Aggressive Behavior in Early Childhood

The role of prenatal risk and self-regulation

Jill Suurland

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INDEX

Chapter 1. General introduction 7

Chapter 2. Aggressive behavior in early childhood: The role of emotional and cognitive self-regulation 19

Chapter 3. The Mother-Infant Neurodevelopment Study (MINDS) – Leiden: Background, design and study population 39

Chapter 4. Aggressive behavior in early childhood: The role of prenatal risk and physiological self-regulation 63

Chapter 4. 1. Interaction between prenatal risk and physiological self-regulation in infancy and physical aggression at 20 months 63

Chapter 4. 2. Interaction between prenatal risk and physiological self-regulation in infancy and physical aggression and oppositional behavior at 30 months 79

Chapter 5. Effects of prenatal risk on physiological self-regulation in infancy 103

Chapter 6. Summary and general discussion 135

References 149

Nederlandse samenvatting (Summary in Dutch) 179

Dankwoord (Acknowledgements) 193

Curriculum vitae 195

List of publications 197

CHAPTER 1

General introduction

1

Central to this study is the search for mechanisms that predict the risk for aggressive behavior in early human development. Aggressive behavior is part of typical development of young children (Tremblay, 2000), and is seen as a natural way to express negative emotions such as anger and frustration. Physical aggression typically peaks around age two and three, and then declines from the fourth year onwards (Alink et al., 2006). However, studies investigating trajectories of aggressive behavior over time, have found that a high level of aggressive behavior in early childhood is a strong predictor of delinquency and antisocial behavior later in life (Côté, Vaillancourt, LeBlanc, Nagin, & Tremblay, 2006; Nagin & Tremblay, 1999; NICHD Early Child Care Research Network, 2004). Young children with persistent high levels of aggressive behavior may face negative long-term outcomes including low academic achievement, poor interpersonal relationships, alcohol and drug abuse, accidents, violent crimes, depression, suicide attempts, spouse abuse, and neglectful and abusive parenting (Campbell et al., 2006; Farrington, 1994; Fergusson & Horwood, 1998; Kokko & Pulkkinen, 2000; Nagin & Tremblay, 1999; Serbin et al., 1998; Stattin & Magnusson, 1989). Chronic aggressive behavior is difficult to treat, and current successful psychosocial intervention programs only achieve a moderate effect size at best (Fossum, Handegard, Martinussen, & Morch, 2008; Smeets et al., 2015). Severe aggressive behavior during childhood and adolescence may come at high costs for individuals, their family and society. In 2015, the Dutch government stated in a report that the consequences of criminal behavior cost our society more than €20 billion euros per year (Moolenaar, Vlemmings, Van Tulder, & De Winter, 2016). Research on factors that help to identify early mechanisms that predict the risk for aggression at a very early age may eventually help to diminish the impact of early aggression on later development.

Longitudinal studies have identified a number of maternal prenatal risk factors associated with high levels of aggressive behavior during (early) childhood, such as low socioeconomic status, low educational attainment, early entry into parenthood (Côté et al., 2006; NICHD Early Child Care Research Network, 2004; Tremblay et al., 2004), smoking during pregnancy (Huijbregts, Seguin, Zoccolillo, Boivin, & Tremblay, 2008; Tremblay et al., 2004), mothers' own history of antisocial behavior (Hay, Pawlby, Waters, Perra, & Sharp, 2010; Tremblay et al., 2004), and high levels of stress, anxiety or depression during pregnancy (Hay et al., 2011; O'Connor, Heron, Golding, Beveridge, & Glover, 2002). Others have established the role of stressful, adverse home environments and negative parenting practices during the first years of life in the development of aggressive behavior (Tremblay et al., 2004). However, current theoretical models stress that the influence of these risk factors on the development of aggressive behavior is influenced in part by the child's ability for

self-regulation (Boyce & Ellis, 2005; Calkins & Keane, 2009; El-Sheikh & Erath, 2011), which in turn might be sensitive to the negative influences of the aforementioned risk factors itself (Dawson, Ashman, & Carver, 2000).

Self-regulation can be defined as the capacity to control physiological, emotional, cognitive, and behavioral responses (Baumeister & Vohs, 2004), and is directly and interactively linked with the development of aggressive behavior (Calkins & Keane, 2009). The development of self-regulation is relatively protracted due to its dependence on the maturation of prefrontal and limbic brain systems (Beauregard, Lévesque, & Paquette, 2004), and emerges in the form of basic and automatic regulation of physiological processes in infancy and gradually develops into more self-conscious and intentional regulation of emotion, cognition and behavior from the second year onwards that requires, and is supported by physiological processes (Ochsner & Gross, 2004). Although early self-regulatory processes may moderate the effects of environmental risk on aggressive behavior, other, complementary developmental models emphasize that individual physiological vulnerabilities that are associated to aggressive behavior are fostered by environmental stressors during prenatal and early postnatal development (Dawson et al., 2000; Van Goozen, Fairchild, Snoek, & Harold, 2007).

Infancy is a period of increased sensitivity to the effects of environmental stressors on the biological systems involved in self-regulation (Dawson et al., 2000). Further, brain structures underlying cognitive self-regulation show immense development during early childhood. Research on early predictors and processes that lead to early forms of aggression is critical to enable identification of children at risk at an early age and to intervene timely, before developmental trajectories leading to aggression begin to be set. However, most research has focused on childhood and adolescence and much less is known about these factors in infancy and early childhood. The aim of this dissertation is to provide insight into: 1) processes by which early forms of self-regulation and prenatal risk increase vulnerability for aggressive behavior in early childhood and in turn, 2) how prenatal risk predicts early self-regulation at a physiological level (see Figure 1). The studies that comprise the current dissertation focus on self-regulation in very young children measured at a parent-reported emotional and cognitive level (Chapter 5), and an individual experienced physiological level (Chapter 4.1, 4.2, and 5).

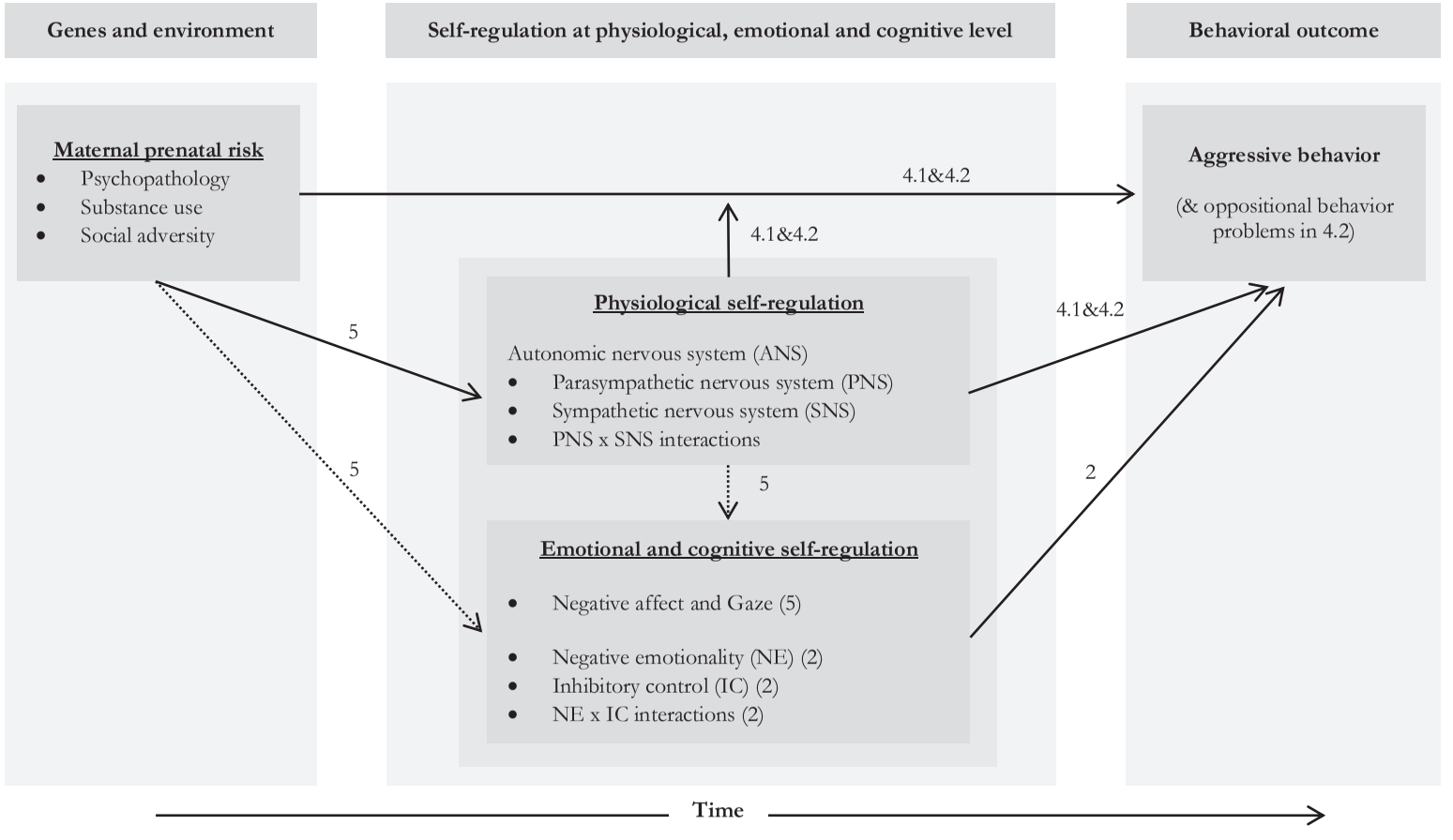

Figure 1. This model illustrates the different associations examined in this dissertation between prenatal risk, self-regulation and aggressive behavior. The numbers refer to the chapters in which these associations are described. This model is derived in part from the self-regulatory framework for understanding early childhood behavior problems (Calkins & Keane, 2009), the neurobiological model of childhood antisocial behavior (Van Goozen et al., 2007), Biological Sensitivity to Context framework (Boyce & Ellis, 2005), and the biopsychosocial model of describing Familial risk x ANS functioning interactions predicting psychopathology (El-Sheikh & Erath, 2011).

Emotional and cognitive self-regulation: Negative emotionality and inhibitory control

Vulnerability for aggressive behavior during childhood is associated with a temperament that is characterized by high levels of negative emotionality (NE) (Calkins & Fox, 2002; Eisenberg et al., 2009). NE is generally defined as the child's tendency to react to stressors with high degrees of emotionality, including anger, irritability, fear or sadness (Rothbart & Bates, 2006). High levels of NE make children prone to develop aggressive behavior, but theoretical models suggest that the negative impact of high NE can be buffered by effortful control (Muris & Ollendick, 2005). Effortful control refers to self-regulative processes that pertain to controlling or regulating one's emotions and behavior. Effortful control has strongly been associated with activity in the anterior cingulate gyrus and regions of the prefrontal cortex (Posner & Rothbart, 2007), and encompasses both inhibitory control (IC), defined as the ability to inhibit a dominant response to perform a subdominant response, and attentional control, which can be defined as the ability to focus and shift attention as needed (Rothbart & Bates, 2006). Previous studies have provided evidence for the specific link between IC and aggressive behavior (Raaijmakers et al., 2008; Sterzer & Stadler, 2009).

Although there is evidence that NE and IC each play a unique role in the development of aggressive behavior, according to Muris and Meesters' interactive model (2005), studying NE in the context of cognitive regulatory capacities allows for a more specific prediction of (the development of) aggressive behavior. However, NE and (components of) effortful control are mostly studied independently and the few studies that have been conducted in young children that included both factors have shown inconsistent results (Belsky, Friedman, & Hsieh, 2001; Gartstein, Putnam, & Rothbart, 2012; Lawson & Ruff, 2004; Olson, Sameroff, Kerr, Lopez, & Wellman, 2005). Longitudinal and cross-sectional studies have demonstrated initial development of effortful control between 27 and 30 months of age (Kochanska, Murray, & Harlan, 2000; Rothbart, Ellis, Rueda, & Posner, 2003). However, effortful control demonstrates vast improvement from infancy through the end of early childhood (Eisenberg, Spinrad, & Eggum, 2010), with further protracted development of executive function components into early adulthood (Diamond, 2002). Hence, the question is whether effortful control abilities have developed sufficiently to buffer the negative effects of NE on aggressive behavior in younger children, or that this can only be expected at a later stage in early childhood.

1

Physiological self- regulation: Autonomic nervous system (ANS)

The autonomic nervous system (ANS) is part of the peripheral nervous system and provides a physiological window on self-regulatory skills, and maturation of the ANS during the prenatal period and the first year after birth provides the foundation for more complex self-regulation observed later in development (Porges & Furman, 2011). The ANS functions as a complex system of afferent (up) and efferent (down) feedback pathways, that are integrated with other neurophysiological and neuroanatomical processes, and reciprocally link the ANS with central nervous system processes (brain and spinal cord) (Chambers & Allen, 2007). Feedback pathways between the central- and peripheral nervous systems are functional relatively early in development (Porges, 2007); however, individual differences in these systems depend on both genetics and environmental influences. The ANS is comprised of the sympathetic (SNS) and parasympathetic (PNS) nervous system, which perform opposing actions. The SNS initiates the 'fight/flight' response by increasing heart rate and respiration. Sympathetic activation frees and directs metabolic resources in order to support active defense behaviors. In contrast, the PNS has an inhibitory effect on the SNS and its role is to maintain homeostasis and to regulate recovery following challenge by decreasing heart rate and respiration.

PNS activity is often assessed by respiratory sinus arrhythmia (RSA), the heart rate variability at the frequency of respiration (Cacioppo, Uchino, & Berntson, 1994), which is thought to index the neural control of the heart via the vagus nerve (Porges, 2007). In response to emotional challenge, RSA levels are assumed to decline, indicating withdrawal of the 'brake' on the SNS allowing for flexible responding to environmental events, active engagement with the environment, and coping with mild to moderate environmental stressors (see Porges & Furman, 2011 for a review). If withdrawal of the PNS is not sufficient to manage a challenging situation, SNS activity is expected to increase in order to prepare the body for more active stress responses. Research suggests that higher levels of baseline RSA and greater withdrawal of the PNS during challenging conditions reflect more effective emotion regulation in infancy (Bazhenova, Plonskaia, & Porges, 2001; Moore & Calkins, 2004).

The vast majority of studies examining ANS functioning in young children have focused on RSA or global measures of autonomic functioning like heart rate without assessments of the SNS (Propper & Holochwost, 2013). However, adaptive autonomic responses to emotionally challenging situations requires a delicate balance in the operation of both the PNS and SNS (see next paragraph), and in order to fully understand the link between ANS functioning and behavior, both branches need to be considered (El-Sheikh & Erath, 2011; Quas et al., 2014). SNS functioning can be measured by the pre-ejection period (PEP), which represents the time between the

onset of the heartbeat and ejection of blood into the aorta (Cacioppo et al., 1994). Although assessment of SNS activity by skin conductance level (SCL) is more common, PEP is considered to be a more pure and direct indicator of cardiac SNS activity and can be reliably measured in infants (Alkon et al., 2006; Quigley & Stifter, 2006).

Most studies focus on baseline ANS levels and ANS reactivity to stress or challenge. Baseline measures are thought to be indicative of response potential (Beauchaine, 2001), and reactivity measures of the ANS represent an individual's physiological response to a discrete environmental challenge compared to a resting state (Alkon, Boyce, Davis, & Eskenazi, 2011). Although recognized as an important parameter of ANS functioning (Porges, 2007), measures indexing autonomic recovery from stress or challenge (i.e. return to baseline) are underrepresented in the current literature (El-Sheikh & Erath, 2011). A study in late preschool children reported that lower PNS recovery was linked to maladaptive emotion regulation responses to frustration (Santucci et al., 2008). Less effective physiological recovery following challenge may result in high ANS activation, even after the stressor has passed, contributing to allostatic load. In sum, measuring the ability to self-regulate at a physiological level should take into account baseline, response, and recovery measures in order to provide a more complete picture of physiological reactivity and regulation (Fox, 1998).

Interaction between prenatal risk and the ANS

Deficits in ANS functioning have been linked to aggression and externalizing behavior in children, adolescents and adults (Beauchaine, Gatzke-Kopp, & Mead, 2007; Van Goozen et al., 2007). However, the pattern of findings between measures of ANS functioning and aggressive behavior is complex and inconsistent. This is likely due to several factors. For example, findings appear to differ in children from non-clinical versus clinical samples (Beauchaine, 2009). In non-clinical samples, increased PNS withdrawal is associated with reduced externalizing behaviors, whereas increased PNS withdrawal is also documented in children with clinically significant externalizing behaviors. Moreover, as mentioned earlier, several theories posit that the influence of individual differences in ANS functioning on behavior occur not directly but in interaction with early adversity (Boyce & Ellis, 2005; El-Sheikh & Erath, 2011). So far, there have been only a handful of studies that investigated the interaction between early adversity and ANS functioning in predicting aggressive or externalizing behavior in early childhood. These studies have exclusively focused on the moderating role of PNS activity (Conradt et al., 2016; Conradt, Measelle, & Ablow, 2013; Eisenberg et al., 2012), with the exception of one study (Waters, Boyce, Eskenazi, & Alkon, 2016) that

reported that less RSA withdrawal and greater PEP reactivity in infancy each predicted more externalizing problems in childhood in the context of maternal depression.

Furthermore, more recent studies suggest that the coordination of the PNS and SNS is predictive of maladjustment (El-Sheikh & Erath, 2011). According to the autonomic space model (see Table 1), there are different modes of autonomic activation and the synergistic action of both the PNS and SNS determines the effectiveness of self-regulation (Berntson, Cacioppo, & Quigley, 1991). Reciprocal autonomic activation, in which the PNS and SNS are oppositely activated, with increased activation of one system and decreased activation of the other, reflects a coordinated response in which both systems either increase or decrease physiological arousal to support responses to environmental demands. Reciprocal ANS activation in response to stress, is presumed to be normative (Alkon et al., 2011; Salomon, Matthews, & Allen, 2000), and linked to better emotion regulation in young children (Stifter, Dollar, & Cipriano, 2011). However, decreased or increased activation of both the PNS and SNS at the same time, is possible (Berntson et al., 1991).Decreased PNS and SNS activation (i.e. *coinhibition*) or increased activation of the PNS and SNS (i.e. *coactivation*) may indicate a breakdown in stress regulation, in which either the PNS or SNS fails to perform its adaptive function in response to stress (Porges, 2007). Indeed, recent studies in middle childhood (El-Sheikh et al., 2009; Gordis, Feres, Olezeski, Rabkin, & Trickett, 2010) have shown that coinhibition and coactivation are associated with increased risk for aggressive and externalizing behavior problems in the context of adversity, as opposed to reciprocal activation between the two systems (i.e. *reciprocal PNS activation* and *reciprocal SNS activation*).

Relations between ANS functioning and behavioral outcome may differ in infancy and middle childhood (Beauchaine et al., 2007), as patterns of PNS and SNS responding evolve across early childhood (Alkon et al., 2011; Alkon et al., 2003). This underlines the need to study interactions between the PNS and SNS in infancy and to determine which patterns of PNS and SNS regulation are indicative of increased biological sensitivity to prenatal adversity.

Table 1. *Autonomic nervous system profiles.*

Profile	PNS	SNS
Reciprocal PNS activation	Activation	Inhibition
	(high RSA baseline or RSA increase)	(high PEP baseline or PEP increase)
Reciprocal SNS activation	Inhibition	Activation
	(low RSA baseline or RSA decrease)	(low PEP baseline or PEP decrease)
Coactivation	Activation	Activation
	(high RSA baseline or RSA increase)	(low PEP baseline or PEP decrease)
Coinhibition	Inhibition	Inhibition
	(low RSA baseline or RSA decrease)	(high PEP baseline or PEP increase)

Note: PNS = parasympathetic nervous system, SNS = sympathetic nervous system, RSA = respiratory sinus arrhythmia, PEP = pre-ejection period (source: El-Sheikh & Erath, 2011).

Effects of prenatal risk on the developing ANS

Physiological systems underlying self-regulation are especially vulnerable to the effects of environmental stressors during the perinatal period (Dawson et al., 2000). Exposure to the risk factors during sensitive periods of fetal development can alter neurological development through fetal programming (i.e. fetal adjustments to cues from the intrauterine environment), thereby affecting the developing ANS (Barker, 1998). Although, as described in the previous paragraph, moderation of the effects of prenatal risk on aggressive behavior by the ANS is presumed, it is therefore also important to consider the possibility that the ANS is already influenced (to some extent) by risk factors during the prenatal (and early postnatal) period.

In a recent review, Propper and Holochwost (2013) concluded that exposure to risk factors during the prenatal period (i.e. maternal stress and substance use) and early postnatal period (i.e. poor quality parent-child interactions, and disruptions in parenting behavior through maternal depression and marital conflict) was consistently related to an altered pattern of ANS functioning in infancy and early childhood, marked by higher baseline heart rate and reduced baseline PNS activity, and increased heart rate but reduced or absent PNS withdrawal in response to challenge (irrespective of type of risk factor and exposure to risk during the prenatal or postnatal period). Although limited, there is some evidence indicating that exposure to adversity during the early postpartum year(s) is associated with heightened SNS activity in early childhood (Hill-Soderlund et al., 2008; Oosterman, de Schipper, Fisher, Dozier, & Schuengel, 2010). However, the effects of prenatal risk on the SNS in infancy has only been scarcely studied. One longitudinal study reported that exposure to maternal prenatal adverse experience, in the form of low social support, was associated with attenuated trajectories of heart rate and SNS responsivity to challenge from six months to five years of age (Alkon et al., 2014). The results so far are contradictory and it is possible that somewhere during early childhood a switch might take place from SNS *hyper*reactivity to SNS *hypo*reactivity (Miller, Chen, & Zhou, 2007). The current study aims to investigate the effects of prenatal risk on PNS and SNS response and recovery in infancy.

Outline of this dissertation

The studies that comprise the current dissertation aim to provide insight into the processes by which early self-regulation (measured at different levels, i.e. physiological, emotional and cognitive) and prenatal risk increase vulnerability for aggressive behavior, and how prenatal risk predicts early self-regulation at a physiological level (see Figure 1). To investigate these aims, we used data from two different empirical studies.

In the first study (**Chapter 2**), we examined how self-regulation at the emotional and cognitive level (respectively NE and IC) predicted aggressive behavior across the preschool years. We specifically tested whether aggressive behavior could be predicted from the interaction between NE and IC. For this study, we recruited a general population sample of 855 preschool children (aged 2-5 years) at child day care centers, preschools and elementary schools throughout The Netherlands. NE, IC and aggressive behavior were assessed through parental reports.

In the second study (Chapters 4.1, 4.2, and 5), we examined how self-regulation at the physiological level (i.e. PNS and SNS functioning) in infancy, in interaction with prenatal risk, predicted aggressive behavior (specifically physical aggression) in toddlerhood (Chapter 4.1 and 4.2), and next, we addressed the question to what extent this self-regulation at a physiological level is already influenced by prenatal risk (Chapter 5). The analyses for the studies described in these chapters, were based on data from a subsample of the Mother-Infant NeuroDevelopment Study (MINDS) – Leiden (The Netherlands). This is a longitudinal study into neurobiological and neurocognitive predictors of early behavior problems, consisting of six assessment waves starting during pregnancy until 42 months post-partum. A total of 275 women with their first-born child participated in this study. Based on the presence of one or more risk factors for poor parenting practices and child emotional and behavioral problems (e.g. presence of maternal psychopathology, substance use, and social adversity; World Health Organization, 2005, 2016), women were assigned to either the low-risk or the high-risk group. Women in the high-risk group were randomly assigned to the intervention or high-risk control group (data from the intervention group is not included in the studies described in this dissertation). The background, design, and study population of the MINDS-Leiden study are described in **Chapter 3**.

In the studies described in Chapter 4.1, 4.2 and 5, we used data from four of the six assessment waves: third trimester of pregnancy (wave 1; home-visit), six months post-partum (wave 2; home-visit), 20 months post-partum (wave 4; home-visit) and 30 months post-partum (wave 5; lab-visit). The number of participating mothers and children differed somewhat between the studies described in Chapter 4.1,

4.2 and 5, depending on the data available at that point. During the pregnancy assessment, we screened for the presence of risk factors (e.g. maternal psychiatric disorders, substance (ab)use, and social adversity). At six months post-partum, mothers and infants participated in two emotionally challenging tasks: a social stress task (Still Face Paradigm; Mesman, Van IJzendoorn, & Bakermans-Kranenburg, 2009; Tronick, Als, Adamson, Wise, & Brazelton, 1978), and a frustration task (Car seat; Goldsmith & Rothbart, 1999). Infant PNS (i.e. RSA) and SNS (i.e. PEP) activity was measured during baseline, throughout the emotional challenge tasks and during recovery from challenge. At 20 and 30 months post-partum, mothers reported on their child's externalizing behavior problems; physical aggression at 20 and 30 months and oppositional behavior problems at 30 months only.

In **Chapter 4.1**, we examined the interactive effects between cumulative prenatal risk and PNS and SNS response to and recovery from stress at six months as predictors of physical aggression at 20 months. In **Chapter 4.2**, we extended the study in Chapter 4.1 by measuring physical aggression at a later age (30 months) and by investigating interactions within dimensions (e.g. PNS baseline x SNS baseline etc.) and between dimensions (e.g. PNS baseline x SNS response, and PNS response x SNS recovery etc.). Further, we examined whether the interactive effects between early adversity and ANS regulation were specific for physical aggression versus non-aggressive externalizing behavior problems (i.e. oppositional behavior problems) (Burt, 2012).

In **Chapter 5**, we examined the extent to which prenatal risk was associated with infant PNS and SNS response to and recovery from a social stressor (Still Face Paradigm). Different from the studies described in Chapter 4.1 and 4.2, we examined differences in ANS functioning between the low-risk and high-risk group and, in additional analyses, we examined the effects of separate risk factors and the full range of risk by looking at the association between ANS functioning and cumulative prenatal risk. A secondary aim of this study was to investigate the relation between ANS regulation and emotional regulation (e.g. Negative affect and Gaze towards the mother; this reflects the extent to which infants successfully regulated distress and used other-directed emotion regulation strategies) measured during the social stress task.

In **Chapter 6**, the results of the studies are summarized and discussed in the context of previous literature.

CHAPTER 2

Aggressive behavior in early childhood: The role of emotional and cognitive self-regulation

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Abstract

Inhibitory control (IC) and negative emotionality (NE) are both linked to aggressive behavior, but their interplay has not yet been clarified. This study examines different NE x IC interaction models in relation to aggressive behavior in 855 preschoolers (aged 2-5 years) using parental questionnaires. Hierarchical regression analyses revealed that NE and IC predict aggression both directly and interactively. The highest aggression levels were reported in children with high NE and low IC. Interestingly, the protective effect of IC for aggressive behavior increases with rising levels of NE. Analyses focusing on physical aggression revealed a significant NE x IC interaction in boys aged 4/5 years only. These findings shed new light on potential compensatory mechanisms for aggressive behavior in developing children.

Keywords: Aggressive behavior, inhibitory control, negative emotionality, preschool, executive function

Introduction

During the early preschool years, low to moderate levels of aggression are relatively common and are often thought of as a natural way of expressing negative emotions such as anger and frustration (Koot, Van den Oord, Verhulst, & Boomsma, 1997). Developmental trajectory studies have shown that the rate in which children engage in aggressive behavior increases and peaks during the second and third year of life and then gradually declines from the fourth year onwards (Alink et al., 2006; NICHD Early Child Care Research Network, 2004). The decline in aggression is thought to reflect increased brain maturation, as through gradual improvement of executive functions children become increasingly able to regulate emotions and to control their behavior (Posner & Rothbart, 2000). Children who engage in high levels of aggression during the preschool period are at risk of engaging in a pattern of increasingly frequent and intense aggressiveness over childhood (NICHD Early Child Care Research Network, 2004) and a wide range of adaptational problems including academic failure, internalizing problems, and conflicts with family members, peers and teachers (Campbell et al., 2006).

As high levels of aggression pose major psychological and financial costs to society, families, and individuals (Tremblay et al., 1992), it is important to gain more insight in the factors involved in early aggressive behavior, to eventually be able to intervene adequately and timely. A propensity towards negative emotionality, which is the tendency to react to stressors emotionally, constitutes a significant risk for maladaptive development (Rothbart & Bates, 2006). High levels of NE and deficits in executive functioning have been linked to high levels of aggressive behavior (Calkins & Fox, 2002; Eisenberg et al., 2009; Muris & Ollendick, 2005). However, studies that have focused on both constructs and their interaction in relation to aggressive behavior in the early years are currently lacking.

Negative emotionality

NE is in part an early-developing, heritable and stable trait (Durbin, Hayden, Klein, & Olino, 2007), associated with neurophysiological factors such the neurotransmitter serotonin and the neuropeptide oxytocin (Montag, Fiebach, Kirsch, & Reuter, 2011). NE is considered to be the core dimension of the difficult temperament construct (Prior, 1992), and is generally defined as the child's tendency to react to stressors with high degrees of emotionality, including anger, irritability, fear or sadness (Rothbart & Bates, 2006). The construct of NE, is a defining feature of childhood conduct problems and externalizing problems (Calkins & Fox, 2002). However, many children do not develop aggressive behavior despite high levels of NE. In fact, over the preschool years most children learn to modulate their emotions

through deployment of self-regulatory skills, leading to a decrease in the use of aggressive behavior during development (Posner & Rothbart, 2007).

Self-regulation

Deficits in self-regulation are associated with early behavior problems (Eisenberg et al., 2009; Valiente et al., 2003). Self-regulation is a broad and multidimensional construct consisting of cognitive and behavioral processes that allow individuals to control physical, emotional, behavioral, and cognitive responses (Blair & Diamond, 2008). Although the capacity for self-regulation continues to develop throughout childhood until early adulthood (Murphy, Eisenberg, Fabes, Shepard, & Guthrie, 1999), the preschool years constitute a period in which individual differences in the ability to regulate attention and goal-directed behavior develop rapidly (Posner & Rothbart, 2000). This growth is marked by increased connectivity between neural systems and parallels significant maturation of the prefrontostratial circuitry (Carlson & Wang, 2007).

Traditionally, researchers have studied the development of self-regulation focusing on the concepts of, 'effortful control' and 'executive functions'. Effortful control, a construct introduced by Rothbart and colleagues, is defined as "the efficiency of executive attention, including the ability to inhibit a dominant response and/or to activate a subdominant response, to plan, and to detect errors" (Rothbart & Bates, 2006, p. 129). Executive functions refer to a set of higher order cognitive processes, commonly defined as the ability to engage in deliberate goal-directed thought and action via inhibitory control (IC), attention shifting or cognitive flexibility and working memory (Garon, Bryson, & Smith, 2008). Although there are differences between effortful control and executive functions, the two constructs are largely overlapping, with similar underlying neural systems (Nigg, 2006), and with IC as a central component (Zhou, Chen, & Main, 2012).

Inhibitory control

The capacity of IC, the ability to deliberately withhold or suppress a prepotent dominant response (Diamond, Carlson, & Beck, 2005) is one of the first executive functioning components to emerge in development and is seen as functionally different from other executive control components, such as working memory and cognitive flexibility (Garon et al., 2008). Deficits in IC appear to be consistently linked to aggression in school-aged children (Sterzer & Stadler, 2009), and preschoolers (Raaijmakers et al., 2008; Utendale & Hastings, 2011), although the findings in preschool children have not been replicated in other studies (e.g. Brocki, Nyberg, Thorell, & Bohlin, 2007).

In the present study, we specifically focused on the construct of IC, as opposed to broader constructs, such as effortful control or executive functioning, as previous research has shown that IC is of particular importance in understanding the development of aggressive behavior. For example, a self-report survey in nonclinical children showed that different aspects of effortful control are differentially related to specific behavioral problems (Muris, Meesters, & Blijlevens, 2007), such that deficits in IC but not attentional control were linked to externalizing problems. Furthermore, in a sample of four year old children with high levels of aggressive behavior, Raaijmakers and colleagues (2008) found that of the different executive functions only IC was significantly related to aggression.

Evidence for a moderation model

Although NE and IC have been independently linked to the development of aggressive behavior, the combination of high levels of NE and low levels of IC may place children at an even higher risk for aggression (Calkins & Fox, 2002). In their literature review investigating the role of reactive and regulative temperament factors in the pathogenesis of child psychopathology, Muris and Ollendick (2005) put forward two models in which temperamental reactivity and regulative processes either have interactive or additive effects on the development of psychopathology. According to the interactive model, children high in NE may be prone to aggressive behavior, but may be relatively protected if they have adequate IC at the same time, whereas children with high NE and low IC may experience more difficulties in regulating NE and consequently are at greater risk for aggression. Adequate IC may therefore be particularly important for children high in NE.

Neuro-imaging studies of typically developing children and individuals with prefrontal cortex lesions provide support for this interactive model. These studies emphasize the importance of the role of the prefrontal cortex in the expression and regulation of different aspects of emotional reactivity (Beer, Heerey, Keltner, Scabini, & Knight, 2003; Perlman & Pelphrey, 2010), by attenuating activation in subcortical limbic regions, including the amygdala, which are essential to the processing of emotional information (Ochsner et al., 2004). Furthermore, developmental studies in children have reported significant relations between asymmetries of frontal electroencephalographic activations and temperament characteristics such as hostility, negative affect and reactivity to novelty (McManis, Kagan, Snidman, & Woodward, 2002).

Despite its theoretical plausibility, presently there are no studies in preschoolers that have actually examined the interplay between NE and IC in relation to aggressive behavior. Studies that did investigate the interaction, focused on global

constructs of externalizing behavior or behavioral problems and general measures of effortful control using a variety of measures (e.g. Eisenberg et al., 2001; Muris et al., 2007; Valiente et al., 2003). Moreover, most of this research has been conducted in school-aged children and adolescents, and only a few studies included younger children (Belsky, Friedman, & Hsieh, 2001; Gartstein, Putnam, & Rothbart, 2012; Lawson & Ruff, 2004; Olson, Sameroff, Kerr, Lopez, & Wellman, 2005). However, the outcomes of these studies in older children provide support for a model in which NE and (components of) effortful control interact in predicting externalizing problems; the findings in preschool children are rather inconclusive. Belsky et al. (2001) failed to find either direct or interaction effects of observed attentional persistence, a subcomponent of effortful control, and observed NE at 15 months on parental reports of behavior problems at age 3. Similar findings were reported by Lawson and Ruff (2004). However, after creating groups of children showing high and low levels of NE and attentiveness, they found that children with high maternal ratings of NE and low observed attentiveness in the first two years of life had higher levels of parent reported behavior problems at 3.5 years, which is supportive for the moderation of NE by IC. In a sample of 3 year olds, Olson et al. (2005) reported that effortful control abilities and parental perceptions of anger (a subcomponent of NE) were associated with parental reports of externalizing problems, but the interaction between anger and effortful control was not significant. Gartstein et al. (2012) examined the contributions of NE and effortful control to externalizing problems from infancy to preschool at 7 months, 25 months, and 4 years using parental reports. The authors reported a significant interaction effect indicating NE was more closely related to externalizing problems when effortful control was low, but this finding was significant only for the oldest age group.

Overall, the literature suggest that the moderating effect of (components of) effortful control on the relation between NE and aggressive behavior may not occur until children reach the late preschool or elementary school years. One explanation for the absence of an interaction effect in younger preschoolers may be the immaturity of the frontostriatal systems. Due to this immaturity, regulatory skills may not yet exist or be able to affect NE resulting in higher levels of externalizing problems. In fact, over the preschool period, normative increases in IC go hand in hand with marked decreases in aggressive behavior and externalizing problems (Lemery, Essex, & Smider, 2002). This is supported by a recent study in which poorer IC predicted more aggressive behavior in older preschoolers and kindergarten-aged children, but not in younger children (Utendale & Hastings, 2011).

The present study

The present study focused on the role of NE and IC, and their interaction, in the development of aggressive behavior using parental report data in a large sample of preschoolers. The preschool period is an important period to evaluate the impact of this interaction on behavior, given children's rapidly improving regulatory skills. To this end, an important aim was to test whether the interplay between parental perceptions of NE and IC would evolve over the preschool period. To our knowledge, the present study is the first to examine the unique and interactive contributions of NE and IC in relation to aggressive behavior in this age group.

Based on the interactive model put forward by Muris and Ollendick (2005), it was hypothesized that parental perceptions of IC would modulate the relation between parentally reported NE and aggressive behavior, such that the negative impact of NE was either enhanced at low levels of IC, or reduced at high levels of IC. We further predicted that the protective effect of high levels of IC would be stronger for children with high levels of NE. Because regulatory skills develop rapidly in young children, we expected the interplay between report of NE and IC to be more pronounced in older compared to younger preschoolers. We also considered possible sex differences in relations between parental reports of NE, IC and aggressive behavior, as a large number of studies has shown that, in general, boys show higher levels of aggressive behavior than girls from an early age on (Hay, 2007).

