childcare group.

	1-3 months		4-6 months		7-9 months		10-12 months	
	non centre-based	centre-based	non centre-based	centre-based	non centre-based	centre-based	non centre-based	centre-based
Infant health								
Respiratory	4.60 (2.80)	4.75 (2.48)	5.60 (3.12)	7.37 (3.02)	7.37 (3.68)	9.31 (3.08)	6.53 (3.07)	8.00 (3.13)
Digestive	0.92 (1.10)	0.79 (1.04)	0.67 (1.04)	1.12 (1.40)	0.93 (1.66)	1.87 (1.83)	1.20 (1.46)	1.84 (1.78)
General	0.95 (1.04)	1.18 (1.16)	0.90 (1.02)	1.78 (1.45)	1.90 (1.76)	2.39 (1.57)	1.77 (1.35)	2.35 (1.82)
Skin	1.84 (1.78)	1.67 (1.47)	1.29 (1.27)	1.33 (1.38)	1.12 (1.27)	1.75 (1.72)	1.31 (1.23)	1.62 (1.47)
Childcare								
Number of hours	2.55 (3.26)	2.60 (3.77)	11.77 (7.62)	16.89 (8.44)	14.04 (7.01)	19.43 (6.09)	13.88 (6.94)	20.24 (5.98)
Number of arrangements	.29 (0.38)	0.22 (0.34)	1.19 (0.69)	1.35 (0.79)	1.32 (0.58)	1.46 (0.68)	1.28 (0.64)	1.51 (0.69)

Note. Data are expressed as Mean *(SD)*. 'Centre-based' refers to the infants that included centre-based childcare in their childcare arrangements (N=107), and 'Non centre-based' refers to those infants that did not (N=61).

Table 3 shows the descriptive statistics of the health variables and non-parental care predictors at each trimester, also separately for the centre-based and the non centre-based group. Repeated measures ANOVA's show that infants in the centre-based childcare group have more respiratory (F(1,161)=15.971, p=.000), digestive (F(1,161)=8.851, p=.003) and general (F(1,161)=14.636, p=.000) illnesses and complaints, but not skin illnesses and complaints (F(1,161)=.995, p=.320). In addition, the analyses revealed that infants in the centre-based childcare group spent more hours in non-parental care during their first year of life (F(1, 161)=24.060, p=.000), while no significant difference was found between the two groups in their mean number of arrangements (F(1,161)=1.789, p=.183).

Table 4 shows the correlations between number of hours and number of arrangements in each trimester, divided per childcare group. Although some strong correlations can been seen, no problems with multicollinearity occurred as shown by exploratory multiple regression analyses.

Table 4: Correlations between number of hours and number of arrangements at each trimester, divided per childcare group

	Number of arrangements			
	1-3 months	4-6 months	7-9 months	10-12 months
Non centre-based				
Number of hours 1-3 months	.81**	.31*	.12	.18
Number of hours 4-6 months	.34**	.66**	.48**	.44**
Number of hours 7-9 months	.17	.44**	.58**	.36**
Number of hours 10-12 months	.27*	.45**	.49**	.63**
Centre-based				
Number of hours 1-3 months	.87**	.25*	.07	.03
Number of hours 4-6 months	.32**	.57**	.24*	.21*
Number of hours 7-9 months	.17	.35**	.35**	.31**
Number of hours 10-12 months	.07	.27**	.23*	.33**

* = p < .05, ** = p < .01

Table 5: Estimates for the best fitting multilevel models of the infant health data for the infants that included centre-based childcare in their childcare arrangements

	Respiratory		General		Skin	
	Estimate	S.E.	Estimate	S.E.	Estimate	S.E.
Fixed effects						
Number of hours	**.255***	**.122**	**.212*****	**.057**	**-.093**	**.057**
Number of arrangements	**-.625***	**.302**	**-.362***	**.146**	**.295***	**.150**
Intercept	6.343**	2.178	-.163	.422	3.024**	1.066
Time linear	-4.151	3.310	.708	.434	-3.749*	1.576
Time quadratic	3.128*	1.403	-.085	.077	1.629*	.666
Time cubic	-.521**	.183			-.209*	.087
Attendance centre-based childcare[1]	1.241**	.474				
Breastfeeding in months	-.313**	.119				
Alcohol ingestion during pregnancy	-.951*	.052				
Maternal postnatal depression	.072	.480	.093***	.025	.069*	.031
Maternal postnatal daily hassles					.594*	.280
Number of siblings			-.191	.121	-.089	.136
Random effects						
Intercept	.256	.579	.000	.000	.347*	.154
Time linear	.251**	.093	.047**	.015	.046*	.021
Deviance	2023.82		1477.70		1427.31	

†= $p < .10$, * = $p < .05$, ** = $p < .01$, *** = $p < .001$ [1]= Refers to the number of trimesters infants attended centre-based childcare.

3.2 Main analyses

With respect to the centre-based childcare group, the best fitting models are presented in Table 5. Because in the model of digestive illnesses and complaints, adding the childcare predictors complaints did not improve model fit, this model was left out of the table. After correcting for relevant confounders, multilevel analyses showed that more hours in non-parental care were related to more respiratory and general illnesses and complaints, while more arrangements were related to fewer respiratory and general illnesses and

complaints. In contrast, more arrangements were related to more skin illnesses and complaints.

With respect to the non centre-based childcare group, none of the respiratory, digestive, general and skin models improved model fit after adding the non-parental care predictors. Including the infants that did not receive any non-parental care during their first year of life (*N*=20) in this group did not affect the results. These multilevel models are therefore not presented.

4. Discussion

To our knowledge, this study is the first to link the number of concurrent non-parental childcare arrangements to infant illnesses and health complaints early in life. From four months of age onwards, a considerable number of infants in our sample (about 35%) attended two arrangements or more during their first year of life. Analyses showed that infants who were cared for in more concurrent arrangements had more skin illnesses and complaints, but fewer respiratory and general illnesses and complaints. Furthermore, infants who spent more hours in non-parental care had more respiratory and general illnesses. However, these results only applied to infants attending centre-based childcare. In the group of infants that did not attend centre-based childcare, health was not related to either the number of hours or the number of arrangements. Additionally, our results supported earlier findings (Bradley, 2001; Bradley, 2003; Dales et al., 2004; Lu et al., 2004; Kamper-Jorgensen et al., 2006), by showing that infants attending centre-based childcare had more respiratory, digestive and general illnesses and complaints than infants who do not. While this replicates existing findings, it also extends research by looking at all illnesses and health complaints in the infant's first year of life, in stead of focusing on one or a few specific types of illnesses.

The explanation most commonly offered for increased rates of illnesses among infants in centre-based childcare points to increased contagion. Exposure to pathogens carried by other children in childcare settings increases the likelihood of contracting communicable illnesses, especially during infancy (Bradley, 2001). Our analyses showed that infants who spent more time in non-parental care had more respiratory and general illnesses during the first

year of life. This is in line with previous research, which positively related the number of hours to the likelihood of acquiring otitis media and respiratory illnesses (Bradley, 2001; Hagerhed-Engman et al., 2006; Kotch & Bryant, 1990). Moreover, our analyses showed that this positive relation between hours and illnesses was only true for infants attending centre-based childcare. It is plausible that the more hours infants spend in non-parental care, especially in care with large numbers of other children, the more the infants are exposed to common pathogens (Bradley, 2001). In turn, these pathogens could lead to more communicable illnesses, such as respiratory and general illnesses.

Furthermore, our results showed that having more concurrent childcare arrangements decreased the likelihood of contracting respiratory and general illnesses and complaints. However, this was only true for the group of infants that included centre-based childcare in their arrangements. Infants in this centre-based childcare group who attended more than one arrangement were also cared for by relatives and/or non-relatives in the child's home or elsewhere. The number of children typically found in childcare homes and relative care is low, while the number of children typically found in childcare centers is high. Therefore, the infants in the centre-based childcare group who attended more than one arrangement were probably exposed to fewer children during a single week, and thus to fewer pathogens, than infants who attended only centre-based childcare.

Interesting were our divergent findings which showed that infants who were cared for in more concurrent arrangements had less respiratory and general illnesses and complaints, but more skin illnesses and complaints. Although increased contagion appears to be the most important mechanism in contracting communicable illnesses, it appears to be not the only mechanism behind increased rates of illnesses in non-parental care. The exact cause of skin illnesses, such as atopic dermatitis, is unknown. Exposure to different environments with varied household products and chemicals may be a possible mechanism through which skin illnesses can be affected by the number of concurrent arrangements (Becher et al., 1996; Sheriff et al., 2005). Also, there is general agreement that psychological factors are important in the pathogenesis of skin illnesses (Pourpak, Sedighipour, Firooz, Afrooz,

Ghobari, Kazemnejad, et al., 2007). In our study, a possible psychological mechanism behind the positive association between skin illnesses and number of arrangements may be difficulties with the formation of dietary and sleep routines, or difficulties with the adjustment to different settings. Infants who experience transitions on a daily or weekly basis may not have adequate time or opportunity to adapt to different settings.

Moreover, our study indicated that the positive relation between number of concurrent arrangements and skin illnesses and complaints was only true for infants attending centre-based childcare. Adjustment to centre-based childcare may even be more of a challenge, because this type of care differs from other types of care in several respects. While other care arrangements resemble the home-setting more, centre-based childcare differs from other care types of care in group sizes, noise levels, routines and physical settings. Previous research has already shown that infants exhibit higher levels of the stress hormone cortisol on days when they are at centre-based childcare compared with days when they are at home (Dettling et al., 1999; Dettling et al., 2000; Watamura et al., 2000; Watamura et al., 2003). However, the cortisol patterns of infants in multiple concurrent arrangements, especially of infants in arrangements that differ in setting and routines, have yet to be examined (Morrissey, 2009). Naturally, we cannot discard the possibility that a family history of skin illnesses, such as atopic dermatitis, led the parents to choose a greater number of arrangements.

In contrast to respiratory, general and skin illnesses and complaints, digestive illnesses and complaints were not associated with either the number of hours or the number of arrangements. In previous research, the number of children present within each type of childcare was also not a factor associated with enteric tract illnesses (Bradley, 2001). In our study, as in other studies, digestive illnesses were only associated with attending centre-based childcare. The most commonly identified pathogens causing outbreaks of diarrhoea are Shigella, Giardia and rotavirus. These three agents require a very low inoculum to produce disease in susceptible individuals (Brady, 2005). This implies that even low levels of exposure to a centre-based childcare setting are sufficient to increase the probability of contracting diarrhoea. Apparently, this makes

other factors, like the number of hours and number of arrangements, irrelevant additional factors next to centre-based childcare attendance.

In the group of infants that did not attend centre-based childcare, neither the number of hours nor the number of arrangements were related to infant health. Note that the combinations and the type of arrangements in this group were varied. A challenge for future larger scaled (epidemiological) studies will be to look more closely at combinations of arrangements of different nature (e.g. relatives versus non-relatives, maternal versus paternal relatives) in relation to infant health.

Our study has several strengths, including monthly interviews and the focus on, and accurate measuring of, concurrent care arrangements and infant health. However, some limitations of the current study should also be noted. The study population was relatively small, and almost all mothers were from Dutch origin and had relatively high education levels. This makes it difficult to generalize the findings to other populations. Another limitation is that information on the numbers of children and adults that were present in the different child care settings was not available, so that we could not control for these possible confounders in the analyses. Further studies including the numbers of children and adults in each child care setting, may reveal whether children in centre-based care and children who attend more arrangements are exposed to more pathogens through exposure to larger numbers of children. Another aspect of care that was not included in this study is the quality of childcare. It would be interesting to include child care quality, because lower quality care, in terms of health-promotion behaviors and safety practices, may contribute to infant health problems. Finally, it would have been interesting to include measures of maternal and paternal employment, particularly work schedules. Nonstandard work schedules could interrupt care routines, on top of interrupted routines due to multiple concurrent child care arrangements, and thereby contribute to infant health problems.

5. Conclusion

Number of hours spent in non-parental care, and number of concurrent arrangements, do matter in relation to infant health early in life, but only for

infants that include centre-based childcare in their arrangements. Whether these apparent effects will play a role in the future health of these children, either positive or negative, remains to be explored. Also, future research into the underlying role of parenting factors related to the selection of childcare and handling of infant illnesses is warranted, as these factors could help clarify (some of) the mechanisms behind the associations between childcare and infant health.

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Chapter 7

Early Non-parental Care and Toddler Behavior Problems: Links with Temperamental Negative Affectivity and Inhibitory Control

Roseriet Beijers, Marianne Riksen-Walraven, Samuel Putnam, Marjanneke de Jong & Carolina de Weerth

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Abstract

Objectives: The first aim was to investigate inhibitory control as a mediator of the link between multiple aspects of early non-parental care and toddlers' behavior problems. The second aim was to explore temperamental negative affectivity as a moderator.

Methods: Participants were 193 mothers and their infants (91 girls; 79 firstborn). Infant negative affectivity was measured with a temperament questionnaire at 3 months of age. Information on different aspects of non-parental care was obtained through monthly maternal interviews across the first year of life. At 30 months of age, toddlers' inhibitory control was measured with observational tasks, and behavior problem questionnaires were filled in by the mothers and the child care caregivers.