We conducted analyses to test the moderation model for parental perceptions of physical aggression in particular. Physical aggression (e.g. hitting, kicking and fighting) emerges around the first year and has been shown to peak during the second and third year and to gradually decline in the fourth year (Alink et al., 2006; NICHD Early Child Care Research Network, 2004). Over the preschool period, overt physical aggression is gradually replaced by other expressions of aggressive behavior such as verbal and relational aggression. Higher rates of physical aggression have been found in boys (Alink et al., 2006), whereas girls showed more relational aggression (Ostrov & Keating, 2004). Based on these findings, we expected age and sex to have a stronger moderating effect on the interplay between reports of NE and IC for physical aggression than for aggressive behavior in its broader form.

Methods

Participants

Participants in the present study were sampled to represent the general population of children aged between two and five years old in The Netherlands. Recruitment took place over the course of December 2010 until March 2012 through child day care centers (N=8), preschools (N=20) and elementary schools (N=32) in

different urban and rural areas. Children with documented physical or mental disorders were excluded from participation.

The sample consisted of 855 children (455 boys and 400 girls) aged 2.01-5.99 years (*M*=4.19, *SD*=1.08) and their parents. Families were predominantly Caucasian (85%), 5% Turkish or Moroccan, 1% Surinam or Antillean, 6% mixed (Caucasian and other origin) and 3% of other origin. Children were primarily from intact families (90%). In most families (61%) at least one parent completed higher vocational education or a university degree, in 30% of the families at least one parent completed higher secondary school and in 6% of the families at least one parent completed lower secondary school or lower vocational education (in 3% educational status was unknown or at least one parent completed primary school).

Procedures and measures

The primary caretaker (usually the mother) completed a variety of questionnaires concerning the child's emotionality and behavioral regulation. The questionnaires could be completed at home in paper and pencil style or digitally through a link that was sent by email.

Negative emotionality. To assess children's NE, the primary caretaker completed the Dutch version of the Very Short Form of Rothbart's temperament questionnaires: the Early Childhood Behavior Questionnaire (ECBQ; Putnam, Gartstein, & Rothbart, 2006) for 2 year olds and the Children's Behavior Questionnaire (CBQ; Putnam & Rothbart, 2006) for 3 to 5 year olds. The Very Short Forms of the ECBQ and CBQ contain three 12-item scales and were designed to assess the three broad dimensions of temperament (i.e. negative affectivity, surgency and effortful control), which have consistently emerged from scale-level factor analysis of the standard forms of the (E)CBQ. The ECBQ items can be rated on a 7-point Likert scale ranging from 1 (*never*) to 7 (*always*). The CBQ items are to be rated on a 7-point Likert scale ranging from 1 (*extremely untrue of your child*) to 7 (*extremely true of your child*). The CBQ Very Short Form demonstrated both satisfactory internal consistency and criterion validity and exhibited longitudinal stability and cross- informant agreement comparable to that of the standard CBQ (Putnam & Rothbart, 2006). For the present study the subscale Negative affectivity (discomfort, sadness, fear, anger-frustration, and soothability) was used as a measure of the child's NE. The 12 items were averaged into one overall score for NE (Cronbach's alpha = .60 and .73 for the ECBQ and CBQ respectively).

Inhibitory control. IC was measured with the Inhibit subscale of the Dutch translation of the Behavior Rating Inventory of Executive Function – Preschool version (BRIEF-P; Gioia, Espy, & Isquith, 2005). The BRIEF-P is a 63-item rating

scale that assesses children's daily executive functioning in a variety of contexts. The BRIEF-P items are organized into five clinical scales (Inhibit, Shift, Emotional Control, Working Memory, and Plan/Organize), three clinical indexes (Inhibitory Self-Control, Flexibility, and Emergent Metacognition), and a Global Executive Component (GEC). The primary caregiver rated how often their child exhibited various behaviors in the past six months on a 3 -point scale (1 = *never*, 2 = *sometimes*, and 3 = *often*). The BRIEF-P is reverse-scaled, indicating that lower scores represent higher levels of IC.

The Dutch translation of the BRIEF-P (Van der Heijden, Suurland, De Sonneville, & Swaab, 2013) showed sufficient to high internal consistency, test-retest reliability, inter-rater reliability, and construct validity. Furthermore, it showed adequate convergent discrimant, and predictive validity. Scores of the BRIEF-P Inhibit scale correlated significantly (r =-.342, p<.001, n=719), with the Effortful control dimension (measured with the [E]CBQ), indicating that higher levels of effortful control were associated with better IC. In a random subsample of four- and five year olds (N=57, M age = 5.08 years, SD = .58, range 4.05-5.99), a computerized GoNoGo task (Amsterdam Neuropsychological Tasks; De Sonneville, 1999) was administered. Subjects have to press a key if a go signal appears on the screen and refrain from responding if they see a no-go signal (see for a more detailed task description Slaats-Willemse, Swaab-Barneveld, de Sonneville, van der Meulen, & Buitelaar, 2003). Deficits in response inhibition are reflected by a higher percentage of false alarms; this variable was significantly associated with lower levels of BRIEF-P IC (Spearman rank correlation rho (47)= .29, p<.05). The Inhibit scale consists of 16 items measuring the child's ability of impulse control, or the ability of stopping/ modulating actions, responses, and behavior ('has trouble putting the brakes on his/her actions even after being asked' or 'is impulsive'). All items (raw scores) are summed to create a composite index of IC (Cronbach's alpha in the present sample was .89).

Aggression. The primary caregiver completed the Dutch version of the Child Behavior Checklist 1 ½ -5 yr (Achenbach & Rescorla, 2000; Koot et al., 1997). The parent is asked to indicate whether their child displayed any of the 100 behavioral descriptions in the last two months on a 3- point scale (0 = *not true*, 1 = *somewhat true or sometimes true*, and 2 = *very true or often true*), with higher scores indicating higher levels of problem behavior. The instrument yields two broad-band factors (i.e. internalizing and externalizing behavior problems) and seven narrow band factors (i.e., emotionally reactive, anxious depressed, somatic complaints, withdrawn, sleep problems, attention problems and aggressive behavior). The narrow-band subscale Aggressive behavior (sum of 19 items, raw scores) was used for the analyses in the present study.

Additional analyses were conducted with physical aggression as outcome variable. To this end, a subscale for physical aggression was created, consisting of 6 items from the Aggressive behavior subscale that were also derived by the NICHD (2004) to measure overt physical aggression to people, animals and objects.

The reliability and validity of the CBCL have been confirmed in several studies (e.g. Koot et al., 1997). Internal consistency (Cronbach's alpha) in this sample was .88 for Aggressive behavior and .75 for Physical aggression.

Data analyses

All variables were examined for outliers and violations of specific assumptions applying to the statistical tests used. For each variable, observations with values that exceeded three standard deviations from the mean were recoded to the next highest value, within three standard deviations from the mean (0.9% of the total number of observations across NE, IC, Aggressive behavior and Physical aggression). IC was log transformed because of its skewed distribution. Pearson correlations and *t*-tests were used to determine whether age, sex and educational level of the primary caregiver needed to be included in the subsequent analyses as covariates. Educational level showed a non-normal distribution and was therefore dichotomized into low (no or primary education and lower vocational training to intermediate vocational training) or high (higher vocational training and university degree or higher) educational level.

Subsequently, hierarchical linear regression analyses were conducted to examine the associations between NE and IC and the two aggression scales (i.e. Aggressive behavior and Physical aggression), and the potential moderation by IC of the NE and aggression relation. All variables were centered prior to analyses (Aiken & West, 1991). The control variables (e.g. age, sex and/or educational level), NE and IC were entered in Step 1, and all relevant two-way interactions were entered in Step 2. The three-way NE x IC x Age and NE x IC x Sex interaction terms were entered in Step 3 to test whether the interaction between NE and IC was moderated by age or sex. In order to test the presence of potential age or sex differences that did not appear in the three-way interactions, the hierarchical regression analyses were rerun for 2/3 and 4/5 year olds (without interaction terms for Age) and boys and girls separately (without interaction terms for Sex).

Significant interaction effects were further examined following procedures recommended by Aiken and West (1991) NE was regressed onto aggression at three different levels of IC (at 1.5 SD below the mean, at the mean, and at 1.5 SD above the mean). A score of 1.5 SD above the mean for IC represents the recommended cutoff score for abnormal elevation and potential clinical significance (Sherman & Brooks, 2010). In order to gain a better (visual) understanding of the NE x IC interaction, IC

was also regressed at three different levels of NE (at 1 *SD* below the mean, at the mean, and at 1 *SD* above the mean). Significant interactions involving sex were examined by regressing IC onto aggression separately for boys and girls.

All analyses were conducted using the Statistical Package for Social Sciences (SPSS for Windows, version 19.0, SPSS Inc, Chicago).

Results

The descriptive statistics are presented in Table 1. Preliminary analyses (*t*-tests) revealed that boys, compared to girls, showed significantly more parent reported Aggressive behavior ($t(740)=5.31$, $p<.001$), Physical aggression ($t(668)=7.36$, $p<.001$) and lower IC ($t(806)=6.41$, $p<.001$). However, boys and girls did not differ on NE ($t(728)=-.26$, $p=.799$). Children of higher educated families, compared to children of lower educated families, showed significantly less Aggressive behavior ($t(739)=3.11$, $p<.001$), Physical aggression ($t(728)=-.26$, $p=.799$), NE ($t(632)=3.34$, $p<.001$) and higher IC ($t(803)=3.17$, $p<.01$).

NE and IC were significantly associated ($r=.303$, $p<.01$). Both NE and IC were significantly related to Aggressive behavior (respectively $r=.314$, $p<.001$ and $r=.694$, $p<.001$), and Physical aggression (respectively $r=.157$, $p=.01$ and $r=.496$, $p=.001$). Age was not associated with any of the variables except for NE ($r=.208$, $p<.01$). Because Age was significantly related to NE, and Sex and Educational level were significantly related to both the predictor and outcome variables, these variables were included as covariates in subsequent analyses.

Table 1. *Descriptives.*

N= 713	*M*	*SD*	Min	Max
CBCL Aggressive behavior	8.56	5.84	0	26
CBCL Physical aggression	.85	1.35	0	6
(E)CBQ Negative Emotionality (NE)	3.08	.89	1.00	5.70
BRIEF-P Inhibitory Control (IC)	23.60	5.43	16	40
Age	4.25	1.06	2.02	5.99
Sex (% boys)	51.9%			
Educational level (% low)	43.8%			

Aggressive behavior

The results of the hierarchical regression analysis for Aggressive behavior are reported in Table 2. Significant effects were found for NE (pr =.175, p<.001) and IC (pr=.636, p<.001) . Children with higher levels of NE and lower levels of IC showed higher levels of Aggressive behavior. There was also a significant negative effect for Age (pr=-.125, p<.01). Further, a significant interaction effect was found for NE and IC (pr=.111, p<.01). The three-way interactions between NE, IC and Age and between NE, IC and Sex were not significant. Additional regression analyses for 2/3 and 4/5 year olds and for boys and girls separately in order to test the consistency of the NE x IC interaction effect across age and sex yielded similar results.

The significant interaction between NE and IC was further examined by plotting the relation between NE and Aggressive behavior separately at three different levels of IC (at 1.5 *SD* above, at the mean and 1.5 *SD* below the mean, see Figure 1 left). The association between NE and Aggressive behavior was found to increase with lower levels of IC. At high levels of IC (at 1.5 *SD* below the mean), the association between NE and Aggressive behavior was non-significant (β=-.020, p=.680). This relation was significant at mean levels of IC (β=.106, p<.001), and became stronger at lower levels of IC (at 1.5 *SD* above the mean; β=.232, p<.001). The NE x IC interaction was also examined by plotting the relation between IC and Aggressive behavior separately at low, mean and high levels of NE (at respectively 1 *SD* below, at the mean and 1 *SD* above the mean). The association between IC and Aggressive behavior increased with higher levels of NE, but was significant at all three levels (standardized beta's for 1 *SD* below the mean, mean and 1 *SD* above the mean NE respectively β=.566, p<.001, β=.650, p<.001, and β=.735, p<.001). As illustrated in Figure 1 (right), the level of Aggressive behavior was similar for different levels of NE when IC was high, while the level of Aggressive behavior increased from low to high levels of NE when IC was low.

Physical aggression

The results of the hierarchical regression analysis with Physical aggression (see Table 2), yielded significant main effects for IC (pr=-.431, p<.001), Sex (pr=-.186, p<.001), Age (pr=-.082, p<.05) and Educational level (pr=.084, p<.05). There was also a significant two-way interaction between IC and Sex (pr=-.103, p<.01). Examination of this interaction effect (see Figure 2) showed that the association between IC and Physical aggression was stronger for boys (β=.558, p<.001) than for girls (β=.327, p<.001).The three-way interactions between NE, IC and Sex and between NE, IC and Age were not significant.

Additional regression analyses in 2/3 year olds, yielded main effects for IC (pr=.465, p<.001) and Sex (pr=-.150, p<.05). In 4/5 year olds, there was a significant interaction effect between NE and IC (pr=.101, p<.05) which was moderated by Sex (pr=-.097, p<.05). Further examination of the three-way interaction between NE, IC and Sex in 4/5 year olds by running separate hierarchical regression analyses for boys and girls, showed that for boys there was a significant effect for IC (pr=.447, p<.001) and an interaction effect between NE and IC (pr=.162, p<.05), whereas for girls there was only a main effect for IC (pr=.346, p<.001).

Plotting the NExIC interaction for 4/5 year old boys (see Figure 3 left) showed that the association between NE and Physical aggression was only significant at lower levels of IC (1.5 *SD* above the mean; β=.242, p<.01). At high (1.5 *SD* below the mean) and mean levels of IC, the association between NE and Aggressive behavior was non-significant. Although the association between IC and Physical aggression was significant for all levels of NE (see Figure 3 right), the strength of the association increased from low to high levels of NE (standardized beta's for 1 *SD* below the mean, mean and 1 *SD* above the mean respectively β=.317, p<.001, β=.446, p<.001, and β=.575, p<.001).

Table 2. *Hierarchical regression analyses predicting aggressive behavior and physical aggression from NE and IC.*

Step	Predictor	*Adjusted* R^2	ΔR^2	ΔF	β	*t*
CBCL Aggressive behavior						
1	Age	.497	.501	141.92***	-.09	-3.36**
	Sex				-.05	-1.78†
	Educational level				-.02	-.89
	NE				.14	4.71***
	IC				.63	21.92***
2	NE x IC	.506	.012	3.55**	.08	-2.95**
	NE x Age				.04	1.47
	ICx Age				-.01	-.44
	NE x Sex				-.04	-1.30
	IC x Sex				-.03	-1.12
3	NE x IC x Age	.505	.000	.02	-.00	-.07
	NE x IC x Sex				-.00	-.17
CBCL Physical aggression						
1	Age	.279	.284	55.85***	-.07	-2.20*
	Sex				-.17	-5.03***
	Educational level				-.07	-2.24*
	NE				.03	.92
	IC				.44	12.69***
2	NE x IC	.290	.016	3.22**	.05	-2.95
	NE x Age				.02	.43
	ICx Age				-.04	-1.17
	NE x Sex				-.04	-1.02
	IC x Sex				-.09	-2.74**
3	NE x IC x Age	.292	.004	1.94	.03	.72
	NE x IC x Sex				-.06	-1.73†

†<.10, *p<.05, **p<.01, ***p<.001

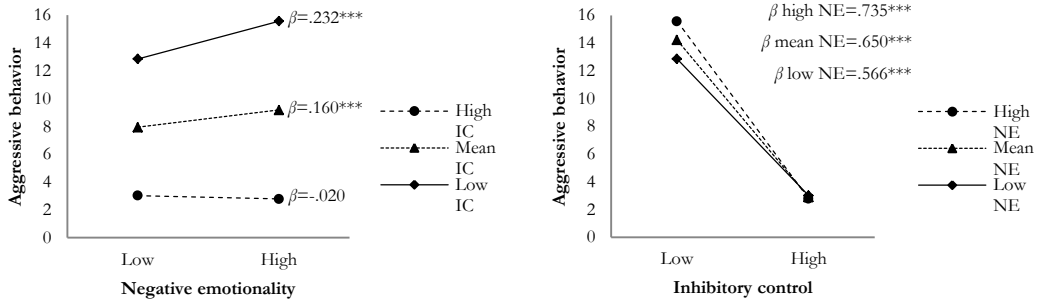

Figure 1. Interaction between NE and IC predicting Aggressive behavior plotted for different levels of IC (left) and NE (right). *Note:* Low IC= 1.5 *SD* above the mean, high IC= 1.5 *SD* below the mean, low NE= 1 *SD* below the mean and high NE= 1 *SD* above the mean. The asterisks (***) indicate $p<.001$.

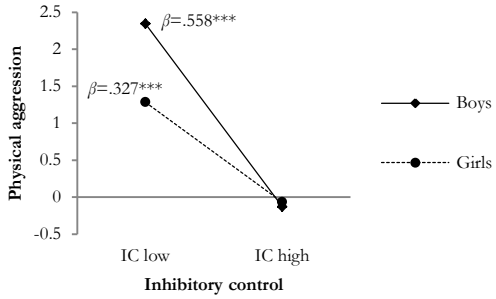

Figure 2. Interaction between IC and Sex predicting Physical aggression. *Note*: Low IC= 1.5 *SD* above the mean, high IC= 1.5 *SD* below the mean. The asterisks (***) indicate $p<.001$.

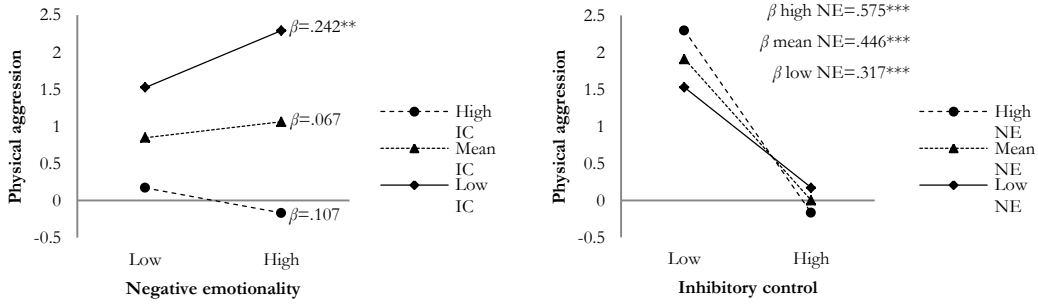

Figure 3. Interaction between NE and IC predicting Physical aggression for 4/5 year old boys plotted for different levels of IC (left) and NE (right). *Note*: Low IC= 1.5 *SD* above the mean, high IC= 1.5 *SD* below the mean, low NE= 1 *SD* below the mean and high NE= 1 *SD* above the mean. The asterisks (**) indicate $p<.01$ and (***) indicate $p<.001$.

Discussion

The aim of the present study was to test a model in which temperamental negative emotionality (NE) interacts with inhibitory control (IC) in predicting aggressive behavior (and physical aggression specifically) using valid parental questionnaires in a community sample of preschoolers. This model is based on a model proposed by Muris and Ollendick (2005) who suggested that the vulnerability to externalizing problems (and psychopathology in general) is determined by high levels of NE and low levels of regulation. The results of this study supported this model, indicating that parental reports of aggressive behavior was predicted by the interaction between NE and IC, such that the negative impact of high levels of NE was reduced by relatively good IC and enhanced by relatively poor IC. Our findings therefore demonstrate that parental perceptions of early emotional and cognitive functioning are linked and interact to predict aggressive behavior in the preschool period.

We first examined the unique relations between NE, IC and aggressive behavior. As expected, parental reports of NE and IC independently predicted aggressive behavior over and above the effects of demographic variables such as age, sex and educational level of the primary caregiver. These findings are in line with existing studies using parental reports and/or direct observations indicating significant contributions of high NE and low IC to the development of aggression (Calkins & Fox, 2002; Raaijmakers et al., 2008; Utendale & Hastings, 2011). We then examined the interaction between NE and IC in predicting aggressive behavior and found that, irrespective of age and sex, higher levels of parental reports of NE were associated with higher levels of aggressive behavior and that this relation became stronger as IC progressed to clinically significant levels (i.e. > 1.5 SD above the mean; Sherman & Brooks, 2010). The association between reports of NE and aggressive behavior was not significant when IC levels were adequate. As expected, children who were characterized by high levels of NE and low levels of IC were rated significantly higher on aggressive behavior compared to children who were characterized by either high NE or low IC, or low NE and high IC. We further expected that the protective effect of better IC would be stronger for children with higher levels of NE. This expectation was confirmed after inspection of plot in which the relation between IC and aggressive behavior was plotted for different levels of NE. The supplementary effect of low levels of IC was larger when children displayed higher levels of NE, underscoring the importance of good regulatory skills in children with high levels of NE.

Previous studies revealed that (components of) effortful control do not become a moderator of the association between NE and behavioral problems until children reach late preschool or early elementary school age. Our study differs from

these earlier studies in several ways, which may explain these contrasting findings. First, whereas our focus was on IC as a regulatory mechanism, Gartstein et al. (2012) examined effortful control (composed of attentional control and IC) in relation to externalizing problems, while Belsky et al. (2001) and Lawson and Ruff (2004) focused on early attention as a regulatory mechanism in relation to behavioral problems. Processes related to attentional control may regulate NE and aggressive behavioral tendencies to a lesser extent. This is in line with literature suggesting that a lack of attentional control is more strongly linked to internalizing problems, whereas poor IC is more clearly related to externalizing problems (Muris & Ollendick, 2005). Second, both Belsky et al. (2001) and Lawson and Ruff (2004) used a longitudinal design in which NE and early attention were measured during the early toddler years whereas behavioral problems were measured around age 3. As both constructs undergo development in early childhood, this may also be a source of variation in results. Third, methodological differences may account for the lack of consistency between our study and previous findings, as NE and/or regulational abilities have mostly been measured through direct observation (e.g. Belsky et al., 2001; Lawson & Ruff, 2004; Olson et al., 2005) instead of parental reports. However, Gartstein et al. (2012) also relied exclusively on parental report data providing evidence against this argument.

Analyses specifically aimed at physical aggression showed results consistent with those for aggressive behavior in general, and indicated that IC moderated the relation between NE and physical aggression. Interestingly, this interaction effect was present for older boys (4-5 years) only. The presence of age and sex effects for physical aggression, and their absence in aggressive behavior in its broader form, may be explained by the fact that physical aggression peaks during the early preschool period (Alink et al., 2006; NICHD Early Child Care Research Network, 2004) and is gradually replaced by verbal and relational forms of aggression. In addition, girls are suggested to be more inclined to show relational aggression (Ostrov & Keating, 2004), whereas boys show higher levels of physical and verbal aggression (Alink et al., 2006; Ostrov & Keating, 2004). Because the CBCL Aggressive behavior scale measures aggression in its broadest form, age and sex effect may not appear. In line with previous studies, we found that sex moderated the association between IC and physical aggression in that this relation was stronger for boys than for girls. This result is consistent with the fact that girls are generally better than boys in self-regulation and inhibitory control, with differences becoming particularly apparent in the 2 - 5 year age range (Kochanska, Murray, & Harlan, 2000).

In the present study, we did not assess relational forms of aggression, therefore we were not able to differentiate between physical and relational forms of aggression. Murray- Close and Ostrov (2009) showed that the association between

physical and relational aggression was low in late preschool age and that both forms of aggression were stable within children over the preschool period. Testing the moderation model for different forms of aggression could be an interesting direction for future studies. In fact, a recent study conducted among adolescents reported differential relations between overt physical and relational forms of aggression and temperamental reactivity and self-regulation (Dane & Marini, 2014). A study conducted by Gower and Crick (2011) in two samples of preschoolers showed that physical and relational aggression were both associated with low baseline autonomic nervous system arousal but only in the context of low effortful control.

The outcomes of this study provide an extension of the research that has so far mainly been conducted in school-aged populations, and support a model in which emotionality and regulation interact in predicting behavioral problems (Eisenberg et al., 2001; Muris et al., 2007; Valiente et al., 2003). Interestingly, for reports of aggressive behavior in general we found that the moderation effect of IC was consistent across the whole age range indicating that in children as young as two years old developing IC capacities may already buffer against the negative effects of high levels of NE. Whereas simple forms of self-regulation such as the inhibition of touching an attractive toy on request of parents, already develop during the first year of life (Kochanska, Tjebkes, & Forman, 1998), more complex and sustained forms of self-regulation develop later during the preschool- and school period. As for the self-regulation of emotions, there is evidence for a similar developmental pattern: simple forms develop during the first two years of life, such as the use of self-soothing, while later on in the preschool period new and more complex use of objects and interactions emerge to regulate emotional state (Diener & Mangelsdorf, 1999). In line with that, our findings reveal that the interaction between self-regulation (inhibition) and emotional reactivity is similar for different preschool ages. However, despite the consistency over ages, there might still be a developmental change in how the self-regulating processes manifest themselves behaviorally.

Considering the relative contributions of NE and IC to aggressive behavior, our findings suggest that the protective effect of high IC (particularly in the presence of high NE) is stronger than the protective effect of low NE. It may be the case that regulatory strategies (already) play a more important role than temperamental factors at this young age. However, this finding may also be explained by the fact that in this study NE was measured as a broad temperamental factor. In fact, subcomponents of NE have been differentially related to externalizing and internalizing problems (Rothbart & Bates, 2006), with anger/frustration making more substantial contributions to externalizing problems, whereas fear/sadness are more predictive of internalizing problems. Hence, the relation between NE and aggression may have

been attenuated by the inclusion of a general measure of NE. Future studies should incorporate more fine-grained measures of NE in order to gain more insight in the specific temperamental characteristics that play a role in the development of aggression and how these interact with development of IC and other regulatory capacities.

Certain limitations regarding this study should be noted. The first issue concerns the possible confounding or overlap between the measures used in this study. Sanson, Prior and Kyios (1990) have argued that relations between temperament and behavior problems may be caused by item overlap. However, recent studies have shown that links between parental reports of temperament and behavior problems remain even after removing overlapping items (Lemery et al., 2002; Oldehinkel, Hartman, De Winter, Veenstra, & Ormel, 2004). Moreover, the interaction between IC and NE in predicting aggressive behavior in the present study argues against simple overlap.

Another limitation is that the independent and dependent measures were drawn exclusively from parental report which may have influenced the strength of the associations between our measures. Parental reports of child functioning include a subjective component reflecting biases associated with differences in parental personality, emotional status, relationship with the child, knowledge of child behavior and inconsistent interpretation of items (Kagan, 1994). There are also studies that have shown that parental perceptions of their child were differentiated in meaningful and consistent ways (Bates, 1990). Nonetheless, future studies should incorporate multiple methods and informants to measure temperament, executive functioning and behavioral problems. Both laboratory and field observations of aggressive behavior by independent raters, as well as behavioral and physiological measures of temperamental reactivity and regulation would provide stronger evidence for the patterns observed in the current study. A third limitation is that the data are correlational and collected at a single time point. Future studies should include prospective, multiple time point assessments of emotionality, regulation and aggressive behavior to more fully understand the dynamic interplay between emotionality and regulation and the persistence of aggressive behavior across development. A final note concerns the generalizability of our results. Although the children in our sample showed the full range of aggressive behavior problems and their scores were in line with normative samples (Achenbach & Rescorla, 2000), only a small portion of children showed aggressive behavior in the extreme range, limiting generalizability to clinical samples.

In conclusion, this is the first study that demonstrates that parental perceptions of aggressive behavior in a large community sample of preschoolers are specifically associated with the interactive effects of IC and NE. Based on the

outcomes of this study, we propose that inhibitory control mechanisms may already play a role in regulating negative emotions in children as young as two years old. Parental reports of low levels of IC, especially in combination with high levels of NE, characterize children with high (borderline to clinical) levels of aggressive behavior. Interestingly, we found moderating effects of age and sex for physical aggression that provide more insight in the specificity of the interaction effects of NE and IC for aggression in preschoolers. High levels of aggression at this age may be predictive for a persistent pattern of aggressive behavior over childhood (NICHD Early Child Care Research Network, 2004), and have been associated with numerous negative adaptational outcomes (Campbell et al., 2006). We want to emphasize that the capacity of a preschool child to deal with his or her (difficult) temperamentally determined emotionality is not only explained by his or her biologically determined capacities: these capacities are and will be constantly influenced by the social environment (predominantly composed by his or her primary caregivers).

This study can have important implications for intervention programs for toddlers and preschoolers whose parents report high levels of (physical) aggression and appear to be at risk for continued externalizing behavior problems. Early interventions aimed at improving children's emotional and behavioral regulation skills through parental perceptions and behaviors may be promising. For instance, previous studies have demonstrated a significant effect of maternal support on the child's reactivity and self-regulation (Robinson & Acevedo, 2001). For children in the late preschool age whose parent report high levels of physical aggression, our findings suggest that interventions in boys should focus on strengthening inhibitory control. This might be particularly effective in boys with higher levels of NE, given our findings that there are direct effects of IC on physical aggression as well as an IC x NE interaction effect. In girls aged 4-5, the direct effect of IC on physical aggression was less strong and the interaction effect was not significant, which suggests that interventions focusing on strengthening inhibitory control would be less effective. Other treatment strategies, for example those focusing on improving prosocial behavior and empathy, and problem solving might be more effective for girls. Further studies incorporating a longitudinal and multi-method design are needed to replicate the findings of the present study and to provide insight in the interaction between emotionality and regulation in relation to the development of aggression over time.

CHAPTER 3

The Mother-Infant Neurodevelopment Study (MINDS) – Leiden: background, design and study population

Manuscript submitted:

Smaling, H. J. A.*, Suurland, J.*, Huijbregts, S. C. J., Van der Heijden, K. B., Van Goozen, S. H. M., & Swaab, H.. The Mother-Infant Neurodevelopment Study (MINDS) – Leiden: background, design and study population.

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Abstract

This paper describes the background, design, and sample characteristics of the Mother-Infant Neurodevelopment Study – Leiden, a longitudinal study investigating 1) mechanisms through which neurobiological, neurocognitive and social-environmental factors increase risk for emotional and behavioural problems in early childhood, 2) effects of an intensive home-visiting program for first-time mothers at high-risk on child neurobiological, neurocognitive and emotional and behavioural development, and 3) which factors (neurobiological, neurocognitive and social-environmental) predict variation in effects of the home-visiting program on child emotional and behavioural outcomes. A total of 275 families (128 low-risk and 147 high-risk) were included in the study. High-risk women were randomly assigned to the intervention (*n*=65) or high-risk control group (*n*=82). Six assessment waves were conducted within a four-year period. Demographic and mental health characteristics of the low-risk and high-risk group, collected during the first assessment at 27 gestational weeks, are presented. This study will help identifying specific biomarkers, precursors of neurocognitive functions and temperamental factors in infancy, facilitating the detection of children at risk for later emotional and behaviour problems. Furthermore, this study may yield insights into effective, targeted, and tailor-made components of prevention programs, ultimately reducing the psychological and economic costs of mental health problems to society.

Key words: High-risk, neurobiology, reflective functioning, infants, home-visiting program

Introduction

Children growing up in families struggling with multiple complex issues, including maternal psychiatric problems, substance (ab)use, single parenthood, and poverty, are at high risk for developing emotional and behavioural problems (Cabaj, McDonald, & Tough, 2014; Côté, Vaillancourt, LeBlanc, Nagin, & Tremblay, 2006; Hay, Mundy, et al., 2011; Huijbregts, Seguin, Zoccolillo, Boivin, & Tremblay, 2008). As these problems raise major public health concerns and increase costs to society, it is important to gain insight into developmental mechanisms and effectiveness of prevention approaches. Recent theoretical models have emphasized the complex interactions between neurobiological vulnerabilities and environmental risk factors (Beauchaine, Neuhaus, Brenner, & Gatzke-Kopp, 2008; Belsky & Pluess, 2009; Boyce & Ellis, 2005), and the mediating role of neurocognitive and neurobiological factors in the link between early adversity and emotional and behavioural outcomes (Van Goozen, Fairchild, Snoek, & Harold, 2007).

However, there are several important gaps in the current literature. First, most studies so far have involved toddlers, school-aged children and adolescents, and little is known about earlier manifestations of neurobiological and neurocognitive vulnerabilities, and how these might increase risk for emotional and behavioural problems later in life. This is particularly important given that the neurobiological systems underlying later emotional and behavioural regulation rapidly develop during the prenatal period and first years of life, resulting in increased sensitivity to environmental influences (Beauchaine et al., 2008; Laurent, Harold, Leve, Shelton, & Van Goozen, 2016). Prevention efforts initiated early in life are therefore presumed to be more effective (Beauchaine et al., 2008). Second, most longitudinal studies investigating the neurobiological and neurocognitive mechanisms underlying emotional and behavioural problems in early childhood involved community samples. Because evidence is accumulating that shows that different neurodevelopmental processes underlie emotional and behavioural development in normative versus high-risk or clinical samples (Beauchaine, 2001; Beauchaine et al., 2008), it is of critical importance to extend the current body of literature with studies focusing on high-risk samples in order to fully understand the mechanisms that are related to the development of emotional and behavioural problems.

The overarching aim of the Mother-Infant Neurodevelopment Study (MINDS) – Leiden study is to examine which neurobiological, neurocognitive and social-environmental factors increase risk or confer protection for developing emotional and behavioural problems in the first years of life in a heterogeneous sample of low- and high-risk families. Based on integrative models of the neurobiological bases of early-onset antisocial behaviour (Van Goozen et al., 2007),

and theories of differential susceptibility (Belsky & Pluess, 2009) and biological sensitivity to context (Boyce & Ellis, 2005), we considered neurobiological and neurocognitive factors as potential mediating and moderating mechanisms that lead to emotional and behavioural problems. We adopted a rigorous and systematic approach, assessing a wide range of social-environmental (i.e. maternal psychiatric problems, substance [ab]use, poverty, social support, parenting, maternal reflective functioning), neurocognitive (i.e. precursors of executive functioning, Theory of Mind, language, empathy) and neurobiological factors (i.e. autonomic nervous system and hypothalamic pituitary adrenal axis) that may either directly, indirectly, or in interaction increase risk for emotional and behavioural problems in young children.

Families at risk need substantial support to promote good enough care for their child. However, it is often difficult to engage these families in intervention programs. Home-visiting programs for first-time mothers at high-risk have the advantage of serving families at their home, thereby increasing the likelihood that they will (continue to) participate. Home-visiting programs have been found to be effective in improving maternal prenatal health behaviours (e.g. reductions in tobacco use), maternal life course (e.g. fewer rapid second pregnancies, returning to school/ seeking education), sensitive parenting behaviour and parenting attitudes, and child physical abuse (e.g. number of emergency room visits, injuries or ingestions treated, and accidents requiring medical attention) (Mejdoubi et al., 2014; Mejdoubi et al., 2015; Olds, Sadler, & Kitzman, 2007; Ordway et al., 2014; Sweet & Appelbaum, 2004). Further, positive effects of home-visiting programs have been reported for a wide range of child outcomes (Avellar & Supplee, 2013; Filene, Kaminski, Valle, & Cachat, 2013; Mejdoubi et al., 2015; Olds et al., 2007; Ordway et al., 2014; Peacock, Konrad, Watson, Nickel, & Muhajarine, 2013; Sweet & Appelbaum, 2004). For example, home-visiting programs have been found to positively affect cognitive development (e.g. academic and arithmetic achievement, intellectual functioning, executive functioning, receptive language), socio-emotional development (e.g. attachment security, social development, emotional vulnerability), and to reduce behavioural problems (e.g. externalizing and internalizing behaviour problems). Also, favourable results have been reported for birth outcomes (e.g. birth weight, gestational age) and physical health, although these effects have been less consistent among different home-visiting programs (Avellar & Supplee, 2013; Filene et al., 2013; Robling et al., 2016). Moreover, at 15 year follow-up, adolescents who had received home visitation early in life had fewer arrests and convictions, and committed fewer violations of probation (Olds et al., 1998).

Despite these promising results, the overall effect sizes of studies evaluating home-visiting programs are relatively small (Filene et al., 2013), and many studies

report non-significant findings (Avellar & Supplee, 2013). Currently, an important question is which factors predict this variability in the effects of home-visiting programs on child outcomes? While it is generally acknowledged that effectiveness of prevention programs may vary as a function of neurobiological and neurocognitive vulnerabilities (Beauchaine et al., 2008), there are no studies that have examined child neurobiological and neurocognitive factors as moderators of the effects of home-visiting programs. Moreover, surprisingly little is known about the effects of home-visiting programs on child neurobiological and neurocognitive development. Therefore, a second overarching aim of MINDS – Leiden was to evaluate the effects of an intensive home-visiting program for first-time mothers at high-risk on child neurobiological, neurocognitive and emotional and behavioural development, and to evaluate the predictive value of these child neurobiological and neurocognitive factors on the effectiveness of such a program.

Study aims

MINDS – Leiden is part of a large research program funded by the Dutch government called 'Brain & Cognition – Social innovation in health, education, and safety' (http://www.nwo.nl and http://www.nwo.nl/en/research-and-results/programmes/nihc/hcmi/index.html). The purpose of this Brain & Cognition program is to promote neurobiological and neurocognitive research to develop and improve intervention efforts aimed at reducing aggression and violence in society.

In the present study three important questions are addressed. First, which neurobiological and neurocognitive factors predict (directly, indirectly or in interaction with social-environmental factors) emotional and behavioural problems – specifically aggressive behaviour – in the first three-and-a-half years of life? Second, what are the effects of an intensive home-visiting program for first-time mothers at high-risk on neurobiological, neurocognitive and emotional and behavioural development in children in the first years of life? Third, which factors (neurobiological, neurocognitive and social-environmental) predict variation in effects of the home-visiting program on child emotional and behavioural outcomes? This paper describes the design of the study, the measures used, the intervention program and the sample characteristics.

Methods

Study design

The MINDS – Leiden study was designed as a longitudinal randomized control trial (see Figure 1 for an overview of the design of the study). Based on elaborate screening for the presence of risk factors during the first prenatal home visit (27 weeks gestation), pregnant women were assigned to either the high-risk (HR) or low-risk (LR) group (see *screening for risk factors* for a description of allocation criteria). Women in the HR group were randomly assigned to the high-risk intervention (HR-I) group (see *The intervention* for more details) or the high-risk control (HR-C) group. All participating families were followed over a period of approximately four years, consisting of six assessment waves, (the first assessment took place in the third trimester of pregnancy and the last assessment took place when the children were 42 months of age). A total of 65 families were included in the HR-I group. Families in the HR-I group took part in a home-visiting program starting within 2 weeks after the prenatal assessment until 30 months post-partum.

Recruitment

Recruitment of pregnant women took place between February 2011 and April 2015, via hospitals, midwifery clinics, prenatal classes, pregnancy fairs, and social workers. Dutch-speaking primiparous women between 17 and 25 years old with uncomplicated pregnancies were eligible to participate. Exclusion criteria were heavy drug addiction or severe psychiatric or psychotic disorder, an intelligence quotient (IQ) below 70, major acute or significant chronic illness in the mother or a disorder or syndrome in the child, which would affect normal development. We oversampled families from a high-risk background in order to obtain sufficient variance in risk factors that might influence children's early socio-emotional and cognitive development. This was done by collaborating with midwifery/obstetric clinics in areas with a low average social-economic status and/or by recruitment through social workers. All participating women provided written informed consent. The study was approved by the Medical Research Ethics Committee at the Leiden University Medical Centre (NL39303.058.12), and by the ethics committee of the Department of Education and Child Studies at the Faculty of Social and Behavioural Sciences, Leiden University (ECPW-2011/025).

Screening for risk factors

Classification to the HR-group was based on the following risk factors (Smaling et al., 2015; World Health Organization, 2005, 2016): 1) positive screening on current psychiatric disorder(s) or substance use (alcohol, tobacco and/or drugs)

during pregnancy; or 2) presence of two or more of the following risk factors: single status (biological father not involved), unemployment, financial problems, no secondary education, limited social support network (<4 individuals listed in network), and young maternal age (<20 years). In case only one risk factor was present - other than an indication for current psychiatric disorder(s) or substance use - women were discussed in a clinical expert meeting to determine whether placement in the HR-group was appropriate (*n*=6).

Positive screening on current psychiatric disorder(s) was established by the Mini-International Neuropsychiatric Interview – plus (M.I.N.I. - plus; Sheehan et al., 1997, Van Vliet, Leroy, & Van Megen, 2000) by screening for the following disorders: depressive disorder (current), dysthymic disorder (past 2 years), suicidality, mania (current), panic disorder (current and lifetime), agoraphobia (current), social phobia (current), other type of phobia (current), obsessive-compulsive disorder (current), generalized anxiety disorder (GAD; current), posttraumatic stress disorder (current), alcohol dependence and abuse (current and lifetime), drug dependence and abuse (non-alcohol, current and lifetime), attention-deficit/hyperactivity disorder (lifetime), and antisocial personality disorder (lifetime). Current is defined as 'in the past month' for all diagnoses except GAD, which has a 6-month time frame, and alcohol abuse/dependence and drug abuse/dependence for which a 12-month time frame is used.

The size of the social support network was established by using the Norbeck Social Support Questionnaire (NSSQ; Norbeck, Lindsey, & Carrieri, 1981, 1983). The presence of other risk factors (i.e. substance use during pregnancy, no secondary education, unemployment, financial problems, single status, and maternal age) were assessed by means of the Dutch translation of the 'Becoming a mother' questionnaire (Hay et al., 2011; Smaling et al., 2015).

Procedures

The study comprised of six assessment waves (see Figure 1). During the third trimester of pregnancy (T1), at 6 months (T2) and at 20 months (T4) post-partum 2-to-2.5 hour home visits were carried out by two female researchers. One leading researcher conducted all the tasks with the infant and guided the mother-infant interaction tasks, while a second researcher digitally recorded the whole session and administered the questionnaires to the mother. At 12 months (T3), 30 months (T5) and 42 months (T6), the mother-infant dyads visited the Baby lab at the Faculty of Social and Behavioural Sciences, Leiden University. The lab test sessions were carried out by one researcher, while a second researcher was seated behind a one-way screen, recorded the session and provided observational records.

Table 1 gives an overview of the main research areas of the study. In addition, Table 2 and give a detailed overview of instruments and its content used in the study. T1 included an interview regarding the emotional impact of the pregnancy to assess prenatal reflective functioning (RF), a structured interview to assess current psychiatric disorders, and various questionnaires to assess demographic information, mental and physical health, life style, social support, self-efficacy in the nurturing role, executive functioning, emotion regulation, life events, and antenatal attachment. T2 to T5 started with a free play session to measure maternal interactive behaviour. For T2, T3, and T5, this was followed by attachment of the cardiac monitoring equipment to the child to measure their autonomic nervous system (ANS) reactivity and regulation during mildly stressful events (see Table 2 and 3 for the paradigms that were used to measure stress reactivity and regulation). In addition, during the lab visits saliva samples were taken several times (before and after stress) from mother and child to measure hypothalamic pituitary adrenal (HPA)-axis functioning. Moreover, each wave consisted of various age-appropriate tasks to measure precursors of executive functioning, temperament and emotion, empathy, Theory of Mind, and language skills, and ended with the mother completing various questionnaires to assess demographic information and multiple mother (i.e. mental and physical health, obstetric characteristics, life style, social support, self-efficacy in the nurturing role, executive functioning, emotion regulation, life events, parenting cognitions and parenting stress) and child domains (language, temperament, aggression, behavioural problems, executive functioning) (see Table 1, 2 and 3 for more details). Mother-child interaction during normal, playful interaction, teaching tasks and following mildly stressful events was observed at T2 to T5. T4 also included an interview regarding maternal representations of the relationship with their child and the emotional impact of parenting to measure postnatal RF. At T6, children were assessed for approximately 1 hour in individual testing rooms, and then brought together with their mother and one or two other families for a simulated birthday party and a 20 minute free play session with peers, designed to provide an acceptable yet emotionally arousing setting in which to observe children's social behaviour (and specifically aggressive behaviour) with their peers.

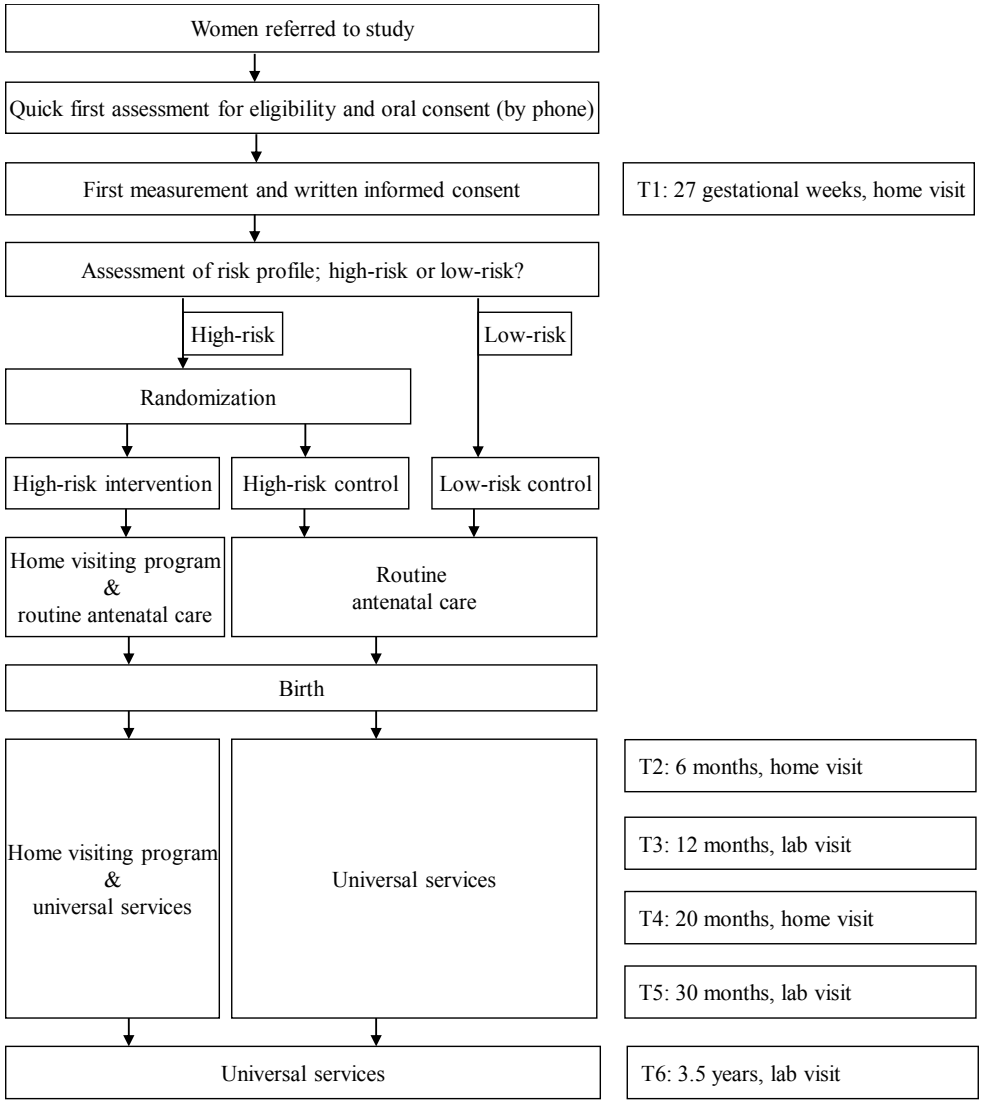

Figure 1. Design of the MINDS-Leiden study.

Table 1. *The main research areas of the MINDS- Leiden Study.*

Maternal variables	Wave 1: pregnancy	Wave 2: 6 months	Wave 3. 12 months	Wave 4. 20 months	Wave 5: 30 months	Wave 6: 42 months
Demographics	X	X	X	X	X	X
Reflective functioning	X			X		X
Mental and physical health	X	X	X	X	X	X
Life style	X	X			X	X
Social support	X				X	X
Self-efficacy in the nurturing role	X	X		X		
Executive functioning	X				X	
Emotion regulation	X				X	
Life events	X					X
Antenatal attachment	X					
Obstetric characteristics		X				
Parenting		X	X	X	X	X
Parenting stress					X	
IQ estimate		X				
Stress reactivity and regulation			X		X	

Note. IQ = intelligence quotient.

Table 1 cont.

Child variables	Wave 1: pregnancy	Wave 2: 6 months	Wave 3. 12 months	Wave 4. 20 months	Wave 5: 30 months	Wave 6: 42 months
Physical health		X	X	X	X	X
Cognitive development		X				
Stress reactivity and regulation		X	X		X	X
Language development			X	X	X	X
(Precursors of) executive functioning			X	X	X	X
(Precursors of) Theory of Mind			X	X	X	X
(Precursors of) empathy				X	X	X
Aggression		X	X	X	X	X
Behavioural problems				X	X	X
Temperament		X	X	X		
Peer social interaction						X

Table 2. *Research domains, instruments, and contents: mother.*

Domain (mother)	Instrument / data source	Content
Demographics, lifestyle, physical health & obstetric characteristics	'Becoming a mother'-questionnaire and 'Being a mother'-questionnaire (Hay, Mundy, et al., 2011; Smaling et al., 2015)	Key information regarding demographics, obstetric characteristics, life style, and physical health
Life events	List of Threatening Experiences – questionnaire (LTE-Q; Brugha & Cragg, 1990)	Stressful life events over the past year to self and close others, and whether they currently have impact
Social support	Norbeck Social Support Questionnaire (NSSQ; Norbeck, Lindsey, & Carrieri, 1981, 1983)	Functional variables (affect, aid, affirmation) and total network variables of social support (number of persons in the network, duration of relationships, frequency of contact), and loss of support within past year
	Multidimensional Scale of Perceived Social Support (MSPSS; Zimet, Dahlem, Zimet, & Farley, 1988)	Subjective assessment of social support adequacy
Self-efficacy in the nurturing role	Self-efficacy in the nurturing role scale (SENR; Pedersen, Bryan, Huffman, & Del Carmen, 1989)	Mothers' perceptions of their competence on basic skills required in caring for an infant
Mental health	Mini-International Neuropsychiatric Interview (MINI)– plus (Sheehan et al., 1997)	Screening for psychiatric disorders
	Beck depression inventory (BDI)-II (Beck, Steer, & Brown, 1996)	Intensity of depression
	State-Trait Anxiety Inventory (STAI; Spielberger, 2010; Spielberger, Gorsuch, & Lushene, 1970)	Indication of transient anxiety and tendency to experience general anxiety
	Aggression Questionnaire (Buss & Perry, 1992)	Verbal aggression, physical aggression, anger, and hostility
	Borderline personality checklist (Arntz et al., 2003; Giesen-Bloo, Arntz, & Schouten, 2005)	Severity of borderline-related symptoms during past month

Emotion regulation	Difficulties in Emotion Regulation Scale (DERS; Gratz & Roemer, 2004)	Clinically relevant difficulties in emotion regulation.
Antenatal attachment	Maternal antenatal attachment scale (MAAS; Condon, 1993; Van Bussel, Spitz, & Demyttenaere, 2010)	Quality of mother's affective experiences towards the foetus and intensity of preoccupation with the foetus
Reflective functioning	Pregnancy Interview (PI) – Revised (Slade, 2007; Slade, Patterson, & Miller, 2007) and Parent Development Interview (PDI) – Revised (Slade et al., 2003; Slade et al., 2005)	Parental reflective functioning
Parenting	Adapted version of the Mother Infant Coding System (Miller, McDonough, Rosenblum, & Sameroff, 2002) to code maternal behaviour during Free play, Teaching tasks, and Still-Face Paradigm (SFP; Tronick et al., 1978)	Maternal interactive behaviour (e.g. sensitivity, intrusiveness) with her child during various contexts.
	Parental Cognitions and Conduct Toward the Infant Scale (PACOTIS; Boivin et al., 2005)	Parental perceptions and behavioural tendencies towards infant
Parenting stress	Nijmeegse Ouderlijke Stress Index (NOSI; Abidin, 1990; De Brock, Vermulst, Gerris, & Abidin, 1992)	Parenting stress
IQ	WAIS-III-NL (Wechsler, 2005) – subtests Vocabulary, Matrix Reasoning, and Digit Span	Global indicators of intellectual functioning (IQ estimate) - mother
Executive functioning	Behavior Rating Inventory of Executive Function (BRIEF) – adult version (Roth, Isquith, & Gioia, 2005)	Difficulties in executive functioning in daily life
Stress reactivity and regulation – baseline, stress and recovery	Maternal response to mildly stressful situation (i.e. short mother-child separation and child exposure to fear paradigm (see child variables))	*Hypothalamic pituitary adrenal (HPA)-axis*: Cortisol and Alpha-amylase (saliva) *Subjective experience of stress (mother):* Visual Analogue Scale (VAS) (Lesage, Berjot, & Deschamps, 2012)

Table 3. *Research domains, instruments, and contents: child.*

Domain (child)	Instrument / data source	Content
Cognitive development	Infant Mental Development Index of the Bayley Scales of Infant Development (BSID), 2nd version (Bayley, 1993)	Global measure of infant cognitive development
Stress reactivity and regulation – baseline, stress and recovery	Stressors: - *Social*: Still Face Procedure (Mesman, Van IJzendoorn, & Bakermans-Kranenburg, 2009; Tronick et al., 1978) - *Frustration*: Laboratory Temperament Assessment Battery (Lab-TAB; Goldsmith & Rothbart, 1999a, 1999b): Car seat, Gentle arm restraint - *Fear*: Adapted version of unpredictable-mechanical-toy from the Lab-TAB (Baker, Shelton, Baibazarova, Hay, & van Goozen, 2013; Goldsmith & Rothbart, 1999a)	*Autonomic nervous system (child)*: Heart rate, pre-ejection period, skin conductance, respiratory sinus arrhythmia with the Vrije Universiteit Ambulatory Monitoring System (VU-AMS; De Geus, Willemsen, Klaver, & Van Doornen, 1995; Willemsen, De Geus, Klaver, Van Doornen, & Carrofl, 1996) *Hypothalamic pituitary adrenal (HPA)-axis*: Cortisol and Alpha-amylase (saliva) *Behavioural stress reactivity and regulation (child)*: observation (coding based on Lab-TAB)
Aggression	Cardiff Infant Contentiousness Scale (CICS; Hay et al., 2010)	Early manifestations of aggression
	Physical Aggression Scale for Early Childhood (PASEC; Alink et al., 2006)	Physical aggression
	Peer Interaction Coding System (PICS; Caplan, Vespo, Pedersen, & Hay, 1991; Hay, Nash, et al., 2011)	Peer aggression

Behavioural problems	Child Behavior Check List (CBCL) 1 ½ -5 year (Achenbach & Rescorla, 2000; Achenbach & Ruffle, 2000)	e.g. internalizing and externalizing behavioural problems
Temperament	Short versions of the Infant Behavior Questionnaire (IBQ; Rothbart, 1981) and Early Childhood Behavior Questionnaire (ECBQ; Putnam & Rothbart, 2006)	Dimensions of temperament (i.e. negative emotionality, effortful control)
(Precursors of) executive functioning	BRIEF – preschool version (Gioia, Espy, & Isquith, 2003)	Difficulties in executive functioning in daily life
	Music box (Goldsmith & Rothbart, 1999a)	Sustained attention
	Don't Paradigm (adapted from Kochanska, Tjebkes, and Fortnan (1998))	Infants' spontaneous restraint to maternal prohibition
	Self-restraint (Friedman, Miyake, Robinson, & Hewitt, 2011), and Inhibitory control tasks (Carlson & Moses, 2001)	Inhibitory control
	Snack task and Gift task (Kochanska, Coy, & Murray, 2001; Spinrad et al., 2007)	Delay of gratification
	Hide the pots (Bernier, Carlson, & Whipple, 2010), and Spin the pots (Hughes & Ensor, 2005)	Working memory
	Dimensional Change Card Sort (Zelazo, 2006)	Mental flexibility
(Precursors of) Theory of Mind	Pleasure-disgust task (Slaughter & McConnell, 2003)	Social referencing
	Discrepant desires tasks (Atance, Bélanger, & Meltzoff, 2010; Laranjo, Bernier, Meins, & Carlson, 2010; Repacholi & Gopnik, 1997)	Understanding of discrepant desires
	Early Social Communication Scales (ESCS) – subtask joint attention (Mundy et al., 2003)	Joint attention

	Visual Perspectives (Bigelow & Dugas, 2009; Carlson, Mandell, & Williams, 2004; Laranjo et al., 2010)	Visual perspective taking
	Subtest functional and symbolic imitation - Autism Diagnostic Observation Schedule (ADOS; Luyster et al., 2009)	Imitation
	False belief tasks (Bigelow & Dugas, 2009)	False belief
(Precursors of) empathy	Pain task (Young, Fox, & Zahn-Waxler, 1999; Zahn-Waxler, Robinson, & Emde, 1992)	Empathy
	Mishaps (Kochanska, Gross, Lin, & Nichols, 2002)	Guilt
	Emotion recognition task (Pollak, Cicchetti, Hornung, & Reed, 2000)	Emotion recognition
	Emotion eliciting film clips of ecologically valid situations (e.g. fear, sadness)	Social attention with the Tobii T120 eye-tracker
Language development	MacArthur communicative development inventory (Fenson et al., 2000)	Communicative skills in infants and toddlers
	Reynell Developmental Language Scales (Reynell, 1985), Peabody Picture Vocabulary Test (PPVT; Dunn & Dunn, 1997)	Receptive language skills
	Schlichting Expressive Language Test (Schlichting, Van Eldik, Lutje Spelberg, Van der Meulen, & Van der Meulen, 1995)	Expressive vocabulary skills

The intervention

Families in the HR-I group (n=65) participated in an intensive home-visiting program based on 'Minding the baby' (MTB) (Sadler et al., 2013; Slade et al., 2005; Slade, Sadler, & Mayes, 2005). MTB is an interdisciplinary home-visiting program developed at Yale University Child Study Center and Yale University School of Nursing (Slade et al., 2010). MTB focuses on young vulnerable first-time parents, primarily mothers, who are at high risk due to multiple complex issues, including psychiatric problems, poverty, young maternal age, single motherhood, or limited social support. MTB combines two well-researched early-intervention models; home visiting and infant-parent-psychotherapy, in order to meet the holistic, complex, multi-layered care needs of vulnerable families (Sadler et al., 2013). The program specifically aims to enhance maternal reflective functioning (RF) and the development of secure attachment relationships, as well as to address maternal (mental) health issues. RF refers to the mother's capacity to 'keep the baby in mind', to make sense of his/her internal states, emotions, thoughts, and intentions, as well as her own (Slade, 2002). Particularly in high-risk mothers, RF is often compromised, leading to disrupted interactions, insecure attachment relationships, and long term emotional difficulties. In the present study (see Table 3), RF skills were significantly lower among women in the HR-group compared to women in LR-group. In MTB, parents are encouraged to be curious, to try and figure out what the child needs or is thinking or feeling even in early infancy. RF is seen as a key to maternal sensitivity and plays an important role in the development of the child's capacity for Theory of Mind and adaptive socio-emotional development in young children (Ordway et al., 2014; Laranjo, Bernier, Meins, & Carlson, 2010; Sadler et al., 2013; Sadler, Slade, & Mayes, 2006; Slade, Sadler, & Mayes, 2005). Programs that are specifically aimed at improving parental RF in 'at-risk' parents indeed appear to improve RF-skills and parenting behaviour (Katznelson, 2014; Suchman et al., 2010). For a more detailed description of the conceptual framework underlying the MTB-model, see Sadler and colleagues (2013).

The MTB-program offers a treatment manual with a set of well-developed protocols and guidelines (Slade et al., 2010). At the same time, the program can be adapted to the individual needs of the family and the circumstances of each home visit. For implementation of the MBT-program in The Netherlands, one of the PI's (HS) and social workers of the MINDS-Leiden team were thoroughly trained in the basic constructs and techniques of the reflective parenting model used in MTB. This MTB 'Introductory training institute' provides the basis for implementation of the MTB-program in other settings. A difference between the original MTB and the intervention used in this study is that we chose to work only with clinical social workers ("coaches"), instead of alternating the home visits between a nurse

practitioner and clinical social worker. This decision has been based on the fact that in The Netherlands, mother and child pay regular visits to a paediatric nurse in the first four years of life. In the MTB-program, the visits by the nurse practitioner are mostly centered on health-related issues, which in The Netherlands are monitored by paediatric nurses at child health and welfare centres.

Home visits generally lasted about one hour, although at times of crisis home visits could be extended or increased in frequency. The home visits were conducted by a trained coach, starting during the last trimester of pregnancy until the child was 30 months old, and were scheduled weekly during the first year and continued two-weekly after that. Apart from the planned home visits, the coaches were available for their families when needed (by phone or 'WhatsApp'). The main objective of the coaches was to promote parental RF, support the mother-infant attachment relationship, and stimulate adequate parenting skills. Further, the coaches aimed to reinforce prenatal health care and health education, supported both mother's and child's health and development, helped mothers to extend or build a stronger social support network, educated mothers about the safety of their child, referred to a range of treatments as appropriate when psychiatric complaints were detected, and helped mothers negotiate issues involving legal, financial and housing problems. However, the coaches conferred regularly about their families during monthly supervision meetings, and maintained close contact with each other and their supervisor in case of crisis, or other family problems.

Group characteristics

Table 3 provides an overview of demographic and obstetric characteristics of the LR-and HR-groups. Compared to women in the LR-group, women in the HR-group were significantly younger, lower educated, had a lower income, were more often non-Caucasian and single, and had a smaller social support network (ps<.01). Further, pregnancies in the HR-group were more often unplanned, and women in the HR-group more often experienced miscarriages or had undergone abortion (ps<.05).

Among women in the LR-group (n=128), 9% (n=11) had one risk factor present, which was mostly limited social support (n=5), followed by single parenthood (n=2), young maternal age (n=2), unemployment (n=1) or no secondary education (n=1). In the HR-group, 64% of the women (n=94) had an indication for current psychiatric disorder(s), with 26% (n=38) having two or more diagnoses on the M.I.N.I.-plus. See Table 4 for an overview of the diagnoses on the M.I.N.I.-plus in the HR-group. Substance use during pregnancy was the second most frequent observed risk factor in the HR-group. Of the women who used substances during pregnancy, 33% (n=48) continued to smoke, 5% (n=8) drank alcohol, 1% (n=1) continued to use

(other) drugs, 2% (*n*=3) were smoking and drinking alcohol, 3% (*n*=5) were smoking and using drugs, and 1% (*n*=1) used all these substances (smoking, alcohol and drugs). Drugs used during pregnancy were cannabis (*n*=4), cocaine (*n*=1), methadone (*n*=1), and cocaine and cannabis (*n*=1).

Table 3. *Demographic characteristics of the low-risk and high-risk groups.*

	LR (*n*=128)		HR (*n*=147)		Group comparisons[a]
Variables	*M*	*SD*	*M*	*SD*	
Maternal age (years)	23.42	1.74	21.56	2.32	$F(1,273)=55.59^{***}$
Maternal education (% high[b])	41%		7%		$\chi^2(1)=44.10^{***}$
Monthly family income (euro's)	2,944.02	964.50	1,609.00	1,145.00	$F(1,273)=107.47^{***}$
Ethnicity (% Caucasian)	91%		76%		$\chi^2(1)=10.06^{**}$
Married or living with partner (%)	98%		61%		$\chi^2(1)=54.59^{***}$
First-time pregnant (%)	87%		76%		$\chi^2(1)=5.53^{*}$
Unplanned pregnancy (%)	23%		72%		$\chi^2(1)=65.15^{***}$
Expecting twins (%)	4%		5%		*ns*
Gestational weeks at assessment	30.03	3.77	29.91	3.68	*ns*
Size social support network	9.19	3.84	6.94	3.54	$F(1,273)=25.40^{***}$
Prenatal reflective functioning	4.27	1.03	3.53	0.86	$F(1,273)=42.58^{***}$
Number of risk factors	0.09	0.29	2.24	1.35	$F(1,273)=311.72^{***}$
Current psychiatric disorder(s) (%)	0%		64%		$\chi^2(1)=124.36^{***}$
Substance use pregnancy (%)	0%		44%		$\chi^2(1)=70.78^{***}$
Alcohol	0%		7%		$\chi^2(1)=7.36^{**}$
Tobacco	0%		39%		$\chi^2(1)=62.61^{***}$
(Other) drugs	0%		5%		$\chi^2(1)=6.25^{*}$
Single parenthood (%)	2%		21%		$\chi^2(1)=24.70^{***}$
Unemployed (%)	1%		24%		$\chi^2(1)=31.89^{***}$
No secondary education (%)	1%		9%		$\chi^2(1)=9.21^{**}$
Financial problems (%)	0%		25%		$\chi^2(1)=37.23^{***}$
Limited social support (<4 persons)	4%		12%		$\chi^2(1)=6.21^{*}$
Young maternal age (<20 years)	2%		20%		$\chi^2(1)=23.63^{***}$

Note: $^{*}p<.05$, $^{**}p<.01$, $^{***}p<.001$ [a]ANOVA or Chi-square test, [b]Maternal education (% high) represents percentage with a bachelor's or master's degree, LR = low-risk group, HR = high-risk group.

Table 4. *Overview of the current psychiatric problems in the HR-group.*

Current psychiatric problems*	N
Depressive disorder	17
Dysthymic disorder	8
Suicidality	11
Mania	1
Panic disorder	15
Agoraphobia	17
Social phobia	7
Other type of phobia	8
Obsessive-compulsive disorder	5
Generalized anxiety disorder	4
Posttraumatic stress disorder	4
Alcohol dependence / abuse	9
Drug dependence / abuse (non-alcohol)	18
Attention-deficit/hyperactivity disorder	7
Antisocial personality disorder	14

Note: N high-risk group = 147; * = positive screening based on the M.I.N.I.-plus (Sheehan et al., 1997; Van Vliet & De Beurs, 2007).

Discussion

This paper presented an overview of the background, methods and study population of the MINDS-Leiden study, a longitudinal study investigating social-environmental, neurobiological, and neurocognitive mechanisms predicting emotional and behavioural problems in young children and variation in effects of an intensive RF-based home-visiting program for first-time mothers with a high-risk background. The scope for changing behaviour is greatest in the early years because neurobiological systems involved in emotional and behavioural regulation are presumed to be most sensitive for environmental influences early in development (Beauchaine et al., 2008). The outcomes of this study may result in the identification of specific biomarkers, precursors of neurocognitive functions and temperamental factors in infancy, which directly, indirectly or interactively with social-environmental risk factors, may help to detect children who are at risk for later emotional and behaviour problems.

Home-visiting programs hold considerable promise for improving child outcomes (Avellar & Supplee, 2013; Filene et al., 2013; Olds et al., 2007; Peacock et al., 2013; Sweet & Appelbaum, 2004). From a socio-economic and ethical perspective, intervention programs should only be offered to those families who are expected to benefit and an alternative should be offered to those who are not expected to benefit. The outcomes of our study may result in a better understanding of the individual

(neurocognitive and neurobiological) factors that explain variation in effects of home-visiting programs. Ultimately, this may contribute to more efficient matching of families to intervention programs while at the same time provide relevant information to further enhance the effectiveness of the home-visiting program.

A considerable strength of the study includes the random controlled trial and the use of multi-method approach, including a combination of (semi-) structured interviews, questionnaires, behavioural observations, and physiological measures. An important aspect of the current study, compared to previous studies evaluating the effects of home-visiting programs (Olds et al., 2007; Ordway et al., 2014; Robling et al., 2016), is the use of both a high-risk and a low-risk control group when examining the effects of home-visitation, which allows us to more thoroughly determine the extent to which neurobiological and neurocognitive development in children in the HR-I group normalizes (as observed deviations in HR-children compared to LR-children throughout development may, in part, depend on their 'starting levels').

A limitation of this study is that we lack information about the number of subjects that refused to participate before the research team tried to contact them for scheduling the first assessment. This is due to our sampling strategy in which we relied on external parties for the recruitment of potential participants. It cannot be ruled out that non-participants may differ from participants in several respects, especially as participation in a longitudinal study with 6 assessments requires a strong commitment of the mothers and might be perceived as a burden. This may have resulted in the loss of more severe cases. For example, in our high-risk sample, only a small group of participants had three or more risk factors present (17%). We do have information about the women who were recruited by their healthcare provider and gave permission to contact them for partaking in the study, but declined participation when they were contacted (16%). The most common reasons for not participating were: too busy/too time-consuming, partner does not want to participate, personal problems, medical problems, or miscarriage.

A special point of interest is the potential loss of participants to follow-up over time. Although some loss to follow-up is inevitable, we used various strategies for maximizing retention in order to retain as many participants as possible. For example, mothers received gift certificates (respectively 15, 20, 25, 30, 35, and 25 EUR) and a present for their child after each assessment. After completion of the fifth assessment, they received a personalized DVD with video material from all five assessments. Also, over the course of the study regular contact was maintained by telephone calls after birth, birth- and birthday cards, and a newsletter (every 3 months). Travel expenses were covered for lab visits (wave three, five, and six). Moreover, we offered to pick mother and child up from their homes and drive them

to our baby lab when they had difficulties coming to the lab, and mothers in the HR-intervention group were often accompanied to the lab by their coach. With data collection for the first assessment completed, attrition rates between the first and second assessment were 5%. Therefore, we are optimistic that our strategies are working and our attrition rates in the long run will be limited.

At this moment, funding has been obtained for the first five waves of follow up, and we plan to seek funding for additional follow-up cycles. Now that the study is well underway, efforts are being made to disseminate the results of our study to obstetric care providers, health care workers, paediatricians and policymakers on a regular basis. Further, once we have gained insight into factors that predict the effectiveness of the home-visiting program, we will look for ways to implement the program in clinical practice. For this, we have already sought cooperation with health care organizations in the area of Leiden, The Hague, and Amsterdam.

Taken the potential restraints into account, we believe that this study program may provide detailed insight in the factors associated with (very) early child development and treatment success for interventions aiming to reduce and/or prevent emotional and behavioural problems. We hope that by effectively addressing behavioural problems from infancy onwards, improvements in (mental) health status (of children and their mothers), and public health policy and decision making may be achieved.

CHAPTER 4.1

Interaction between prenatal risk and physiological self-regulation in infancy in predicting physical aggression at 20 months

Manuscript invited to revise and resubmit:
Suurland, J., Van der Heijden, K. B., Huijbregts, S. C. J., Van Goozen, S. H. M., & Swaab, H.. Interaction between prenatal risk and infant parasympathetic and sympathetic stress reactivity predicts early aggression.

Abstract

A breakdown in stress regulation, as reflected in nonreciprocal activation of the parasympathetic (PNS) and sympathetic (SNS) nervous systems, increases susceptibility to emotional and behavioral problems in children exposed to adversity. Little is known about the PNS and SNS in interaction with early adversity during infancy. Yet this is when the physiological systems involved in emotion regulation are emerging and presumably most responsive to environmental influences. We examined whether parasympathetic respiratory sinus arrhythmia (RSA) and sympathetic pre-ejection period (PEP) response and recovery at six months, moderate the association between cumulative prenatal risk and physical aggression at 20 months (*N*=113). Prenatal risk predicted physical aggression, but only in infants exhibiting coactivation of PNS and SNS (i.e. increase in RSA and decrease in PEP in response to stress). These findings indicate that coactivation of the PNS and SNS in combination with prenatal risk is a biological marker for the development of aggression.

Keywords: Aggression, stress reactivity, autonomic nervous system, prenatal risk, infancy

Introduction

Exposure to adversity during the prenatal period, such as maternal psychiatric problems, substance (ab)use, single parenthood and poverty, has been shown to predict aggression in children persisting into adolescence and adulthood (Côté, Vaillancourt, LeBlanc, Nagin, & Tremblay, 2006; Hay et al., 2011; NICHD Early Child Care Research Network, 2004). Yet, not all children seem to be equally affected by adversity. Guided by theories of differential susceptibility (Belsky & Pluess, 2009) and biological sensitivity to context (Boyce & Ellis, 2005), a number of studies have demonstrated that individual differences in stress reactivity, as measured by indices of the autonomic nervous system (ANS), can predispose or protect against the effects of adversity on children's behavioral maladjustment (e.g. El-Sheikh & Erath, 2011). Although these studies provide important insights into physiological measures of susceptibility, they have focused mostly on older children. Little is known about the role of the ANS in interaction with early adversity during infancy when the physiological systems involved in emotion regulation are emerging and presumably most responsive to environmental influences (Beauchaine, Neuhaus, Brenner, & Gatzke-Kopp, 2008; Laurent, Harold, Leve, Shelton, & Van Goozen, 2016).

Altered ANS functioning has been consistently linked to aggression in children, adolescents and adults (Van Goozen, Fairchild, Snoek & Harold, 2008). The ANS is comprised of a sympathetic (SNS) and parasympathetic (PNS) branch. The SNS initiates the 'fight/flight' response, whereas the PNS has opposing effects and promotes rest and restorative behavior (Porges, 2007). Low baseline PNS activity, as indicated by respiratory sinus arrhythmia (RSA), has been identified as a vulnerability factor that exacerbates the relation between adversity (e.g. marital conflict, parental drinking problems) and children's externalizing behavior (El-Sheikh, 2005a; El-Sheikh, Harger, & Whitson, 2001). Other studies have measured RSA reactivity to stress, with decreases in RSA in response to stress considered to be indicative of better adaptation (El-Sheikh & Erath, 2011). RSA withdrawal in response to stress has been associated with lower levels of externalizing behavior in the context of adversity (El-Sheikh, 2001; Katz, 2007), although findings have been inconsistent (Obradovic, Bush, Stamperdahl, Adler, & Boyce, 2010). Studies investigating interactions between adversity and SNS activity (measured as skin conductance level [SCL] in most studies) indicate that either very low or very high baseline levels of SCL and high SCL reactivity may increase the risk of aggression and externalizing behavior in the context of adversity (El-Sheikh, 2005b; El-Sheikh, Keller, & Erath, 2007).