Results: Early non-parental care was not related to inhibitory control in toddlerhood, but greater observed inhibitory control was related to less caregiver-reported internalizing and externalizing behavior. Furthermore, negative affectivity moderated the effect of early non-parental care on behavior problems. Non-parental care was unrelated to behavior problems in toddlers who displayed low or mean levels of negative affectivity as infants. For infants high in negativity, however, centre-based care was associated with higher mother-rated internalizing and externalizing problems, while higher number of hours in care was associated with lower mother-rated internalizing problems.

Conclusion: The link between non-parental care during the first year of life and toddlers' behavior problems was not mediated through inhibitory control. Instead, inhibitory control and non-parental care, in conjunction with negative affectivity, appear to be two independent predictors of toddlers' internalizing and externalizing behavior problems.

1. Introduction

Over the past years, scores of studies have been published investigating the effects of non-parental care on children's socio-emotional development, demonstrating that several aspects of early non-parental care are related to increased levels of behavior problems during the first years of life (Belsky,

Booth-LaForce, Bradley, Brownell, Burchinal, Campbell, et al., 2006), and that these effects extend into late childhood and adolescence (Belsky, Vandell, Burchinal, Clarke-Stewart, McCartney & Owen, 2007; Vandell, Belsky, Burchinal, Steinberg & Vandergrift, 2010). Especially early and extensive non-parental care, and in particular center-based care, has been found to be a robust predictor of behavior problems (Belsky et al., 2006; Bradley & Vandell, 2007), as have the number of concurrent care arrangements and long-term instability of care arrangements (Morrissey, 2009; NICHD, 1998; De Schipper, Van IJzendoorn & Tavecchio, 2004; De Schipper, Tavecchio, Van IJzendoorn & Van Zeijl, 2004). Questions remain about the underlying mechanisms linking early non-parental care to problem behavior. In the present study, child inhibitory control was examined as a possible mediator of this link. Furthermore, we examined whether temperamental negativity moderated the effects of multiple aspects of early non-parental child care on problem behavior.

Inhibitory control can be defined as the ability to inhibit and override dominant responses and behaviors in favour of more appropriate or subdominant responses (Goldsmith, 1996; Rothbart, Ellis, Rueda & Posner, 2003). Inhibitory control starts to emerge during the first postnatal year and undergoes rapid development across the toddler period and into the preschool years, a pattern coinciding with age-related changes in frontal lobe maturation and connectivity (Kochanska, Murray, Jacques, Koenig & Vandegeest, 1996; Morasch & Bell, 2011). Delayed or diminished inhibitory control could make it difficult for children to generate autonomously controlled and appropriate responses to challenging or goal-directed situations, which might result in problems with socio-emotional development (Kieras, Tobin, Graziano & Rothbart, 2005; Utendale & Hastings, 2011).

A growing body of research has linked inadequate regulation, including inadequate ability to inhibit behavior, to externalizing problems (e.g. Eisenberg, Sadovsky, Spinrad, Fabes, Losoya, Valiente et al., 2005; Gagne, Saudino & Asherson, 2011; Utendale & Hastings, 2011). However, findings regarding the relation between inhibitory control and internalizing problems have been more mixed. Because internalizing problems often involve the inability to control negative emotionality, as reflected in relatively high levels

of sadness, anxiety, and depression, it can be theorized that children with internalizing problems are relatively low in control (Kochanska, Coy, Tjebkes & Husarek, 1998). However, it has also been suggested that overcontrolled children are especially prone to internalizing problems (Robins, John, Caspi, Moffitt & StouthamerLoeber, 1996). Indeed, while in some studies children with internalizing symptoms appear to have low levels of control (Dennis, Brotman, Huang & Gouley, 2007; Rhoades, Greenberg & Domitrovich, 2009), in other studies they have average or high levels of control (Huey & Weisz, 1997; Krueger, Caspi, Moffitt, White & StouthamerLoeber, 1996).

Animal models provide evidence that early life stress might compromise the development of the neurobiological systems that are responsible for later regulatory capacities including inhibitory control (Loman & Gunnar, 2010), potentially impacting the likelihood of behavior problems (Heim & Nemeroff, 2001). According to Levine (2005), deprivation or loss of parental care is among one of the most potent stressors early in life. Non-parental care is characterized by separation from the parents, and might therefore be seen as a source of stress for children. In addition, non-parental care, and especially centre-based care, is characterized by confrontation with an unfamiliar setting, different routines, different caregivers, larger numbers of peers, and higher levels of noise than parental care at home, potentially constituting a source of early life stress for young children. In turn, exposure to stress in the first years of life is suggested to negatively affect the development of children's capacity for self-regulation, including inhibitory control (Loman & Gunnar, 2010; Schore, 2001). To our knowledge, no studies have examined the relations between (multiple aspects of) early non-parental care and inhibitory control.

The effects of stress, however, may not be the same for all children, but instead might be moderated by early-appearing individual differences. It is suggested that children with a more difficult temperament might encounter more challenges in non-parental care than children with a less difficult temperament, increasing the risk of behavior problems. For instance, Crockenberg and Leerkes (2005) found that easily-frustrated infants spending more hours in center-based care displayed more externalizing behavior at the age of 30 months, while no such effect appeared for children manifesting less

distress in the face of frustration. Nevertheless, Pluess and Belsky (2009, 2010) did not find temperament to moderate the relation between centre-based care or number of hours in care with behavior problems. Regarding internalizing, De Schipper et al., (2004) found a higher number of care arrangements to predict greater internalizing problems in difficult, but not in temperamentally easy children. Suggesting that child care may serve a preventative role, another study found amount of time in centre care to be associated with lower internalizing, while amount of time in care was associated with less positive adjustment (Bates, Marvinney, Kelly, Dodge, Bennett & Pettit, 1994). This study also found that temperament did not function as a moderator of child care experience upon adjustment. Due to the inconsistent nature of findings concerning temperament as a moderator of child care aspects, further replications are warranted. In addition, no prior studies have examined the moderating effect of temperament on the relation between other aspects of early non-parental care, such as long-term instability of care, on children's internalizing and externalizing problems, and the present study involves a more comprehensive array of different aspects of early non-parental care than previous studies.

To summarize, we examined the link between multiple aspects of non-parental care during the first year of life (i.e. centre-based care, number of hours spent in centre-based care and in non-parental care, age of entry, number of concurrent arrangements, and long-term instability of care) and internalizing and externalizing behavior problems at 30 months of age. More specifically, we examined whether this link was mediated by the children's inhibitory control and moderated by their temperamental negative affectivity.

2. Methods

2.1 Participants

This study is part of an ongoing prospective longitudinal project investigating maternal and other caregiving influences on children's development during their first years of life. The participants were healthy children living in the Netherlands, whose mothers were recruited during pregnancy through midwife practices in the cities of Nijmegen, Arnhem and surrounding areas.

Table 1: Descriptive statistics of the demographics, confounders, early non-parental care predictors and toddlers' outcome measures (N's ranging between 134 and 193).

	Mean *(SD)*	Range
Demographics characteristics		
Maternal marital status (wedlock or living together)	97.9%	
Maternal educational level (%)		
Primary education	3.8%	
Secondary education	20.4%	
College or university	75.8%	
Infant sex (%)		
Girl	46.0%	
Boy	54.0%	
Birth order (%)		
First	41.7%	
Second or more	58.3%	
Confounders		
Maternal postnatal depression		
During the first year of life	5.00 (*2.95*)	0.00-14.33
At 30 months of age	4.63 (*3.59*)	0.00-15.00
Non-parental care at 30 months of age		
Centre-based care (% attending centre-based care)	63.5%	
Number of hours	19.32 (*7.34*)	0.00-40.00
Number of concurrent arrangements	1.79 (*.88*)	0.00-5.00
Non-parental care during the first year of life		
Centre-based care (% attending centre-based care)	57.8%	
Number of hours in centre-based care	8.53 (8.80)	0.00-31.11
Number of hours in non-parental care	15.44 (8.27)	0.00-32.89
Number of concurrent arrangements	1.27 (0.72)	0.00-3.00
Long-term stability of type of care	1.39 (0.97)	0.00-5.00
Age of entry in months	4.52 (2.94)	1.00-12.00
Temperamental negative affectivity at 3 months of age	2.54 (*0.57*)	1.38-4.67
Toddlers' observed inhibitory control	0.00 (0.67)	-2.22-1.92
Toddlers' behavior problems		
Mother-reported internalizing problems	48.05(9.29)	29.00-74.00
Mother-reported externalizing problems	50.88 (9.26)	28.00-71.00
Caregiver-reported internalizing problems	41.26 (8.93)	29.00-63.00
Caregiver-reported externalizing problems	40.25 (8.52)	28.00-62.00

The study was approved by the faculty ethical committee and informed consent was obtained from each mother. Inclusion criteria were an uncomplicated, singleton pregnancy, no drug use, and no current physical or mental health problems.

Of the 220 women that enrolled in the study, 8 were excluded because of medical reasons, such as preterm birth. Of the remaining 212 mothers, a further 19 discontinued the study during the first 3 postpartum months due to personal circumstances. This resulted in a final sample of 193 mothers and their infants, for which demographic characteristics are provided in Table 1. No differences in these demographic characteristics were found between participating mothers and those that dropped out (*n*=19).

2.2 Procedure

Infant negative affectivity at 3 months of age was measured with a temperament questionnaire filled out by the mother (Infant Behavior Questionnaire-Revised; IBQ-R; Gartstein & Rothbart, 2003). Information on early non-parental care during the first year of life was obtained through monthly maternal interviews (3 in person, 9 by phone). These interviews provided information about centre-based care (yes or no), the number of hours the child spent in different types of care, the number of concurrent arrangements, the long-term instability of care over time and the age of entry into non-parental care.

At 30 months of age (*M*=30.16, *SD*=.62), toddlers' inhibitory control was recorded on videotape during a home visit using three tasks: snack delay, wrap a gift and whisper tasks (Kochanska et al., 1996). Furthermore, at this time a questionnaire about behavior problems (Child Behavior Checklist for ages 1.5-5; CBCL 1.5-5, Achenbach & Rescorla, 2000) was filled out by the mothers, and by a non-parental caregiver (no relatives included). Also, at 30 months of age information about current non-parental care was obtained by a maternal questionnaire (centre-based care, number of hours and number of arrangements).

Furthermore, to control for maternal depression throughout the first postnatal years, mothers filled in a depression questionnaire at 3, 6, 12 and 30

months postpartum. Finally, additional information on mother and infant was obtained with questionnaires filled in during the last trimester of pregnancy, immediately after birth and when the infant was 30 months of age.

2.3 Non-parental care

During monthly interviews during the first postnatal year, mothers reported on the type(s) of early non-parental care arrangements the child experienced during the week, and the hours per week the child spent in each arrangement. Non-parental care was defined as any type of care by caregivers other than the parents for at least 4 hr per week, and lasting for at least one month. Nine types of non-parental care arrangements were distinguished: centre-based childcare, care in the child's home by a non-relative, care by a non-relative elsewhere (i.e., a child care home), four types of care by grandparents (care by maternal grandparents in the child's home, care by maternal grandparents elsewhere, care by paternal grandparents in the child's home, care by paternal grandparents elsewhere), and two types of care by a relative other than the grandparents (care by relative in the child's home, care by relative elsewhere). For each month, the centre-based care hours per week, the total non-parental care hours per week and the number of non-parental care arrangements per week, were summed.

The following early non-parental care measures were used in this study (see also Beijers, Jansen, Riksen-Walraven & de Weerth, 2011): centre-based care, the number of hours the child spent in centre-based care, the number of hours the child spent in non-parental care, the number of concurrent arrangements, the long-term instability of care over time, and the age of entry into non-parental care. *Centre-based care* is a dichotomous variable indicating whether the child did or did not receive centre-based care during the first year of life. The *number of hours spent in centre-based care and non-parental care*, and the *number of concurrent arrangements* were computed by averaging the number of hours, and the number of concurrent arrangements, over the months in which the child was in care (i.e., the number of months after a child first entered care). The *long-term instability* of care is the total number of structural changes in type of non-parental care during

the first year of life. A change is defined as an alteration in type of care which lasted for at least two months. The period of two months was chosen in order to avoid temporary changes due for example to vacations or illnesses. Finally, a*ge of entry* is the month in which the child first experienced non-parental care. If a child did not experience non-parental care during the first year of life, a score of 12 (months) was assigned in order to prevent exclusion of these children from the analyses.

2.4 Inhibitory control

Snack delay (Kochanska et al., 1996). Children were presented with a placemat that had pictures of hands and were told to put their hands on the pictures of the hands. Then, a self-chosen treat (e.g. raisin, chocolate pastille) was placed at the top center of the mat, and a transparent plastic cup was placed over the snack. The toddler was instructed to wait to pick up the cup and eat the snack until the experimenter rang a bell. Practice trials were conducted to ensure that the child understood the task. After the practice trials, four consecutive trials were conducted. The delays were 10, 20, 30, and 15 seconds, respectively. Every five seconds the waiting behavior of the child was coded with a score from 0 to 4 (0 = eats snack before the bell rings; 1 = touches or grasps snack before the bell rings; 2 = touches or grasps cup before the bell rings; 3 = waits for the bell to ring without hands on the placemat; 4 = waits for the bell to ring with hands on the placemat). The mean scores over all trials was used.