It is clear that ANS functioning has important implications for the association between adversity and the development of aggression. However, such associations may be less straight forward in infancy. For example, recent studies indicated a

stronger positive relation between higher (rather than lower) baseline RSA and (externalizing) problem behavior in infants and toddlers exposed to a more negative caregiving environment (Conradt, Measelle, & Ablow, 2013; Eisenberg et al., 2012). Measures of RSA reactivity and SNS functioning in infants have not been studied as moderators of relations between early adversity and aggression before, although there is one study in toddlers reporting no effects of RSA reactivity (Eisenberg et al., 2012).

Although the PNS and SNS are generally thought to operate in a reciprocal manner, with increased activation of one system and decreased activation of the other, nonreciprocal activation of the PNS and SNS, with increased or decreased activation of both systems at the same time, is possible (Berntson, Cacioppo, & Quigley, 1991). Reciprocal modes of PNS and SNS activation may indicate more evolutionarily advanced response strategies in response to stress, whereas nonreciprocal activation of the PNS and SNS may indicate a breakdown in stress regulation, in which either the PNS or SNS fails to perform its adaptive function in response to stress (Porges, 2007). Recently, this has led to the acknowledgement that the interaction between the PNS and SNS should be examined (Bauer, Quas, & Boyce, 2002; El-Sheikh & Erath, 2011). Findings from recent studies indicate that adversity interacts with both PNS and SNS measures to predict children's externalizing problems (El-Sheikh et al., 2009; Gordis, Feres, Olezeski, Rabkin, & Trickett, 2010). Specifically, decreased PNS and SNS activation (i.e. *coinhibition*) and increased PNS and SNS activation (i.e. *coactivation*) predicted higher levels of aggression and externalizing problems in the context marital conflict (El-Sheikh et al., 2009). Conversely, coordinated action between the two systems (i.e. *reciprocal PNS activation* and *reciprocal SNS activation*) operated as protective factors. Similar findings were reported in the context of maltreatment predicting aggression among girls (Gordis et al., 2010).

Nonreciprocal PNS or SNS activation may develop as a result from exposure to intense or chronic stress (Bauer et al., 2002), and exacerbate the effects of early adversity on aggression over time. So far, there have been no studies that we know of that have examined measures of both PNS and SNS functioning in infancy as potential moderators of the effects of early adversity on outcome in toddlerhood. Elucidating how early physiological systems increase or decrease susceptibility to aggression, may enhance our ability to identify children at risk of aggression at an early age, before developmental trajectories begin to be set.

In the present study, we investigated the interaction between ANS response to and recovery from stress measured in six-month-old infants, taking into consideration both the PNS and SNS, and prenatal risk in predicting physical aggression at 20 months of age. We were specifically interested in cumulative risk as previous work has shown a dose-dependent relation between the presence of multiple

risk factors and child adjustment, with increases in the number of risk factors being associated with increased levels of problems (Appleyard, Egeland, van Dulmen, & Sroufe, 2005). We measured parasympathetic RSA and sympathetic pre-ejection period (PEP) response and recovery from stress. Although previous studies involving PNS and SNS interactions have focused on SCL (El-Sheikh et al., 2009; Gordis et al., 2010), PEP is considered to be a purer measure of cardiac SNS activity (Cacioppo, Uchino, & Berntson, 1994), that can be reliably measured in infants (Alkon et al., 2006; Quigley & Stifter, 2006). We hypothesized that higher levels of *coactivation* and *coinhibition* would exacerbate the relation between cumulative prenatal risk and physical aggression, whereas, *reciprocal PNS activation* and *reciprocal SNS activation* would attenuate the association between cumulative risk and physical aggression.

Methods

Participants

The participants in this study were part of an ongoing longitudinal study into neurobiological and neurocognitive predictors of early behavior problems (Mother-Infant NeuroDevelopment Study in Leiden, The Netherlands [MINDS – Leiden]). We oversampled women based on the presence of one or more risk factors (see criteria under Cumulative risk). The sample was composed of 113 mothers and their infants (55.8% males) who had completed the prenatal home-visit during the third trimester of pregnancy (T1), and the postpartum home-visits at six (T2) and 20 months (T3). The mean age of the children was 6.03 months (*SD*=.41, range 5-7 months) at T2 and 19.94 months (*SD*=.81, range 18-24 months) at T3. The mothers were on average 22.96 years (*SD*=2.12, range 17-27 years) at T1. Approximately 96% of the mothers had a partner (87.6 % was married or living with a partner) and 32.7% of the mothers had a high educational level (Bachelor's or Master's degree). Families were predominantly Caucasian (88.5%).

Of the 136 mothers originally enrolled in the study at T1, 10 did not participate at T2, and another 13 dropped out between T2 and T3. Main reasons for families dropping out were inability to contact, moving away or too busy. Sample attrition was unrelated to demographic variables (i.e. maternal age, ethnicity, marital status, educational level; ps>.05). However, mothers who dropped out were more often single ($\chi^2(1) = 8.41$, p=.013).

The study was approved by the ethics committee of the Department of Education and Child Studies at the Faculty of Social and Behavioral Sciences, Leiden University, and by the Medical Research Ethics Committee at Leiden University Medical Centre. Informed consent was obtained from all parents of infants included

in the study. Mothers were compensated for each completed home or laboratory visit and children were given a small present for their participation.

Procedures

The protocol during the six-month home-visit (2hrs), included attachment of cardiac monitoring equipment to the infant's chest and back after which they watched a 2-minute relaxing movie while lying on a blanket, followed by two procedures designed to elicit physiological responses to social stress (Still Face Paradigm) and frustration (Car seat). The social stress and frustration tasks were administered with a break in between to limit carry over effects. Infants were only assessed in the next procedure when they were calm and displayed no distress. The home-visits were scheduled at a time of the day when mothers deemed their infant to be most alert.

The Still Face Paradigm (SFP; Mesman, Van IJzendoorn, & Bakermans-Kranenburg, 2009) is a well-established social stress paradigm comprising a sequence of three 2-minute episodes during which the mother is asked to interact normally with the infant (SFP baseline), then withhold interaction (SFP social stress), and then resume interaction (SFP recovery) (for a more detailed description of the SFP, see Suurland, Van der Heijden, Smaling, Huijbregts, Van Goozen, & Swaab, 2016). The Car Seat (CS) task, adapted from the Laboratory Temperament Assessment Battery Pre-locomotor version (Lab-TAB; Goldsmith & Rothbart, 1999a), was used to measure infant physiological response to a frustrating event. Following a 2-minute baseline (CS baseline), mothers placed their infants in a car seat and stood 1 meter away from their child. After 1 minute of restraint (CS frustration), a 2-minute recovery period (CS recovery) followed in which mothers were allowed to hold their child and interact as they normally would. Mothers were instructed to remain neutral and refrain from comforting or speaking to the child during the CS frustration episode.

During the challenge episodes, infant distress (i.e. whining, fussing or crying) was coded by trained raters from videotaped recordings according to scales of the Mother Infant Coding System (Miller, McDonough, Rosenblum, & Sameroff, 2002) for the SFP; the Lab-TAB coding system (Goldsmith & Rothbart, 1999a) was used for the CS. During the SFP social stress and the CS frustration episodes respectively 26.8% and 25.5% of the infants showed signs of distress.

Measures

Physiological measures. Parasympathetic RSA and sympathetic PEP were monitored continuously with the Vrije Universiteit Ambulatory Monitoring System (VU-AMS 5fs; De Geus, Willemsen, Klaver, & Van Doornen, 1995; Willemsen, De Geus, Klaver, Van Doornen, & Carroll, 1996). The VU-AMS device continuously

recorded electrocardiogram (ECG), and impedance cardiogram (ICG) measures; basal thorax impedance (Z_0), changes in impedance (dZ), and the first derivative of pulsatile changes in transthoracic impedance (dZ/dt). The ECG and dZ/dt signal were sampled at 1000 Hz, and the Z_0 signal was sampled at 10Hz. The VUDAMS software suite version 2.0 was used to extract mean values of heart rate (HR), RSA, and PEP across SFP baseline (2 minutes), SFP social stress (2 minutes), and SFP recovery (2 minutes), and CS baseline (2 minutes), CS frustration (1 minute), and CS recovery (2 minutes).

R-peaks in the ECG, scored by the software, were visually checked and adjusted manually when necessary. RSA was derived by the peak-trough method (De Geus et al., 1995; Grossman, Van Beek, & Wientjes, 1990), which combined the respiration (obtained from filtered [0.1 – 0.4 Hz] thoracic impedance signal) and inter beat interval (IBI) time series to calculate the shortest IBI during heart rate acceleration in the inspiration phase and the longest IBI during deceleration in the expiration phase (De Geus et al., 1995). RSA was defined as the difference between the longest IBI's during expiration and shortest IBI's during inspiration. Automatic scoring of RSA was checked by visual inspection of the respiratory signal from the entire recording.

PEP is the time interval between the onset of the ventricular depolarization (Q-wave onset) and the onset of left ventricular ejection of blood into the aorta (B-point on the Dz/dt complex (De Geus et al., 1995). Average dZ/dt waveforms were derived by the software. PEP was automatically scored from the Q-wave onset (opening of the aortic valve) on the ECG and the B-point on the dZ/dt waveform. Each automated scoring was checked and corrected manually when necessary (Riese et al., 2003). Wave forms which were morphologically distorted and could not be visually corrected, were discarded. The procedure of interactive visual scoring was done independently by two trained raters; inter-rater reliability (intraclass correlation ICC) was .949.

Cumulative risk. During the third trimester of pregnancy (between 26 and 40 weeks gestation, M = 29.78, SD = 3.63), mothers were screened for the presence of risk factors (see for a more elaborate description of these criteria: Smaling et al., 2015; Suurland et al., 2016), including current psychiatric disorder(s) with the Dutch version of the Mini- International Neuropsychiatric Interview (MINI-plus; Van Vliet, Leroy, & Van Megen, 2000), substance use (alcohol, tobacco and/or drugs) during pregnancy, no secondary education, unemployment, self-reported financial problems, limited or instable social support network, single status, and maternal age <20 years. The cumulative risk score was computed as the sum of risk factors present (maximum number of risk factors was 10), with M=.67, SD=.93 (range 0-3). There were 66

mothers with no risk factors, 25 with one risk factor, 15 with two risk factors, and 7 with three risk factors. The prevalence of the different risk factors among mothers with one or more risk factors (41.6%) was: 55.3% current psychiatric diagnosis, 4.3% alcohol, 44.7% smoking, 2.1% drugs, 10.6% single status, 10.6% unemployed, 4.3% no secondary education, 8.5 % financial problems, 8.5% limited social support, 14.9% age <20 years.

Maternal reports of physical aggression. Mothers reported on their child's physical aggression at 20 months using the 11-item Physical Aggression Scale for Early Childhood (PASEC; Alink et al., 2006). Mothers indicated whether their child had shown physically aggressive behaviors (e.g. 'hits', 'kicks', 'destroying things') in the past two months on a 3-point Likert scale (0 = 'not true to 2 = 'very true or often true'). A total score for physical aggression was calculated by summing item scores (range 0-22). Internal consistency (Cronbach's alpha) was .73.

Missing data

Approximately 12% of ANS data were missing across the SFP and CS episodes. Missing data was due to dyads that did not complete the SFP or CS because the infant became too fussy (3.8%), loose electrodes (5.7%), equipment failure (1.9%), or excessive child movement in which case PEP and/or RSA could not be scored (88.6%). Missing data was not systematically related to demographic and obstetric variables (i.e. sex, ethnicity, gestational age, and birth weight; *p*s>.250) or cumulative risk and physical aggression (*p*s>.250). Main analyses were conducted based on the number of infants for which there was data (see Table 1 for available ANS data across SFP and CS episodes).

Data analysis

All variables were examined for outliers and violations of specific assumptions applying to the statistical tests used. Variables with values that exceeded >3*SD* from the group mean were recoded to the next extreme value within 3*SD* from the mean (across all SFP and CS episodes there were 14 outliers for RSA and two outliers for PEP). Because RSA was skewed at baseline, the emotional challenge tasks, and recovery, its natural logarithm (lnRSA) was used in the analyses.

Baseline levels of lnRSA and PEP were significantly correlated with lnRSA and PEP challenge scores (*rs*=.27 to .84, *ps*<.001). Further, lnRSA and PEP challenge scores were significantly correlated with lnRSA and PEP recovery scores (*rs*=.53 to .87, *ps*<.001). To control for initial levels of arousal, response and recovery variables for lnRSA and PEP were computed as standardized residualized change scores (Eisenberg et al., 2012; El-Sheikh et al., 2009). The standardized residualized change

scores for response to challenge were obtained by regressing the challenge scores on the baseline levels and for recovery from challenge by regressing the recovery scores on the challenge scores. This was done separately for the SFP and the CS. The standardized residualized change scores for lnRSA and PEP during response and recovery on the SFP were significantly correlated with the standardized residualized change scores for lnRSA and PEP during response and recovery on the CS (rs=.24 to .28, with ps=.021 to .009). Therefore, the residualized change scores of lnRSA and PEP on the SFP and CS were averaged to create four indices: lnRSA response and PEP response (average SFP and CS) and lnRSA recovery and PEP recovery (average SFP and CS). Negative values reflect lnRSA and PEP decreases (i.e. greater PNS suppression and greater SNS activation respectively), while positive values reflect lnRSA and PEP increases (i.e. greater PNS activation and greater SNS suppression respectively).

Preliminary analyses (independent *t*-tests, and Pearson correlations) tested for potential covariates (demographic and obstetric characteristics). Hierarchical linear regression analyses were conducted to examine the interactive effects of cumulative risk and ANS response and recovery on physical aggression. Two sets of regression analyses were conducted: (1) lnRSA and PEP response measures, and (2) lnRSA and PEP recovery measures. All variables were centered to their mean prior to analyses (Aiken & West, 1991). Step 1 included cumulative risk, Step 2 included lnRSA and PEP, Step 3 included all two-way interactions between cumulative risk, lnRSA and PEP, and Step 4 included the three-way interaction between cumulative risk, lnRSA, and PEP. Significant interaction effects were examined following procedures recommended by Aiken and West (Aiken & West, 1991) by plotting regression lines of the relation between cumulative risk and physical aggression at 0 risk factors and 1.6 risk factors (i.e. mean number of risk factors for the group of infants with ≥1 risk factors) and 1 *SD* above and below the mean for the moderators (lnRSA response/lnRSA recovery, and PEP response/PEP recovery).

We also tested whether the main and interactive effects were moderated by sex. Because this was not the case, we do not report these findings. All analyses were conducted using the Statistical Package for Social Sciences (SPSS for Windows, version 21.0, SPSS Inc., Chicago).

Table 1. *Descriptives for stress response and recovery variables.*

	N	*M*	*SD*	Min.	Max.
LnRSA					
SFP Baseline	107	3.37	.36	2.39	4.33
SFP Social stress	106	3.21	.41	2.38	4.18
SFP Recovery	106	3.27	.47	1.97	4.57
CS Baseline	104	3.27	.37	2.28	4.16
CS Frustration	101	3.25	.50	1.92	4.49
CS Recovery	98	3.17	.40	2.21	4.13
PEP					
SFP Baseline	96	62.87	6.39	44.13	76.89
SFP Social stress	100	61.75	7.16	43.02	76.89
SFP Recovery	91	61.63	7.51	40.99	79.01
CS Baseline	102	63.42	6.18	45.06	76.89
CS Frustration	91	62.01	6.82	45.00	76.00
CS Recovery	93	63.95	6.51	46.00	83.00

Note: lnRSA = natural logarithm of respiratory sinus arrhythmia, PEP = pre-ejection period, SFP = Still Face Paradigm, CS = Car seat.

Results

Descriptive analyses

Descriptive statistics for lnRSA and PEP baseline, challenge episodes and recovery are presented in Table 1. LnRSA and PEP response and recovery levels on the SFP and CS were significantly different from zero ($t(105)=4.33$, $p<.001$ for lnRSA SFP response, $t(97)=3.68$, $p<.001$ for lnRSA CS recovery, $t(91)=2.56$, $p<.05$ for PEP SFP response, $t(87)=2.87$, $p<.01$ for PEP CS response, and $t(82)=-2.28$, $p<.05$ for PEP CS recovery), except for lnRSA CS response ($t(98)=.23$, $p=.816$), lnRSA SFP recovery ($t(105)=-1.15$, $p=.140$), and PEP SFP recovery $t(87)=.14$, $p=.889$).

Averaged across the SFP and CS challenge episodes, 63% of the sample showed a decrease in lnRSA (i.e. PNS suppression) and 62% exhibited a decrease in PEP (i.e. SNS activation) from baseline. Averaged across the SFP and CS recovery episodes, 44.5% of the sample showed an increase in lnRSA (i.e. PNS activation) and 54.4% showed an increase in PEP (i.e. SNS suppression) from the challenge episode. Thus, there was sufficient variability in infant lnRSA and PEP response to and recovery from challenge.

Preliminary analyses

Means, SDs, and correlations for the potential covariates and main study variables are presented in Table 1. For interpretation purposes, lnRSA and PEP raw change scores are used for means and SDs in Table 2; however, as noted, residualized change scores are used in the correlation and regression analyses. The demographic characteristics (ethnicity, sex) and obstetric characteristics (gestational age, birth weight) were not significantly related to the main study variables (ps>.05). Higher levels of cumulative risk were associated with higher physical aggression scores (r=.31, p<.01). Cumulative risk was not related to response and recovery measures of lnRSA and PEP.

Hierarchical regression analyses

LnRSA and PEP response. Results of the hierarchical regression analysis for lnRSA and PEP response are shown in Table 3. There was a significant main effect of cumulative risk (b = .65, SE = .27, p<.05). Higher cumulative risk predicted higher levels of physical aggression. There were no significant main effects for lnRSA response or PEP response. There were no significant two-way interaction effects between cumulative risk, lnRSA and PEP on physical aggression. However, a significant three-way interaction between cumulative risk x lnRSA response x PEP response was found (b = -1.23, SE = .53, p<.05), explaining 4.7% of the variance in physical aggression over and above the variance explained by cumulative risk, lnRSA and PEP response and all two-way interactions.

Examination of simple slopes (see Figure 1) revealed that for infants exhibiting coactivation (i.e. lnRSA response at 1 *SD* above the mean and PEP response at 1 *SD* below the mean) in response to challenge, higher cumulative risk predicted higher levels of physical aggression (β = .74, p<.001). Conversely, for infants exhibiting coinhibition, reciprocal PNS activation and reciprocal SNS activation in response to challenge, cumulative risk was unrelated to physical aggression (β = .34, p=.199, β = -.05, p=.836, and β = -.02, p=.929 for respectively coinhibition, reciprocal PNS activation and reciprocal SNS activation).

LnRSA and PEP recovery. Results of the hierarchical regression analysis for lnRSA and PEP recovery are shown in Table 3. The main effect for cumulative risk was the same as in the hierarchical regression analysis for lnRSA and PEP response. There were no significant main effects for lnRSA recovery or PEP recovery, and none of two-way or three-way interactions were significant.

Table 2. *Means, standard deviations and correlations among study variables* .

Variable	1.	2.	3.	4.	5.	7.	8.	9.	10.	*M*	*SD*	*range*
1. Cumulative risk	-									.67	.93	0-3
2. Ethnicity (% Caucasian)	.10	-								88.5%		
3. Infant sex (% male)	.03	.07	-							55.8%		
4. Gestational age (weeks)	-.03	-.07	.02	-						39.21	1.85	32-42
5. Birth weight (kg)	-.15	-.05	-.17†	.62***	-					3.4	.53	1.9-4.5
7. LnRSA response	.05	.15	-.08	-.01	.09	-				.09	.35	-.61-1.14
8. PEP response	-.12	-.08	.18†	-.06	-.10	-.09	-			1.27	3.28	-5.81-11.25
9. LnRSA recovery	-.10	.08	.09	-.16†	-.14	-.15	-.03	-		.02	.26	-.60-.89
10. PEP recovery	-.10	.16	.01	.16	.16	.20*	-.30**	.12	-	-.60	3.69	-14.99-9.67
11. Physical aggression	.31**	.06	-.17†	-.02	-.01	.02	-.18†	.04	-.02	2.85	2.28	0-10

Note: lnRSA = natural logarithm of respiratory sinus arrhythmia, PEP = pre-ejection period. †<.10, *p<.05, **p<.01, p<.001.

Table 3. *Hierarchical regression analyses predicting physical aggression from lnRSA and PEP response and recovery and cumulative risk.*

		LnRSA and PEP response					LnRSA and PEP recovery				
Step	Predictor	*Adjusted* R^2	ΔR^2	ΔF	β	*t*	*Adjusted* R^2	ΔR^2	ΔF	β	*t*
1	Cumulative risk	.096	.105	11.51**	.32	3.39**	.089	.089	10.81**	.31	3.29**
2	Cumulative risk	.094	.016	.90	.30	3.11**	.076	.006	.31	.32	3.33
	lnRSA				.04	.39				.08	.79
	PEP				-.12	-1.25				.02	.18
3	Cumulative risk	.096	.029	1.07	.25	2.38*	.058	.010	.37	.35	3.45**
	lnRSA				.07	.74				.09	.89
	PEP				-.14	-1.40				.04	.39
	lnRSA x PEP				.02	.21				.05	.45
	Cumulative risk x lnRSA				.17	1.66				.09	.84
	Cumulative risk x PEP				-.08	-.76				-.02	-.22
4	Cumulative risk	.137	.047	5.39*	.25	2.5*	.055	.007	.75	.37	3.56**
	lnRSA				.03	.27				.09	.84
	PEP				-.12	-1.21				.05	.50
	lnRSA x PEP				-.04	-.37				.07	.64
	Cumulative risk x lnRSA				.09	.82				.07	.62
	Cumulative risk x PEP				-.10	-.97				.01	.10
	Cumulative risk x lnRSA x PEP				-.24	-2.32*				.10	.87

Note: *p<.05, **p<.01.

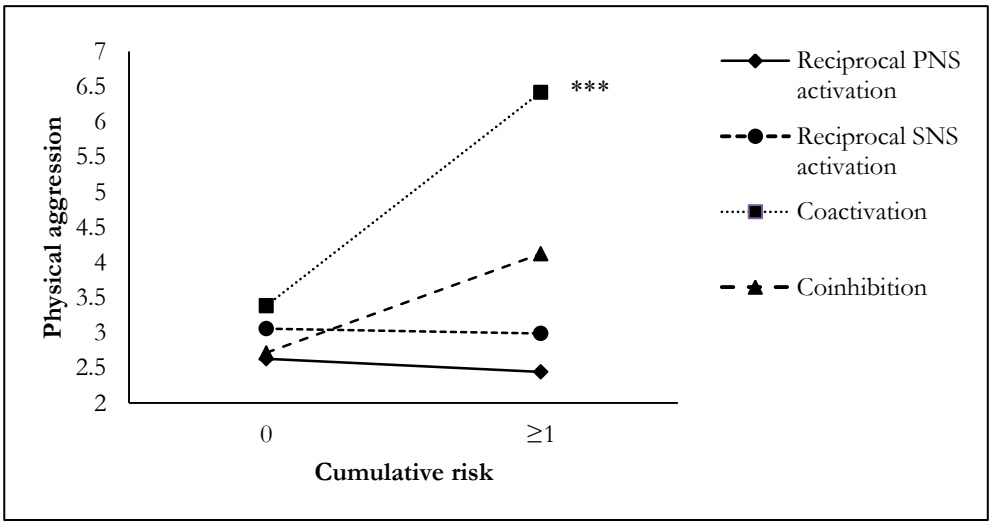

Figure 1. Three-way interaction between lnRSA response, PEP response and cumulative prenatal risk, predicting mother reported physical aggression. LnRSA response and PEP response are plotted 1 *SD* above and 1 *SD* below the mean. Cumulative risk is plotted at 0 risk factors and 1.6 risk factors (this is the average number of risk factors present in infants with one or more risk factors). *Reciprocal PNS activation* refers to PNS activation and SNS inhibition (i.e. lnRSA and PEP response at 1 *SD* above the mean), *reciprocal SNS activation* refers to PNS inhibition and SNS activation (i.e. lnRSA and PEP response at 1 *SD* below the mean), *coactivation* refers to PNS and SNS activation (i.e. lnRSA response at 1 *SD* above the mean and PEP response at 1 *SD* below the mean), and *coinhibition* refers to PNS and SNS inhibition (i.e. lnRSA at 1 *SD* below the mean and PEP response at 1 *SD* above the mean). ***p<.001.

Discussion

Our findings showed that higher levels of coactivation of the PNS and SNS in response to stress at 6 months increase vulnerability for physical aggression at 20 months, but only in the presence of higher levels of cumulative prenatal risk. Cumulative risk was not associated with physical aggression for infants who exhibited reciprocal PNS activation, reciprocal SNS activation or coinhibition in response to stress. We found no effects for PNS and SNS recovery from stress.

In previous studies, coactivation has been found to operate as a vulnerability factor for aggressive behavior and externalizing behavior problems in school-aged children exposed to adversity (e.g. marital conflict and maltreatment; El-Sheikh et al., 2009; Gordis et al., 2010). Our results extend these findings and indicate that coactivation of the PNS and SNS is already a risk factor for aggression at age 6 months. In fact, 20-month old children who, at six months of age, exhibited coactivation and were exposed to higher levels of prenatal risk, had physical aggression scores more than one standard deviation above the mean of physical

aggression scores reported in an community sample of 24-month old children (Alink et al., 2006).

Coactivation of the PNS and SNS indicates that both branches of the ANS are activated, promoting opposing physiological outcomes. In situations without challenge or stress, nonreciprocal modes of ANS activity may operate to preserve the baseline functional state of an organ or system (Berntson et al., 1991). However, in novel or challenging situations, the outcome may be ambiguous. Whereas the SNS accelerates heart rate and activates the 'fight/flight' response, the PNS decelerates heart rate and modulates SNS input to the heart and other target organs, regulating recovery and restoring autonomic homeostasis (Porges, 2007). Activation of the PNS (i.e. RSA augmentation) in response to stressful challenges has been associated with poor emotion regulation (e.g. Suurland et al., 2016). In the context of SNS activation, activation of the PNS may be especially harmful, reflecting poor regulation of high negative emotional reactivity. Interpreted within the framework of biological susceptibility to context (Boyce & Ellis, 2005), this pattern of physiological overarousal may reflect infants' conditional adaptation to a stressful prenatal and early postnatal environment. Moreover, in adverse early postnatal environments patterns of coactivation may lead to consolidation of less adaptive regulation strategies contributing to higher levels of aggression, even more so because in these environments it may be more adaptive for children to act aggressively (i.e. to get what they want, or to get attention from others).

The current study did not provide empirical support for previous findings indicating that coinhibition of the PNS and SNS also acts as a vulnerability factor for externalizing behavior problems (El-Sheikh et al., 2009; Gordis et al., 2010). It should be noted that these results were obtained in older children exposed to adversity compared to the infants/toddlers in our study. It is possible that coinhibition acts as a biomarker for aggression only later in development, for example after more prolonged exposure to adverse, threatening or stressful situations.

The findings of this study provide insight into the mechanisms by which prenatal adversity interacts with biological susceptibilities to explain early aggression. However, it is unknown to which extent early adversity has already exerted its influence on the ANS earlier in development, in utero and the first six months of life, and thus influenced this early biological susceptibility for aggression. In the present study, cumulative risk was not related to PNS and SNS activity. However, as noted by Boyce (Boyce, 2016), biology x environment interactions are probably 'both the originating source and the functional mechanism' of biological susceptibility to early environments. Future studies should therefore consider both mediating and

moderating processes in the study of early adversity, biology and developmental outcomes.

Although this study has some important strengths, including the longitudinal design, the use of both PNS and SNS measures, and two different stress tasks, there are also limitations that should be discussed. First, the range of cumulative risk was somewhat restricted, with 42% having one or more risk factors, but only 6% having three risk factors, which may limit the generalizability of our results to samples with higher levels of risk. Another limitation is the reliance on maternal reports of physical aggression. Although the PASEC has shown sufficient validity and reliability in earlier studies, future studies should use multiple informants and methods including behavioral observations of early physical aggression.

The first signs of aggression can already be observed in the first year of life (Hay et al., 2011). Although higher rates of aggression are common around age two (Alink et al., 2006), children who show high levels of aggression as toddlers are at risk for severe and persistent aggressiveness over the course of childhood (Côté et al., 2006; NICHD Early Child Care Research Network, 2004). Understanding the biological mechanisms underlying the earliest forms of aggression is of critical importance, particularly because the ANS still undergoes strong development in the first years of life, which may also render it more malleable and a good target for intervention programs (Beauchaine et al., 2008). Our findings underline the importance of studying patterns of stress reactivity across systems, specifically their interplay, in interaction with adversity during the prenatal period. The results of this study show that coactivation may be a biological marker that, already by 6 months of age, increases vulnerability for aggression during toddlerhood. Future studies of how patterns of coactivation develop and whether these can be influenced by intervention are necessary.

CHAPTER 4.2

Interaction between prenatal risk and physiological self-regulation in infancy in predicting physical aggression and oppositional behavior at 30 months

Manuscript invited to revise and resubmit:
Suurland, J., Van der Heijden, K. B., Huijbregts, S. C. J., Van Goozen, S. H. M., & Swaab, H.. Infant parasympathetic and sympathetic activity during baseline, stress and recovery: interactions with prenatal adversity predict aggressive behavior in toddlerhood.

Abstract

Exposure to prenatal adversity is associated with aggression later in life. Individual differences in autonomic nervous system (ANS) functioning, specifically nonreciprocal activation of the parasympathetic (PNS) and sympathetic (SNS) nervous systems, increases susceptibility to aggression, especially in the context of adversity. Previous work examining interactions between early adversity and ANS functioning in infancy is scarce and has not examined coordination between PNS and SNS. This study examined whether the PNS and SNS moderate the relation between cumulative prenatal risk and early aggression in 101 children. Cumulative risk (e.g. maternal psychiatric disorders, substance (ab)use, and social adversity) was assessed during pregnancy. Parasympathetic respiratory sinus arrhythmia (RSA) and sympathetic pre-ejection period (PEP) at baseline, in response to and during recovery from emotional challenge were measured at 6 months. Physical aggression and oppositional behavior problems were measured at 30 months. The results showed that cumulative prenatal risk predicted elevated aggression and oppositional behavior problems in toddlerhood; however, the effects on aggression were moderated by specific profiles of autonomic nervous system functioning. Specifically, the effects of cumulative risk on aggression were particularly evident when the ANS-profile was characterized by low baseline PNS activity and/or by nonreciprocal activity of the PNS and SNS, characterized by decreased activity (i.e. coinhibition) or increased activity (i.e. coactivation) of both systems at baseline and/or in response to emotional challenge. These findings extend our understanding of the interaction between early adversity and infant ANS functioning on developmental outcome.

Keywords: Aggression, stress reactivity, respiratory sinus arrhythmia, pre-ejection period, prenatal risk, infancy

Introduction

The earliest expressions of aggression are already apparent in infancy (Hay, Perra, et al., 2010; Tremblay et al., 2004). Although aggressive behavior is known to peak at age two and three, and then to decline over the preschool period (Alink et al., 2006), there is evidence that relatively high levels of aggressive behavior during early development predict persistent and severe aggressive and antisocial behavior over the course of childhood (NICHD Early Child Care Research Network, 2004), and a range of other problems including low academic achievement and poor social relationships (Campbell, Spieker, Burchinal, Poe, & The NICHD Early Child Care Research Network, 2006). Researchers have linked children's aggression and externalizing problems to the presence of maternal risk factors such as low socioeconomic status, low educational attainment, early entry into parenthood (NICHD Early Child Care Research Network, 2004; Tremblay et al., 2004), smoking during pregnancy (Huijbregts, Seguin, Zoccolillo, Boivin, & Tremblay, 2008), mothers own history of antisocial behavior (Hay, Pawlby, Waters, Perra, & Sharp, 2010), and high levels of stress, anxiety or depression during pregnancy (O'Connor, Heron, Golding, Beveridge, & Glover, 2002). Notably, previous work has shown a dose-dependent relation between the presence of multiple risk factors and child adjustment (Appleyard, Egeland, van Dulmen, & Sroufe, 2005).

It is generally acknowledged that children differ in their physiological susceptibility to these early adversities (Boyce & Ellis, 2005). A growing number of studies in children and adolescents have examined interactions between adversity and measures of autonomic nervous system (ANS) functioning in predicting the development of aggression (El-Sheikh & Erath, 2011). The ANS plays an important role in emotion regulation (Porges, 2007), and abnormal ANS functioning has been linked to aggression and externalizing behavior (Van Goozen, Fairchild, Snoek, & Harold, 2007). During infancy, the ANS is rapidly developing which is associated with increased responsiveness to environmental influences (Porges & Furman, 2011). Yet, we know little about how the ANS interacts with early adversity in infancy. In the present study, we examined whether measures of ANS functioning in infancy moderated the relation between maternal prenatal risk and early aggression.

The autonomic nervous system and aggression

Maturation of the ANS during infancy provides the foundation for emotional and behavioral regulation observed later in development (Porges & Furman, 2011). The ANS is comprised of the sympathetic (SNS) and parasympathetic (PNS) nervous system. The SNS initiates the 'fight/flight' response by increasing heart rate and respiration. In contrast, the PNS has an inhibitory effect on the SNS and its role is to

maintain homeostasis and to regulate recovery following stress by decreasing heart rate and respiration. PNS activity is often assessed by respiratory sinus arrhythmia (RSA), the heart rate variability at the frequency of respiration (Cacioppo, Uchino, & Berntson, 1994), which is thought to index the neural control of the heart via the vagus nerve (Porges, 2007). In response to stress, RSA levels are assumed to decline, indicating withdrawal of the 'brake' on the SNS allowing for flexible responding to stress, active engagement with the environment, and coping with mild to moderate stressors (see Porges & Furman, 2011 for a review). If withdrawal of the PNS is not sufficient to manage a stressor, SNS activity is expected to increase in order to prepare the body for more active stress responses.

The majority of research examining stress reactivity in young children has focused on RSA or global measures of autonomic functioning like heart rate without specific assessments of the SNS. SNS functioning can be measured by the pre-ejection period (PEP), which represents the time between the onset of the heartbeat and ejection of blood into the aorta (Cacioppo et al., 1994). Although assessment of SNS activity by skin conductance level (SCL) is more common, PEP is considered to be a more pure and direct indicator of cardiac SNS activity and can be reliably measured in infants (Alkon, Boyce, Davis, & Eskenazi, 2011; Quigley & Stifter, 2006).

Reduced parasympathetic control, as indicated by low baseline RSA and low RSA reactivity to stress, and attenuated SNS activity (measured by SCL or PEP) at baseline and in response to stress and reward, have been associated with externalizing problems in children and adolescents (Beauchaine, Gatzke-Kopp, & Mead, 2007; El-Sheikh & Erath, 2011; Graziano & Derefinko, 2013). However, these associations may be different in clinical samples as increased RSA reactivity has been reported in children with clinical externalizing problems (Beauchaine et al., 2007). Further, the link between RSA and externalizing behavior is less clear in infants and toddlers, and higher baseline RSA has been linked to more negative reactivity (Fox, Schmidt, & Henderson, 2000). Furthermore, there is some evidence that relations between RSA and externalizing problems do not emerge until after the preschool age (Beauchaine et al., 2007).

Several theoretical frameworks posit that the effects of ANS functioning on developmental outcome occur not directly, but in interaction with environmental factors (Boyce & Ellis, 2005; El-Sheikh & Erath, 2011). Indeed, empirical evidence shows that low baseline RSA and low RSA reactivity exacerbate the relation between environmental risk (e.g. marital conflict, parental drinking problems, domestic violence) and children's externalizing behavior (El-Sheikh, 2001, 2005a; El-Sheikh, Harger, & Whitson, 2001). Studies investigating interactions between adversity and SNS activity indicate that either very low or very high baseline levels of SCL and high

SCL reactivity may increase the risk of aggression and externalizing behavior in the context of adversity (El-Sheikh, 2005b; El-Sheikh, Keller, & Erath, 2007).

It is clear that ANS functioning has important implications for the association between adversity and the development of aggression. However, few studies to date have investigated this issue in infancy and the findings have been inconsistent. Two recent studies suggest that higher (rather than lower) baseline RSA and RSA reactivity predict the development of problem behavior in infants exposed to a more negative caregiving environment (Conradt et al., 2016; Conradt, Measelle, & Ablow, 2013). One other study examined interactions between chronic maternal depression, overcrowded housing and infant RSA and PEP reactivity in predicting externalizing problems at age 7 (Waters, Boyce, Eskenazi, & Alkon, 2016). The results showed that low RSA reactivity in combination with chronic maternal depression was related to more externalizing problems, whereas high PEP reactivity was associated with lower levels of externalizing problems in the context of chronic maternal depression. However, a study in toddlers found no evidence of an interaction between environmental quality and RSA reactivity in the prediction of aggressive behavior (Eisenberg et al., 2012).

Interaction between stress systems

Adaptation to stressful contexts requires a delicate balance in the operation of both the PNS and SNS (Porges, 2007), and the synergistic action of both systems determines the effectiveness of regulation (Berntson, Cacioppo, & Quigley, 1991). Reciprocal autonomic activation, in which the PNS and SNS are oppositely activated, with increased activation of one system and decreased activation of the other, reflects a coordinated response in which both systems either increase or decrease physiological arousal to support responses to environmental demands. However, nonreciprocal activation of the PNS and SNS, with increased or decreased activation of both systems at the same time, is possible (Berntson et al., 1991).

Reciprocal ANS activation, particularly reciprocal SNS activation (i.e. increased SNS activation and decreased PNS activation) in response to stress, is presumed to be normative (Alkon et al., 2011; Salomon, Matthews, & Allen, 2000), and linked better emotion regulation in young children (Stifter, Dollar, & Cipriano, 2011). Conversely, nonreciprocal activation of PNS and SNS may indicate a breakdown in stress regulation, in which either the PNS or SNS fails to perform its adaptive function in response to stress (Porges, 2007). Indeed, El-Sheikh et al. (2009) have shown that children with decreased PNS and SNS activation (i.e. *coinhibition*) or increased PNS and SNS activation (i.e. *coactivation*) exhibited higher levels of externalizing problems in the context of marital conflict, compared to children

showing reciprocal activation of the two systems (i.e. *reciprocal PNS activation* and *reciprocal SNS activation*). Similar findings were reported in the context of maltreatment predicting aggression among girls (Gordis, Feres, Olezeski, Rabkin, & Trickett, 2010).

Until now, there have been no studies that have examined the interaction between the PNS and SNS in infancy as potential moderator of the effects of early adversity on developmental outcome in toddlerhood. Because there may be differences in autonomic influence across development from infancy to childhood (Beauchaine et al., 2007), there is a need to further understand how the coordination between the PNS and SNS in infancy may increase or decrease susceptibility to early adversity.

The present study

In the present study we examined the interactive effects of prenatal adversity and infant ANS regulation as longitudinal predictors of aggression in toddlerhood. The study adds to the existing literature in several ways: 1) We measured both PNS and SNS functioning and their interaction. Previous studies in infants have primarily examined baseline RSA as a moderator of early adversity on developmental outcome. As far as we know, only one previous study examined PNS and SNS reactivity in infants, but this study did not test interactive effects (Waters et al., 2016). 2) We also investigated whether the expected interactions between early adversity and both PNS and SNS functioning were specific for aggressive behavior as opposed to oppositional behavior problems. Aggressive and oppositional behavior problems are both part of the externalizing spectrum representing correlated constructs of behavior problems. However, as there is evidence that aggression and oppositional behavior problems are associated with different developmental processes (Burt, 2012), and alterations in ANS functioning are linked specifically to aggression but not to non-aggressive or oppositional behavior problems (Baker, Shelton, Baibazarova, Hay, & van Goozen, 2013), it is important to consider the possibility of differential physiological susceptibility between these two constructs. 3) We were specifically interested in cumulative prenatal risk since cumulative risk models are considered to be more powerful than single risk models in predicting problem behavior (Appleyard et al., 2005). 4) We measured parasympathetic RSA and sympathetic PEP at baseline, in response to and during recovery from stress. Baseline (or resting) measures of RSA and PEP are thought to reflect neural integrity and readiness to respond to environmental stressors (Beauchaine, 2001). However, reactivity and recovery measures may be stronger predictors of later behavioral outcomes (Fox et al., 2000). Notably, measures indexing autonomic recovery from stress have been underrepresented in the current literature (El-Sheikh & Erath, 2011). 5) We

investigated interactions between RSA and PEP within dimensions (i.e. RSA baseline x PEP baseline etc.) and across dimensions (e.g. RSA baseline x PEP response, and RSA response x PEP recovery) as baseline and reactivity measures of RSA and PEP can combine in different ways to buffer or exacerbate effects of early adversity (El-Sheikh et al., 2009; Gordis et al., 2010). This approach allows us to examine a diverse set of profiles of PNS x SNS interactions that may moderate the effects of adversity on aggression and oppositional behavior later in development.

We hypothesized that the interaction between PNS and SNS functioning would moderate the association between cumulative prenatal risk and aggression, such that nonreciprocal activation of the PNS and SNS (i.e. increased or decreased activation of both systems), would exacerbate the relation between cumulative prenatal risk and aggression, whereas reciprocal activation of the PNS and SNS (i.e. increased activation of one system and decreased activation of the other), would attenuate the relation between cumulative prenatal risk and aggression. Further, we expected that these moderating effects would be specific for aggressive behavior as opposed to oppositional behavior problems. Finally, in the analyses we controlled for the effects of temperament and behavioral distress and demographic and obstetric characteristics.

Methods

Participants

Data were collected as part of the Mother-Infant Neurodevelopment Study (MINDS) – Leiden, which is an ongoing longitudinal study of Dutch mothers and their first-born children focusing on neurobiological and neurocognitive predictors of early behavior problems. We oversampled families based on the presence of one or more risk factors (see criteria under Cumulative risk) to obtain sufficient variance in children's early behavioral problems. Detailed information about the study and sample selection has been reported elsewhere (Smaling et al., 2015; Suurland, Van der Heijden, Huijbregts, Van Goozen, & Swaab, 2016). The study was approved by the ethics committee of the Department of Education and Child Studies at the Faculty of Social and Behavioral Sciences, Leiden University, and by the Medical Research Ethics Committee at Leiden University Medical Centre. Informed consent was obtained from all individual participants included in the study.

The sample for this study consisted of 101 mothers and their infants (57.4% males) who had completed the home-visits at T1 (third trimester of pregnancy) and T2 (six months post-partum) and a laboratory session at T3 (30 months post-partum). The mean age of the children was 6.01 months (*SD*=.41, range 5-7 months) at T2 and 30.05 months (*SD*=1.00, range 28-33 months) at T3. At T1, mothers were on average

23.04 years (*SD*=2.14, range 17-27 years), approximately 95% had a partner (87.1 % was married or living with a partner), and 33.7% had a high educational level (Bachelor's or Master's degree). Families were predominantly Caucasian (89.1%). Of the 136 mothers originally enrolled in the study at T1, 10 did not participate at T2, and another 23 dropped out between T2 and T3. The main reasons for families dropping out were inability to be contacted, moving away or too busy. Sample attrition was unrelated to demographic variables or any dependent measures (*ps*>.05). However, mothers who dropped out were more often single ($\chi^2(1) = 8.41$, $p<.05$).

Procedures

During the prenatal home-visit (between 26 and 40 weeks gestation, *M* = 29.78, *SD* = 3.63), mothers were screened for the presence of risk factors based on an interview and multiple questionnaires (Smaling et al., 2015). The protocol during the six-month home-visit, included attachment of cardiac monitoring equipment to the infant's chest and back. Baseline ANS functioning while at rest was measured during a two-minute relaxing movie while the infant was lying on a blanket, followed by two procedures designed to elicit physiological responses to social stress (Still Face Paradigm) and frustration (Car seat). The social stress and frustration tasks were administered with a break in between to limit carry over effects. Infants were only assessed in the next procedure when they were calm and displayed no distress. The home-visits were scheduled at a time of the day when mothers deemed their infant to be most alert.

The Still Face Paradigm (SFP; Mesman, Van IJzendoorn, & Bakermans-Kranenburg, 2009) is a well-established social stress paradigm comprising a sequence of three 2-minute episodes during which the mother is asked to interact normally with the infant (SFP baseline), then withhold interaction (SFP social stress), and then resume interaction (SFP recovery) (for a more detailed description of the SFP, see Suurland et al., 2016). The Car Seat (CS) task, adapted from the Laboratory Temperament Assessment Battery Pre-locomotor version (Lab-TAB; Goldsmith & Rothbart, 1999a), was used to measure infant ANS and behavioral response to a frustrating event. Following a 2-minute baseline (CS baseline), the mothers placed their infants in a car seat with straps firmly attached and stood 1 meter away from their child. After 1 minute of restraint (CS frustration), a 2-minute recovery period (CS recovery) followed in which mothers were allowed to hold their child and interact as they normally would. Mothers were instructed to remain neutral and refrain from comforting or speaking to the child during the CS frustration episode.

During the 30-month laboratory visit, several tasks were performed and mothers completed multiple questionnaires. For the purpose of the current study,

only maternal reports of aggression and oppositional behavior problems were examined.

Measures

Cumulative risk (T1). Cumulative prenatal risk consisted of 10 criteria that were scored as present (1) or absent (0); current psychiatric disorder(s) with the Dutch version of the Mini- International Neuropsychiatric Interview (MINI-plus; Van Vliet, Leroy, & Van Megen, 2000), substance use (alcohol, tobacco and/or drugs) during pregnancy, no secondary education, unemployment, self-reported financial problems, limited or instable social support network, single status, and maternal age <20 years (see for a more elaborate description of these criteria Smaling et al., 2015). The cumulative risk score was computed as the sum of risk factors present (maximum number of risk factors was 10), with *M*=.67, *SD*=.93 (range 0-3). There were 61 mothers with no risk factors, 21 with one risk factor, 12 with two risk factors, and 6 with three risk factors. The prevalence of the different risk factors among mothers with one or more risk factors (39.6%) was: 60.0% current psychiatric diagnosis, 2.5% alcohol, 37.5% smoking, 12.5% single status, 10.0% unemployed, 2.5% no secondary education, 10.0 % financial problems, 10.0% limited social support, 17.5% age <20 years.

ANS parameters (T2). Parasympathetic RSA and sympathetic PEP were monitored with the Vrije Universiteit Ambulatory Monitoring System (VU-AMS 5fs; De Geus, Willemsen, Klaver, & Van Doornen, 1995; Willemsen, De Geus, Klaver, Van Doornen, & Carroll, 1996). The VU-AMS device continuously recorded electrocardiogram (ECG), and impedance cardiogram (ICG) measures; basal thorax impedance (Z_0), changes in impedance (dZ), and the first derivative of pulsatile changes in transthoracic impedance (dZ/dt). The ECG and dZ/dt signal were sampled at 1000 Hz, and the Z_0 signal was sampled at 10Hz. The VUDAMS software suite version 2.0 was used to extract mean values RSA and PEP across baseline (2 minutes), SFP baseline (2 minutes), SFP social stress (2 minutes), and SFP recovery (2 minutes), and CS baseline (2 minutes), CS frustration (1 minute), and CS recovery (2 minutes).

R-peaks in the ECG, scored by the software, were visually checked and adjusted when necessary. RSA was derived by the peak-trough method (De Geus et al., 1995; Grossman, Van Beek, & Wientjes, 1990), which combined the respiration (obtained from filtered [0.1 – 0.4 Hz] thoracic impedance signal) and inter beat interval (IBI) time series to calculate the shortest IBI during heart rate acceleration in the inspiration phase and the longest IBI during deceleration in the expiration phase (De Geus et al., 1995). RSA was defined as the difference between the longest IBI's

during expiration and shortest IBI's during inspiration. Automatic scoring of RSA was checked by visual inspection of the respiratory signal from the entire recording. Because RSA was skewed at baseline, the emotional challenge tasks, and recovery, its natural logarithm (lnRSA) was used in the analyses.

PEP is the time interval between the onset of the ventricular depolarization (Q-wave onset) and the onset of left ventricular ejection of blood into the aorta (B-point on the Dz/dt complex; De Geus et al., 1995). Average dZ/dt waveforms were derived by the software. PEP was automatically scored from the Q-wave onset on the ECG and the B-point on the dZ/dt waveform. Each automated scoring was checked and corrected manually when necessary (Riese et al., 2003). Wave forms which were morphologically distorted and could not be visually corrected , were discarded. The procedure of interactive visual scoring was done independently by two trained raters; inter-rater reliability (intraclass correlation ICC) was .949.

LnRSA and PEP response and recovery scores on the SFP and CS were computed as standardized residualized change scores which represent the standardized residuals from the linear regressions of response and recovery scores on the preceding score to provide a simple change score adjusted for their initial value (El-Sheikh et al., 2009). The standardized residualized change scores for lnRSA and PEP during response and recovery on the SFP were significantly correlated with the standardized residualized change scores for lnRSA and PEP during response and recovery on the CS (rs=.24 to .28, with ps=.021 to .009). Therefore, the residualized change scores were averaged to create four indices: lnRSA response and PEP response (average SFP and CS) and lnRSA recovery and PEP recovery (average SFP and CS). Negative values reflect lnRSA and PEP decreases (i.e. greater PNS suppression and greater SNS activation respectively), while positive values reflect lnRSA and PEP increases (i.e. greater PNS activation and greater SNS suppression respectively).

Behavioral distress (T2). Infant behavioral distress (i.e. intensity of whining, fussing or crying) was coded by four trained raters from videotaped recordings according to scales of the Mother Infant Coding System (Miller, McDonough, Rosenblum, & Sameroff, 2002) for the SFP, and the Lab-TAB (Goldsmith & Rothbart, 1999a) for the CS. The scores for distress on both stressors correlated significantly (r= .240, p<.05) and a composite score was created based on the standardized average of both scales. A subset of recordings (15% of the sample) was double-coded to assess inter-rater reliability. Intraclass correlation (ICC) was .999 on the SFP social stress episode and .950 on the CS frustration episode.

Temperament – distress to limitations (T2). The short form of the Revised Infant Behavior Questionnaire (IBQ-R; Gartstein & Rothbart, 2003) assesses 14 domains of temperament and was completed by the mother. We used the 'Distress

to limitations' subscale (7 items) as a measure of fussing, crying or showing distress. The items were scored on a 7-point scale from never (1) to always (7). Internal consistency (Cronbach's alpha) in the present sample was .74.

Aggression (T3). Mothers reported on their child's physical aggression using the 11-item Physical Aggression Scale for Early Childhood (PASEC;(Alink et al., 2006). The PASEC items were originally derived from Tremblay et al. (1999) and the physical aggression items of the Child Behavior Checklist (CBCL) 1 ½ -5 yr (Achenbach & Rescorla, 2000). Mothers scored whether their child has shown certain physically aggressive behaviors (e.g. 'hits', 'kicks', 'destroying things') during the past two months on a 3-point Likert scale (0 = 'not true to 2 = 'very true or often true'). A total score for physical aggression was calculated by summing item scores (range 0-22). The PASEC showed sufficient reliability in a sample of 2,253 children recruited at 12, 24 and 36 months (Alink et al., 2006). The reported mean scores for the 24-month cohort were 3.20 (*SD*=3.06), and 2.99 (*SD*= 3.07) for the 36-month cohort. Internal consistency (Cronbach's alpha) in the present sample was .73.

Oppositional behavior problems (T3). The CBCL 1 ½-5 yr (Achenbach & Rescorla, 2000) was used to assess oppositional behavior problems. Mothers indicated whether their child displayed any of the 100 behavioral descriptions in the last two months on a 3- point Likert scale (0 = 'not true' to 2 = 'very true or often true'), with higher scores indicating higher levels of problem behavior. We used the DSM-oriented Oppositional Defiant disorder subscale, consisting of six items (range 0-12) measuring oppositional and hard-to-manage behavior (e.g. 'stubborn', 'temper tantrums', 'uncooperative'). The reliability and validity of the CBCL have been confirmed in several studies (e.g. Koot, Van den Oord, Verhulst, & Boomsma, 1997). Internal consistency (Cronbach's alpha) for the Oppositional Defiant problems subscale in this sample was .77.

Missing data

Approximately 9% of ANS data were missing across baseline and/or the SFP and CS episodes. Missing data was due to dyads that did not complete the SFP or CS because the infant became too fussy (3.8%), loose electrodes (5.7%), equipment failure (1.9%), or excessive child movement in which case PEP and/or RSA could not be scored (88.6%). Missing data was not systematically related to demographic and obstetric characteristics (ethnicity, sex, gestational age; *p*s>.250) or cumulative risk, aggression and oppositional behavior problems (*p*s>.250). However, infants with more missing ANS data had a higher birth weight (r=.20, p<.05). Main analyses were conducted based on the number of infants for which there was data (see Table 1 for available ANS data across baseline and SFP and CS episodes).

Data analysis

All variables were examined for outliers and violations of specific assumptions applying to the statistical tests used. Variables with values that exceeded >3*SD* from the group mean were recoded to the next extreme value within 3*SD* from the mean (0.7% of the ANS data across all SFP and CS episodes). Preliminary analyses (Pearson correlations) tested for potential covariates (demographic and obstetric characteristics, behavioral distress and temperamental anger). Hierarchical regression analyses were conducted to examine the interactive effects among cumulative risk, lnRSA (baseline, response or recovery) and PEP (baseline, response or recovery) on aggression and oppositional behavior problems. In separate regression analyses the following interaction effects between lnRSA and PEP were examined: 1) lnRSA baseline x PEP baseline, 2) lnRSA response x PEP baseline, 3) lnRSA baseline x PEP response, 4) lnRSA response x PEP response, 5) lnRSA recovery x PEP response, 6) lnRSA response x PEP recovery, and 7) lnRSA recovery x PEP recovery. All variables were centered to their mean prior to analyses (Aiken & West, 1991). Step 1 included cumulative risk, Step 2 included lnRSA and PEP, Step 3 included all two-way interactions between cumulative risk, lnRSA and PEP, and Step 4 included the three-way interaction between cumulative risk, lnRSA, and PEP. We reported and interpreted the main and interaction effects of cumulative risk and ANS variables from the full interaction model. Significant interaction effects were examined following procedures recommended by Aiken and West (Aiken & West, 1991) by plotting regression lines at 0 risk factors and 1.6 risk factors (i.e. mean number of risk factors for the group of infants with ≥1 risk factors) and 1 SD above and below the mean for the moderators (lnRSA baseline/lnRSA response/lnRSA recovery, and PEP baseline/ PEP response/PEP recovery). We also tested whether the main and interactive effects were moderated by sex. Because this was not the case, we did not report these findings. All analyses were conducted using the Statistical Package for Social Sciences (SPSS for Windows, version 21.0, SPSS Inc., Chicago).

Results

Descriptive analyses

Descriptive statistics for lnRSA and PEP baseline, response and recovery variables are presented in Table 1. LnRSA and PEP response and recovery levels on the SFP and CS were significantly different from zero: $t(94)=3.47$, $p<.01$ for lnRSA SFP response, $t(87)=3.62$, $p<.001$ for lnRSA CS recovery, $t(84)=2.71$, $p<.01$ for PEP SFP response, and $t(76)=2.77$, $p<.01$ for PEP CS response), except for lnRSA CS response ($t(87)=.12$, $p=.907$), lnRSA SFP recovery ($t(94)=-.91$, $p=.364$), PEP SFP recovery $t(79)=-.21$, $p=.835$, and $t(71)=-1.85$, $p=.068$ for PEP CS recovery.

Averaged across the SFP and CS challenge episodes, 59.8% of the sample showed a decrease in lnRSA (i.e. PNS suppression) and 61.1% exhibited a decrease in PEP (i.e. SNS activation) from baseline. Averaged across the SFP and CS recovery episodes, 40.2% of the sample showed an increase in lnRSA (i.e. PNS activation) and 52.2% showed an increase in PEP (i.e. SNS suppression) from the challenge episode. Thus, there was sufficient variability in infant lnRSA and PEP response to and recovery from challenge.

Table 1. *Descriptives for lnRSA and PEP baseline, stress response and recovery variables.*

	N	*M*	*SD*	Min.	Max.
LnRSA					
Baseline	96	3.40	.43	2.47	4.50
SFP Baseline	96	3.37	.36	2.39	4.33
SFP Social stress	95	3.24	.41	2.43	4.18
SFP Recovery	95	3.27	.47	1.97	4.57
CS Baseline	93	3.26	.37	2.28	4.16
CS Frustration	90	3.25	.52	1.92	4.49
CS Recovery	91	3.14	.38	2.22	4.02
PEP					
Baseline	91	64.31	6.09	45.67	76.00
SFP Baseline	87	62.73	6.21	46.06	76.89
SFP Social stress	91	61.52	7.01	43.02	76.89
SFP Recovery	81	61.49	7.47	40.99	79.01
CS Baseline	90	63.31	6.28	45.06	76.89
CS Frustration	80	61.91	6.42	45.00	76.00
CS Recovery	81	63.70	6.54	46.00	83.00

Note: lnRSA = natural logarithm of respiratory sinus arrhythmia, PEP = pre-ejection period, SFP = Still Face Paradigm, CS = Car seat.

Preliminary analyses

Means, SDs, and correlations for the potential covariates and main study variables are presented in Table 2. For interpretation purposes, lnRSA and PEP raw change scores are used for means and SDs in Table 2; however, as noted, residualized change scores are used in the correlation and regression analyses. Cumulative risk was significantly associated with aggression (r=.40, p<.001), and oppositional behavior problems (r=.30, p<.01). Cumulative risk, aggression and oppositional behavior problems were not significantly related to baseline, response and recovery measures of lnRSA and PEP, although there was a marginally significant correlation between cumulative risk and PEP baseline (r=-.20, p=.054).

Behavioral distress was significantly related to lnRSA response and recovery (respectively r=-.29, p<.01, and r=.22, p<.05). Further, there were marginally significant correlations between ethnicity and lnRSA response (r=.19, p=.054), birth weight and PEP recovery (r=.20, p=.062), gestational age and PEP recovery (r=.17, p=.078), and temperamental anger and lnRSA response (r=-.18, p=.079). In preliminary analyses, we tested whether inclusion of these covariates changed the results from main regression analyses. Because this was not the case, we reported the analyses without the covariates.

Regression analyses

Aggression. In the hierarchical regression analyses predicting aggression (see Table 3), significant main effects, controlling for the effects of the other predictors included in step 1-4, were present for cumulative risk (prs = .38-.44, ps<.001). Higher cumulative risk predicted higher levels of aggression. There were no significant main effects for lnRSA or PEP baseline, response or recovery. A significant two-way interaction effect was revealed between cumulative risk x lnRSA baseline (pr = -.32, p<.01) (see Table 3). Examination of simple slopes (see Figure 1) revealed that for infants with lower baseline lnRSA (-1 SD), higher cumulative risk predicted higher levels of aggression (β = .66, p<.001). Cumulative risk was not associated with aggression for infants with higher baseline lnRSA (+1 SD; β = .19, p=.152). None of the other two-way interaction effects between cumulative risk, lnRSA and PEP on aggression were significant.

Significant three-way interactions were found between cumulative risk x lnRSA response x PEP baseline (pr = -.26, p<.05) and cumulative risk x lnRSA response x PEP response (pr = -.32, p<.01) (see Table 3). Further examination of the three-way interaction between cumulative risk x lnRSA response x PEP baseline (see Figure 2) revealed that higher cumulative risk predicted higher levels of aggression for infants exhibiting greater PNS suppression in response to stress (-1 SD; i.e. a decrease in lnRSA) combined with lower baseline SNS activity (+1 SD; high baseline PEP) (β =1.08, p<.01), and for infants exhibiting greater PNS activation in response to stress (+1 SD; i.e. increase in lnRSA) combined with higher baseline SNS activity (-1 SD; high baseline PEP) (β = .69, p<.01). Conversely, for infants exhibiting greater PNS activation in response to stress (+1 SD) combined with lower baseline SNS activity (+1 SD) and greater PNS suppression in response to stress (-1 SD) in combination with higher baseline SNS activity (-1 SD), cumulative risk was not significantly related to aggression (respectively β = .33, p=.055, and β = .26, p=.143). Examination of the three-way interaction between cumulative risk x lnRSA response x PEP response (see Figure 3) revealed that for infants exhibiting greater coinhibition (i.e. lnRSA response

at -1 SD and PEP response at +1 SD) and coactivation (i.e. lnRSA response at +1 SD and PEP response at -1 SD) in response to challenge, higher cumulative risk predicted higher levels of aggression (respectively β = 1.09, p<.01, and β = .62, p<.01). Conversely, for infants exhibiting greater reciprocal PNS activation and SNS activation in response to challenge, cumulative risk was unrelated to aggression (respectively β = .10, p=.692, and β = .07, p=.722).

Oppositional behavior problems. Results of the hierarchical regression analyses predicting oppositional behavior problems are shown in Table 3. The main effects for cumulative risk were in the same direction as in the hierarchical regression analyses predicting aggression, however the regression models were not significant after inclusion of the other predictors in steps 2-4.

Table 2. *Means, standard deviations and correlations among study variables* .

Variable	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.
1. Cumulative risk	-														
2. Ethnicity[a]	.10	-													
3. Infant sex[b]	.05	.02	-												
4. Gestational age	-.03	-.07	-.03	-											
5. Birth weight (kg)	-.16	-.06	-.19†	.58***	-										
6. Behavioral distress	-.09	-.01	-.03	.09	.03	-									
7. Distress (IBQ-R) [c]	.14	.12	-.02	-.12	-.04	.18†	-								
8. lnRSA baseline	-.01	-.05	-.02	-.13	-.14	.09	-.07	-							
9. lnRSA response	.06	.19†	-.04	-.04	.04	-.29**	-.18†	.31***	-						
10. lnRSA recovery	-.14	.02	.05	-.14	-.13	.22*	-.06	.42***	-.15	-					
11. PEP baseline	-.20†	.00	.03	.10	.17	-.01	-.13	.09	.22*	-.06	-				
12. PEP response	-.06	-.01	.14	-.08	-.10	-.04	-.00	-.19†	-.05	.00	-.00	-			
13. PEP recovery	-.07	.17	.02	-.19†	-.20†	.03	-.17	.14	.22*	-.10	.44***	-.30**	-		
14. Aggression	.40***	.02	-.11	.07	-.11	-.04	.07	-.06	-.02	-.05	-.10	.05	-.04	-	
15. Oppositional behavior problems	.30**	.04	-.08	-.05	-.08	.04	.08	.06	.08	.14	-.13	-.06	-.05	.41***	-
N	101	101	101	101	101	101	99	96	91	97	91	90	90	101	101
M	.64	89.1%	57.4%	39.3	3.4	.06	2.78	3.40	.07	.03	64.31	1.29	-.58	2.46	4.86
SD	.92			1.80	.52	.81	.88	.43	.36	.26	6.09	3.19	3.66	2.09	2.07

Note: lnRSA = natural logarithm of respiratory sinus arrhythmia, PEP = pre-ejection period. [a] % male, [b] % Caucasian, [c] 'Distress to limitations' subscale of the Revised Infant Behavior Questionnaire (IBQ-R) - short form. †<.10, *p<.05, **p<.01, ***p<.001.

Table 3. *Interactions between lnRSA and PEP baseline and response moderate the association between cumulative risk and aggression.*

		Aggression					Oppositional behavior problems				
Step	Predictor	*Adjusted* R^2	ΔR^2	ΔF	β	*t*	*Adjusted* R^2	ΔR^2	ΔF	β	*t*
LnRSA baseline x PEP baseline											
1	Cumulative risk	.149	.159	16.41***	.44	4.14***	.071	.081	7.72**	.26	2.28*
2	lnRSA	.131	.002	.11	-.06	-.61	.060	.011	.51	.15	1.31
	PEP				-.01	-.01				-.11	-.96
3	lnRSA x PEP	.188	.083	2.98*	-.10	-.92	.074	.045	1.43	-.24	-2.14*
	Cumulative risk x lnRSA				-.32	-3.04**				-.10	-.91
	Cumulative risk x PEP				.21	1.95†				.00	.01
4	Cumulative risk x lnRSA x PEP	.210	.029	3.25†	.19	1.80†	.083	.019	1.80	.15	-1.34
LnRSA baseline x PEP response											
1	Cumulative risk	.137	.147	14.82***	.36	3.07**	.080	.090	8.52**	.43	3.50**
2	lnRSA	.122	.005	.27	-.13	-1.16	.063	.005	.25	.14	1.21
	PEP				.03	.23				.04	.39
3	lnRSA x PEP	.142	.049	1.65	-.08	-.72	.050	.020	.60	.17	1.45
	Cumulative risk x lnRSA				-.29	-2.28*				.10	.76
	Cumulative risk x PEP				.03	.26				.13	1.06
4	Cumulative risk x lnRSA x PEP	.146	.014	1.41	-.16	-1.19	.067	.027	2.52	.22	1.59

Table 3 cont.

LnRSA response x PEP baseline										
1	Cumulative risk	.149	.159	16.41***	.60	4.24***	.071	.081	7.72**	.39	2.61*
2	lnRSA	.130	.001	.03	-.11	-.97	.065	.015	.73	.04	.34
	PEP				-.05	-.42				-.12	-1.06
3	lnRSA x PEP	.106	.008	.26	-.09	-.87	.061	.028	.89	-.21	-1.86†
	Cumulative risk x lnRSA				-.07	-.50				-.12	-.93
	Cumulative risk x PEP				.13	1.02				.03	.23
4	Cumulative risk x lnRSA x PEP	.154	.054	5.64*	-.29	-2.38*	.064	.013	1.27	-.15	-1.13
LnRSA response x PEP response											
1	Cumulative risk	.137	.147	14,82***	.48	433***	.080	.090	8.52**	.34	2.89**
2	lnRSA	.121	.005	.23	-.12	-1.10	.067	.009	.42	.05	.42
	PEP				.12	1.21				.02	.20
3	lnRSA x PEP	.105	.016	.50	-.08	-.72	.045	.012	.36	.01	.07
	Cumulative risk x lnRSA				-.10	-.87				-.01	-.64
	Cumulative risk x PEP				.11	1.05				.10	.87
4	Cumulative risk x lnRSA x PEP	.185	.084	8.94**	-.34	-2.99**	.040	.006	.53	-.09	-.73

Note: *p<.05, **p<.01, ***p<.001.

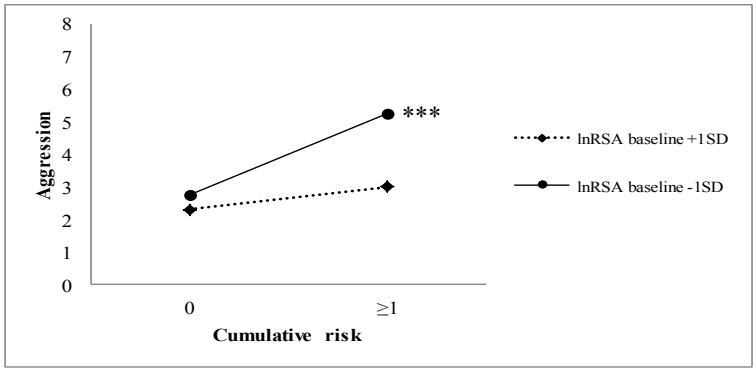

Figure 1. Two-way interaction between lnRSA baseline and cumulative risk, predicting aggression. Cumulative risk is plotted at 0 risk factors and 1.6 risk factors (this is the average number of risk factors present in infants with one or more risk factors), ****p*<.001.

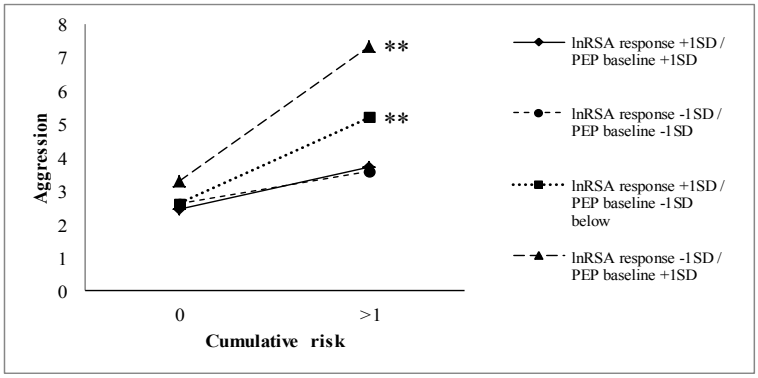

Figure 2. Three-way interaction between lnRSA response and PEP baseline, and cumulative risk, predicting aggression, ***p*<.01.

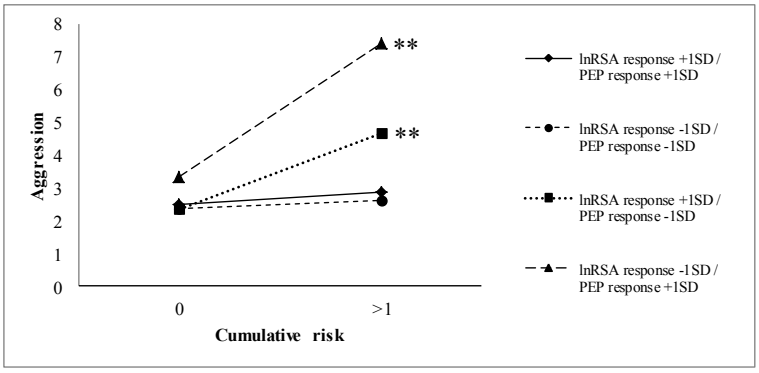

Figure 3. Three-way interaction between lnRSA and PEP response, and cumulative risk, predicting aggression, ***p*<.01.

Discussion

The present study examined interactions between infant PNS and SNS functioning and prenatal adversity in predicting developmental outcome in toddlerhood. Our results align with theoretical models indicating that the complex associations between physiological functioning and behavior may be better understood as interactions with (early) adversity (Boyce & Ellis, 2005; El-Sheikh & Erath, 2011). In the context of higher cumulative prenatal risk infant ANS functioning and particularly the (nonreciprocal) interaction of PNS and SNS increased risk for later aggression, but not for oppositional behavior problems,. Specifically, moderating effects were found for 1) low baseline PNS activity, 2) low baseline SNS activity and PNS suppression in response to stress, 3) high baseline SNS activity and PNS activation in response to stress, 4) PNS and SNS suppression in response to stress, and 5) PNS and SNS activation in response to stress. We found no interaction effects between ANS recovery measures and cumulative risk.

We found a significant two-way interaction between cumulative risk and baseline PNS activity predicting aggression. Consistent with previous work in school-aged children exposed to marital conflict and parental drinking problems (El-Sheikh, 2001, 2005a), the infants in this study who exhibited lower baseline PNS activity and were exposed to higher cumulative prenatal risk showed higher levels of aggression. Our findings suggest that high baseline PNS activity may buffer against the effect of (early) adversity. However, others have argued that high baseline PNS activity may increase susceptibility to environmental influence, resulting in higher levels of problem behavior in the context of unsupportive environments (Conradt et al., 2013), and even lower (aggressive) problem behavior in more supportive environments (Conradt et al., 2013; Eisenberg et al., 2012). Although this seems inconsistent, it may be an effect of the type of risk factors with which the ANS interacts. The aforementioned studies (Conradt et al., 2013; Eisenberg et al., 2012) focused on the quality of the environment or caregiving context as adversity factor, whereas in the present study (and other previous studies in school-aged children; e.g. (El-Sheikh, 2005a; El-Sheikh et al., 2001) most infants were exposed to maternal psychiatric problems and substance (ab)use. Although we had a clear rationale for examining risk as a cumulative variable, different types of risk factors may impact or interact with the ANS in different ways. For example, Waters et al. (2016) found an interaction between ANS functioning and maternal chronic depression on externalizing behavior problems but not with overcrowded housing. Future studies should explore how different maternal and environmental risk factors interact with ANS functioning.

Our results extend prior research in school-aged children (El-Sheikh et al., 2009; Gordis et al., 2010) by demonstrating that coinhibition (i.e. PNS suppression

accompanied with low baseline SNS activity or SNS suppression) and coactivation (i.e. PNS activation accompanied with high baseline SNS activity or SNS activation) at six months of life, predict aggression at 30 months, but only among infants exposed to elevated levels of prenatal adversity. Notably, our results indicate that coinhibition in context of adversity confers higher risk for aggression than coactivation in context of adversity. The group mean for infants exhibiting coinhibition was more than one standard deviation above the average aggression level reported in a large community sample of 24- and 36-month old children (Alink et al., 2006), whereas the group mean of infants exhibiting coactivation lay within one standard deviation of the mean reported by Alink et al. (2006).

The interaction effects of coinhibition and coactivation with prenatal adversity suggests that infants with a less adaptive ANS profile at six months of age, may be more sensitive to negative effects of maternal depression and anxiety and substance (ab)use, and maternal psychological and caregiving distress due to limited social support, single parenthood, unemployment and financial problems. Nonreciprocal activation of the PNS and SNS may yield an ambivalent physiological response in which one branch of the ANS increases arousal whereas the other branch dampens arousal (Berntson et al., 1991). Coinhibition of the PNS and SNS in the present study was evident by PNS suppression in response to stress accompanied by low baseline SNS activity or SNS suppression in response to stress. According to the Polyvagal theory (Porges, 2007; Porges & Furman, 2011), PNS suppression equips the infant for action by withdrawing its inhibitory influence on the SNS. However, without joint activation of the SNS, there may be insufficient metabolic output to mobilize an effective behavioral self-regulatory response (El-Sheikh & Erath, 2011). Conversely, in case of coactivation, where PNS activation in response to stress was accompanied by high baseline SNS activity or SNS activation in response to stress, the PNS fails to withdraw its brake on the SNS and instead stimulates the body into a calm state, reflecting poor regulation of high emotional and physiological arousal (El-Sheikh & Erath, 2011). Over time, these patterns of coinhibition and coactivation may promote aggressive behavior, especially in environments that tend to elicit these behaviors more often.