Wrap a gift (Kochanska et al., 1996). The child was told that he or she would receive a gift, but that the gift was not wrapped yet. The child was asked to put his hands in front of his eyes and wait. The child sat in this way in front of the experimenter while she noisily wrapped a gift for the child during one minute. The child's behavior was coded every 10 seconds using a four-point scale (0 = watches wrapping/gift; 1 = peeks; 2 = looks away from wrapping/gift; 3= closed eyes and/or hands in front of the eyes). Mean scores were used.

Whisper task (Kochanska et al., 1996). The child was asked to whisper the names of 12 consecutively presented animals pictures. Two practice trials were conducted to ensure that the child understood the task. Responses were

coded 0 to 2 for every picture (0=shout; 1=normal or mixed tone; 2=whisper). Non-responding was coded as missing. Mean scores were used.

Reliability of coding. All tasks were coded from the videotapes and observed by three independent observers. To determine inter-rater reliability, 36 of the tapes were scored by all three observers. The Intra-class Correlation Coefficients for the inhibitory control tasks were: .98 for the snack delay, .82 for wrap a gift, and .96 for the whisper task. To obtain one score for inhibitory control, the three task scores were standardized and averaged.

2.5 Negative affectivity

The IBQ-R consists of 191 items. We used an adaptation of the original instrument, wherein items were scored on a 4-point scale. The 191 items constitute 14 scales that have been factor analyzed to yield 3 broad temperament dimensions (Gartstein et al., 2003). The infant's score for the temperamental dimension of negative affectivity (average of sadness, distress to limitations, fear, and reversed falling reactivity scales) was used in this study as a moderator. Cronbach's alphas for the scales (.74 to .82) as well as the dimension of negative affectivity (.71) were sufficient.

2.6 Behavior problems

The CBCL 1.5-5 (Achenbach & Rescorla, 2000) requires the parent/caregiver to indicate whether certain problem behaviors have occurred within the last 2 months. The internalizing and externalizing subscales were used for the analyses.

2.7 Potential covariates

Adjustments were made for the following covariates: maternal educational level, child sex, and firstborn (yes or no). To examine the specific effects of early non-parental care, this study controlled for non-parental care at 30 months of age, i.e. centre-based care (yes or no), the number of hours spent in non-parental care and the number of concurrent arrangements at 30 months of age. Finally, to control for maternal postnatal depression, the Edinburgh Postnatal Depression Scale (Cox, Holden & Sagovsky, 1987) was filled in by the

mother at 3, 6, 12 and 30 months postpartum. Because of inter-correlations over the first year's scores (*r*s ranging from .51 to .63, all *p*-values <0.01), mean depression scores over the first year of life were calculated. The depression scores over the first year of life and at 30 months postpartum were used as covariates.

2.8 Data analysis

Missing data. Infants that missed four or more monthly maternal interviews during the first year of life were excluded from the study (*N*=6). Of the remaining 187 infants, 125 infants had no missing data, 50 infants missed 1 month, 9 infants missed 2 months and 3 infants missed 3 months. From the 187 infants, data of 184 infants was also obtained when they were 30 months of age.

Statistical analyses. Square root or logarithm transformations were applied to skewed data. The mediation model was tested to investigate observed inhibitory control as a mediator of the link between a history of non-parental care during the first year of life and internalizing and externalizing behavior problems at 30 months of age, according to the procedure outlined by Baron and Kenny (1986). According to this procedure, three conditions must hold in order to establish mediation: (a) the predictor is significantly associated with the outcome, (b) the predictor is significantly associated with the mediator, and (c) the mediator is significantly associated with the outcome. If these conditions hold in the predicted direction, mediation is proven when the effect of the predictor on the outcome is shown to decrease when the mediator is also entered as a predictor in the regression equation. These conditions were all tested with standard multiple hierarchical regression models. The first model contained all covariates and non-parental care predictors. To eliminate irrelevant confounders and predictors, and to increase power, the second model only contained the variables which individually explained at least 1 percent of the variance in the first model (see also Beijers, Jansen, Riksen-Walraven & de Weerth, 2010). These models, with all maintained confounders in hierarchical step 1 and all maintained predictors in hierarchical step 2, are presented in the results and Table 3.

To explore early temperamental negativity as a moderator of non-parental care, interactions terms were computed according to the procedure outlined by Aiken and West (1991). Five interaction terms were computed representing negative affectivity times centre-based care, number of hours in centre-based care, number of hours in non-parental care, number of concurrent arrangements, long-term instability of care, and age of entry. Next, multiple regression models were computed in the same way as described earlier, with all maintained interaction terms in hierarchical step 2. In the case of an interaction term explaining at least 1 percent of the variance in the first model, both main effects were also included in the final model.

3. Results

3.1 Descriptive statistics

Means and standard deviations of the non-parental care predictors, child variables, and potential confounders are presented in Table 1. In total, 167 children went to non-parental care in their first year of life. Table 2 shows the correlations between the non-parental care predictors and the child variables. Furthermore, internalizing and externalizing problems as reported by the mother and by the caregiver were inter-correlated. Also, mother-reported internalizing problems were significantly related to caregiver-reported internalizing problems, and the same was the case for externalizing problems. Finally, as number of hours spent in centre-based care was strongly correlated to centre-based care and total number of hours spent in non-parental care, separate analyses for number of hours spent in centre-based care, and for centre-based care together with number of hours spent in non-parental care, were carried out.

3.2 Main analyses

Non-parental care and behavior problems.

As can be seen in Table 2, two significant correlations appeared between the non-parental care predictors and toddlers' behavior problems. More caregiver-reported externalizing problems were related to attending centre-based childcare and to spending more hours in centre-based care in the first

Table 2: Pearson correlations between the non-parental care predictors and the outcome measures.

		1	2	3	4	5	6	7	8	9	10	11	12
	Early non-parental care												
1	Centre-based care	-											
2	Number of hours in centre-based care	-.83**	-										
3	Number of hours in non-parental care	-.56**	-.67**	-									
4	Number of concurrent arrangements	-.35**	-.16*	-.64**	-								
5	Stability of type of care	-.06	-.03	-.30**	-.46**	-							
6	Age of entry	-.35**	-.30**	-.65**	-.63**	-.46**	-						
7	**Early negative affectivity**	-.03	-.00	-.07	-.00	-.04	-.05	-					
8.	**Toddlers' inhibitory control**	-.09	-.10	-.13†	-.08	-.01	-.05	-.06					
	Toddlers' behavior problems												
9	Internalizing problems - M	-.08	-.00	-.04	-.10	-.11	-.08	-.12	-.01	-			
10	Externalizing problems - M	-.09	-.02	-.02	-.06	-.02	-.03	-.13†	-.02	.60**	-		
11	Internalizing problems - C	-.09	-.00	-.02	-.01	-.05	-.00	-.01	-.20*	.18*	.02	-	
12	Externalizing problems - C	-.21*	-.20*	-.12	-.01	-.01	-.06	-.03	-.17†	.06	.25**	.40**	-

Note †=p<.10, *=p<.05, **=p<.01, M = behaviour problems as reported by the Mother, and C = behaviour problems as reported by the Caregiver

year of life. Hierarchical multiple regression analysis, however, showed no significant main effects of the non-parental care measures on externalizing and internalizing problems as reported by mothers and caregivers.

Non-parental care and behavior problems: inhibitory control as a mediator.
Next, the procedure outlined by Baron and Kenny (1986) was used to test whether inhibitory control mediated the relation between early non-parental care and later behavior problems. Early non-parental care was not related to observed inhibitory control in toddlerhood, such that the mediational model was not supported. Greater observed inhibitory control, however, was related to less caregiver-reported internalizing and externalizing behavior. These results are presented in Table 3. No main effects of inhibitory control were found for mother-reported internalizing and externalizing behavior.

Non-parental care and behavior problems: infant temperament as a moderator.
Finally, interactions terms were computed according to the procedure outlined by Aiken and West (1991) to explore temperamental negativity as a moderator of early non-parental care. Significant interaction effects were obtained for negative affectivity X centre-based care and negative affectivity X hours of non-parental care on toddlers' internalizing and externalizing problems as reported by the mother. These results are summarized in Table 3, with the interactions graphically represented in Figures 1 and 2 (effects of negative affectivity X centre-based care on internalizing and externalizing problems, respectively), and Figure 3 (effect of negative affectivity X hours of care on internalizing problems). The single slopes of the regression lines reflecting the relation between early non-parental care and behaviour problems for infants with different levels of negative affectivity (low, mean, or high) were tested for significance, using the method of Hayes and Matthes (2009).

Figure 1 and 2 depict the interaction effects of negative affectivity X centre-based care on internalizing and externalizing problems, respectively. In both figures, only the slopes for infants with high levels of negative affectivity (mean+1SD) were significant, indicating that attending centre-based care in the

Table 3: Final multiple hierarchical regression models early non-parental care, inhibitory control, and behavior problems.

		B	β	R^2_{model}	F_{change}	R^2_{change}
Inhibitory control and behavior problems						
Internalizing problems - C						
Step 1	Maternal educational level	1.03	.16†*			
	Maternal depression (1st year)	.26	.08**			
	Number of hours non-parental care (1st year)	-.01	-.01**	.04**		
Step 2	Inhibitory control	-2.49	-.18**	.07**	4.61	.03
Externalizing problems - C						
Step 1	Maternal educational level	.72	.12**			
	Number of hours in non-parental care at 30 months of age	-2.67	-.29**			
	Number of concurrent arrangements at 30 months of age	.22	.17†*	.09**		
Step 2	Inhibitory control	-2.26	1.08**	.12*	4.42	.03
Non-parental care, including non-parental care hours, and behavior problems						
Internalizing problems - M						
Step 1	Maternal depression (1st year)	.60	.19**			
	Number of concurrent arrangements at 30 months of age	1.83	.17**			
	Early negative affectivity	-2.44	-.15**	.06**		
Step 2	Centre-based care (1st year – no=0, yes=1)	2.82	.15**			
	Number of hours non-parental care (1st year)	-.07	-.06**			
	Negative affectivity X centre-based care	8.30	.37**			
	Negative affectivity X number of hours non-parental care	-.38	-.19**	.13**	3.25	.07
Externalizing problems - M						
Step 1	Maternal depression (30 months of age)	.53	.21**			
	Early negative affectivity	-1.43	-.09**	.05**		
Step 2	Centre-based care (1st year – no=0, yes=1)	2.47	.13**			
	Number of hours non-parental care (1st year)	-.08	-.06**			
	Negative affectivity X centre-based care	7.22	.32**			
	Negative affectivity X number of hours non-parental care	-.33	-.16†*	.11**	2.55	.05

		B	β	R^2_{model}	F_{change}	R^2_{change}
Non-parental care, including centre-based care hours, and behavior problems						
Internalizing problems - M						
Step 1	Maternal depression (1st year)	.58	.18**			
	Number of arrangements (30 months of age)	1.65	.16**			
	Early negative affectivity	1.10	.07**	.06*		
Step 2	Number of hours centre-based care (1st year)	.08	.07**			
	Negative affectivity X number of hours centre-based care	.17	.11**	.08	1.49	.02
Externalizing problems - M						
Step 1	Maternal depression (30 months of age)	.50	.20**			
	Early negative affectivity	1.63	.10**	.05**		
Step 2	Number of hours centre-based care (1st year)	.04	.04**			
	Negative affectivity X number of hours centre-based care	.21	.13†*	.07	1.72	.02

Notes: †=*p*<.10, *=*p*<.05, **=*p*<.01, M = behaviour problems as reported by the mother, and C = behaviour problems as reported by the caregiver. Repetition of the analyses with the group infants for whom both problem behaviour reports of the mother and the child care caregiver were available (N=134), were not different from the analyses for the total group (N=180).

first year of life predicted more internalizing (*t*=3.39, *p*<.01, see Figure 1) and externalizing problems (*t*=2.96, *p*<.01, see Figure 2) in children who were high in negative affectivity as infants. Figure 3 depicts the interaction effect of negative affectivity X hours of care on internalizing problems. Only the slopes for infants with high levels of negative affectivity (mean+1SD) were (marginally) significant, indicating that spending more hours in non-parental care in the first year of life predicted less internalizing (*t*=-2.04, *p*<.05, see Figure 3) and marginally less externalizing problems (*t*=-1.69, *p*<.10, not in figure, but see Table 3) in children who were high in negative affectivity as infants. On the contrary, spending more hours in centre-based care in the first year of life was marginally related to more internalizing problems (*t*=1.66, *p*<.10, not in figure, but see Table 3) in children who were high in negative affectivity as infants.

Furthermore, in all interaction analyses, the slopes for the infants with low or mean levels of negative affectivity were not significant, indicating

that non-parental care was not related to mother-reported internalizing or externalizing problems in children who were low or average in negative affectivity as infants. No negative affectivity X non-parental child care interaction effects were found on toddlers' externalizing and internalizing behavior problems, as reported by the caregiver.