It should be noted that the precise pattern of interactions between prenatal adversity and PNS and SNS measures of baseline and response differed from previous studies. Whereas El-Sheikh et al. (2009) and Gordis et al. (2010) reported significant interactions between baseline and response values of the PNS and response values of the SNS, our findings revealed the opposite, namely, significant interactions between PNS response and baseline and response values of the SNS. Noteworthy is that the interaction between PNS response and SNS baseline could not be tested in the study

of Gordis et al. (2010) due to multicollinearity problems, so we do not know whether they might have found the same interaction effect as we did. However, given the scarcity of studies looking into PNS and SNS interactions, and the fact that the children in this study were much younger, this suggests that the pattern of interactions between baseline and response measures of the PNS and SNS needs further research.

Another point worth mentioning is that, although it was beyond our scope, and not possible due to statistical power limitations, it is important to also examine interactions among baseline and response levels within one system (e.g. PNS baseline x PNS response). In fact, previous studies in older samples have demonstrated that low baseline PNS activity in combination with PNS activation in response to stress predicted the highest level of delinquency (Hinnant, Elmore-Staton, & El-Sheikh, 2011). Including both between-system and within-system interactions in one model would potentially better reflect the complexity of the ANS in interaction with adversity in predicting developmental outcome.

Contrary to our expectations, ANS recovery measures did not moderate the impact of prenatal adversity on aggression. Although few studies to date have addressed ANS recovery from stress, there is some evidence that blunted PEP recovery increases the positive association between adversity between ages 0-15 years and antisocial behavior in boys at age 16 (Sijtsema et al., 2015). Further, a study in 4-7 year old children showed that impaired vagal recovery predicted poor emotion regulation to frustration (Santucci et al., 2008), underlying the importance of studying ANS recovery measures in future research..

The present study has a number of strengths including the longitudinal design, the use of a heterogeneous sample consisting of low and high(er) risk families, the measurement of both PNS and SNS activity and their interaction early in life, the examination of resting, reactivity and recovery measures, and the focus on both aggressive behavior and oppositional behavior problems. However, our findings should be interpreted in light of several limitations. First, we relied on maternal reports of physical aggression and oppositional behavior problems. Future studies should use multiple informants and include behavioral observations of early behavioral problems. Second, the physiological measures were only assessed at six months of age. Although previous studies (e.g. Alkon et al., 2011) have reported moderate stability of PEP and RSA during resting and challenging conditions from 6 to 60 months, lower stability was reported for reactivity measures and ANS reactivity profiles. This indicates that during the first few years of life, autonomic responses to stress are not yet fully developed, and therefore may be influenced by repeated exposure to environmental stressors. Future longitudinal investigations should examine the stability of coinhibition and coactivation across development and their

association with early adversity and later aggression. Third, we do not know to what extent the physiological susceptibility to early adversity in our study was already influenced by continuous exposure to higher levels of adversity during the prenatal and early postnatal period. Finally, it should be noted that it is unsure whether our findings generalize to higher risk samples, given that the level of cumulative risk in our sample was relatively low with only 39.6% having more than one risk factor and 18.8% with two or more risk factors.

In sum, our findings indicate that low baseline PNS activity and nonreciprocal activation of the PNS and SNS in infancy, with increased or decreased activity within both branches of the ANS at the same time, increase vulnerability for early aggression in the context of higher cumulative prenatal risk. Further, these effects were found to be specific for aggression, as opposed to a broader spectrum of difficult behavior (see also Baker et al., 2013; Burt, 2012), possibly indicating a stronger biological basis for aggressive behavior, whereas oppositional behavior problems may be more environmentally determined. Notably, the interactions between the ANS and early adversity predicted aggression over and above the effects of observed behavioral distress and mother-reported temperament at six months. The results of this study add to our understanding of how physiological systems measured early in development increase susceptibility to early adversity and highlight the need to incorporate indices of both PNS and SNS functioning in order to elucidate its role in developmental processes leading to early aggression. The ANS is rapidly developing in the first year after birth (Porges & Furman, 2011), thereby marking an important period of increased susceptibility to environmental influences, which, in turn, creates opportunities for interventions to prevent the development of aggressive behavior.

CHAPTER 5

Effects of prenatal risk on physiological self-regulation in infancy

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Abstract

This study examined whether risk status and cumulative risk were associated with autonomic nervous system reactivity and recovery, and emotion regulation in infants. The sample included 121 six-month-old infants. Classification of risk status was based on World Health Organization-criteria (e.g. presence of maternal psychopathology, substance use, and social adversity). Heart rate, parasympathetic respiratory sinus arrhythmia (RSA), and sympathetic pre-ejection period (PEP) were examined at baseline and across the Still Face Paradigm (SFP). Infant emotion regulation was coded during the SFP. Infants in the high-risk group showed increased heart rate, parasympathetic withdrawal and sympathetic activation during recovery from the Still Face episode. Higher levels of cumulative risk were associated with increased SNS activation. Moreover, increased heart rate during recovery in the high-risk group was mediated by both parasympathetic and sympathetic activity, indicating mobilization of sympathetic resources when confronted with socio-emotional challenge. Distinct indirect pathways were observed from maternal risk to infant emotion regulation during the SFP through parasympathetic and sympathetic regulation. These findings underline the importance of specific measures of parasympathetic and sympathetic response and recovery, and indicate that maternal risk is associated with maladaptive regulation of stress early in life reflecting increased risk for later psychopathology.

Keywords: Autonomic nervous system, respiratory sinus arrhythmia, pre-ejection period, infants, risk

Introduction

Developmental trajectories resulting in emotional and behavioral problems are established early in life and are predicted by numerous prenatal, perinatal, and postnatal risk factors that reflect environmental adversity (e.g. Campbell, Shaw, & Gilliom, 2000; Cicchetti & Rogosch, 1996; Huijbregts, Seguin, Zoccolillo, Boivin, & Tremblay, 2008). Disruptions in functioning of the autonomic nervous system (ANS), consisting of the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS), are proposed to be one mechanism through which exposure to early adversity affects emotional and behavioral outcomes (McLaughlin et al., 2015). The prenatal period and first two years after birth constitute a sensitive period during which exposure to early adversity is particularly likely to alter the development of the ANS (McLaughlin et al., 2015; Porges & Furman, 2011). Although there is an increasing number of studies providing evidence for effects of early adversity on infant ANS functioning through measures of heart rate (HR) and PNS activity (Propper & Holochwost, 2013), very few studies focused on SNS functioning. Moreover, studies that have examined the effects of early adversity on simultaneous measurements of PNS and SNS functioning in infants are lacking. The present study presents a comprehensive assessment of both PNS and SNS functioning in infants exposed to early adversity and their counterparts from low-risk backgrounds. The resultant findings may provide insight into the mechanisms by which early adversity affects developmental outcomes through altering physiology and eventually may lead to identification of children at risk for psychopathology at an early age.

Stress regulation through the ANS

The ANS consists of the PNS and the SNS, which are generally thought to act in complementary ways to respond and adapt to environmental challenges. While the PNS is active during rest and functions to maintain homeostasis, the SNS is activated during periods of perceived threat ('fight or flight' response) by increasing HR and mobilizing metabolic resources. According to Porges' polyvagal theory (Beauchaine, 2001; Porges, 2007), disengagement of the PNS during mildly challenging situations marks an evolutionary advance in the control of arousal, which allows individuals to attend to environmental demands without activating the more costly SNS. PNS activity is commonly measured by respiratory sinus arrhythmia (RSA), a component of heart rate variability influenced by the vagal system and related to rhythmic increase and decrease of heart rate that coincides with respiration (Beauchaine, 2001). Research in infants, toddlers and preschoolers has demonstrated that high levels of baseline RSA at rest and/or the ability to suppress PNS activity in challenging situations (RSA withdrawal) are related to better state regulation, greater

self-soothing, more attentional control, and greater capacity for social engagement (Blair & Peters, 2003; Calkins, Dedmon, Gill, Lomax, & Johnson, 2002; Calkins & Keane, 2004; Degangi, Dipietro, Greenspan, & Porges, 1991). In contrast, failure to withdraw PNS activity or lower levels of RSA suppression have been related to both externalizing and internalizing behavior problems (Beauchaine, 2001; Beauchaine, Gatzke-Kopp, & Mead, 2007; Boyce et al., 2001; El-Sheikh, Arsiwalla, Hinnant, & Erath, 2011).

An important component that determines whether an individual will activate the PNS or SNS is the perception of threat. An environment perceived as safe allows the expression of the PNS whereas the evolutionarily more primitive SNS is inhibited. However, the degree to which the PNS and SNS are activated during stressful conditions differs between individuals (Beauchaine, 2001), and may depend on early experiences (Oosterman, De Schipper, Fisher, Dozier, & Schuengel, 2010).

Effects of early adversity on the developing ANS

During the last trimester and continuing through the first two years postpartum, the ANS is rapidly developing (Porges & Furman, 2011). Prenatal exposure to adversity during sensitive periods of fetal development can have lasting effects on neurological development through processes of fetal programming (Barker, 1998), and alter maturation of the ANS (Alkon et al., 2014; Jacob, Byrne, & Keenan, 2009). For example, prenatal exposure to psychosocial risk factors, such as poverty or low social support, has been found to impact ANS trajectories from six months to five years of age (Alkon et al., 2014). Postnatal exposure to early adversity may exert its influence on the developing ANS either directly or indirectly through limiting the mother's ability to exhibit sensitivity parenting behavior. In a recent review, Propper and Holochwost (2013) conclude that prenatal exposure to maternal stress and substance use, and postnatal exposure to a low quality parent-child relationship, maternal depression and marital conflict were consistently related to lower basal levels of PNS activity and higher basal HR. Moreover, exposure to these risk factors was associated with increased cardiac arousal and reduced or absent vagal withdrawal in response to challenge (see also Conradt & Ablow, 2010; Graziano & Derefinko, 2013; Haley & Stansbury, 2003).

The literature on the effects of early adversity on early SNS functioning is not as complete as the corresponding literature on the PNS (Propper & Holochwost, 2013). Preliminary evidence, using independent measures of SNS activity (e.g. salivary α-amylase [sAA] or pre-ejection period [PEP]),suggests that exposure to early adversity is associated with heightened SNS reactivity in infancy and early childhood (Frigerio et al., 2009; Hill-Soderlund et al., 2008; Oosterman et al., 2010; Propper &

Holochwost, 2013; Repetti, Taylor, & Seeman, 2002). However, most studies in infancy used HR as a measure of SNS activity (Propper & Holochwost, 2013). Because HR is autonomically controlled by both the SNS and PNS, it represents a more global measure of autonomic functioning rather than a specific measure of SNS activation.

PEP represents the sympathetically mediated time between the onset of the heartbeat and ejection of blood into the aorta (Cacioppo, Uchino, & Bernston, 1994). It has been suggested that PEP is a 'relatively pure' measure of SNS activity, as the myocardial tissue of the heart's left ventricle is innervated primarily by sympathetic inputs, and shorter PEP indicates increased SNS activity (Randall, Randall, & Ardell, 1991). Although previous research has established PEP as a good indicator of SNS activity in infants and children (Alkon et al., 2006; Quigley & Stifter, 2006), so far very few studies in infants have included PEP as a measure of SNS activity (Alkon et al., 2011; Alkon et al., 2014).

Infant stress response patterns to a social stress paradigm

In this study, we investigate infant ANS response patterns to a well-established social stressor, the Still Face Paradigm (SFP) , during which the mother is asked to normally interact with the infant (Play episode), then withhold interaction holding a neutral expression (Still Face episode), and then resume interaction (Reunion episode) (Mesman, Van IJzendoorn, & Bakermans-Kranenburg, 2009; Tronick, Als, Adamson, Wise, & Brazelton, 1978). The SFP has shown to reliably produce a stress response in infants, as reflected in increases in negative affect and HR and decreases in positive affect, gaze and RSA from baseline or the Play episode to the Still Face episode (Bazhenova, Plonskaia, & Porges, 2001; Conradt & Ablow, 2010; Bosquet Enlow et al., 2014; Haley & Stansbury, 2003; Mesman, Van IJzendoorn, & Bakermans-Kranenburg, 2009; Moore & Calkins, 2004; Moore et al., 2009; Weinberg & Tronick, 1996), and increases in cortisol output following the SFP (Enlow et al., 2014; Grant et al., 2009; Haley & Stansbury, 2003). The transition from the Still Face episode to the Reunion episode allows us to investigate individual differences in recovery from stress. Although decreases in negative affect and heart rate and increases in RSA and positive affect have been reported, there is evidence of partial carry-over effects of stress into the Reunion episode, indicating infants' stress levels do not always return to baseline Play episode levels (Bazhenova et al.. 2001; Bosquet Enlow et al., 2014; Conradt & Ablow, 2010; Mesman et al., 2009; Moore & Calkins, 2004; Weinberg & Tronick, 1996).

Limited research has been conducted on SNS response patterns across the SFP. One recent study among 35 six-month-old infants found that, using a modified

SFP (with an additional Still Face - Reunion sequence), greater infant SNS activation (indexed by T-wave amplitude) during periods of stress was associated with greater maternal insensitivity (Bosquet Enlow et al., 2014). Another study reported increases in skin conductance levels across the SFP in a sample of 12 five-month old infants (Ham & Tronick, 2009). To date, there are no studies that we know of that have examined PEP reactivity across the different episodes of the SFP.

Biobehavioral associations

Individual variation in ANS recovery patterns on the SFP have been associated with infants' early emotion regulation in previous studies (Bazhenova et al., 2001; Haley & Stansbury, 2003; Moore & Calkins, 2004; Weinberg & Tronick, 1996). For example, Conradt and Ablow (2010) reported differential associations between specific aspects of infant regulatory behavior during the Reunion episode and changes in cardiac arousal versus PNS activity during recovery from the Still Face episode, such that greater increases in RSA were associated with infant attention to the mother, whereas resistant behavior was related to greater HR increases. This study provides empirical evidence for Porges' model of social engagement, a model derived from the Polyvagal theory (Beauchaine, 2001; Porges & Furman, 2011), describing how individual differences in ANS regulation, specifically vagal regulation, underlie social engagement with the environment. Conversely, when the vagal system is compromised, activation of the SNS mediates the expression of strong negative emotions (Beauchaine, 2007). However, empirical accounts investigating differential associations between infant PNS versus SNS reactivity and emotion regulation are currently lacking. Moreover, given the vulnerability of the ANS for prenatal and early postnatal adverse influences (Holochwost & Propper, 2013; Porges & Furman, 2011), investigating mediating pathways from exposure to adversity to infant emergent emotion regulation capacities through PNS and SNS functioning, may contribute to existing theories concerning the physiological underpinnings of emotion (dys)regulation in infants.

The present study

Using both SNS and PNS measures, a primary aim of the present study was to examine the effects of exposure to early adversity on infant ANS response to and recovery from stress. To this end, we examined ANS (HR, RSA and PEP) reactivity across the SFP, and more specifically, in response to and recovery from the Still Face episode, in a high-risk group of infants exposed to prenatal and early postnatal adversity and a low-risk control group. We hypothesized that infants in the high-risk group, compared to infants in the low-risk group, would show a pattern of ANS

reactivity across the SFP indicative of less efficient PNS-mediated regulation of stress. Specifically, in response to the Still Face episode, infants in the high-risk group were expected to show stronger increases in HR and SNS activity (i.e. larger decrease in PEP) and lower PNS withdrawal (i.e. decreases in RSA). During recovery from the Still Face episode, infants in the high-risk group were expected to show poorer recovery than their low-risk counterparts, indicated by more limited decreases in HR and SNS activity, and more limited increases in PNS activity. Follow-up analyses within the high-risk group were conducted to examine the associations between cumulative risk (i.e. the sum of maternal risk factors present) and infant ANS response and recovery. In addition, we investigated independent contributions of the PNS and SNS to HR response and recovery. Taking into account that PNS and SNS influences on HR often operate in considerable independence (Cacioppo et al., 1994), and that exposure to early adversity may impact the integrity of the ANS (Porges & Furman, 2011), we hypothesized that the contribution of PNS and SNS to the change in HR in response to and recovery from stress would be different in infants in the high-risk versus the low-risk group. As the SFP is a relatively mild stressor, presumably requiring minimal SNS activation, we expected HR response and recovery to be mainly PNS mediated in infants in the low-risk group, whilst these would be mediated by both the PNS and SNS in infants in the high-risk group.

A secondary aim of our study was to examine associations between ANS response and recovery and emotion regulation during the Still Face and Reunion episode. We expected that emotion regulation during the Still Face and Reunion episode, specifically the extent to which infants show negative affective expressions such as whining, fussing or crying, or the extent to which infants were attending to their mother, would be differentially associated to PNS and SNS response and recovery. We hypothesized that greater PNS withdrawal in response to the Still Face episode and increases in PNS activity during recovery from the Still Face episode would be related to more attentional engagement towards the mother during the Still Face and Reunion episode respectively. In contrast, we hypothesized that greater increases in SNS activity in response to the Still Face episode and greater SNS activity during recovery from the Still Face episode would be related to more negative affect during the Still Face and Reunion episode respectively. We also investigated whether the effect of risk status on emotion regulation was mediated through ANS response and recovery. Based on previous research, we expected that infants in the high-risk group, compared to infants in the low-risk group, would exhibit more negative affect and attend less towards their mother during the Still Face episode and the Reunion episode (e.g. Bosquet Enlow et al., 2014; Conradt & Ablow, 2010; Haley & Stansburry, 2003), and that these associations would be mediated through less efficient

PNS regulation of stress indexed by lower PNS withdrawal and increased SNS activity in response to the Still Face episode and more limited increases in PNS activity and decreases in SNS activity during recovery from the Still Face episode respectively.

Methods

Participants

The present study is part of the Mother- Infant Neurodevelopment Study in Leiden, The Netherlands (MINDS - Leiden). MINDS – Leiden is a large ongoing longitudinal study into neurobiological and neurocognitive predictors of early behavior problems. The study was approved by the ethics committee of the Department of Education and Child Studies at the Faculty of Social and Behavioral Sciences, Leiden University, and by the Medical Research Ethics Committee at Leiden University Medical Centre. All participating women provided written informed consent. Women were recruited during pregnancy via midwifery clinics, hospitals, prenatal classes and pregnancy fairs. Dutch speaking primiparous women between 17 and 25 years old with uncomplicated pregnancies were eligible to participate. We chose to oversample women from a high-risk background (see criteria below) to obtain sufficient variance in children's early behavioral problems.

After completing the prenatal visit in the third trimester of pregnancy, women were allocated to the high-risk or low-risk control group. Classification in the high-risk group was based on the following criteria (Mejdoubi et al., 2011; World Health Organization, 2005): positive screening on current psychiatric disorder(s) using the Dutch version of the Mini- International Neuropsychiatric Interview (MINI-plus; Van Vliet, Leroy & Van Megen, 2000) or substance use (alcohol, tobacco and drugs) during pregnancy, or presence of two or more of the following psychosocial risk factors: no secondary education, unemployment, self-reported financial problems, limited or instable social support network, single status, and maternal age <20 years. In case only one risk factor was present - other than positive screening for current psychiatric disorder(s) or substance use - women were discussed in a clinical expert meeting to determine whether placement in the high-risk group was appropriate. See Smaling et al. (2015) for a more detailed description of classification criteria used in this study.

The sample for this study consisted of 121 mothers (79 low-risk and 42 high-risk) and their six-month-old infants who had completed both the first (prenatal home visit) and second wave (home visit at six months port partum) of the study. A total of 9 women (6.2%) originally enrolled in the study did not participate in the second wave of the study. Attrition was due to emigration or moving house (n=2), inability to contact (n=3), refusal (n=2), and withdrawal due to premature delivery (<36 weeks,

n=2). Sample attrition was unrelated (*ps*>.10) to demographic variables such as maternal age, marital status, ethnicity, and educational level.

Mean age of the infants (56.2% males) was 27.6 weeks (*SD*=2.07, range 24-38 weeks), and mean age of the mothers was 23.6 years (*SD*=2.12, range 18-27 years). Approximately 93% of the mothers had a partner (84.3 % was married or living with a partner) and 29.8% of the mothers had a high educational level (Bachelor's or Master's degree). Families were predominantly Caucasian (86.8%), 5% Surinam or Antillean, 4.1% mixed (Caucasian and other origin), and 4.1% other origin. There were 71 mothers with no risk factors, 25 mothers with one risk factor (of which 17 mothers were assigned to the high-risk group), 15 mothers with two risk factors, 8 mothers with three risk factors, and 2 mothers with respectively four and five risk factors. For an overview of the cumulative prevalence as well as the specific combinations of risk factors present within the total sample, see Table 1.

Procedures and instruments

Home visits at six months post-partum were carried out by trained female experimenters and scheduled at a time of the day when mothers deemed their infant to be most alert. After some time to get familiar with the experimenters, cardiac monitoring equipment was attached to the infant. During a 2-minute relaxing movie, baseline ANS measures were taken while the infant was lying on a blanket. Subsequently, the mother-infant dyads participated in the SFP.

Still Face Paradigm. The SFP consists of three 2-minute episodes (respectively Play, Still Face and Reunion). Following the baseline, infants were seated in an infant seat placed on a table. Mothers sat on a chair approximately 1 meter from the infant at eye level. Mothers were instructed to play with their child as they normally would (no toys). Immediately following the Play episode, the Still Face episode started. Mothers were instructed to adopt and maintain a neutral facial expression, remain still and not to touch or respond to their infant. The procedure ended with the Reunion episode in which mothers could resume play and respond to their child in any way they felt was appropriate, but without taking the child out of the seat. The beginning and end of each episode was prompted by the experimenter. Mothers were informed that they could terminate the Still Face episode and resume playing when the child became overly distressed. If the infant was unable to be soothed at any point during the procedure, the SFP was stopped by the experimenter. The entire procedure was recorded with one camera focused on the infant. A wooden frame with a mirror was placed behind the infant seat, through which the mother's facial expression and behavior was recorded.

Infant autonomic nervous system (ANS) parameters. Infant ANS parameters were measured with the Vrije Universiteit Ambulatory Monitoring System (VU-AMS 5fs; De Geus, Willemsen, Klaver, & Van Doornen, 1995; Willemsen, De Geus, Klaver, Van Doornen, & Carroll, 1996) during a 2-minute baseline and the SFP. After removing oil with alcohol wipes, seven disposable pre-gelled silver-silver chloride (Ag-AgCl) snap electrodes (ConMed Huggable 1620-001, New York) were attached to the skin. The VU-AMS device continuously recorded electrocardiogram (ECG), and impedance cardiogram (ICG) measures; basal thorax impedance (Z_0), changes in impedance (dZ), and the first derivative of pulsatile changes in transthoracic impedance (dZ/dt). The ECG and dZ/dt signal were sampled at 1000 Hz, and the Z_0 signal was sampled at 10Hz. The VUDAMS software suite version 2.0 was used to extract mean values of HR, RSA, and PEP across the baseline and SFP Play episode (each lasting 2 minutes), and per minute across Still Face and Reunion episodes.

Table 1. *Cumulative prevalence of risk factors.*

N risk factors						N (%)
0						**71 (58.7)**
1						**25 (20.6)**
	Psychiatric diagnosis					8
	Smoking					8
	Alcohol					1
	Psychosocial risk[a]					8
2						**15 (12.4)**
	Psychiatric diagnosis	Smoking				4
	Psychiatric diagnosis	Psychosocial risk				7
	Smoking	Psychosocial risk				2
	Smoking	Alcohol				1
	Psychosocial risk	Psychosocial risk				1
3						**10 (12.1)**
	Psychiatric diagnosis	Smoking	Drugs			1
	Psychiatric diagnosis	Smoking	Psychosocial risk			2
	Psychiatric diagnosis	Alcohol	Psychosocial risk			1
	Psychiatric diagnosis	Psychosocial risk	Psychosocial risk			2
	Smoking	Psychosocial risk	Psychosocial risk			2
4						**1 (0.8)**
	Psychosocial risk	Psychosocial risk	Psychosocial risk	Psychosocial risk		1
5						**1 (0.8)**
	Psychiatric diagnosis	Smoking	Psychosocial risk	Psychosocial risk	Psychosocial risk	1

Note: [a] Psychosocial risk factors are single status, unemployment, no secondary education, self-reported financial problems, limited social support, and age <20 years.

All R-peaks in the ECG, scored by the software, were visually checked and when necessary were adjusted manually. RSA was derived by the peak-trough method (De Geus et al., 1995; Grossman, Van Beek, & Wientjes, 1990), which combined the respiration (obtained from filtered [0.1 – 0.4 Hz] thoracic impedance signal) and inter beat interval (IBI) time series to calculate the shortest IBI during heart rate acceleration in the inspiration phase and the longest IBI during deceleration in the expiration phase (De Geus et al., 1995). RSA was defined as the difference between the longest IBI's during expiration and shortest IBI's during inspiration. Automatic scoring of RSA was checked by visual inspection of the respiratory signal from the entire recording, leading to rejection of fewer than 6% of the data.

PEP is the time interval between the onset of the ventricular depolarization (Q-wave onset) and the onset of left ventricular ejection of blood into the aorta (B-point on the Dz/dt complex; De Geus et al., 1995). Average dZ/dt waveforms were derived by the software. PEP was automatically scored from the Q-wave onset (opening of the aortic valve) on the ECG and the B-point on the dZ/dt waveform. Each automated scoring was checked and corrected manually when necessary (Riese et al., 2003). In case wave forms were morphologically distorted in such a way visual correction of automated scoring was not possible, those wave forms were discarded (fewer than 17% of the wave forms were discarded). The procedure of interactive visual scoring was done independently by two trained raters. Post-scoring, the raters chose a consensus for the points where their judgment did not overlap, and these were retained for the analyses. Inter-rater reliability (intraclass correlation ICC) was .949.

Approximately 8% of ANS data were missing across the baseline and SFP episodes. Missing data was due to dyads that did not complete the SFP because the infant became too fussy (n=3), loose electrodes (n=2), or equipment failure (n=4). The remainder of ANS data was missing because of noisy data due to excessive child movement in which case HR data was available but PEP and/or RSA could not be scored. Data were not missing systematically by maternal risk status, ethnicity, infant sex, or maternal educational level. Main analyses were conducted based on the number of infants for which there was data (see Table 4 for available data for HR, PEP and RSA across baseline and SFP episodes).

Coding of infant behavior. Infant Negative affect and Gaze (reflecting the extent to which infants successfully regulated distress and used other-directed emotion regulation strategies) were coded during the Play episode and per minute during the Still Face and Reunion episodes. Coders rated infant behavior with an adapted version of the 4-point global rating scale (0=absent – 3=high levels or predominantly present) of the Mother Infant Coding System (Miller, McDonough, Rosenblum, & Sameroff,

2002). *Negative affect* was defined as the intensity of negative affective expressions (e.g. whining, fussing, crying). *Gaze* was defined as the extent to which infants were engaged with their mothers through looking at their mother's face or making eye contact. All coders were trained extensively until the ICC was .700 or higher on a subset of 20 recordings. A subset of recordings (15% of the sample) was double-coded to assess ongoing inter-rater reliability. ICC was .999 on both dimensions.

Cumulative risk. In order to analyze the effects of cumulative risk within the high-risk group, maternal risk factors present during the third trimester of pregnancy were summed to create a cumulative risk score (maximum number of risk factors was 10), with M=1.76, SD=.94 (Range 1-5). Because there were only two participants with respectively four and five risk factors, the presence of three, four or five risk factors was collapsed into one group with ≥3 risk factors.

Data analysis

All variables were examined for outliers and violations of specific assumptions applying to the statistical tests used. For each variable, observations with values that exceeded three standard deviations from the mean were deleted (0.4% of the total number of observations across the ANS variables). Because RSA was skewed at baseline and all episodes of the SFP, its natural logarithm (lnRSA) was used in the analyses.

For all analyses, the second minute of the Still Face and Reunion episode was chosen as reference to examine the infant stress response and recovery because we found cumulative effects of stress experienced in the Still Face and Reunion episode, as well as carry-over effects of stress into the Reunion episode, with group differences being more pronounced during the second minute of the Still Face and Reunion episode compared to the first minute. More specifically, in line with suggestions made by Mesman et al. (2009), we found that it took some time for infants to become stressed during the Still Face episode, as evidenced by a significant increase in HR from the first to the second minute of the Still Face (t(113)=-1.83, p=.07), especially for infants in the low-risk group (t(72)=-2.36, p<.05). Further, significant increases in PEP from the first to the second minute of the Reunion episode for infants in the low-risk group (t(43)=-2.50, p<.05) indicated that recovery took place mainly during the second part of the Reunion episode (see also Mesman et al., 2009). Moreover, we found stress levels to increase across the Still Face and Reunion episode, as evidenced by a significant (further) decrease in RSA from the first to the second minute of the Reunion episode (t(102)=2.09, p<.05), especially for infants in the high-risk group (t(36)=2.86, p<.01).

For each infant, difference scores were computed to examine the ANS stress response (Δ Play – Still Face episode), and the ANS stress recovery (Δ Still Face – Reunion episode). Negative values for HR indicate HR acceleration. Positive values for PEP and lnRSA indicate respectively SNS activation and PNS withdrawal.

Preliminary analyses. Prior to conducting the main analyses, preliminary analyses (independent t-tests, Chi-square and Pearson correlations) were carried out to test for potential covariates (maternal and infant demographic and obstetric characteristics) and to test whether there were there were effects of risk status (high-risk vs low-risk) on baseline ANS measures. In addition, paired t-tests were used to compare mean levels of Negative affect and Gaze across the SFP in order to check the validity of the SFP (i.e. to examine whether infant behavior changed in the expected direction (see meta-analyses Mesman et al., 2009) from Play to the Still Face episode, from the Still Face to the Reunion episode and from the Play to the Reunion episode).

Risk status and ANS response and recovery. Repeated measure ANOVAs were conducted to examine whether ANS variables (HR, PEP and lnRSA) changed across the SFP episodes and whether there were effects of risk status on these variables across the SFP. The corrected degrees of freedom using the Greenhouse-Geisser ($\varepsilon<.75$) or the Huynh- Feldt ($\varepsilon>.75$) correction were reported if the sphericity assumption was violated. Planned contrasts were used to further examine effects of risk status on the ANS stress response (Play to the Still Face episode), the ANS stress recovery (Still Face to the Reunion episode), and ANS activity across the SFP (Play to the Reunion episode).

Cumulative risk and ANS response and recovery. Spearman's rank correlations were used to examine the association between Cumulative risk and HR, PEP and lnRSA response and recovery within the high-risk group.

Risk status and independent contributions of the SNS and PNS to HR response and recovery. To examine whether the independent contributions of the PNS and SNS to the HR response and recovery differed between high-risk versus low group, partial correlations were examined between HR response and recovery and both PEP response and recovery and LNRSA response and recovery for high-risk versus low-risk group separately. These analyses enabled us to determine the independent contribution to the HR response and recovery of the PNS while controlling for SNS influences and of the SNS while controlling for PNS influences.

Associations between ANS response and recovery and emotion regulation. Pearson correlations were computed among ANS (HR, PEP and lnRSA) response and recovery variables and behavior (Negative affect and Gaze) during the

Still Face and Reunion episode, to investigate whether ANS response and recovery were associated with emotion regulation.

ANS response and recovery as mediator between risk status and emotion regulation. Using the "indirect" macro designed for SPSS (Preacher & Hayes, 2008), bootstrapping procedures with 5000 bootstrapped samples were applied to test whether the PEP and lnRSA response mediated the effect of risk status on emotion (Negative affect and Gaze) regulation during the Still Face episode, and whether PEP and lnRSA recovery mediated the effect of risk status on emotion regulation during the Reunion episode. ANS variables that were significantly related to Negative affect and Gaze during the Still Face or Reunion episode were added as potential mediators to the model. The bootstrapping strategy quantifies the indirect effect and makes no assumptions of multivariate normal distribution in the sampling of indirect effects. In addition, these bootstrapping analyses can be applied to smaller samples with more confidence, provide a direct test of mediation and have more power. As discussed elsewhere (Hayes, 2009), it is not necessary for the independent variable to be significantly related with the dependent variable to show mediation. Direct and indirect effects and 95% bias-corrected and – accelerated (BCA) confidence intervals (CI) are reported. The indirect effect is significant if zero does not fall within the confidence interval.

All analyses were conducted using the Statistical Package for Social Sciences (SPSS for Windows, version 21.0, SPSS Inc., Chicago). Statistical significance was established a priori at $p<.05$.

Results

Preliminary analyses

Demographic and obstetric characteristics of the high-risk and low-risk group are presented in Table 2. Independent t-tests showed that there were no baseline differences between the high-risk and low-risk group on the different ANS measures (p values >.85). Infant's HR, PEP and lnRSA were not associated with the maternal and infant demographic variables or obstetric characteristics as listed in Table 2 (p values >.10); however, boys were found to have lower PEP values on all episodes of the SFP (p values <.05). Therefore, infant sex was included as a covariate in the analyses with PEP.

Table 2. *Demographic and obstetric characteristics for the high-risk and low-risk group.*

	Low-risk (n=79)		High-risk (n=42)		Group comparisons[a]
Variables	*M*	*SD*	*M*	*SD*	
Maternal age (years)	24.3	1.7	22.3	2.2	$t(119)=4.85$, $p<.001$
Maternal education (% high[b])	41.8%		7.1%		$\chi^2(1)=15.73$, $p<.001$
Ethnicity (% Caucasian)	91.1%		78.6%		*ns*
Relationship status (% partner)	96.2%		85.7%		$\chi^2(1)=4.38$, $p<.05$
APGAR scores (5-min)	9.5	1.0	9.6	0.7	*ns*
Gestational age (weeks)	39.3	1.8	39.0	2.5	*ns*
Infant birth weight (kg)	3.9	0.5	3.3	0.6	*ns*
Sex (% male)	60.8%		47.6%		*ns*
Infant age (weeks)	27.5	2.0	27.7	2.1	*ns*

Note: [a]*t*-test or χ^2 test, [b]Maternal education (% high) represents percentage with a bachelor's or master's degree.

The means and standard deviations for Negative affect and Gaze across episodes of the SFP for the high-risk and low-risk group separately and the sample as a whole are presented in Table 3. Paired t-tests revealed significant increases in Negative affect and decreases in Gaze from the Play to the Still Face episode (respectively $t(117)=-3.18$, $p<.01$, and $t(117)=5.14$, $p<.001$), and from the Play to the Reunion episode (respectively $t(116)=-5.77$, $p<.001$, and $t(116)=-1.97$, $p=.052$). From the Still Face to the Reunion episode, infants exhibited significant increases in Negative affect ($t(116)=-2.46$, $p<.05$), and Gaze ($t(116)=-2.95$, $p<.01$). These results, except for the increase in Negative affect from the Still Face to the Reunion episode, were consistent with the results of the meta-analyses of Mesman et al. (2009). However, it should be noted that Mesman et al. (2009) reported no significant change in negative affect from the Still Face to the Reunion episode, and significant heterogeneity among studies that included recovery effects for negative affect. For example, a study among infants prenatally exposed to alcohol (Haley et al., 2006) reported increases in negative affect from the Still Face to the Reunion episode, suggesting that the extent to which recovery effects are reported for negative affect may be dependent on the nature of the sample included (high versus low-risk).

Table 3. *Means and standard deviations for negative affect and gaze across SFP episodes.*

	Low-risk			High-risk			Total		
	N	*M*	*SD*	*N*	*M*	*SD*	*N*	*M*	*SD*
Negative affect									
Play	78	.58	.83	42	.93	.92	120	.70	.88
Still Face	77	1.05	1.21	41	.98	1.11	118	1.03	1.17
Reunion	76	1.18	1.09	41	1.46	1.25	117	1.28	1.15
Gaze									
Play	78	1.58	.75	42	1.62	.76	120	1.59	.75
Still Face	77	1.12	.74	41	1.15	.79	118	1.13	.76
Reunion	76	1.46	.87	41	1.32	.88	117	1.41	.87

Note: Play = Play episode, Still Face = Still Face episode, Reunion = Reunion episode.