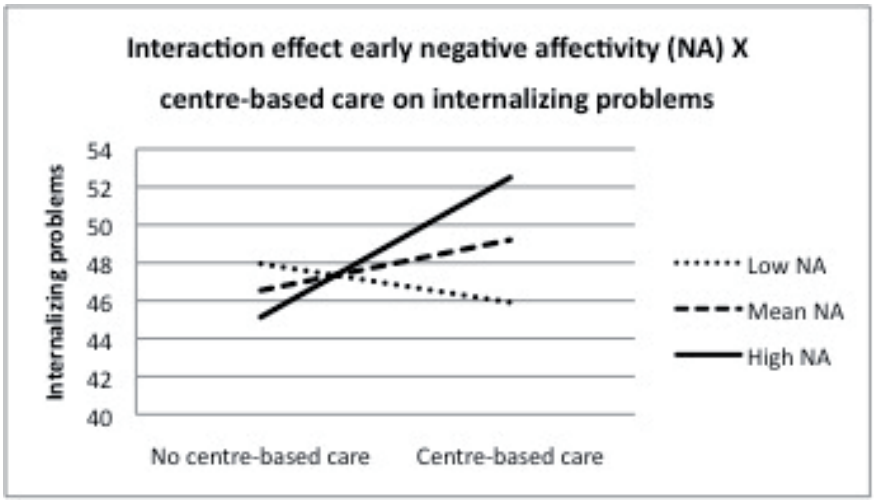

Figure 1: Interaction effect early negative affectivity (NA) X centre-based care on internalizing problems

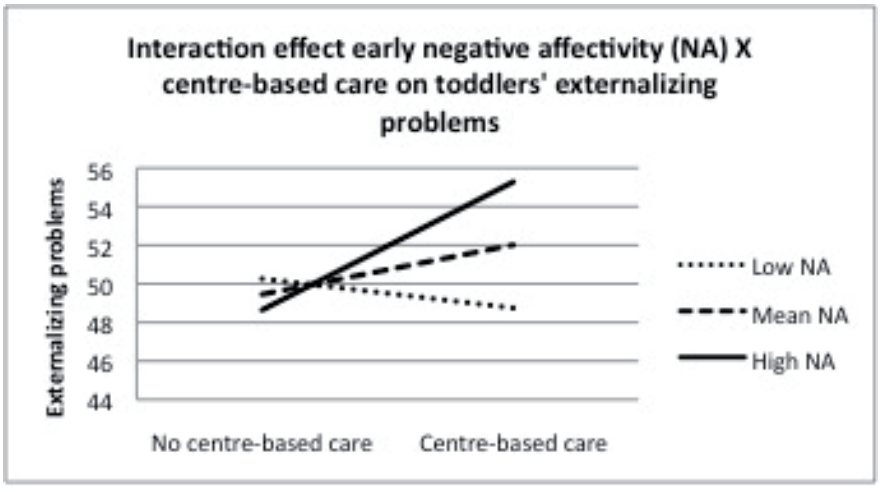

Figure 2: Interaction effect early negative affectivity (NA) X centre-based care on externalizing problems

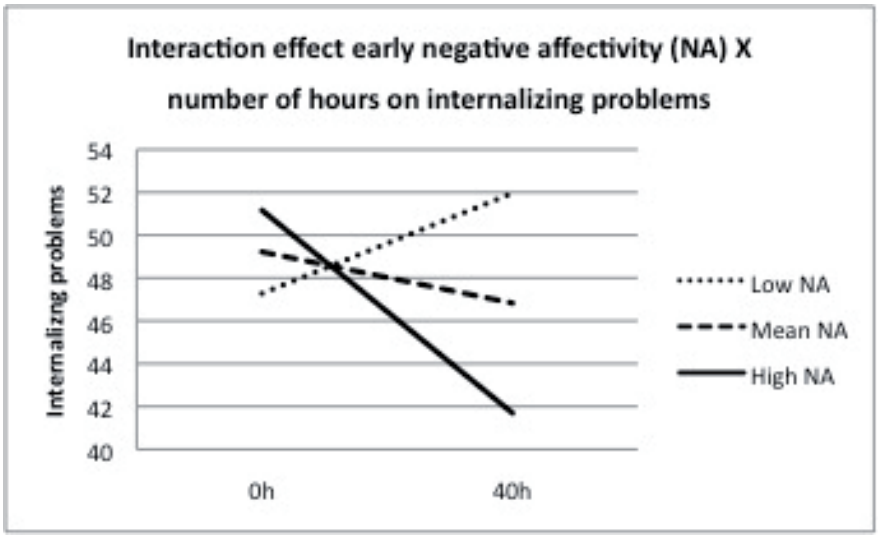

Figure 3: Interaction effect early negative affectivity (NA) X number of non-parental care hours on internalizing problems

4. Discussion

The aims of this study were two-fold. The first aim was to investigate inhibitory control as a mediator of the link between non-parental care during the first year of life and behavior problems at 30 months of age. As early non-parental care was not related to observed toddlers' inhibitory control, the mediation model was not supported. Greater observed inhibitory control, however, was related to less caregiver-reported internalizing and externalizing behavior.
The second aim was to explore early temperament as a moderator of the link between early non-parental care, and toddlers' inhibitory control and behavior problems. Significant moderation effects were obtained between early negative affectivity and non-parental care on toddlers' behavior problems. Children high in negative affectivity and attending centre-based care in their first year of life showed more maternal-reported internalizing and externalizing problems than children not attending centre-based care. Contrarily, children high in negative affectivity and attending non-parental care for more hours during the first year showed less maternal-reported internalizing problems than children attending care for fewer hours.

To our knowledge, this study was the first to examine inhibitory control as a possible mechanism underlying the link between early non-parental care and later behavior problems. Early non-parental care was not related to toddlers' inhibitory control, and also no main effects were found of early non-parental care on toddlers' behavior problems. These latter findings contrast several studies, mostly those from the large NICHD study of early child care, reporting the adverse consequences of non-parental care on child outcomes across all ages. However, some of these studies also point to possible 'sleeper effects' of non-parental care, as they also found less evidence that non-parental care was related to 2- and 3-year olds compliance and behavior problems (NICHD, 1998; Youngblade, 2003). Possibly, the effects of non-parental care take some time to become visible at later ages (NICHD, 1998). Furthermore, the previous studies mainly concentrated on populations in the U.S. where children attend non-parental care for substantial amounts of time, e.g. for 30 hours or more. In the Netherlands it is very common for one or both of the parents to work part-time after having a child, enabling them to make part-time, instead of full-time, use of non-parental care. In our study, the mean number of non-parental care hours that infants spent was 15.44 hours (around 2 days) with a standard deviation of 8.27 hours. Although non-parental care might be seen as a source of early life stress for infants and children, possibly impacting the likelihood of regulation and behavior problems (Loman & Gunnar, 2010), it is very well possible that little to modest exposure to non-parental care is manageable and absent of negative effects on later child outcomes for most children.

In line with this notion, not all children appeared to be unaffected by early non-parental care. Whereas infants of low or mean levels of negative affectivity seemed to be unaffected by different aspects of early non-parental care, infants high in negativity and attending centre-based care showed more internalizing and externalizing problems at 30 months of age. In addition, infants high in negativity and attending non-parental care for more hours showed less internalizing problems. However, if only hours in centre-based care were considered, infants high in negativity and attending centre-based care for more hours showed a trend to engage in more internalizing problems.

These latter findings are in line with the findings by Crockenberg and Leerkes (2005). They also saw that easily frustrated children who spent more hours in center-based care at 30 months of age had more behavior problems than easily frustrated children who spent more hours in other types of non-parental care. Apparently, the effects of number of care hours appear to depend on the context in which care occurs. As centre-based care is often characterized by extra challenges, e.g. larger group sizes, more noise and different caregivers, it makes sense that infants high in negative affectivity will be more affected by these challenges than easy-going infants, especially when these challenges are cumulative such as in the case of spending more hours in centre-based care (Crockenberg & Leerkes, 2005).

Less clear is why attending more hours in all types of non-parental care is related to less internalizing problems in toddlers who were more negative as infants. This finding partially replicates that of Bates et al. (1994), who showed more hours in care to be linked to lower internalizing, although Bates et al. (1994) explored but did not find an interaction with temperament. The first possible explanation is in line with the argument mentioned above that children in the Netherlands often spend fewer hours in non-parental care than children of other countries, including the U.S. Children who spend only few hours in non-parental care might have trouble getting used to the care situation and adapting to the dietary and sleep routines required by the occasional care situation, which in turn could contribute to increased internalizing problems. Another explanation could be that more hours provide the opportunity for caregivers to get to know the infant better, and to adjust their behavior and routines to the child's needs, which might be especially important for infants with a more difficult temperament. Finally, attending care for more hours possibly unburdens the family of a more negative infant, so that at the moments when the parents themselves are taking care of the infant they have more resources to behave sensitively and responsively.

The hypothesized mediation effect could not be found in this study, because inhibitory control was unrelated to child care. However, inhibitory control did show the expected relation to toddlers' internalizing and externalizing problems. This result replicates findings from previous

studies on older children relating low inhibitory control to more externalizing and internalizing problems (e.g. Dennis et al., 2007; Eisenberg et al., 2005; Rhoades et al., 2009; Utendale & Hastings, 2011), and add to this previous research by showing that this relation already exists for both externalizing and internalizing problems at a very young age, namely in toddlerhood. As such, it provides possible implications for interventions and parenting to reduce both externalizing as internalizing problems. Interventions designed to reduce behavior problems can benefit from attempts to foster inhibitory control (Eisenberg, Valiente, Spinrad, Cumberland, Liew, et al., 2009).

This study has several strengths, including the longitudinal design, multiple observational measures of inhibitory control and the independent ratings by multiple informants of toddlers' behavior problems. Nevertheless, there are also some limitations. First, most of the mothers in our sample were highly educated. This could have implications for the generalizability of our results. A second limitation is that no measure of quality of care was included. Several studies and reviews have pointed to the importance of quality of care as predictor of child outcomes, although positive effects of quality mostly pertain to cognitive and language developmental outcomes than to behavioral problems (Allhusen, Belsky, Booth, Bradley, Brownell, Burchinal, et al., 2002; Belsky et al., 2006; Vandell et al., 2010). The feasibility of determining quality of care with observations in relative large samples and with infants attending up to 3 concurrent arrangements is very low. Nevertheless, future studies should consider observational methods for determining the quality of care, as this aspect of care might be related to inhibitory control.

Summarizing, the link between non-parental care during the first year of life and toddlers' behavior problems was not shown to be mediated through inhibitory control. Instead, inhibitory control and non-parental care, in conjunction with negative affectivity, appear to be two independent predictors of both toddlers' internalizing as externalizing behavior problems. As such the results provide two different and independent entries for parenting and interventions to prevent the early development of internalizing and externalizing problems.

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Chapter 8

Conclusion and General Discussion

8.1 Summary of the research thesis

Environmental factors in utero and during early life are predictive of cognitive, behavioural and socio-emotional development and physical health in children and adults. However, questions remain about the underlying mechanisms linking these early experiences to child outcomes later in life (Loman & Gunnar, 2010; Taylor, Way & Seeman, 2011).

The aim of the studies presented in this thesis was to investigate the effects of the early maternal and caregiving environment on regulation and health of infants and young children. Regulation problems are increasingly acknowledged to play a key role in the development of psychopathology. Unveiling the development of regulation, in conjunction with determining the role of the early maternal and caregiving environment, not only gives us information about normal development, but might also help to reveal the mechanisms underlying the link between early experiences and later psychopathology (Posner & Rothbart, 2000). This thesis focused on multiple levels of regulation, including the regulation of physiological states, i.e. cortisol, and the regulation of behaviour and emotions, i.e. the frequency of night wakings and inhibitory control.

Next to infant regulation, this thesis also studied the role of the early environment in infants' physical health. Empirical studies show that children that experience forms of early adversity are at risk for immune system dysfunctioning and disease in adulthood, including cardiovascular disease (Galobardes, Smith & Lynch, 2006; Miller, Chen & Cole, 2009). However, less is known about the relationship between early adversity and physical health within younger populations, including infants. It remains unclear whether adverse experiences already lead to illnesses early in life, or whether the negative effects are stored in a latent form and emerge later on (Ben-Shlomo & Kuh, 2002). This thesis focused on the links between the early environment and infant respiratory, general, digestive and skin illnesses within the first year of life.

Fortunately, the role of the early environment is not limited to adverse effects. Positive environments, including maternal and caregiving environments of high quality, have been related to positive outcomes. Both aspects of the

maternal and caregiving environment associated with adversity or benefits are the topic of this thesis. The following early environmental aspects were studied in relation to infant regulation and health: maternal prenatal stress and anxiety, breastfeeding, parent-infant co-sleeping, mother-infant attachment-in-the-making, and non-parental care (see Figure 1). The studies presented in this thesis were all part of the BIBO study (BIBO: Basale Invloeden op de Baby Ontwikkeling; Basal Influences on Infant Development): a prospective longitudinal study that followed 193 mothers and their children from pregnancy through their first years of life. For the present thesis, the data from the assessment during pregnancy, from the multiple assessments during the infant's first year of life, and from the 30-months assessment were used.

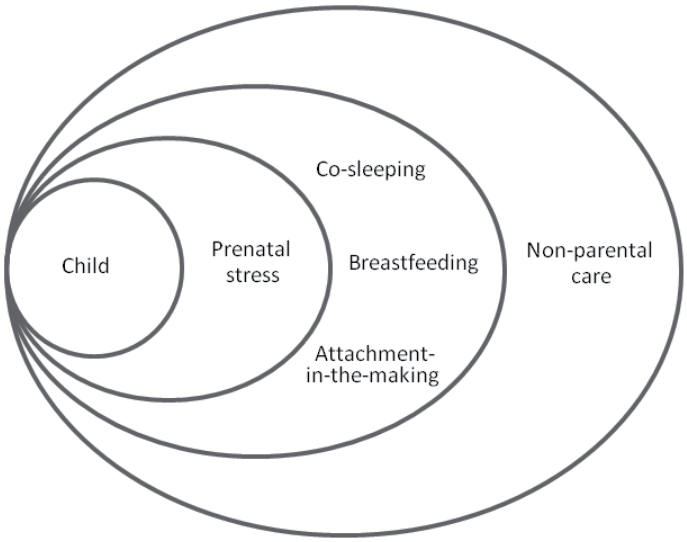

Figure 1: The maternal and caregiving environmental factors studied in the present thesis. The ovals on the right side represent the expanding environment by increasing infant age.

Study 1. The goal of the first study, described in Chapter 2, was to examine whether maternal anxiety and stress during pregnancy were related to more illnesses and antibiotic use during the infant's first year of life. Although animal research has shown that prenatal stress has a negative impact on the offspring's immune system, previous human studies investigating physical

health outcomes are often restricted to birth complications, including preterm delivery and low birth weight (Merlot, Couret & Otten, 2008).

To measure maternal prenatal stress and anxiety, 174 mothers filled out questionnaires on general and pregnancy-related anxiety and stress during the last trimester of pregnancy. In addition, mothers collected saliva samples on two consecutive days for determination of circadian cortisol levels. Information on infant illnesses and antibiotic use was obtained by the use of semi-structured interviews that were conducted at monthly intervals during the first year of life. Furthermore, information about many relevant confounders was collected, including postnatal anxiety and stress experienced by the mother at 3, 6 and 12 months postnatally.

As expected, our study showed that maternal prenatal anxiety and stress were related to more infant illnesses and antibiotic use during the first year of life. Even after controlling for relevant confounders, prenatal anxiety and stress predicted a considerable amount of variance: 9.3% for respiratory illnesses, 10.7% for general illnesses, 8.9% for skin illnesses, and 7.6% for antibiotic use. Digestive illnesses were not related to maternal prenatal anxiety and stress.

In conclusion, this study is the first to link maternal prenatal anxiety and stress, measured both by self report and by cortisol physiology, to infant illnesses and antibiotic use early in life. The suggested underlying mechanisms point to the possibility that prenatal anxiety and stress affect the infant's immune system. While this hypothesis is supported by extended animal research and a few human studies on immunity, there is no direct evidence that the infants were indeed immune compromised. As such, this study taps into a new area for future research incorporating infant health and the immune system when studying maternal prenatal anxiety and stress.

Study 2. The second study, reported in Chapter 3, examined the relationship between sleeping arrangements and infant cortisol reactivity to two stressors in the first two months of life. Co-sleeping, as compared to solitary sleeping, is hypothesized to provide more parental external regulation by night. Parental co-regulation protects the infant from excessive levels of distress and promotes

the infant's emergent capacity to regulate physiological arousal (Schore, 2001). We therefore expected lower cortisol reactivity to stressors in infants who co-slept more regularly.

Information on sleeping arrangements was collected for 163 infants with the use of daily sleep diaries in the first two months of life. The mothers received the diaries and accompanying instructions at the end of pregnancy, so they could start immediately after birth. We classified co-sleeping as sleeping in the parents' bed and/or sleeping in the parents' room. The percentage of co-sleeping for each night was calculated. The infants were divided into 3 groups: solitary sleepers (co-sleeping 0-10% of the time), partial co-sleepers (11-90% of the time) and full co-sleepers (91-100% of the time). Cortisol reactivity was measured twice: to a bathing session at 5 weeks of age and to a vaccination at 2 months of age.

Longitudinal regression analyses showed that infants with a solitary sleeping arrangement showed a heightened cortisol response to the bathing session at 5 weeks of age compared to infants that co-slept part-time or full-time. This effect was not explained by confounding variables, including breastfeeding practices, quality of maternal caregiving behaviour, or infants' sleep duration. No effects were found of co-sleeping on the cortisol response to the vaccination at 2 months of age.

This is the first study to prospectively investigate relations between parent-infant sleeping arrangements and cortisol reactivity in early infancy. The results suggest that solitary sleeping, as compared to co-sleeping, in the first month of life is associated with heightened sensitivity of the HPA-axis to a mild stressor, possibly due to less nocturnal parental availability as an external stress regulator.

Study 3. The study presented in Chapter 4 aimed to examine whether feeding and sleeping arrangements during the first six months of life were associated with cortisol regulation, i.e. cortisol reactivity and recovery, to a stressor at 12 months of age. Breastfeeding and co-sleeping are hypothesized to support parental co-regulation of the infant. Therefore, it was expected that co-sleeping and breastfeeding for a longer period would predict better cortisol regulation

in the infant, i.e. a less strong reaction to the stressor and a quicker recovery afterwards.

Information on breastfeeding was collected with the use of a weekly diary for the first 6 months of life. The number of weeks that an infant received exclusive breastfeeding or breastfeeding in combination with bottle-feeding during the first six months of life was used as a predictor. Furthermore, information on co-sleeping was collected using a daily diary for the first 6 months of life (see also study 2). The weeks in which 10-100% of the time co-sleeping had taken place were classified as co-sleeping weeks. Information on feeding and sleeping arrangements was adequately collected by 173 mothers. At 12 months of age, infants were subjected to a stressor that consisted of the arrival of a stranger and two brief separations from the mother (Strange Situation Procedure, Ainsworth, Blehar, Waters & Wall, 1978). Saliva was sampled to measure infant cortisol reactivity and recovery.

Regression analyses showed that more weeks of co-sleeping indeed predicted lower infant cortisol reactivity to the Strange Situation Procedure, but only for children who had also received breastfeeding for a relatively long period (six months). More weeks of breastfeeding and co-sleeping by themselves did not predict cortisol reactivity. Also, more weeks of breastfeeding, but not co-sleeping, predicted quicker cortisol recovery. These effects were found after correcting for several mother, infant and environmental characteristics, including maternal sensitivity, infant attachment security, and feeding and co-sleeping practices at 12 months of age.

These results suggest that early breastfeeding and co-sleeping contribute positively to cortisol regulation at 12 months of age. As such, these findings support the hypothesis that early environmental factors, including breastfeeding and co-sleeping, contribute positively to cortisol regulation. One explanation for this finding might be that breastfeeding and co-sleeping support parental co-regulation of the infant, and as such promote the infant's own emergent capacity to regulate physiological arousal later in life.

Study 4. The study presented in Chapter 5 examined the early history of night waking in infants who were later classified as securely or insecurely (avoidantly,

resistantly, or disorganized) attached. Although a link between infant night waking and attachment to the primary caregiver, usually the mother, has been previously proposed, empirical support is limited so far. Moreover, the few published studies mostly concentrated on one-year-olds. The quality of the attachment relationship cannot be reliably assessed before 12 months of age, when a "clear-cut attachment" has emerged (Ainsworth et al., 1978). It is therefore not surprising that previous research examined night waking in relation to attachment in infants beyond the first year of life. However, the attachment relationship develops gradually over the first year of life: this process is referred to as attachment-in-the-making. During this process, infants build up expectations of how their needs will be met by their caregivers, and these expectations may be reflected in signaling and other contact-promoting behaviours. Hence, infants' reaction to their waking up at night could reflect the emerging attachment relationship.

Information on the number of infant night wakings was collected with the use of daily sleep diaries for the first 6 months of life (see also study 2). Infant-mother attachment was assessed when the infants were 12 months of age using the Strange Situation Procedure (Ainsworth et al., 1978). Additionally, information about confounding variables, including maternal sensitivity and co-sleeping, was collected during the first year of life.

Although there were no apparent differences in number of wakings in the first five to six weeks of life, and all infants decreased their night waking frequency, infant attachment at 12 months of age was related to different night waking patterns from around seven weeks onwards. Longitudinal regression analyses showed that, after controlling for many covariates, infants with an insecure-resistant attachment at 12 months of age awoke more during the night in their first 6 months of life than the other infants. Furthermore, infants with different attachment classifications developed different patterns of night wakings over the first 6 months, with the insecure-avoidant infants waking the least towards the end of the 6 months.

Summarizing, this study is the first in showing that infant attachment at 12 months of age was related to night waking patterns in the first six months

of life. As such, the development of the attachment relationship is apparently reflected in the regulation of signalling infant wakings during the night.

Study 5. The goal of the fifth study, described in Chapter 6, was to determine whether more hours spent in non-parental care and more concurrent non-parental care arrangements were related to more illnesses and health complaints during the first year of life. Previous research already found that children who attend centre-based childcare experience more communicable illnesses. However, studies about the influence of the amount of time spent in non-parental care on children's illnesses have been few and less consistent. Furthermore, research looking at whether children's health status is affected by the number of concurrent care arrangements is lacking. Number of concurrent care arrangements is defined here as the number of separate non-parental care arrangements a child regularly experiences during a single week. Moving among different settings not only exposes an infant to different environments with varied household products and chemicals, but could also be burdening for infants. Frequent transitions may delay or prevent the adjustment to the different care situations and the formation of dietary and sleep routines, which in turn could contribute to increased health problems (Morrissey, 2009).

During the first year of life, information on the frequency of infant illnesses and health complaints was obtained through monthly maternal interviews (see also study 1). Furthermore, these interviews provided information about the type of non-parental care used, the amount of time the child spent in non-parental care, and the number of concurrent arrangements. Analyses were done separately in infants for whom centre-based childcare was included in their arrangements (n=107), and for those in which it was not (n=61).

A considerable number of infants in our sample (about 35%) had two or more concurrent arrangements during their first year of life. Longitudinal regression analyses showed that infants who were cared for in more concurrent arrangements had more skin illnesses, but fewer respiratory and general illnesses. Furthermore, infants who spent more hours in non-parental care had more respiratory and general illnesses. However, these results only

applied to infants attending centre-based childcare. In the group of infants that did not attend centre-based care, health was not related to either the number of hours or the number of arrangements. Additionally, our results support earlier findings, by showing that infants attending centre-based childcare had more respiratory, digestive and general illnesses and complaints than infants who did not.

In conclusion, the number of hours spent in non-parental care and the number of concurrent arrangements do matter in relation to infant health early in life, but only for infants that include centre-based childcare in their arrangements. Interesting are our findings that show that infants who were cared for in more concurrent arrangements had less respiratory and general illnesses and complaints, but more skin illnesses and complaints. Although increased contagion appears to be the most important mechanism in contracting illnesses during the first years of life, it appears not to be the only mechanism behind increased rates of illnesses in non-parental care.

Study 6. The aim of the last empirical study presented in Chapter 7 was to investigate inhibitory control as a mediator of the link between early non-parental care and toddler behaviour problems. Several studies suggest that early non-parental care impacts different aspects of children's development, including their socio-emotional adjustment (i.e. behaviour problems). However, questions remain about the underlying mechanisms linking early non-parental care to internalizing and externalizing behaviour. As non-parental care, and especially centre-based care, is often characterized by challenges, it might be seen as a source of early life stress for young children. In turn, exposure to stress early in life might compromise the development of the neurobiological systems that are responsible for later regulatory capacities, including inhibitory control, potentially impacting the likelihood of behaviour problems (Loman & Gunnar, 2010). Therefore, we investigated inhibitory control as a mediator in the link between non-parental care within the first year of life and behaviour problems at 30 months of age. The effects of non-parental care, however, may not be the same for all children, but might be moderated by early-appearing individual differences. It is suggested that infants with a more

difficult temperament might encounter more challenges in non-parental care than infants with a less difficult temperament, increasing the risk of behaviour problems. Therefore, we additionally explored early temperamental negative affectivity as a moderator.

Information on early non-parental care was obtained for 187 infants through monthly maternal interviews across the first year of life (see also study 5). The following non-parental care measures were used in this study: type of non-parental care, the number of hours in centre-based care and non-parental care, the number of concurrent arrangements, the instability of care arrangements over time, and the age of entry into non-parental care. Negative affectivity was measured with a temperament questionnaire at 3 months of age (i.e. the age at which most infants began non-parental care). At 30 months of age, inhibitory control was measured with three observational tasks, and behaviour problem questionnaires were filled in by the mothers and the child care caregivers.

Early non-parental care was not related to observed inhibitory control in toddlerhood, so that the mediational model was not supported. Greater observed inhibitory control, however, was related to less caregiver-reported internalizing and externalizing behaviour. Furthermore, negative affectivity moderated the effect of early non-parental care on behaviour problems. Non-parental care was unrelated to behaviour problems in toddlers who displayed low or mean levels of negative affectivity as infants. For infants high in negativity, however, centre-based care was associated with more mother-rated internalizing and externalizing problems, while more hours in non-parental care were associated with less mother-rated internalizing problems.

To summarize, the link between non-parental care during the first year of life and toddlers' behaviour problems was not mediated by inhibitory control. Instead, inhibitory control and non-parental care in conjunction with negative affectivity, independently predicted toddlers' internalizing and externalizing behaviour problems.