Risk status and ANS response and recovery

The means and standard deviations for HR, PEP and lnRSA across episodes of the SFP for the high-risk and low-risk group separately and the sample as a whole are presented in Table 4. Repeated measure AN(C)OVAs to examine changes in ANS variables (HR, PEP and lnRSA) across the different episodes of the SFP (Play, Still Face and Reunion episode), showed significant within-subjects effects for HR and lnRSA (respectively $F(2,218)=15.83$, $p<.001$, $\eta^2=.13$ and $F(1.90, 192.23)=7.93$, $p<.01$, $\eta^2=.07$). Follow-up planned contrasts from Play to the Still Face and Reunion episode showed significant increases in HR (respectively $F(1, 109)=24.27$, $p<.001$, $\eta^2=.18$ and $F(1, 109)=26.17$, $p<.001$, $\eta^2=.19$) and decreases in lnRSA (respectively $F(1, 101)=10.94$, $p<.01$, $\eta^2=.10$ and $F(1, 101)=16.86$, $p<.001$, $\eta^2=.14$). Planned contrasts for HR and lnRSA from the Still Face to the Reunion episode were not significant, indicating no significant changes during recovery for the whole sample.

No significant effects for risk status were found. However, significant risk status x episode interactions for HR ($F(2,218)=4.89$, $p<.01$, $\eta^2=.04$), PEP ($F(2,138)=3.63$, $p<.05$, $\eta^2=.05$), and lnRSA ($F(1.90, 192.23)=3.25$, $p<.05$, $\eta^2=.03$) indicated that the ANS response patterns of the high-risk and low-risk group differed significantly. None of the covariate effects for sex were significant (for analyses concerning PEP only).

Planned contrasts revealed significant differences between the high-risk and low-risk group in HR response ($F(1, 109)=5.22$, $p<.05$, $\eta^2=.05$) and HR recovery ($F(1, 109)=8.20$, $p<.01$, $\eta^2=.07$), but not for HR activity across the SFP. As illustrated in Figure 1, infants in the low-risk group showed a larger increase in HR from the Play to the Still Face episode compared to infants in the high-risk group. Further, infants in the low-risk group showed a decrease in HR in recovery from the Still Face episode, whereas infants in the high-risk group showed a further increase in HR. Planned

contrasts for lnRSA revealed significant differences between the high-risk and low-risk group in lnRSA recovery ($F(1, 101)=4.96$, $p<.05$, $\eta^2=.05$) and lnRSA activity across the SFP ($F(1, 101)=4.27$, $p<.05$, $\eta^2=.04$), but not for lnRSA response (see Figure 1). Specifically, infants in the low-risk group were found to show increases in lnRSA from the Still Face to the Reunion episode whereas infants in the high-risk group showed decreases in lnRSA. Furthermore, infants in the high-risk group showed a larger lnRSA decrease across the SFP compared to infants in the low-risk group. Planned contrasts for PEP revealed significant differences between the high-risk and low-risk group for PEP recovery ($F(1, 69)=6.10$, $p<.05$, $\eta^2=.08$), but not for PEP response and PEP activity across the SFP. As illustrated in Figure 1, infants in the low-risk group showed an increase in PEP from the Still Face to the Reunion episode, whereas infants in the high-risk group showed a decrease in PEP.

Table 4. *Means and standard deviations for HR, PEP and lnRSA across SFP episodes.*

	Low-risk			High-risk			Total		
	N	*M*	*SD*	*N*	*M*	*SD*	*N*	*M*	*SD*
HR									
Baseline	73	135.30	13.51	42	134.74	12.74	115	135.09	13.18
Play	73	140.21	11.40	41	139.72	12.02	114	140.03	11.57
Still Face	73	150.40	14.31	41	143.96	14.73	114	148.08	14.73
Reunion	71	146.39	16.50	41	148.38	18.05	112	147.12	17.03
PEP									
Baseline	67	64.45	6.70	40	64.42	6.42	107	64.44	6.57
Play	65	63.58	6.79	35	62.92	7.52	100	63.35	7.02
Still Face	59	62.72	7.43	35	62.13	8.16	94	62.50	7.67
Reunion	49	63.87	7.80	33	60.71	8.49	82	62.59	8.18
lnRSA									
Baseline	71	3.35	.45	39	3.41	.36	110	3.37	.42
Play	69	3.36	.38	40	3.42	.33	109	3.38	.36
Still Face	68	3.14	.56	38	3.27	.44	106	3.19	.52
Reunion	67	3.28	.53	38	3.13	.57	105	3.22	.54

Note: HR = heart rate, lnRSA = natural logarithm of respiratory sinus arrhythmia, PEP = pre-ejection period.

Cumulative risk and ANS response and recovery

Cumulative risk was significantly associated with PEP response (r=.418, p<.05), indicating that, within the high-risk group, an increase in the number of risk factors is related to larger decreases in PEP from the Play to the Still Face episode. The correlation between cumulative risk and PEP recovery approached significance (r=-.358, p=.052). The correlations between cumulative risk and HR and lnRSA response and recovery were not significant.

Risk status and independent contributions of the SNS and PNS to HR response and recovery

Partial correlations between HR and lnRSA response, controlling for PEP response and between HR and PEP response, controlling for lnRSA response, showed that for the low-risk group, only the PNS (lnRSA response) made an independent contribution to the HR response (partial r=-.693, p<.001); the SNS (PEP response) did not. For the high-risk group, both the PNS and SNS made an independent contribution (partial r=-.534, p<.01, and partial r=-.371, p<.05 for the lnRSA response and PEP response respectively).

The independent contributions of the PNS and SNS to HR recovery also differed between the high-risk and low-risk group. Whilst for infants in the low-risk group only the PNS (lnRSA recovery) made a significant contribution to the HR recovery (partial r=-.773, p<.001), both partial correlations were significant for infants in the high-risk group (partial r=-.602, p<.01 and partial r=-.505, p<.01 for the lnRSA recovery and PEP recovery respectively), indicating independent contributions to HR recovery from both the PNS and the SNS.

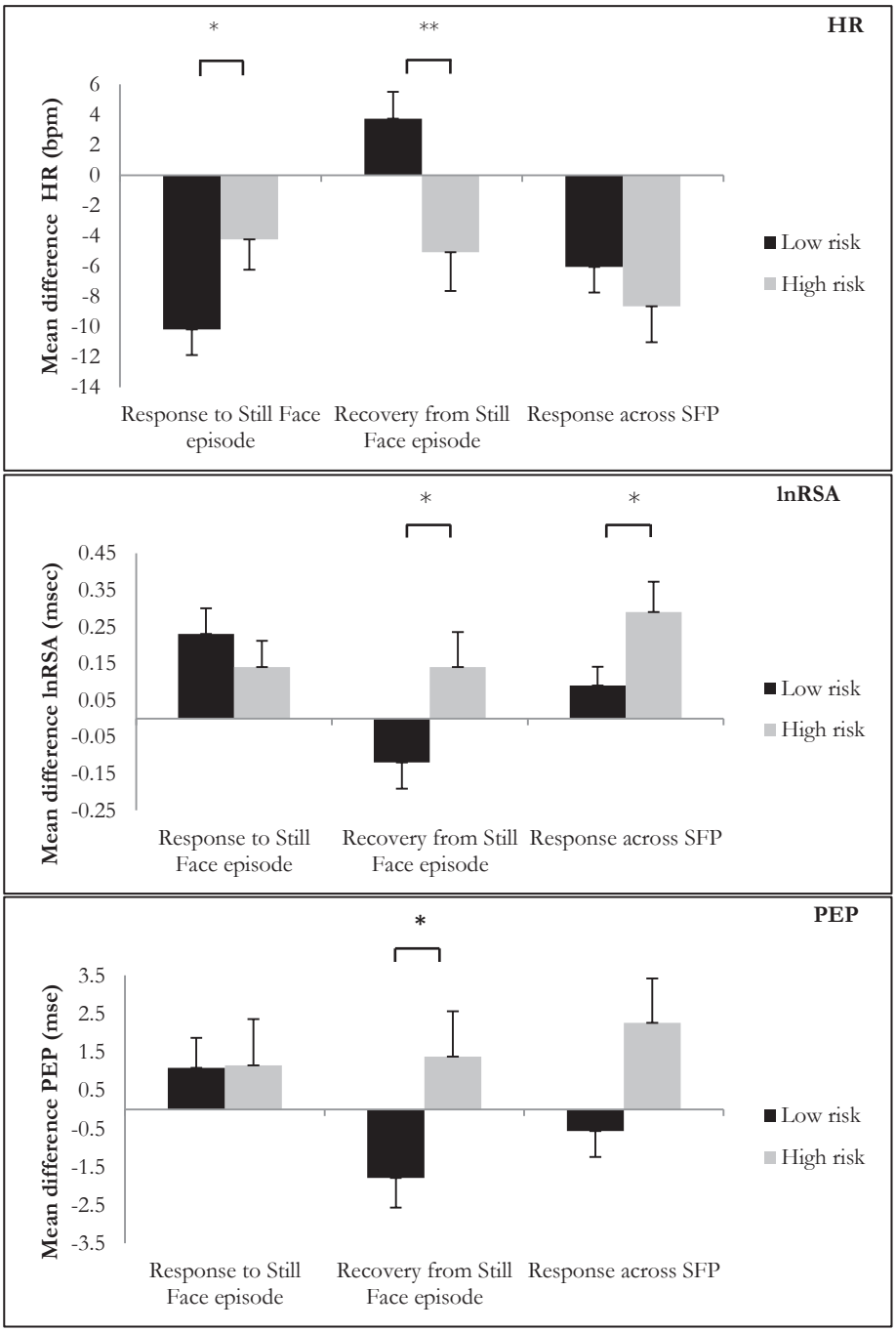

Figure 1. HR, lnRSA and PEP mean differences between infants in the high-risk group versus the low-risk group in response to the Still Face episode, during recovery from the Still Face episode and across the SFP. *Note:* *p<.05, and **p<.01.

Associations between ANS response and recovery and emotion regulation

Correlations among ANS response and recovery variables and Negative affect and Gaze during the Still Face and Reunion episode are presented in Table 5. Negative affect during the Still Face episode showed significant correlations with the HR and lnRSA response (respectively r=-.571, p<.001, and r=.334, p<.001), but not to the PEP response, indicating that larger increases in HR and decreases in lnRSA from Play to the Still Face episode were associated with higher levels of Negative affect during the Still Face episode. There were no significant correlations between Gaze during the Still Face episode and the ANS response variables.

Negative affect during the Reunion episode was significantly associated with HR and PEP recovery (respectively r=-.397, p<.001, and r=.329, p<.01), indicating that larger increases in HR and decreases in PEP from the Still Face to the Reunion episode were associated with higher levels of Negative affect during the Reunion episode. The correlation between Negative affect during the Reunion episode and lnRSA recovery approached significance (r=-.175, p=.076). Gaze during the Reunion episode was significantly associated with HR and lnRSA recovery (respectively r=.334, p<.001, and r=-.265, p<.01), but not PEP recovery, indicating that larger decreases in HR and increases in lnRSA from the Still Face to the Reunion episode were associated with higher levels of Gaze during the Reunion episode.

Table 5. *Correlations among HR, PEP and lnRSA response and recovery and Negative affect, and Gaze during the Still Face and Reunion episode.*

		1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
1.	HR response	-									
2.	HR recovery	-.538***	-								
3.	lnRSA response	-.642***	.323**	-							
4.	lnRSA recovery	.500***	-.712***	-.617***	-						
5.	PEP response	-.219*	.227*	-.029	.004	-					
6.	PEP recovery	.001	-.277*	.132	-.100	-.584***	-				
7.	Negative affect Still Face	-.571***	.295**	.334***	-.216*	.011	.131	-			
8.	Negative affect Reunion	-.155	-.397***	.133	.175 †	-.176	.329**	.462***	-		
9.	Gaze Still Face	.113	-.141	-.114	.117	.041	-.104	-.071	-.022	-	
10.	Gaze Reunion	-.044	.334***	-.002	-.265**	.070	-.120	.005	-.279**	.206*	-

Note: †p<.10, *p<.05, **p<.01, and ***p<.001.

ANS response and recovery as mediator between risk status and emotion regulation

Since there were no significant effects of risk status on the PEP and lnRSA response (see planned contrasts and Figure 1), the mediation model could not be tested for the indirect effect between risk status and emotion regulation during the Still Face episode through the PEP and lnRSA response. Based on the correlations between the PEP and lnRSA recovery and Negative affect and Gaze during the Reunion episode, bootstrapping procedures were carried out for the indirect effect between risk status and Negative affect during the Reunion episode through PEP and lnRSA recovery, and for risk status and Gaze during the Reunion episode through lnRSA recovery.

Although there was no direct effect of risk status on Negative affect and Gaze during the Reunion, the total effect model was significant for both Negative affect ($F(3,69)=3.24$, $p<.05$, $R^2=.08$) and Gaze ($F(2,101)=3.84$, $p<.05$, $R^2=.05$) (see also Figure 2). The mediation model for Negative affect showed a significant indirect effect of PEP recovery (95% CI=.04, .48), indicating that infants in the high-risk group showed larger decreases in PEP from the Still Face to the Reunion episode which in turn predicted more Negative affect during the Reunion episode, whereas infants in the low-risk group showed larger increases in PEP from the Still Face to the Reunion episode which in turn predicted less Negative affect during the Reunion. The mediation model for Gaze showed a significant indirect effect of lnRSA recovery (95% CI=-.26, -.01), indicating that infants in the high-risk group showed larger decreases in lnRSA from the Still Face to the Reunion episode which in turn predicted less Gaze during the Reunion episode, while infants in the low-risk group showed larger increases in lnRSA from the Still Face to the Reunion episode which in turn predicted increased Gaze during the Reunion episode.

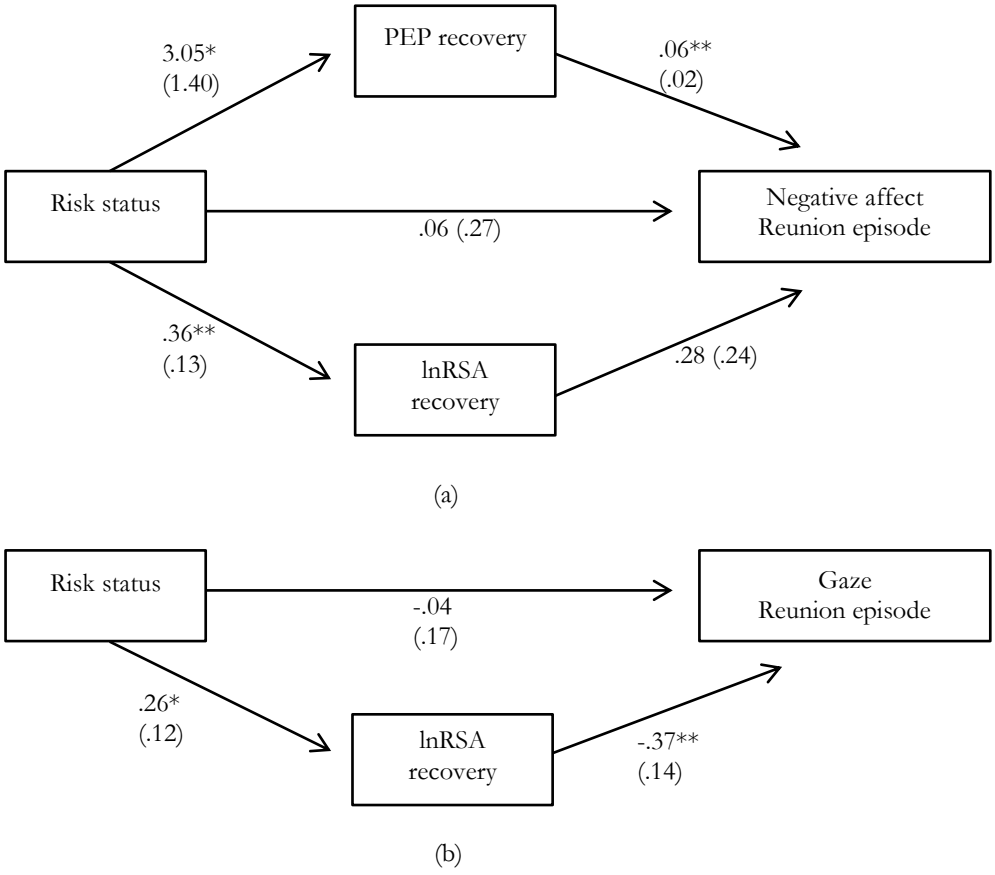

Figure 2. Bootstrapping results testing the mediation model for (a) Risk status and Negative affect during the Reunion episode via PEP and lnRSA recovery and (b) Risk status and Gaze during the Reunion episode via lnRSA recovery. Numbers within parentheses show standard error. Note*:* **p*<.05, and ***p*<.01.

Discussion

The aim of the present study was to examine infant autonomic response to and recovery from emotional challenge, using both PNS and SNS measures, in a sample of infants at risk for the development of psychopathology and a low-risk control sample. A second aim was to investigate associations between ANS response and recovery and emotion regulation, and to examine whether the association between risk status and emotion regulation was mediated by ANS reactivity. Our results showed that maternal risk status was associated with infant ANS response to and recovery from stress. Infants in the high-risk group showed less parasympathetic regulation, indicated by greater RSA withdrawal, and increased SNS activity specifically during recovery from stress compared to infants in the low-risk group. While for infants in the low-risk group HR recovery was primarily mediated by the PNS, for infants in the high-risk group the (lack of) recovery in HR was both PNS and SNS mediated. Finally, distinct indirect pathways from maternal risk status to infant emotion regulation via infant PNS and SNS regulation were observed.

As expected, our findings showed more efficient PNS- mediated regulation of stress, specifically during recovery, among infants in the low-risk group compared to infants in the high-risk group. Whereas infants in the low-risk group showed decreases in HR and increases in RSA upon recovery from the Still Face episode, infants in the high-risk group showed the opposite response, that is, HR increased and RSA decreased during recovery, indicating further inhibition of the PNS. These findings are consistent with studies that examined associations between quality of maternal caregiving and infant physiological regulation during the SFP. For example, Conradt and Ablow (2010) and Haley and Stansbury (2003) reported that infants of less sensitive and less responsive mothers were characterized by greater cardiac arousal and less PNS regulation during the Reunion episode. It should be noted that these two studies did not include measures of SNS activity, so it remains unclear whether higher levels of cardiac arousal are solely due to reduced PNS input or a joint result of reduced PNS and increased SNS activity.

Infants in the high-risk group showed increased SNS activity from the Still Face to the Reunion episode, indicated by decreases in PEP, while infants in the low-risk group showed decreases in SNS activity. Similar findings were reported by Oosterman, De Schipper, Fisher, Dozier and Schuengel (2010) with respect to 2- to 7-year old foster children with disordered attachment and a background of neglect, who showed increased PEP reactivity across the Strange Situation Procedure, compared to foster children with ordered attachment. Furthermore, Bosquet Enlow et al. (2014) reported that greater maternal insensitivity was associated with greater SNS activation in the Still Face episode relative to the Reunion episode on a repeated version of the

SFP. Our findings concerning increased SNS activity upon recovery from stress in infants in the high-risk group were corroborated by the observed differences between the high-risk and low-risk group in contributions of the PNS and SNS to the HR recovery. The decrease in HR during recovery in infants in the low-risk group was mediated by an increase in RSA from the Still Face to the Reunion episode, reflecting efficient vagal regulation. In contrast, the increase in HR upon recovery in infants in the high-risk group was mediated by a (further) decrease in both RSA and PEP, indicating that infants in the high-risk group mobilized additional sympathetic resources when confronted with (prolonged) emotional challenge. The effect sizes in our study were small to medium which is comparable to other studies investigating physiological measures in clinical and at risk populations (Graziano & Derefinko, 2013).

A stress response marked by excessive or sustained activation of the SNS is hypothesized to be one of the major harmful components of the stress response (e.g. Nesse & Young, 2000). Heightened SNS reactivity in children has been linked to a range of negative physical and mental health outcomes including adjustment problems, increased anxiety, greater reactive aggression, and impaired immune functioning (Bakker, Tijssen, van der Meer, Koelman, & Boer, 2009; El-Sheikh, Erath, Buckhalt, Granger, & Mize, 2008; Hubbard et al., 2002; Kiecolt-Glaser & Glaser, 1995). Children born in high-risk families, suffering from early adversity both prenatally and postnatally, are likely to be exposed to risk factors frequently and continuously. If resources offered by the PNS are deficient, those offered by the SNS will be drawn upon, perhaps more heavily than in children born to a less stressful environment.

Contrary to expectations, differences between infants from the high-risk and low-risk group in RSA and PEP were only found across the SFP and during recovery and not in response to the Still Face episode. Based on previous research (Graziano & Derefinko, 2013; Propper & Holochwost, 2013), we expected to find lower PNS withdrawal in response to stress among infants in the high-risk group. Although we did find a significant difference in HR response, with infants in the low-risk group showing greater cardiac arousal in response to the Still Face episode compared to infants in the high-risk group, both groups showed RSA withdrawal indicative of parasympathetic regulation. A small number of studies have suggested that children exposed to early adversity show heightened SNS reactivity to stress (Frigerio et al., 2009; Hill-Soderlund et al., 2008; Repetti et al., 2002). Although we did not find evidence for this suggestion in the high-risk versus low-risk comparisons, the partial correlations within the high-risk group showed independent contributions of both the PNS and SNS to the HR response. Moreover, our results suggest that the effects of

early adversity on the SNS are more pronounced with higher levels of cumulative risk. In sum, although our findings are not unequivocal, our results support the notion of increased SNS reactivity to stress in infants exposed to early adversity.

Our findings demonstrated significant distinct associations between PNS and SNS recovery and aspects of emotion regulation. Consistent with the Polyvagal theory (Porges & Furman, 2011) and previous research (Bazhenova et al., 2001; Conradt & Ablow, 2010), we found that larger increases in PNS activity (and decreases in HR) upon recovery from the Still Face episode were associated with increased attention towards the mother during the Reunion episode, whereas larger increases in HR were associated with increased negative affect. However, none of these studies specifically examined SNS activity. Therefore, a novel finding is that larger decreases in PEP (indicating increases in SNS activity) from the Still Face to the Reunion episode were associated with increased negative affect during the Reunion, but not with attentional engagement with the mother.

Evidence for a significant role of the ANS in associations between risk status and behavioral outcomes stems from the results of the mediation analyses. Maternal risk status was associated with infant gaze through RSA recovery, while PEP recovery mediated the effect of maternal risk status on infant negative affect. Infants in the low-risk group showed more efficient PNS mediated regulation of stress by increasing PNS activity upon termination of the Still Face episode which was associated with more attentional engagement with the mother during the Reunion. The Polyvagal theory (Beauchaine, 2001; Porges & Furman, 2011) states that social behavior and the capacity to manage emotional challenge are dependent on effective modulation of the PNS. In this regard, more attentional engagement toward the mother may reflect low-risk infants' capacity to engage and use their mother to regulate arousal following stress. The PNS is only partially developed at birth and continues to develop during the first few months postpartum. As such, the PNS is especially susceptible to adversity during the late prenatal and postnatal period. Infants in the high-risk group exhibited further PNS withdrawal and increased SNS activity in recovery from the Still Face episode which was related to less gaze towards the mother and higher levels of negative affect during recovery. The present findings suggest that in infants exposed to early adversity, the development of the PNS may have been compromised, leading to increased activity within the SNS. Without an efficiently working vagal system, negative affective expressions are more frequently exhibited in times of stress, thereby limiting opportunities for these infants to develop effective emotion regulation strategies in interaction with their mother.

Notably, we found that boys had lower PEP values on all episodes of the SFP. These results corroborate to some extent with studies reporting sex differences

in infant regulation of distress. More specifically, boys have been found to show more irritability and fewer self-regulatory behaviors, such as hand-to-mouth activity and attention skills (Stifter & Spinrad, 2002; Weinberg, Tronick, Cohn, & Olson, 1999), and were less able to regulate distress physiologically, indexed by decreased RSA withdrawal (Calkins et al., 2002). Although we found no sex differences in emotion regulation across the SFP, the results of these studies may reflect increased proneness to distress in boys, explaining increased SNS activity among boys in our study. Although scarce, the available literature on PEP resting and reactivity measures in infancy (6 and 12 months) did not report such sex effects (Alkon et al., 2006), and sex-related findings in older child samples are inconsistent (Alkon et al., 2003; Alkon et al., 2011; Alkon et al., 2014; Hinnant, Elmore- Staton, & El-Sheikh, 2011; Matthews, Salomon, Kenyon, & Allen, 2002; Van Dijk, Van Eijsden, Stronks Gemke, & Vrijkotte, 2012). These contrasting findings may be caused to some extent by differences in samples (age, ethnicity), protocols used to assess physiological reactivity, and design (cross-sectional versus longitudinal). More research is necessary to shed more light on the role of sex on infant and child PEP developmental trajectories.

In the current study, we used the second minute of the Still Face- and Reunion-episodes as reference to examine the infants' stress response and recovery. In line with the meta-analyses of Mesman et al. (2009), we found evidence for cumulative effects of stress experienced during the Still Face and Reunion episode, and carry-over effects of stress into the Reunion-episode. As a result, differences between the low and high-risk group were more pronounced during the second minute compared to the first minute. Notably, analysis of the Still Face- and Reunion-episodes as a whole, did not reveal group differences. Although this approach is not uncommon in studies using other stress paradigms than the SFP (e.g. Reijman et al., 2014), it should be noted that (most) previous work using the SFP examined stress responses during whole episodes (including the first minute) which may limit the possibilities for comparing our results with previous work on the SFP. However, based on our findings it may be valuable for future studies to examine differences in stress measures between the first and second halves the Still Face- and Reunion-episodes as it may provide more insight in individual differences in stress reactivity across the SFP.

This study is not without limitations. First, the physiological measures were only assessed at six months of age. Although previous studies (e.g. Alkon et al., 2011; Alkon et al., 2006) have reported moderate stability of autonomic measures (HR, PEP and RSA) during resting and challenging conditions from 6 to 60 months, this was not found for reactivity measures (representing the difference between resting and challenging conditions). This indicates that during the first few years of life, autonomic responses to stress are not yet fully developed, and therefore may be

influenced by repeated exposure to environmental stressors. Future longitudinal investigations should examine whether the early patterns of decreased vagal regulation and increased sympathetic activation found in this study remain stable across development and whether they are associated with increased risk for later psychopathology (Repetti et al., 2002). Second, we were not able to assess the effects of timing of exposure (prenatal versus postnatal) to risk. Although Propper and Holochwost (2013) have shown that a broad range of pre- and postnatal risk factors have been associated with a general pattern of ANS activity characterized by lower basal levels of PNS activity and vagal withdrawal and higher basal HR, there is evidence that prenatal and postnatal exposure adversity may involve distinct causal pathways (Hickey, Suess, Newlin, Spurgeon, & Porges, 1995). In addition, we did not differentiate between different types of risk in our analyses. For the results from additional analyses exploring associations between specific maternal risk factors and infant ANS response and recovery, we refer to the *Supplement of Chapter 5 (Table A and B)*. Third, it should be noted that most mothers within the high-risk group either had one or two risk factors, and that approximately 24% had three or more factors (i.e. there were two mothers with respectively four and five risk factors). Although almost all mothers within the high-risk group had a psychiatric diagnosis or used substances during pregnancy, it is important to emphasize that the relatively low level of cumulative risk within the high-risk group may limit the generalizability of our results to samples with higher levels of cumulative risk. Finally, previous studies have shown that children's autonomic responses can vary across different challenging tasks (e.g. Bazhenova et al., 2001; Calkins & Keane, 2004). We do not know whether the observed pattern of autonomic regulation is dependent on the type of stressor used. Since PNS regulation is associated with social engagement behavior (Porges, 2007), it may be possible that the effects found in this study are specific to social situations or to the Still Face Paradigm. However, there is some evidence that other emotion eliciting tasks yield similar results. For example, less vagal withdrawal in response to a gentle arm restraint task, a well-validated paradigm designed to elicit anger/frustration (Goldsmith & Rothbart, 1999), was reported among nine-month old infants exposed to nicotine compared to non-exposed infants (Schuetze, Eiden, Colder, Gray, & Huestis, 2013). Although further research using different emotional challenges in different contexts is necessary to replicate our findings, the results of these studies provide some evidence that the results of the current study may be generalizable across contexts and different types of emotional challenges.

Most studies in the field of early adversity and infant ANS functioning have focused on global measures of HR or parasympathetic RSA. One of the strengths of this study is the inclusion of specific measures to assess both PNS and SNS

functioning. Our findings show that maternal risk status, as established during pregnancy, is associated with an altered pattern of both PNS and SNS regulation of stress in six-month-old infants, contributing to less effective regulation of emotional distress. Future empirical studies investigating links between early adversity and ANS functioning, as well as prevention and intervention studies aimed at improving prenatal and postnatal circumstances in order to prevent the development of psychopathology, should therefore take into account measures of the parasympathetic and sympathetic branch of the ANS. Furthermore, given that the maturation of the ANS during the prenatal period and first year(s) of life lays the foundation for adaptive cognitive and emotional functioning (Porges, 2003) and that the developing ANS is sensitive to early environmental influences, the findings have important implications for future research and clinical practice, underscoring the importance of identifying women with a high-risk profile during pregnancy in order to offer preventive intervention programs aimed at improving prenatal and postnatal circumstances.

Chapter 5: Supplement

Associations between specific maternal risk factors and infant ANS response and recovery

The analyses regarding the influence of specific maternal risk factors on infant ANS response and recovery were conducted in two steps. First, correlations were computed between infant ANS activity (HR, RSA and PEP response and recovery variables) and specific maternal risk factors (Psychiatric diagnosis, Smoking, Financial problems, No secondary education, Unemployment, Limited social support, Single status, and Age <20 years) were computed, see Table A. Because the prevalence of the variables Alcohol and Drugs was low (respectively N=3 and N=1), these variables were not included in the analyses. Second, to determine which specific maternal risk factors were associated with infant ANS variables, six backward stepwise regression analyses with p>.05 as the criterion for removal were conducted. The results of the final regression models are presented in Table B. Note that the same results (i.e. the same unique predictors) were obtained with multiple regression analyses with all maternal risk variables entered as predictors to the model simultaneously. However, since all multiple regression models were non-significant except for the PEP response, we decided that backward regression analyses were more appropriate as risk factors that were not associated with the dependent variables would be removed from the model.

The backward stepwise regression analyses indicated that the HR response was predicted by maternal smoking during pregnancy. Specifically, smoking during pregnancy was associated with smaller increases in HR from the Play to the Still Face episode. Both HR and PEP recovery were predicted by maternal psychiatric diagnosis, such that maternal psychiatric diagnosis was associated with larger increases in HR and decreases in PEP from the Still Face to the Reunion episode. Furthermore, maternal age <20 years predicted lnRSA recovery, such that younger maternal age was associated with larger decreases in lnRSA from the Still Face to the Reunion episode. None of the maternal risk factors were uniquely associated with PEP and lnRSA response.

Table A. *Correlations between HR, PEP and lnRSA response and recovery and maternal risk factors.*

	ANS response			ANS recovery		
	HR	PEP	lnRSA	HR	PEP	lnRSA
Psychiatric diagnosis	.183†	-.101	-.054	-.253**	.233*	.161
Smoking	.264**	.053	-.142	-.150	.002	.132
Single status	.004	.104	-.046	-.039	.022	.028
Unemployment	.041	.100	-.021	-.041	.166	.033
No secondary education	-.064	.063	.088	-.097	-.062	.029
Financial problems	.036	.013	.035	-.110	.208†	.006
Limited social support	.013	-.122	-.078	-.136	.145	.125
Age <20 years	.005	.044	-.064	-.114	-.122	.204*

Note: †p<.10, *p<.05, and **p<.01.

Table B. *Backward regression analyses predicting infant ANS response and recovery from maternal risk factors.*

Predictor	*B*	*SE*	*β*	*t*	*p*
HR response					
Smoking	9.78	3.37	.264	2.90	.005
HR recovery					
Psychiatric diagnosis	-9.55	3.49	-.253	-2.73	.007
lnRSA response					
Smoking	-.20	.14	-.142	-1.45	.149
lnRSA recovery					
Age <20 years	.48	.23	.204	2.11	.037
PEP response					
Limited social support	-3.20	2.82	-.122	-1.14	.259
PEP recovery					
Psychiatric diagnosis	3.18	1.54	.233	2.06	.043

CHAPTER 6

Summary and general discussion

Deficits in self-regulation across multiple domains of functioning, from the physiological to the cognitive, have been linked to the development of early aggressive behavior (Calkins & Keane, 2009). The studies described in this dissertation aimed to provide insight into mechanisms by which early self-regulation (measured at different levels of analysis, i.e. physiological, emotional and cognitive) and prenatal risk increase vulnerability for aggressive behavior, and into the effects of prenatal risk on early self-regulation (measured at a physiological level).

In short, summarizing our findings in light of the model described in Figure 1 (see General introduction), we found that at the emotional and cognitive level, higher levels of negative emotionality (NE) were associated with higher levels of aggressive behavior in preschool children, especially in preschoolers characterized by lower inhibitory control (IC) (**Chapter 2**). At the physiological level, we found that nonreciprocal patterns of parasympathetic (PNS) and sympathetic (SNS) activity in infancy, demonstrated by coinhibition and coactivation of the PNS and SNS, at baseline and/or in response to emotional challenge, increased vulnerability for physical aggression in toddlerhood, in the context of exposure to prenatal risk (**Chapter 4.1 and 4.2**). In turn, we found negative effects of prenatal risk on physiological self-regulation, indicated by increased PNS suppression and SNS activation in infants exposed to prenatal risk, specifically during recovery from emotional challenge (**Chapter 5**).

The main findings of the studies described in Chapter 2, 4.1, 4.2, and 5 are discussed in more detail below, followed by a discussion of the strengths and limitations of the research, and the presentation of some recommendations for future research and the concluding remarks.

Aggressive behavior in early childhood: The role of emotional and cognitive self-regulation

In **Chapter 2**, we examined how self-regulation at the emotional and cognitive level predicted aggressive behavior across the preschool years in a community sample of children aged 2-5 years. This study was based on Muris and Ollendick's interactive model (Muris & Ollendick, 2005), which states that the vulnerability for externalizing problems (and psychopathology in general) is determined by emotional and cognitive self-regulation, and that the highest levels of externalizing problems are present in children with both low emotional (i.e. NE) and cognitive (i.e. effortful control) self-regulation. We tested whether IC (a subcomponent of effortful control, specifically linked to aggressive behavior), moderated the effects of NE on aggressive behavior, and whether this effect would be consistent across the preschool years.

In addition to significant main effects of NE and IC on aggressive behavior, the results showed that aggressive behavior was indeed predicted by the interaction between NE and IC, such that the negative impact of higher levels of NE was reduced in the absence of IC deficits and enhanced by relatively poor IC. The highest levels of aggressive behavior (borderline to clinical levels) were reported in children characterized by high NE and low IC. These findings provide an extension of the research that has so far mainly been conducted in school-aged children and adolescents focusing on the broader construct of externalizing behavior or behavior problems, and using rather general assessments of effortful control (Eisenberg et al., 2001; Muris, Meesters, & Blijlevens, 2007; Valiente et al., 2003). Importantly, as opposed to some previous studies in early childhood (Belsky, Friedman, & Hsieh, 2001; Gartstein, Putnam, & Rothbart, 2012; Olson, Sameroff, Kerr, Lopez, & Wellman, 2005), we found that the interaction effect between NE and IC was consistent across the age range of 2-5 years. Although the behavioral representations of IC may develop across the preschool years, we conclude that cognitive self-regulation (IC), separately and in interaction with emotional self-regulation (NE), may already play a role in the development of aggressive behavior in children as young as two years old. It is important to note that the sample in this study was of relatively low-risk (with the children's parents having high educational levels and living in intact families), and it is therefore possible that our findings would be more pronounced in children with a higher-risk background.

Additional analyses with physical aggression (as opposed to aggressive behavior which includes also verbal and relational aggression) as outcome variable revealed a somewhat different pattern of results. Overall, there was a significant main effect of IC, but not of NE, on physical aggression. This indicates the importance of cognitive self-regulation in the development of physical aggression, whereas emotional self-regulation may be more predictive of other forms of aggressive behavior such as verbal or relational aggression. More detailed analyses revealed that the interaction between NE and IC in relation to physical aggression was present only in boys aged 4-5 years old, as opposed to 2-3 year old children and 4-5 year old girls, underlining the role of sex and age in the development of physical aggression, and indicating that cognitive self-regulation is especially important for older preschool boys with weaker emotional self-regulation. The absence of an interaction effect between IC and NE on physical aggression, as opposed to aggressive behavior measured in a broader form, for younger children and for girls may be explained in several ways. First, physical aggression is relatively normal in two-and three-year old boys and girls (Alink et al., 2006; NICHD Early Child Care Research Network, 2004). Individual differences in physical aggression and protective effects of IC may therefore become more apparent

in older children. Second, boys exhibit more physical aggression (Alink et al., 2006), whereas girls generally show more relational aggression (Ostrov & Keating, 2004), especially as they grow older (Hay, 2007). Third, girls generally have better effortful control skills (Kochanska, Murray, & Harlan, 2000). Because the CBCL Aggressive behavior scale (Achenbach & Rescorla, 2000) measures aggression in its broadest form, age and sex effects may not appear.