8.2 Conclusions

- Maternal anxiety and stress during pregnancy are related to infant illnesses and antibiotic use during the first year of life.
- Solitary sleeping in young infants is associated with heightened cortisol reactivity to a bathing session at five weeks of age, but not to a vaccination at two months of age.
- More weeks of co-sleeping during the first six months of life predict lower infant cortisol reactivity to a maternal separation at 12 months of age, but only for children who also receive breastfeeding for at least six months.
- More weeks of breastfeeding during the first six months of life predict quicker cortisol recovery from a maternal separation at 12 months of age.
- Mother-infant attachment status at 12 months of age is related to the development of different night waking patterns in the first six months of life.
- For infants that attend centre-based childcare, more hours in non-parental care are related to more respiratory and general illnesses, while more concurrent arrangements are related to fewer respiratory and general illnesses, but more skin illnesses.
- Non-parental care during the first year of life is related to increased levels of toddlers' internalizing and externalizing behaviour problems, but only for children who were high in negative affectivity as infants.
- Non-parental care during the first year of life is not related to toddlers' inhibitory control.
- Less inhibitory control is related to more internalizing and externalizing behaviour problems at 30 months of age.

8.3 General Discussion

Positive environmental influences on infant cortisol regulation.

The findings in the present thesis showed that higher levels of breastfeeding and co-sleeping during the first months of life are, in general, positively associated with infant cortisol regulation, i.e. less strong reactions to a stressor

and a quicker recovery afterwards. While previous studies concentrated on stressful life experiences and quality of maternal care on infant cortisol regulation, influences of other caregiving factors, including breastfeeding and co-sleeping, were less well documented. Study 2 showed that infants sleeping alone in the first weeks of life respond with higher cortisol reactivity to a bathing session at 5 weeks of age, but their cortisol responses to a vaccination at 2 months of age are similar to those of young infants that co-sleep. Study 3 showed that more weeks of co-sleeping during the first six months of life predict lower infant cortisol reactivity to a maternal separation at 12 months of age, but only for children who also received breastfeeding for six months. Furthermore, this study showed that more weeks of breastfeeding predict quicker cortisol recovery from a maternal separation at 12 months of age.

While these studies point to the positive role of co-sleeping and breastfeeding on infant cortisol regulation, the effects of these caregiving factors varied somewhat depending on the age at which cortisol regulation was assessed. One reason for these alternating effects could be that caregiving influences on cortisol regulation change with infant age. However, in our study, the bathing session at 5 weeks of age and the vaccination at 2 months of age were close together in time, making such a developmental change an unlikely explanation. Another explanation might be the nature of the stress tasks. This may affect how and where stressors are processed in the brain, hence, leading to different effects for different stressors (Tollenaar, Beijers, Jansen, Riksen-Walraven & de Weerth, 2011). Moreover, cortisol regulation in reaction to the vaccination at 2 months of age yielded no results with respect to breastfeeding and co-sleeping. As the vaccination is known to elicit relatively strong cortisol responses, it might be a less optimal stressor to show differences in reactivity as compared to the bathing session and the maternal separation (Keenan, Gunthorpe & Grace, 2007). Finally, the last explanation for the alternating effects could be that the effects depend on the timing and duration of the caregiving factors. This thesis found main effects for co-sleeping on cortisol reactivity at 5 weeks of age, while an interaction effect between co-sleeping and breastfeeding on cortisol reactivity and a main effect of breastfeeding on cortisol recovery was found at 12 months of age. In line with the hypothesis

that co-sleeping facilitates parental co-regulation during infant night wakings, the role of co-sleeping might be the most important when night wakings are signaled the most, i.e. during the first weeks of life. Breastfeeding, on the other hand, has also been hypothesized to support maternal co-regulation, but in this case co-regulation might develop with increased duration of breastfeeding. Some aspects of breastfeeding hypothesized to be related to increased maternal co-regulation may take time to develop postnatally, such as the quality of the mother-infant interaction and the improved maternal mood when breastfeeding. If this were the case, the positive role of breastfeeding in cortisol regulation might be best seen when the infant receives breastfeeding for a longer period. However, as this explanation is speculative, future research is clearly needed.

In sum, these findings support the hypothesis that positive early experiences, including breastfeeding and co-sleeping, contribute to infant cortisol regulation. Hence, important empirical evidence for Hofer's (1994) concept of hidden regulators is provided. He argued that a number of components of typical parent-infant interactions have long-term regulatory effects on infant physiology. The regulatory role of the caregiving environment, especially very early in life, is also acknowledged in the 'Early Life Stress' model by noting its positive role in the development of the stress systems, including the HPA-axis (Loman & Gunnar, 2010). The 'Early Life Stress' model, however, mainly points to sensitive and responsive care by the parents as the critical aspect of the early caregiving environment. Importantly, this thesis shows the merit of breastfeeding and co-sleeping for infant cortisol regulation as well, possibly because breastfeeding and co-sleeping support parental co-regulation and as such promote the infant's own emergent capacity to regulate physiological arousal. However, as this latter explanation needs testing, underlying mechanisms of the links found should be topic of future research.

What happens during the night? Parent-infant nighttime interactions

This thesis also points to the importance of early environmental conditions during the night for the development of infant regulatory capacities. Studies 2 and 3 showed that co-sleeping during the night is related to better cortisol

regulation, i.e. lower infant cortisol reactivity to a bathing session at 5 weeks of age and, in conjunction with breastfeeding, to lower cortisol reactivity to a maternal separation at 12 months of age. Co-sleeping, as compared to solitary sleeping, is hypothesized to provide more parental external stress regulation during the night. Parental co-regulation, in turn, would protect the infant from excessive levels of distress and promote the infant's emergent capacity to regulate physiological arousal (Schore, 2001). Although our findings of lower cortisol reactivity to stressors support this hypothesis, it remains unclear if co-sleeping indeed is related to increased parental co-regulation by night, i.e. it remains unclear what exactly happens during the night.

The same goes for Study 4, which showed that infant attachment at 12 months of age is related to infant night waking patterns in the first six months of life. It was hypothesized that night waking patterns reflect the experiences of the infant with the primary caregivers and, as such, the emerging attachment relationship. Our results showed that infants who are resistantly attached at 12 months of age showed more night wakings in their first 6 months of life than the other infants. Furthermore, infants with different attachment classifications develop different patterns of night waking, with the avoidantly attached infants ending up waking the least toward the end of the 6 months. These findings are in line with what one would expect according to the attachment theory (Ainsworth et al., 1978). Moreover, as the different night waking patterns become apparent from around seven weeks onwards, they constitute a remarkably early sign of the type of attachment bond that is being formed. Nevertheless, what exactly happens during the night also remains unknown in this study. In other words, are the differences seen in night waking patterns by attachment classification related to differences in infant behavior, differences in parent behavior, or differences in parent perceptions of infant behavior? Future studies using observational measures of parent-infant nighttime interactions can help unravel how differences in night waking patterns emerge and how attachment bonds are formed.

Summarizing, these studies suggest that the way parents respond to and co-regulate infant night wakings is important for the development of infant cortisol regulation, behavioural regulation (i.e. coping with night wakings)

and the formation of the parent-infant attachment bond. Although maternal sensitivity during daytime interactions has been frequently linked to child outcomes, developmental studies including observational measures of parent responses to infant night wakings are limited and therefore clearly needed. This new area of research would not only shed more light upon the possible consequences of parent-infant nighttime interactions for child development, but they would also provide clues as to how to best deal with infant night wakings. This is relevant for clinical practice as night wakings can cause distress and exhaustion for parents and are among the most common complaints to pediatricians and other professionals (Sadeh, Tikotzky & Scher, 2010).

What about non-parental care? Moderators and mediators

The findings in the present thesis showed that multiple aspects of non-parental care are related to infant health and toddler behavior problems. However, these effects were shown not to affect all children in the same way. Study 5 showed that infants who were cared for in more concurrent arrangements had more skin illnesses, but fewer respiratory and general illnesses. Furthermore, infants who spent more hours in non-parental care had more respiratory and general illnesses. However, these results only applied to infants attending centre-based childcare. Study 6 showed that the link between non-parental care during the first year of life and toddlers' behaviour problems was moderated by temperamental negative affectivity. For infants high in negativity, centre-based care was associated with more internalizing and externalizing problems, while more non-parental care hours were associated with less internalizing problems at 30 months of age. These two studies point to the importance of taking moderators into account when studying the role of non-parental care, as the relations between non-parental care and child outcomes apparently differ for children attending centre-based care or not, and for children varying in early negativity.

Contrary to our expectations, Study 6 showed that early non-parental care was not related to toddlers' inhibitory control and therefore could not mediate the link between non-parental care and behaviour problems. Although the ability to inhibit and override dominant responses in favour of

more appropriate responses is a fundamental and perhaps the most studied aspect of behavioural regulation (Eisenberg, Spinrad & Eggum, 2010), it might be that other aspects of behavioural regulation are affected by non-parental care. For example the ability to focus and reorient attention also develops early in life and is related to later externalizing problems (Crockenberg, Leerkes & Jo, 2008). Another possible explanation for the lack of a relationship between non-parental care and inhibitory control could be that children in the Netherlands spend, on average, fewer hours in non-parental care than children of other countries, including the U.S. Although non-parental care might be seen as a source of early life stress for infants and children, it is very well possible that little to modest exposure to non-parental care is manageable for most children and absent of negative effects on regulatory capacities. Nevertheless, as inhibitory control was not found to be a mediator, it remains unclear how non-parental care is related to internalizing and externalizing problems in toddlers that were high in negativity as infants. One possibility could be that infants with a more difficult temperament experience difficulties adapting to non-parental care, including the dietary and sleep routines, which in turn could contribute to increased behaviour problems.

In conclusion, when studying the effects of non-parental care on child health and socio-emotional development, this thesis highlights the importance of looking at multiple aspects of non-parental care, including type and number of hours, and taking moderator effects into account, including infant temperament and centre-based care. Furthermore, as the underlying mechanisms remain unclear, future developmental studies unraveling how multiple aspects of non-parental care are related to child health and development are clearly needed. These future studies might also expand empirical information relevant for clinical practice. When developing optimal childcare advices and policies, the results in this thesis suggests that the most optimal decision about non-parental care is not the same for all families, but that children might benefit from a tailored choice of non-parental care based upon child characteristics, including temperamental negative affectivity, and multiple aspects of non-parental care, including attendance of a centre-based care or not.

The role of the early environment in infant health

Two types of early life experiences were linked to illnesses and health complaints during the first year of life, namely prenatal maternal stress and anxiety (Study 1) and non-parental care (Study 5). Study 1 linked maternal stress and anxiety during pregnancy to more infant illnesses and antibiotic use early in life. The explained variance was considerable: 9.3% for respiratory, 10.7% for general, 8.9% for skin, and 7.6% for antibiotic use. Study 5 showed that infants attending centre-based care had more respiratory, digestive and general illnesses and complaints. Also, for the group of infants that attended centre-based childcare, the number of hours and the number of arrangements mattered. Infants who spent more hours in non-parental care had more respiratory and general illnesses, and infants who were cared for in more concurrent arrangements had fewer respiratory and general illnesses, but more skin illnesses.

These findings support the hypothesis that early experiences associated with challenges are related to more illnesses and health complaints early in life. The data collected for this thesis do not uncover the underlying mechanisms behind the links found. However, it is possible to speculate on these mechanisms. Although contagion appears to be the most important mechanism in contracting communicable illnesses, there are other possible mechanisms through which the early environment could be related to infant health problems. One possible mechanism is that exposure to abnormal cortisol levels in utero programs the functioning of the immune system of the fetus (Sapolsky, Romero & Munck, 2000), resulting in increased susceptibility to illnesses early in life. The role of stress in contracting illnesses is also underlined in the non-parental care study. A possible mechanism behind the positive association between skin illnesses and number of arrangements lies in difficulties with the establishment of dietary and sleep routines, or difficulties with the adjustment to different settings. Infants who experience frequent transitions on a weekly basis may not have adequate time or opportunity to adapt to different routines and settings.

In sum, it can be concluded that early life experiences, such as prenatal maternal stress and multiple aspects of non-parental care, are related

to illnesses early in life. As such, this thesis taps into a new area for future research incorporating infant illnesses and health complaints when studying the early environment. Whether these apparent effects will play a role in the future health of these children, either positive or negative, remains to be explored. Also, future studies into the underlying mechanisms, including the role of stress, cortisol and the immune system, are warranted.

8.4 Strengths and limitations

The studies presented in this thesis are all part of the prospective and longitudinal BIBO study that distinguishes itself from other studies by multiple strengths. Both environmental factors and infant outcomes are intensively measured, such as by using daily and weekly diaries for the first six months of life, monthly maternal interviews and repeated exposure to stressors during the first year of life, and the use of observational tasks and multiple-informants ratings at 30 months of age. In addition, several relevant confounding variables were also extensively measured and subsequently controlled for. This prospective, multidisciplinary and longitudinal design enables us to carefully investigate the development of infant regulation and health, in conjunction with determining the role of the early maternal and caregiving environment, within the first years of life.

Nevertheless, some limitations should also be noticed. First, almost all mothers were highly educated and lived together with their partner, which limits the generalizability of the study. Second, due to the relatively large sample size and intensive longitudinal design, a part of the data was necessarily based on maternal report. Although maternal report is a valid and well-accepted method for the assessment of infant outcomes, including sleep and health (Monette, Séguin, Gauvin & Nikiéma, 2007; Müller, Hemmi, Wilhelm, Barr & Schneider, 2011), it could be influenced by maternal characteristics. Precautions were taken in this study to reduce a possible reporter bias to the minimum. First, we controlled for several maternal characteristics, including postnatal depression and stress, educational level, and quality of caregiving behavior. Second, scores were based on data from multiple time points. Nevertheless, future research should also consider other (complementary)

methods that could help shed more light on the mechanisms behind the findings, such as observational methods of infant wakings during the night.

8.5 Directions for future research

This thesis generates new questions that could be addressed in future studies. Some of these questions were already discussed above, but other questions remain. First, while this thesis adds importantly to research by showing relations between the early environment and infant regulation and health, the relations and interactions between the behavioral and physiological levels of regulation and health remain unclear. For example, there are indications that high cortisol responses might increase the susceptibility to physical illnesses in adults (Sheridan, Dobbs, Jung, Chu, Konstantinos, Padgett, et al., 1998), but little is known about the association between cortisol reactivity and vulnerability to physical illnesses in younger populations, including infants.

Second, this thesis focused on the maternal and caregiving environment, while the role of the father remained underexposed. Evidence is accumulating to support the positive effect of father engagement on child development. Active and regular engagement of the father with the child predicts a range of positive child outcomes, including less behavioural problems, less psychological problems, and enhanced cognitive development (Sarkadi, Kristiansson, Oberklaid & Bremberg, 2008). Hence, it can also be hypothesized that the father plays an important role in the development of regulatory capacities.

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Nederlandse samenvatting

Summary in Dutch

Samenvatting van het project

Omgevingsfactoren tijdens de zwangerschap en de postnatale periode hebben invloed op de cognitieve, sociaal-emotionele en gedragsontwikkeling van kinderen en volwassenen. Daarnaast hebben deze factoren ook invloed op de fysieke gezondheid. De vraag blijft echter wat de onderliggende mechanismen zijn die deze vroege omgevingsfactoren relateren aan uitkomsten later in het leven (Loman & Gunnar, 2010; Taylor, Way & Seeman, 2011).

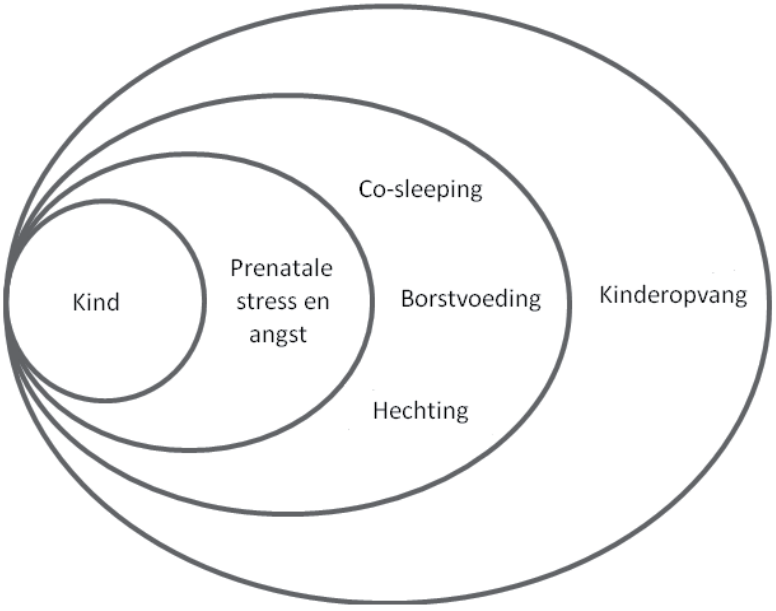
Het doel van dit proefschrift was het onderzoeken van de effecten van de vroege maternale en zorgomgeving op de regulatie en gezondheid van baby's en peuters. Regulatie kan worden gemeten op meerdere niveaus en omvat zowel de regulatie van gedrag en emoties als de regulatie van fysiologie (Haley & Stansbury, 2003). Regulatieproblemen spelen een belangrijke rol in de ontwikkeling van psychopathologie. Onderzoek naar de ontwikkeling van regulatie, in combinatie met het bepalen van de rol van de vroege maternale en zorgomgeving, geeft inzicht in de normale ontwikkeling van kinderen en de mechanismen die ten grondslag liggen aan de relatie tussen de vroege omgeving en psychopathologie (Posner & Rothbart, 2000).

Naast regulatie is ook de fysieke gezondheid van baby's onderzocht. Empirische studies hebben laten zien dat negatieve ervaringen vroeg in het leven gerelateerd zijn aan het disfunctioneren van het immuunsysteem en ziekten in de volwassenheid, zoals cardiovasculaire ziekten (Galobardes, Smith & Lynch, 2006; Miller, Chen & Cole, 2009). Minder is bekend over de relatie tussen vroege ervaringen en de fysieke gezondheid in jongere populaties. Het blijft onduidelijk of negatieve ervaringen leiden tot ziekten vroeg in het leven of dat de negatieve effecten worden opgeslagen in een latente vorm om later naar voren te treden (Ben-Shlomo & Kuh, 2002).

De invloed van de vroege omgeving is gelukkig niet beperkt tot negatieve effecten. Positieve omgevingsfactoren, waaronder een hoge kwaliteit van de zorg door de moeder, zijn gerelateerd aan positieve kinduitkomsten. De volgende omgevingsfactoren zijn in dit proefschrift bestudeerd: maternale stress en angst tijdens de zwangerschap, borstvoeding, co-sleeping, moeder-kind hechting en kinderopvang (zie figuur 1). De studies maken allen deel uit van het BIBO-onderzoek (BIBO staat voor Basale Invloeden op de Baby

Ontwikkeling). Het BIBO-onderzoek is een prospectief onderzoek dat 193 moeders en hun kinderen heeft gevolgd vanaf de zwangerschap en tijdens de eerste levensjaren. Voor dit proefschrift zijn de data gebruikt van de meetronde tijdens de zwangerschap, van de meervoudige meetrondes tijdens het eerste levensjaar en van de meetronde op 30 maanden.

Studie 1. Het doel van de eerste studie, beschreven in hoofdstuk 2, was het onderzoeken of angst en stress bij de moeder tijdens de zwangerschap zijn gerelateerd aan meer ziekten en antibiotica gebruik tijdens het eerste levensjaar van de baby. Dieronderzoek heeft laten zien dat prenatale stress een negatieve invloed heeft op het immuunsysteem van de nakomelingen. Humane studies hebben zich echter doorgaans beperkt tot geboortecomplicaties als gevolg van prenatale stress en angst, waaronder zwangerschapsduur en geboortegewicht (Merlot, Couret & Otten, 2008).



Figuur 1: De onderzochte maternale en zorgfactoren in dit proefschrift. De ovalen aan de rechterkant vertegenwoordigen de groter wordende omgeving van het kind met toename in leeftijd.

Om maternale prenatale angst en stress te kunnen meten hebben 174 moeders tijdens het laatste trimester van de zwangerschap vragenlijsten ingevuld met betrekking tot algemene en zwangerschapsgerelateerde angst en stress. Daarnaast hebben de moeders speeksel verzameld op twee opeenvolgende dagen om het cortisol circadiaan ritme te bepalen. Informatie betreffende de gezondheid van de baby werd verkregen met behulp van maandelijkse interviews tijdens het eerste levensjaar. Verder werd aanvullende informatie verzameld over mogelijk storende variabelen, waaronder angst en stress ervaren door de moeder op 3, 6 en 12 maanden postnataal.

Zoals verwacht liet deze studie zien dat angst en stress ervaren door de moeder tijdens de zwangerschap gerelateerd waren aan ziekten en antibioticagebruik tijdens het eerste jaar. Prenatale angst en stress voorspelden een aanzienlijke hoeveelheid variantie: 9.3% voor ziekten van de luchtwegen, 10.7% voor algemene ziekten, 8.9% voor ziekten van de huid, en 7.6% voor antibiotica gebruik. Ziekten van de spijsvertering waren niet gerelateerd aan prenatale angst en stress.

Samengevat is deze studie de eerste studie die prenatale angst en stress, gemeten door zowel zelfrapportage als cortisol fysiologie, aan meer ziekten en antibiotica gebruik tijdens het eerste jaar van de baby relateert. De veronderstelde onderliggende mechanismen wijzen op de mogelijkheid dat prenatale angst en stress het immuunsysteem van de baby beïnvloeden. Ook al wordt deze hypothese ondersteund door uitgebreid onderzoek bij dieren en enkele humane studies met betrekking tot immuniteit, is er geen direct bewijs dat het immuunsysteem van de baby's inderdaad is aangetast. Als zodanig boort deze studie een nieuw gebied aan voor toekomstig onderzoek en wordt het belang van de gezondheid en het immuunsysteem onderstreept wanneer maternale prenatale angst en stress wordt onderzocht.

Studie 2. De tweede studie, gerapporteerd in Hoofdstuk 3, onderzocht de relatie tussen de slaapplek van baby's en cortisol reactiviteit op twee stressoren. Vergeleken met afgezonderd slapen van de ouders werd verondersteld dat co-sleeping zou zorgen voor meer externe regulatie van de baby door de ouders tijdens de nacht. Externe regulatie door de ouders

beschermt de baby tegen overmatige niveaus van stress en bevordert de ontwikkelende vaardigheid van het kind om fysiologische arousal te reguleren (Schore, 2001). Verwacht werd dan ook dat baby's die samen slapen met hun ouders lagere cortisol reactiviteit lieten zien.

Informatie over de slaapplek werd verzameld voor 163 baby's met behulp van dagelijkse slaapdagboeken in de eerste twee maanden van het leven. De moeders ontvingen de dagboeken en bijbehorende instructies aan het eind van de zwangerschap, zodat onmiddellijk kon worden gestart na de geboorte. Co-sleeping werd geclassificeerd als het slapen van de baby in het ouderlijk bed en/of in een eigen bed in de kamer van de ouders. Het percentage co-sleeping werd berekend per nacht en vervolgens werden de baby's verdeeld in 3 groepen: alleen slapen (co-sleeping voor 0-10% van de tijd), gedeeltelijke co-sleeping (11-90% van de tijd) en volledige co-sleeping (co-sleeping voor 91-100% van de tijd). Cortisol reactiviteit werd twee keer gemeten: in reactie op een badsessie op 5 weken en in reactie op een vaccinatie op 2 maanden.

Uit de longitudinale regressie analyses bleek dat baby's die alleen sliepen een hogere cortisol reactie lieten zien op de badsessie op 5 weken, dan baby's die een gedeelte of de volledige tijd samen sliepen met hun ouders. Dit effect werd niet verklaard door storende variabelen, waaronder borstvoeding, maternale sensitiviteit en slaapduur. Co-sleeping was niet gerelateerd aan de cortisol reactie op de vaccinatie op 2 maanden.

Dit is de eerste studie die prospectief de relaties heeft onderzocht tussen de slaapplek van de baby en cortisol reactiviteit. De resultaten suggereren dat afgezonderd slapen van de ouders in de eerste maand van het leven, vergeleken met co-sleeping met de ouders, gepaard gaat met verhoogde reactiviteit van de HPA-as van de baby op een milde stressor. Dit is mogelijk te verklaren door verminderde beschikbaarheid van de ouders als externe stress regulator tijdens de nacht.

Studie 3. De studie gepresenteerd in Hoofdstuk 4 had als doel het onderzoeken of type voeding en de slaapplek gedurende de eerste 6 maanden van het leven waren geassocieerd met cortisol regulatie bij baby's op 12 maanden.

Er is gekeken naar zowel cortisol reactiviteit als herstel op een stressor. Borstvoeding en co-sleeping werden verondersteld de externe regulatie van de baby door de ouders te bevorderen. Daarom werd verwacht dat borstvoeding en co-sleeping voor een langere tijd gerelateerd zouden zijn aan betere cortisol regulatie, dat wil zeggen een minder sterke cortisol reactie op de stressor en een sneller herstel na afloop.

Informatie met betrekking tot type voeding werd verzameld door middel van een wekelijks logboek voor de eerste 6 maanden van het leven. Het aantal weken dat een baby exclusief borstvoeding had gekregen, of borstvoeding in combinatie met flesvoeding, werd gebruikt als onafhankelijke variabele. Informatie met betrekking tot de slaapplek werd verzameld door middel van een slaapdagboek voor de eerste 6 maanden van het leven (zie ook studie 2). Het aantal weken waarin sprake was van 10-100% van de tijd co-sleeping werd gebruikt als onafhankelijke variabele. Deze informatie werd verzameld door 173 moeders. Op 12 maanden werden de baby's onderworpen aan een stressor die bestaat uit de komst van een vreemde en twee korte separaties van de moeder (de Strange Situation Procedure; Ainsworth, Blehar, Waters & Wall, 1978). Er werd speeksel verzameld bij de baby's om cortisol reactiviteit en herstel te meten.

Regressie analyses lieten zien dat meer weken co-sleeping inderdaad lagere cortisol reactiviteit op de Strange Situation Procedure voorspelde. Dit gold echter alleen voor kinderen die ook borstvoeding hadden gekregen voor een relatief lange tijd (6 maanden). Daarnaast voorspelde meer weken borstvoeding, maar niet co-sleeping, een sneller herstel na afloop van de stressor. Deze effecten werden gevonden na corrigeren voor storende variabelen, waaronder maternale sensitiviteit, hechtingstatus en het type voeding en slaapplek op 12 maanden.

Deze resultaten suggereren dat borstvoeding en co-sleeping vroeg in het leven positief bijdragen aan de cortisol regulatie van baby's op 12 maanden. Als zodanig steunen deze bevindingen de hypothese dat vroege omgevingsfactoren, waaronder borstvoeding en co-sleeping, een positieve bijdrage leveren aan de ontwikkeling van cortisol regulatie. Eén verklaring voor deze bevinding zou kunnen zijn dat borstvoeding en co-sleeping de

externe regulatie van de baby door de ouders bevorderen, en als zodanig de ontwikkelende vaardigheid bevorderen om fysiologische arousal te reguleren later in het leven.

Studie 4. De studie gepresenteerd in Hoofdstuk 5 onderzocht de geschiedenis van het aantal keer wakker worden tijdens de nacht bij baby's die later werden geclassificeerd als veilig of onveilig gehecht (vermijdend, afwerend of gedesorganiseerd). De kwaliteit van de hechtingsrelatie kan niet betrouwbaar worden gemeten voor de leeftijd van 12 maanden, wanneer een 'duidelijke hechtingsstijl' zich heeft ontwikkeld (Ainsworth et al., 1978). De hechtingsrelatie ontwikkelt zich echter gradueel gedurende het eerste levensjaar: dit proces wordt ook wel hechting-in-wording genoemd. Tijdens dit proces bouwen baby's verwachtingen op over de wijze waarop verzorgers hun behoeften zullen voldoen. Deze verwachtingen worden vervolgens gereflecteerd in het signaleren van stress en ander contact bevorderend gedrag. De afwezigheid van de hechtingsfiguur 's nachts kan stressvol zijn voor baby's. Verwacht werd daarom dat de reactie van een baby op het wakker worden 's nachts mogelijk de ontwikkelende hechtingsrelatie reflecteert.

Informatie over het aantal keer wakker worden 's nachts werd verzameld door middel van een slaapdagboek voor de eerste 6 maanden van het leven (zie ook studie 2). Moeder-kind hechting werd bepaald aan de hand van de Strange Situation Procedure (Ainsworth et al., 1978) toen de kinderen 12 maanden oud waren. Informatie over storende variabelen, waaronder maternale sensitiviteit en co-sleeping, werd verzameld tijdens het eerste levensjaar.

Tijdens de eerste 5 tot 6 weken van het leven zijn er geen ogenschijnlijke verschillen in het aantal keer wakker worden 's nachts tussen baby's, en de frequentie van wakker worden neemt bovendien af voor alle baby's. Echter vanaf ongeveer 7 weken is de kwaliteit van hechting gerelateerd aan verschillende patronen van wakker worden. Longitudinale regressie analyses lieten zien dat, na het controleren voor verschillende storende variabelen, kinderen met een onveilig-afwerende hechting op 12 maanden meer wakker werden tijdens de eerste 6 maanden van het leven dan andere

kinderen. Daarnaast bleken kinderen met verschillende hechtingsclassificaties op 12 maanden, verschillende patronen van wakker worden te ontwikkelen tijdens de eerste 6 maanden van het leven, waarbij de onveilig-vermijdende kinderen het minst wakker werden aan het einde van de eerste 6 maanden.

Samenvattend liet deze studie als eerste zien dat de moeder-kind hechtingsrelatie op 12 maanden is gerelateerd aan verschillende patronen van wakker worden tijdens de eerste 6 maanden van het leven. Blijkbaar wordt de ontwikkeling van de hechtingsrelatie gereflecteerd in het signaleren van het wakker worden door baby's tijdens de nacht.

Studie 5. Het doel van de vijfde studie, beschreven in Hoofdstuk 6, was het onderzoeken of het aantal uren doorgebracht in kinderopvang, en het aantal gelijktijdige kinderopvang regelingen, waren gerelateerd aan meer ziekten en gezondheidsklachten bij baby's tijdens het eerste jaar. Het aantal gelijktijdige kinderopvang regelingen is gedefinieerd als het aantal afzonderlijke kinderopvang regelingen waar een kind regelmatig naar toe gaat gedurende een week. Voorbeelden van kinderopvang regelingen zijn opvang door oma's en opa's, het kinderdagverblijf en een gastgezin. Voorgaand onderzoek liet al zien dat kinderen die naar het kinderdagverblijf gaan vaker ziek worden dan kinderen die niet naar het kinderdagverblijf gaan. Er is echter minder onderzoek gedaan naar de invloed van het aantal uren in kinderopvang op het krijgen van ziekten. Daarnaast ontbreekt onderzoek dat kijkt of de gezondheid van kinderen wordt beïnvloed door het aantal gelijktijdige kinderopvang regelingen. Het gaan naar meerdere opvangplekken stelt een baby niet alleen bloot aan verschillende omgevingen met verschillende huishoudelijke producten en chemicaliën, maar kan ook belastend zijn voor baby's. Zo kunnen frequente transities het aanpassingsproces aan verschillende opvangplekken en de vorming van eet- en slaaproutines vertragen of verhinderen, wat kan leiden tot een toename in gezondheidsproblemen (Morrissey, 2009).

Gedurende het eerste levensjaar werd informatie verzameld over het aantal ziekten en gezondheidsklachten bij baby's door middel van maandelijkse interviews met de moeder (zie ook studie 1). Deze interviews leverde ook informatie over het type opvang, het aantal uren opvang en het aantal

gelijktijdige opvang regelingen. Analyses werden apart gedaan voor baby's die naar het kinderdagverblijf gingen (n=107), en voor baby's die niet naar het kinderdagverblijf gingen (n=61).

Een aanzienlijk aantal kinderen in onze steekproef (ongeveer 35%) had twee of meer gelijktijdige kinderopvang regelingen tijdens het eerste levensjaar. Longitudinale regressie analyses lieten zien dat kinderen met meer gelijktijdige opvang regelingen meer huidziekten hadden, maar minder ziekten van de luchtwegen en algemene ziekten. Verder bleken kinderen die meer tijd doorbrachten in opvang, meer ziekten van de luchtwegen en algemene ziekten te hebben. Deze bevindingen waren echter alleen van toepassing op de kinderen die naar het kinderdagverblijf gingen. Voor de kinderen die niet naar het kinderdagverblijf gingen, was gezondheid niet gerelateerd aan het aantal uren opvang of gelijktijdige opvang regelingen. Daarnaast ondersteunden onze resultaten eerder onderzoek door te laten zien dat kinderen die naar het kinderdagverblijf gingen meer ziekten van de luchtwegen, spijsvertering en algemene ziekten hadden dan kinderen die niet naar het kinderdagverblijf gingen.

Samengevat zijn het aantal uren kinderopvang en het aantal gelijktijdige kinderopvang regelingen van belang voor de gezondheid van baby's, maar alleen voor de baby's die naar het kinderdagverblijf gaan. Interessant waren onze bevindingen dat baby's met meer gelijktijdige opvang regelingen minder ziekten van de luchtwegen en algemene ziekten hadden, maar meer ziekten van de huid. Hoewel besmetting het meest belangrijke mechanisme lijkt te zijn in het krijgen van ziekten vroeg in het leven, lijkt het niet het enige mechanisme te zijn achter de toename van ziekten in het kinderdagverblijf.

Studie 6. Het doel van de laatste studie gepresenteerd in Hoofdstuk 7 was het onderzoeken of inhibitie een mogelijk onderliggend mechanisme zou kunnen zijn in de relatie tussen kinderopvang tijdens het eerste jaar en gedragsproblemen op 30 maanden. Meerdere studies hebben al laten zien dat kinderopvang invloed heeft op verschillende aspecten van de ontwikkeling, waaronder de sociaal-emotionele ontwikkeling. De vraag blijft echter

wat de onderliggende mechanismen zijn die kinderopvang relateren aan internaliserend en externaliserend gedrag. Kinderopvang, en in het bijzonder het kinderdagverblijf, wordt vaak gekenmerkt door uitdagingen en kan worden gezien als een vorm van stress voor jonge kinderen. Op zijn beurt kan stress vroeg in het leven invloed hebben op de ontwikkeling van de neurobiologische systemen die verantwoordelijk zijn voor de ontwikkeling van regulatie vaardigheden, waaronder de mogelijkheid tot het inhiberen van gedrag, die op hun beurt de kans op gedragsproblemen kunnen vergroten (Loman & Gunnar, 2010). Daarom werd inhibitie onderzocht als een mediator in de link tussen kinderopvang en probleemgedrag. Echter, de invloed van kinderopvang is waarschijnlijk niet hetzelfde voor alle kinderen, maar wordt mogelijk gemodereerd door vroege individuele verschillen. Het wordt gesuggereerd dat baby's met een moeilijker temperament meer uitdagingen ondervinden in kinderopvang, dan baby's met een makkelijker temperament, waardoor het risico op gedragsproblemen toeneemt. Daarom werd temperament onderzocht als moderator in de link tussen kinderopvang en gedragsproblemen.

Informatie over kinderopvang werd verzameld voor 187 baby's door middel van maandelijkse interviews gedurende het eerste jaar (zie ook studie 5). De volgende variabelen werden gebruikt: type opvang, het aantal uren in kinderdagverblijf en opvang, het aantal gelijktijdige opvang regelingen, de instabiliteit van opvang regelingen over tijd en de beginleeftijd in opvang. Negatieve affectiviteit werd gemeten met behulp van een temperamentvragenlijst op 3 maanden. Meer negatieve affectiviteit wordt geassocieerd met een moeilijker temperament. Op 30 maanden werd inhibitie gemeten met behulp van drie observatietaken. Gedragsproblemen werden gemeten met vragenlijsten die zowel door de moeder als door de leidsters van de opvang werden ingevuld.

Opvang tijdens het eerste jaar was niet gerelateerd aan inhibitie in de kleutertijd en dus werd het mediatie model niet ondersteund. Echter, een toename in inhibitie was gerelateerd aan minder leidster-gerapporteerde internaliserend en externaliserend gedrag. Verder modereerde negatieve affectiviteit de relatie tussen opvang en gedragsproblemen. Opvang was namelijk niet gerelateerd aan gedragsproblemen in kleuters die laag of

gemiddeld waren in negatieve affectiviteit als baby's. Echter, voor baby's hoog in negatieve affectiviteit was het gaan naar een kinderdagverblijf geassocieerd met meer moeder-gerapporteerde internaliserende en externaliserende problemen in de kleutertijd, terwijl meer uren opvang was geassocieerd met minder moeder-gerapporteerde internaliserende problemen.

Samengevat werd de relatie tussen kinderopvang tijdens het eerste jaar en gedragsproblemen op 30 maanden niet gemedieerd door inhibitie. In plaats daarvan blijken inhibitie, en kinderopvang in combinatie met temperament, twee onafhankelijke voorspellers van internaliserend en externaliserend probleemgedrag bij kleuters.

Conclusies

- Angst en stress ervaren door de moeder tijdens de zwangerschap zijn gerelateerd aan ziekten en antibiotica gebruik tijdens het eerste levensjaar van de baby.
- Baby's die afgezonderd slapen van hun ouders laten verhoogde cortisol reactiviteit zien op een badsessie op 5 weken, maar niet op een vaccinatie op 2 maanden.
- Meer weken co-sleeping tijdens de eerste 6 maanden van het leven voorspelt lagere cortisol reactiviteit op een maternale separatie op 12 maanden, maar dit geldt alleen voor de kinderen die ook borstvoeding hebben gekregen voor een relatief lange tijd (6 maanden).
- Meer weken borstvoeding tijdens de eerste 6 maanden van het leven voorspelt sneller cortisol herstel na een maternale separatie op 12 maanden.
- Moeder-kind hechting op 12 maanden is gerelateerd aan de ontwikkeling van verschillende patronen van wakker worden 's nachts in de eerste 6 maanden van het leven.
- Voor baby's die naar een kinderdagverblijf gaan geldt dat meer uren opvang is gerelateerd aan meer ziekten van de luchtwegen en algemene ziekten, terwijl meer gelijktijdige kinderopvang regelingen is gerelateerd aan minder ziekten van de luchtwegen en algemene ziekten, maar meer ziekten van de huid.

- Opvang tijdens het eerste jaar is gerelateerd aan meer gedragsproblemen op 30 maanden, maar dit geldt alleen voor kleuters die als baby hoog waren in negatieve affectiviteit.
- Kinderopvang tijdens het eerste jaar is niet gerelateerd aan inhibitie op 30 maanden.
- Minder inhibitie is gerelateerd aan meer internaliserend en externaliserend probleemgedrag op 30 maanden.

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Curriculum Vitae and Publications

Curriculum Vitae

Roseriet Beijers was born on June 19th, 1984, in Deurne, the Netherlands. She graduated from high school (Atheneum level) at the Peelland College Deurne in 2002. Subsequently she studied Psychology at Tilburg University. Within the group of Prof. Anneloes van Baar, she wrote her Master thesis regarding the relations between maternal prenatal stress and preschoolers' development. She finished her Master in Developmental Psychology in September 2006 (Cum Laude).

In March 2007, Roseriet started her PhD at the department of Developmental Psychology, Behavioural Science Institute, at the Radboud University Nijmegen. Her PhD studies focused on investigating the role of the early maternal and caregiving environment on the development and health of infants and young children, and were supervised by Dr. Carolina de Weerth and Prof. Dr. Marianne Riksen-Walraven. In addition, RB initiated collaboration with Dr. Samuel Putnam of Bowdoin College (Brunswick, United States), which has resulted in a joint paper currently in revision.

At this moment, she is working as a junior-docent/postdoctoral researcher at the department of Developmental Psychology, Radboud University Nijmegen.

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