Aggressive behavior in early childhood: role of prenatal risk and physiological self-regulation

In **Chapter 4.1 and 4.2**, we examined how self-regulation at the physiological level (i.e., in terms of PNS and SNS functioning) in six-month-old infants, in interaction with prenatal risk, predicted aggressive behavior (specifically physical aggression) during toddlerhood. We were specifically interested in the interaction between the PNS and SNS as moderators of the effects of prenatal risk on physical aggression, as it is suggested that the effectiveness of physiological self-regulation depends on the coordination between these systems (Berntson, Cacioppo, & Quigley, 1991). PNS and SNS activity was measured at 6 months at baseline and in response to and during recovery from two well- established emotionally challenging tasks; the Still Face Paradigm (SFP) and the Car Seat (CS). Physical aggression was measured through maternal report at 20 months in Chapter 4.1. In Chapter 4.2, we extended the study in Chapter 4.1 by measuring physical aggression at 30 months (also through maternal report) and by investigating interactions between the PNS and SNS within dimensions (e.g. PNS baseline x SNS baseline etc.) and between dimensions (e.g. PNS baseline x SNS response, and PNS response x SNS recovery etc.). Further, in Chapter 4.2 we examined whether the interactive effects between prenatal risk and the ANS were specific for physical aggression as opposed to more broader forms of externalizing behavior problems (i.e. oppositional behavior problems) (Burt, 2012).

The findings described in Chapter 4.1 and 4.2, show significant main effects of prenatal risk on physical aggression in toddlerhood. However, as opposed to the significant associations between emotional and cognitive self-regulation and aggressive behavior in the preschool period (Chapter 2), infant physiological self-regulation was not robustly associated with physical aggression in toddlerhood. While studies in children and adolescents have consistently found main effects of autonomic nervous system (ANS) measures on aggressive and antisocial behavior (Van Goozen, Fairchild, Snoek, & Harold, 2007), our results align with theoretical frameworks suggesting that the complex association between physiology and behavior may be better understood as interactions with early adversity (Boyce & Ellis, 2005; El-Sheikh & Erath, 2011). It is possible that the association between individual difference factors, such as

physiological self-regulation, and aggressive behavior becomes stronger as children grow older, whereas the influence of the (early) environment behavioral development decreases.

The findings described in Chapter 4.1 and 4.2 were not unequivocal, but overall suggested that nonreciprocal activity of the PNS and SNS, specifically patterns of coactivation and coinhibition, in infancy were associated with higher levels of physical aggression in toddlerhood, but only in the context of higher levels of prenatal risk. On the other hand, coordinated activity of the PNS and SNS, specifically reciprocal PNS activation and reciprocal SNS activation, buffered the effects of prenatal risk on physical aggression.

As described in Chapter 4.1, prenatal cumulative risk predicted physical aggression at 20 months, but only in infants who exhibited higher levels of coactivation of the PNS and SNS in response to emotional challenge (i.e. increased activity of both the PNS and SNS) at six months of age, whereas prenatal cumulative risk was not associated with physical aggression for infants who exhibited reciprocal PNS activation, reciprocal SNS activation or coinhibition. In Chapter 4.2, we found that the effects of cumulative risk on physical aggression were particularly evident in infants who exhibited low baseline PNS activity and/or higher levels of coinhibition (i.e. decreased activity of both the PNS and SNS) and coactivation (i.e. increased activity of both the PNS and SNS) at baseline and/or in response to emotional challenge at six months of age.

Our findings converge with a previous study in young children linking reciprocal ANS activity, specifically reciprocal SNS activity, to better emotion regulation (Stifter, Dollar, & Cipriano, 2011), and extend literature demonstrating adversity x ANS interactions that was so far only conducted in school-aged children with RSA and SCL measures (El-Sheikh et al., 2009; Gordis, Feres, Olezeski, Rabkin, & Trickett, 2010). Coinhibition and coactivation of the PNS and SNS may yield an ambivalent physiological response in which one branch increases arousal whereas the other branch dampens arousal (Berntson et al., 1991), resulting in physiological under- or over arousal that may limit a child's ability to generate an adaptive self-regulated behavioral response under challenging environmental conditions. Over time, these patterns of coinhibition and coactivation may consolidate and promote aggressive behavior, especially in environments that tend to elicit these behaviors more often.

Although levels of physical aggression for infants exhibiting coactivation at 20 months were well above one standard deviation of the averages reported in same-aged children from a large community sample (Alink et al., 2006), our findings show that at 30 months, ANS patterns of coinhibition conferred a higher risk of physical aggression than coactivation. The group mean for infants exhibiting coinhibition was

more than one standard deviation above the average aggression level reported by Alink et al. (2006), whereas the group mean of infants exhibiting coactivation lay within one standard deviation of the mean. Possibly, patterns of coinhibition over time confer greater risk for physical aggression than coactivation. In fact, it is suggested that while coinhibition, with reduced activity within the PNS and SNS, is linked to aggressive and antisocial behavior at a later age in children and adolescents, coactivation may promote anxiety or high-anxious externalizing problems (Beauchaine, 2001; Beauchaine, Gatzke-Kopp, & Mead, 2007; El-Sheikh & Erath, 2011). However, to date there is no empirical evidence to support this hypothesis (El-Sheikh et al., 2009).

All of the moderating effects reported in Chapter 4.2 were specific for physical aggression, and not for oppositional behavior problems. Other studies have demonstrated similar specific findings. For example, Baker et al. (2013) reported significant correlations between skin conductance levels at age 1 and aggressive behavior at age 3, but not for nonaggressive externalizing behavior problems (i.e. difficult or hard-to-manage behavior). Further, Raine et al (1997) reported that low resting heart rate at age 3 was specifically associated with aggressive behavior at age 11, as opposed to non-aggressive antisocial behavior. This suggests a stronger biological basis for physical aggression, whereas oppositional behavior problems may be more environmentally determined (Burt, 2012).

We found no main effects of PNS and SNS recovery, nor any interaction effects between (cumulative prenatal risk and) PNS and SNS recovery on physical aggression at 20 and 30 months. This point will be discussed further in the next paragraph.

Effects of prenatal risk on physiological self-regulation in infancy

The findings described in Chapter 4.1 and 4.2 highlight the role of physiological self-regulation in infancy in determining the impact of prenatal risk on physical aggression in toddlerhood. However, evidence suggests that physiological self-regulation itself is also influenced by exposure to risk factors during the prenatal period (Propper & Holochwost, 2013). We addressed this issue in **Chapter 5**, in which we examined the association between prenatal risk and the ANS. Previous literature in infancy has focused mainly on the effects of prenatal risk (factors) on the developing PNS, and much less is known about the effects of prenatal risk on the SNS (Propper & Holochwost, 2013). To address this gap and to more fully understand the effects of prenatal risk on the developing ANS, we examined measures of both PNS and SNS activity in response to and during recovery from an emotionally challenging task, the SFP, in six-month-old infants.

Our findings showed increased heart rate and PNS withdrawal among high-risk infants, compared to low-risk infants. In line with previous studies (Conradt & Ablow, 2010; Haley & Stansbury, 2003), this difference became only apparent during the recovery phase of the SFP. Further, we found increased SNS activation during the recovery phase in high-risk infants compared to low-risk infants. Also, as the number of prenatal risk factors increased, infants in the high-risk group exhibited greater SNS activation. In additional analyses we tested for the potential differential effects of different types of risk factors on ANS activity during recovery. Our findings showed that the presence of maternal psychiatric problems during pregnancy, as opposed to prenatal exposure to maternal substance use or social adversity, was the strongest predictor of increased SNS activation in the high-risk group during recovery. This converges with previous literature linking maternal psychopathology during pregnancy to offspring aggression over and above the effects of other prenatal risk factors such as maternal smoking and sociodemographic factors (Hay et al., 2011; Hay, Pawlby, Waters, Perra, & Sharp, 2010).

Infants in the low-risk group exhibited a typical pattern of ANS reactivity when confronted with emotional challenge (see Mesman, Van IJzendoorn, & Bakermans-Kranenburg, 2009), with decreases in PNS activity and mild increases in SNS activity in response to challenge, and increases in PNS activity and decreases in SNS activity during recovery. However, infants in the high-risk group, on the other hand, showed increasing levels of physiological arousal as the SFP progressed. In other words, infants in the high-risk group continued to suppress PNS activity and mobilized sympathetic resources, even during the recovery phase. Moreover, increased heart rate during the recovery phase in high-risk infants was mediated both by PNS and SNS activity, indicating PNS withdrawal and mobilization of sympathetic resources when confronted with socio-emotional challenge.

Although activation of the SNS when confronted with environmental challenge may reflect an adaptive physiological response in the short term, frequent and chronic activation of the SNS taxes physiological systems in the form of bodily wear and tear resulting in greater risk for psychopathology (McEwen & Gianaros, 2010). Children born in high-risk families, suffering from early adversity both prenatally and postnatally, are likely to be exposed to risk factors frequently and continuously. Eventually, chronic activation of the SNS may alter the integrity of physiological regulatory systems and may eventually lead to attenuated ANS functioning as observed in other studies (Alkon et al., 2014).

How is it possible that we only found differences between low-risk and high-risk infants in the recovery phase given that we expected differences between these groups in response to stress? Perhaps the SFP is more challenging as it progresses for

infants who are less self-regulated than for infants who are better regulated. Well-regulated infants showed better PNS- and SNS regulation during recovery enabling them to use their environment (the mother) to recover from emotional challenge. This is supported by the findings from additional analyses in which we found that low-risk infants looked more often to their mother and cried less, an effect that was mediated by increases in PNS activity and decreases in SNS activity during recovery. Physiological arousal increased across the SFP in high-risk infants as evident by increased PNS withdrawal and SNS activity during recovery which in turn were associated with less attentional engagement with the mother and more distress during the recovery phase, indicating that these infants were less able to use their mother to decrease their physiological arousal.

Contrary to our expectations, we did not find any main or interaction effects for physiological self-regulation during recovery from emotional challenge on the development of physical aggression (as described in Chapter 4.1 and 4.2). This was even more unexpected given the effects of prenatal risk on ANS functioning that were present mainly during recovery from emotional challenge (Chapter 5). Baseline ANS levels and ANS reactivity to emotional challenge have been examined in numerous studies. However, although recognized as an important parameter of ANS functioning (Porges, 2007), ANS recovery following emotional challenge is surprisingly understudied (El-Sheikh & Erath, 2011). One study in adolescents linked blunted SNS recovery to more antisocial behavior (Sijtsema, Van Roon, Groot, & Riese, 2015). Another study in 4-7 year old children showed that lower vagal recovery was associated with maladaptive emotional regulation strategies to frustration (i.e. a negative focus on delay) (Santucci et al., 2008). To our knowledge, no other studies have examined the association between ANS activity during recovery and outcome in infancy or childhood. It is possible that methodological issues explain the absence of effects for physiological recovery on the development of physical aggression. First, in Chapter 4.1 and 4.2, we examined average ANS recovery from challenge across two different tasks, the SFP and the CS task, while in Chapter 5, we examined ANS recovery from challenge only during the SFP. Second, although there were significant correlations between ANS recovery on the SFP and the CS, the level of SNS recovery for the CS was different than for the SFP. Whereas on average, infants showed decreases in SNS activity from challenge to recovery on the CS, they showed hardly any difference in SNS activity from challenge to recovery on the SFP, indicating that on average infants did not recover from the SFP challenge.

Strengths and limitations

Although the studies described in this dissertation have important strengths, including the use of a longitudinal design, the comprehensive assessments of ANS functioning (i.e. baseline, response and recovery, as well as PNS and SNS measures and their interaction), the focus on infancy, and two different emotional challenge tasks, there are also limitations that should be discussed.

First, we measured infant ANS functioning at one time point, at six months of age, but we know relatively little about the stability of PNS and SNS measures (i.e. RSA and PEP) across the first years of life. There is some evidence for moderate stability of RSA and PEP during resting and challenging conditions from 6 to 60 months (Alkon, Boyce, Davis, & Eskenazi, 2011; Alkon et al., 2006), however these studies have reported low stability for reactivity measures (representing the difference between resting and challenging conditions; Alkon et al., 2011; Alkon et al., 2006). Importantly, repeated and chronic exposure to higher levels of stress during postnatal development may have consequences for the stability of ANS measures (Alkon et al., 2014; Conradt et al., 2016). Further, as opposed to available research on the stability of individual RSA and PEP measures, there have been no studies that have investigated the stability of profiles of different ANS measures (i.e. reciprocal SNS activation, reciprocal PNS activation, coinhibition, coactivation) across (early) childhood. Data from two cross-sectional studies demonstrated developmental differences in ANS patterns in early childhood compared to late childhood and adolescence, with patterns of coinhibition being more prevalent in younger children (3-8-year-olds; Alkon et al., 2003) and reciprocal SNS activation more prevalent in older children and adolescents (8-10- and 15-17-year-olds; Salomon, Matthews, & Allen, 2000). This indicates that during the first few years of life the ANS is not yet fully developed and that developmental shifts are possible. Does this mean that measures of early ANS functioning are only predictive of behavioral problems in toddlerhood? Not necessarily. For example, there is evidence that ANS functioning measured early in development is predictive of later behavioral outcome even if there is no relation between that later behavioral outcome and concurrent ANS functioning. One study found that children with lower decreases in RSA during challenge (reflecting less PNS suppression) at age 2 continued to show more aggressive behavior during the preschool years, even if they were able to suppress RSA during challenge as preschoolers, indicating that deficiencies in early physiological regulation may have far reaching effects on development (Calkins, 2009). Future longitudinal investigations should examine the stability of PNS and SNS measures, and specifically the patterns of coinhibition and coactivation, across development in relation to behavioral

outcomes, and as moderators of the effects of early adversity on behavioral outcome in childhood and adolescence.

Second, our measure of toddler aggressive behavior involved maternal report. Parental reports of child functioning include a subjective component potentially reflecting biases associated with differences in parental personality, emotional status, relationship with the child, knowledge of child behavior and inconsistent interpretation of items (Kagan, 1994). Further, since prenatal risk was positively associated with maternal ratings of aggressive behavior, it is possible that mothers with higher levels of cumulative risk rated their child's behavior as more negative. Therefore, future studies should incorporate behavioral ratings and multiple informants to measure aggressive behavior.

Third, the level of cumulative prenatal risk in our sample was low, having on average 1.6 risk factors, and most mothers in the high-risk group had only one or two risk factors, which is not high compared to some high-risk and clinical samples used in other studies (e.g. Lester et al., 2002). Although nearly all mothers in the high-risk group had a psychiatric diagnosis or used substances during pregnancy, it is important to emphasize that the relatively low level of cumulative risk within the high-risk group may limit the generalizability of our results to high-risk or clinical populations. As noted by Beauchaine (2009) and Raine (2002), it is possible that different mechanisms of environmental and physiological risk and resilience operate in clinical populations. Nevertheless, our sample represents a realistic reflection of the general population and the effect sizes in this study are therefore more representative of the relations between prenatal risk, self-regulation and aggressive behavior in the general population.

Recommendations for future research

The results described in Chapter 4.1 and 4.2 underline the importance of studying patterns of stress reactivity across systems in infancy, using specific measures of PNS and SNS functioning, and their interplay with prenatal risk. However, it should be noted that only a small portion of variance in physical aggression in toddlerhood was explained by ANS functioning. Even in combination with prenatal risk, approximately 80% of the variance in physical aggression was left unexplained. It is therefore imperative for future research to include more factors to fully capture the development of aggressive behavior. Interestingly studies that investigate the ANS in conjunction with the other major stress regulation system, hypothalamic-pituitary-adrenal (HPA) axis, show that the interaction of the ANS and HPA axis predict behavioral problems better than measures of ANS or HPA axis alone (Blair, Berry, Mills-Koonce, Granger, & Invest, 2013; Chen, Raine, Soyfer, & Granger, 2015; Nederhof, Marceau, Shirtcliff, Hastings, & Oldehinkel, 2015). Moreover, future

studies should address the relations and the interactions between the different levels of self-regulation described in this dissertation across (early) childhood in the development of aggressive behavior, as it is proposed that more intentional and self-conscious (emotional and cognitive) self-regulation, which emerges during the early preschool years, depends on and may be constrained by physiological regulation of arousal earlier in life (Calkins & Keane, 2009).

We had a clear rationale for examining prenatal risk as a cumulative (Chapter 4.1, 4.2, and 5) or dichotomous (Chapter 5) variable. First of all, risk factors often co-occur, which makes it difficult to separate the effect of one adverse experience from another on ANS development and behavioral outcome. And second, because cumulative risk models are considered to be more powerful than single risk models in predicting problem behavior (Appleyard, Egeland, van Dulmen, & Sroufe, 2005). However, although we examined differential relations between different types of maternal risk factors (e.g. psychiatric diagnosis, differential substance use, social adversity) on the ANS in additional analyses (see Chapter 5), we did not address the potential differential effects of timing (prenatal versus postnatal) of maternal risk factors on the ANS, as well as potential interactive effects between different types of maternal risk factors and the ANS on later behavioral outcome. Support for our approach was found in a recent review (Propper & Holochwost, 2013) that showed that a broad range of specific prenatal and postnatal risk factors were associated with an altered pattern of ANS (re)activity. However, a recent longitudinal study (Alkon et al., 2014) reported significant differential effects of different maternal risk factors during the prenatal period and ANS trajectories from 6 to 60 months of age. Specifically, whereas prenatal exposure to limited social support was found to be associated with attenuated heart rate trajectories, prenatal exposure to socioeconomic adversity was associated with dampened PEP trajectories. Further, in another study (Waters, Boyce, Eskenazi, & Alkon, 2016) maternal chronic depression and overcrowded housing were examined as moderators of the relation between infant ANS functioning and externalizing behavior at age 10. Significant moderating effects of maternal chronic depression were found, but not for overcrowded housing. While prenatal and postnatal environments are often correlated, there is evidence that prenatal and postnatal exposure to risk may involve distinct causal pathways (Hickey, Suess, Newlin, Spurgeon, & Porges, 1995). For example, the comparison of PNS reactivity patterns for children exposed to drugs in utero to those of children whose mothers began using drugs after they were born, shows that prenatal use of drugs is associated with distinctive, adverse patterns of PNS reactivity that suggest that risk exposure during fetal development goes above and beyond risk exposure during infant development (Hickey et al., 1995). In sum, future studies adopting a different

analytical approach investigating the potential independent effects of different types and timing of maternal risk factors on ANS development and its interactive effects with ANS functioning in infancy on behavioral outcome could contribute to a better understanding of the individual differences in biological sensitivity to maternal risk factors and the mechanisms by which this biological sensitivity influences behavioral development. This type of research could also have important implications for future studies on the effect of early adversity on the developing ANS and subsequent (early childhood) aggressive behavior as well as inform intervention (research).

Conclusion

The studies described in this dissertation focused on the role of prenatal risk and self-regulation in infancy and the preschool period in the development of aggressive behavior, and the influence of prenatal risk on self-regulation in infancy. Three main conclusions can be drawn. First, aggressive behavior in the preschool period can be predicted from the interplay between self-regulation at the emotional and cognitive level (Chapter 2). Children who experience difficulties in regulating negative emotions such as anger, sadness and fear, are more prone to aggressive behavior if they have reduced inhibitory control. However, this effect is reduced if they exhibit better inhibitory control. Interestingly, we found moderating effects of age and sex for physical aggression that provide more insight into the specificity of the interaction effects of emotional and cognitive self-regulation for aggressive behavior in preschoolers. Practical implications of these findings suggest that interventions aimed at reducing aggressive behavior in toddlers and preschool children should focus on improving inhibitory control, especially in children with high levels of negative emotionality and boys with high negative emotionality who exhibit physical aggression.

Second, physiological self-regulation in infancy, as reflected by the coordination between the PNS and SNS, determines the impact of prenatal risk on the development of physical aggression in toddlerhood (Chapter 4.1 and 4.2). Infants characterized by low (re)activity of both the PNS and SNS (coinhibition) or high (re)activity of both the PNS and SNS (coactivation), are more vulnerable to the negative effects of prenatal risk (i.e. show higher levels of physical aggression), compared to infants characterized by reciprocal (re)activity of the PNS and SNS. Notably, in the context of higher prenatal risk, children characterized by reciprocal (re)activity of the PNS and SNS exhibit levels of physical aggression similar to those of children who were not exposed to prenatal risk. It should be noted, however, that our measures of physiological self-regulation were only predictive of physical aggression in combination with prenatal risk. It is therefore important to recognize

that vulnerability to aggressive behavior relies on the combination of the specific ANS profile and (prenatal) risk factors (Moore, 2009), and that these physiological measures should be examined together with the developmental context in which children grow up.

Our study is the first to examine the coordination between the PNS and SNS in infancy in relation to later behavioral outcome. The results highlight the need to consider measures of both PNS and SNS functioning and their interaction with the environmental context in order to elucidate its role in developmental processes leading to early aggression. However, caution is warranted in suggesting practical implications of our findings and future research is necessary to replicate our findings. Nevertheless, our findings show that we may be able to identify children who run the highest risk of developing aggressive behavior in toddlerhood based on assessment of their prenatal risk and their ANS profile in infancy. Maturation of the ANS during infancy lays the foundation for adaptive emotional and cognitive self-regulation later in development (Porges & Furman, 2011). Further, the ANS is rapidly developing during the first year (Porges, 2003), which is associated with increased susceptibility to environmental influences, and therefore also entails increased opportunities for early intervention programs focusing on mitigation of early environmental adverse effects (Beauchaine, Neuhaus, Brenner, & Gatzke-Kopp, 2008). A number of studies have shown that the ANS is sensitive to the early postnatal rearing environment. For example, sensitive parenting has been associated with more effective PNS regulation (Conradt & Ablow, 2010; Moore et al., 2009). Infants with less effective physiological self-regulation may therefore benefit from early prevention or intervention programs aimed at promoting maternal sensitivity.

Third, physiological self-regulation in infancy was found to be associated with prenatal risk (Chapter 5). Infants exposed to risk factors, such as maternal psychiatric problems, substance (ab)use, and social adversity during the prenatal period, showed increased PNS withdrawal and SNS activation during recovery from emotional challenge, which is indicative of impaired physiological self-regulation and might contribute to less effective emotional self-regulation. These findings underscore the importance of identifying women with a high-risk profile during pregnancy in order to offer prevention and intervention programs aimed at improving prenatal and postnatal circumstances. Intensive home-visiting programs have been found to positively influence maternal and child development (Avellar & Supplee, 2013; Mejdoubi et al., 2015; Olds, Sadler, & Kitzman, 2007; Ordway et al., 2014; Peacock, Konrad, Watson, Nickel, & Muhajarine, 2013; Sweet & Appelbaum, 2004). However, it is currently unknown whether these programs also affect infant physiological self-regulation. Examination of the infant ANS functioning as part of early intervention research may

indicate not only whether the intervention has an effect on this most basic self-regulatory mechanism, but also consequently affect the later development of emotional and cognitive self-regulation.

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NEDERLANDSE SAMENVATTING

Summary in Dutch

Kinderen die op jonge leeftijd een hoge mate van agressief gedrag laten zien hebben een verhoogd risico op problemen later in de ontwikkeling. Een hoge mate van agressief gedrag in de peuter- en kleuterleeftijd is in verband gebracht met lagere schoolprestaties, conflicten en ruzies met familieleden en leeftijdgenoten, alcohol- en drugsmisbruik, betrokkenheid bij ongelukken, delinquent gedrag, depressie, suïcide, geweld tegen de partner en verwaarlozing en mishandeling in de opvoeding (Campbell et al., 2006; Farrington, 1994; Fergusson & Horwood, 1998; Kokko & Pulkkinen, 2000; Nagin & Tremblay, 1999; Serbin et al., 1998; Stattin & Magnusson, 1989). Buitensporige agressie in de vroege kindertijd kan een voorloper zijn van antisociaal gedrag en criminaliteit in de adolescentie en volwassenheid. Uit onderzoek is gebleken dat de meerderheid van de hoog agressieve kinderen en adolescenten al tot de meest agressieve groep behoorden in de peutertijd (Coté, Vaillancourt, LeBlanc, Nagin, & Tremblay, 2006; NICHD & Early Child Care Research Network, 2004). Persistent agressief gedrag in de kinderleeftijd en adolescentie is moeilijk te behandelen en huidige psychosociale interventie programma's behalen slechts medium effect groottes (Fossum, Handegard, Martinussen, & Morch, 2008; Smeets et al., 2015). Om problemen later in de ontwikkeling te voorkomen is het belangrijk om kinderen met een risico op ernstig agressief gedrag al zo vroeg mogelijk in de ontwikkeling te identificeren zodat er tijdig gepaste (preventieve) behandeling ingezet kan worden.

Prenatale risicofactoren bij moeder die in verband zijn gebracht met de ontwikkeling van agressief gedrag in de kindertijd zijn onder andere, lage sociaaleconomische status, laag opleidingsniveau, tienermoederschap (Coté et al., 2006; NICHD & Early Child Care Research Network, 2004; Tremblay et al., 2004), roken tijdens de zwangerschap (Huijbregts, Seguin, Zoccolillo, Boivin, & Tremblay, 2008; Tremblay et al., 2004), antisociaal gedrag (Hay, Pawlby, Waters, Perra, & Sharp, 2010; Tremblay et al., 2004), en psychische problematiek zoals angst en depressie (Hay et al., 2011; O'Connor, Heron, Golding, Beveridge, & Glover, 2002). Daarnaast zijn een stressvolle, chaotische thuissituatie en insensitief ouderschap in de eerste levensjaren in verband gebracht met de ontwikkeling van agressie in de kinderleeftijd (Tremblay et al., 2004). Echter, de mate waarin deze risicofactoren de ontwikkeling van het kind beïnvloeden hangt voor een deel af van de eigenschappen van het individuele kind (Boyce & Ellis, 2005; Calkins & Keane, 2009; El-Sheikh & Erath, 2011). Een van deze eigenschappen is het vermogen tot zelfregulatie.

Zelfregulatie is het vermogen om de eigen fysiologische, emotionele en cognitieve processen te controleren en te sturen (Baumeister & Vohs, 2004). Een tekort in zelfregulatie speelt daarmee een belangrijke rol in de ontwikkeling van agressief gedrag (Calkins & Keane, 2009). Zelfregulatie is al zichtbaar in de babytijd in de vorm van vrij basale en automatische regulatie van fysiologische processen en

ontwikkelt zich geleidelijk vanaf het tweede levensjaar, ondersteund door deze vroege fysiologische regulatie, tot meer bewuste en intentionele regulatie van emotie, cognitie en gedrag (Ochsner & Gross, 2004). Hoewel vroege vormen van zelfregulatie mogelijk de effecten van omgevingsrisicofactoren op de ontwikkeling van agressief gedrag bepalen, wordt verondersteld dat de ontwikkeling van zelfregulatie zelf ook beïnvloed wordt door omgevingsrisicofactoren tijdens de prenatale en vroege postnatale ontwikkeling (Dawson et al., 2000; Van Goozen, Fairchild, Snoek, & Harold, 2007).

In de babytijd zijn fysiologische systemen die onderliggend zijn aan het vermogen tot zelfregulatie sterk in ontwikkeling en daardoor in grotere mate beïnvloedbaar door (negatieve) invloeden vanuit de omgeving (Dawson et al., 2000). Daarnaast maken hersenstructuren die onderliggend zijn aan cognitieve zelfregulatie een sterke ontwikkeling door in de vroege kindertijd. Om kinderen met een verhoogd risico op het ontwikkelen van agressief gedrag al zo vroeg mogelijk in de ontwikkeling te identificeren, nog voordat ontwikkelingstrajecten die leiden tot een hoge mate van agressie zijn ingezet, is het van belang om onderzoek te doen naar voorspellers en processen die samenhangen met vroege vormen van agressief gedrag. Vooralsnog is het meeste onderzoek uitgevoerd in de kinderleeftijd en adolescentie en weten we veel minder over deze factoren in de babytijd en vroege kindertijd. In dit proefschrift zijn drie vragen onderzocht, namelijk:

1. Op welke wijze voorspellen emotionele en cognitieve zelfregulatie agressief gedrag in de vroege kinderleeftijd?
2. In hoeverre wordt de impact van blootstelling aan risicofactoren tijdens de zwangerschap op de ontwikkeling van agressief gedrag in de vroege kinderleeftijd bepaald door fysiologische zelfregulatie?
3. In hoeverre hangen prenatale risicofactoren samen met de ontwikkeling van fysiologische zelfregulatie?

Voor de beantwoording van de eerste vraag is gebruik gemaakt van een groep van 855 kinderen in de voorschoolse leeftijd (2-5 jaar oud), geworven via kinderdagverblijven, peuterspeelzalen en kleuterklassen in Nederland. Voor de beantwoording van vraag twee en drie is gebruik gemaakt van data afkomstig van een subgroep uit de Mother-Infant NeuroDevelopment Study (MINDS) – Leiden (zie hoofdstuk 3). Dit is een longitudinaal onderzoek waarin neurobiologische en neurocognitieve voorspellers voor de ontwikkeling van vroege gedragsproblemen onderzocht worden. Het onderzoek bestaat uit zes metingen startend tijdens de zwangerschap tot het kind 42 maanden oud is. In totaal hebben 275 moeders en hun eerstgeboren kind deelgenomen. Tijdens de zwangerschap werden aanstaande

moeders gescreend op de aanwezigheid van risicofactoren voor problemen in de opvoeding en (gedrags-)ontwikkeling van het kind. Onder deze prenatale risicofactoren vallen de aanwezigheid van psychiatrische problematiek, middelen gebruik en sociaal-demografische risicofactoren. Prenataal risico is zowel cumulatief (hoofdstuk 4.1, 4.2 en 5) als dichotoom (hoog- versus laag-risicogroep, zie hoofdstuk 5) onderzocht.

Agressief gedrag in de vroege kindertijd: De rol van emotionele en cognitieve zelfregulatie

Kinderen die een hoge mate van agressief gedrag laten zien worden vaak gekenmerkt door een hoge mate van negatieve emotionaliteit (Calkins & Fox, 2002; Eisenberg et al., 2009), ofwel de neiging om met een hoge mate van boosheid, irritatie, angst en verdriet op frustratie vanuit de omgeving te reageren (Rothbart & Bates, 2006). Hoewel kinderen met een hoge mate van negatieve emotionaliteit kwetsbaarder zijn voor de ontwikkeling van agressief gedrag, kan adequaat ontwikkelde cognitieve zelfregulatie, ook wel *effortful control* genoemd, een beschermende rol spelen (Muris & Ollendick, 2005). Met name het vermogen tot inhibitie, het kunnen afstoppen van een dominante respons om een subdominante respons uit te voeren (Rothbart & Bates, 2006), is in belangrijke mate gerelateerd aan de ontwikkeling van agressief gedrag (Raaijmakers et al., 2008; Sterzer & Stadler, 2009). Zowel negatieve emotionaliteit als inhibitie spelen, afzonderlijk van elkaar, een belangrijke rol in de ontwikkeling van agressief gedrag. Echter, het risico op de ontwikkeling van agressief gedrag kan mogelijk beter voorspeld worden door de interactie tussen beide constructen in relatie tot agressief gedrag te onderzoeken (Muris & Meesters, 2005). Het vermogen tot inhibitie ontwikkelt zich vanaf het tweede levensjaar (Kochanska, Murray, & Harlan, 2000; Rothbart, Ellis, Rueda, & Posner, 2003) en maakt daarna een sterke ontwikkeling door in de vroege kinderleeftijd (Eisenberg, Spinrad, & Eggum, 2010). Het is echter onbekend in hoeverre deze vaardigheden in de vroege kinderleeftijd al sterk genoeg ontwikkeld zijn om de negatieve effecten van negatieve emotionaliteit op de ontwikkeling van agressief gedrag te verminderen.

In hoofdstuk 2 van dit proefschrift hebben we negatieve emotionaliteit, inhibitie, en agressief gedrag bij kinderen in de voorschoolse leeftijd (2-5 jaar) gemeten door middel van vragenlijsten ingevuld door ouders. In overeenstemming met het model van Muris en Meesters (2005), waarin beschreven wordt dat het risico op externaliserende gedragsproblemen (en psychopathologie in het algemeen) wordt bepaald door de interactie tussen emotionele en cognitieve zelfregulatie, werd gevonden dat de samenhang tussen negatieve emotionaliteit en agressief gedrag minder sterk was naarmate er minder deficiënties in inhibitie aanwezig waren, terwijl

de samenhang tussen negatieve emotionaliteit en agressief gedrag juist sterker was als er meer deficiënties in inhibitie aanwezig waren. De hoogste mate van agressief gedrag (in de borderline tot klinische range) werd gevonden bij kinderen met zowel een hoge mate van negatieve emotionaliteit als een lage inhibitie (1,5 *SD* beneden gemiddeld op de Behavior Rating Inventory of Executive Function - Preschool version [BRIEF-P]; Gioia, Espy, & Isquith, 2005). Deze resultaten komen overeen met onderzoek dat tot dusver voornamelijk heeft plaatsgevonden onder schoolgaande kinderen en adolescenten en gericht was op de bredere constructen (externaliserende) gedragsproblemen en *effortful control* (Eisenberg et al., 2001; Muris, Meesters, & Blijlevens, 2007; Valiente et al., 2003). Echter, in tegenstelling tot sommige andere studies in de vroege kindertijd die voornamelijk effecten vonden onder oudere kinderne in de voorschoolse leeftijd (Belsky, Friedman, & Hsieh, 2001; Gartstein, Putnam, & Rothbart, 2012; Olson, Sameroff, Kerr, Lopez, & Wellman, 2005), vonden wij dat het interactie effect tussen negatieve emotionaliteit en inhibitie aanwezig was over de gehele voorschoolse leeftijd (2-5 jaar). Hoewel inhibitie gemeten op gedragsniveau zich kan ontwikkelen tijdens de voorschoolse periode, wordt op basis van deze resultaten geconcludeerd dat, al vanaf de leeftijd van twee jaar, cognitieve zelfregulatie, zowel afzonderlijk als in interactie met emotionele zelfregulatie, een rol speelt in de ontwikkeling van agressief gedrag.

Hoewel negatieve emotionaliteit en inhibitie, zowel elk afzonderlijk als in interactie, voorspellend waren voor agressief gedrag in de vroege kindertijd, kwam in aanvullende analyses naar de samenhang met fysieke agressie (in tegenstelling tot agressief gedrag waarin ook verbale en relationele agressie opgenomen is) naar voren dat alleen inhibitie, en niet negatieve emotionaliteit, gerelateerd is aan fysieke agressie en dat de interactie tussen negatieve emotionaliteit en inhibitie alleen aanwezig is voor 4- en 5-jarige jongens (in tegenstelling tot 2- en 3-jarige kinderen en 4- en 5-jarige meisjes). De aanwezigheid van specifieke (interactie-) effecten met fysieke agressie onderstrepen het belang van cognitieve zelfregulatie voor de ontwikkeling van fysieke agressie, terwijl emotionele zelfregulatie mogelijk meer gerelateerd is aan andere vormen van agressief gedrag zoals verbale of relationele agressie. De gevonden sekse- en leeftijdsverschillen geven aan dat cognitieve zelfregulatie met name een belangrijke rol speelt in relatie tot fysieke agressie onder 'oudere' jongens in de voorschoolse leeftijd met een zwakkere emotionele zelfregulatie. Het ontbreken van de invloed van de interactie tussen negatieve emotionaliteit en inhibitie in relatie tot fysieke agressie bij jongere kinderen en meisjes kan op verschillende manieren verklaard worden. Ten eerste is fysieke agressie relatief normaal bij twee- en driejarigen (Alink et al., 2006; NICHD & Early Child Care Research Network, 2004). Individuele verschillen in fysieke agressie en de beschermende effecten van inhibitie komen mogelijk pas naar

voren naarmate kinderen ouder worden. Ten tweede laten jongens meer fysieke agressie zien (Alink et al., 2006), terwijl meisjes over het algemeen meer relationele agressie (bijvoorbeeld andere kinderen buitensluiten) laten zien (Ostrov & Keating, 2004), met name als ze ouder worden (Hay, 2007). Ten derde beschikken meisjes in het algemeen over betere *effortful control* (Kochanska, Murray, & Harlan, 2000). Omdat de CBCL Aggressive behavior schaal (Achenbach & Rescorla, 2000) agressief gedrag in de brede vorm meet, komen deze leeftijds- en geslachtsverschillen mogelijk niet naar voren.

Agressief gedrag in de vroege kindertijd: De rol van prenatale risicofactoren en fysiologische zelfregulatie

Een groot aantal studies onder kinderen, adolescenten en volwassenen heeft laten zien dat problemen in fysiologische zelfregulatie, specifiek als gevolg van het functioneren van het autonome zenuwstelsel (ANS), gerelateerd zijn aan agressief en externaliserend probleem gedrag (Beauchaine, Gatzke-Kopp, & Mead, 2007; Van Goozen et al., 2007). Het ANS is opgebouwd uit het sympathisch (SNS) en het parasympathisch (PNS) zenuwstelsel. In reactie op stress initieert het SNS de zogenaamde 'fight/flight' reactie door de hartslag en ademhaling te verhogen. Het lichaam wordt zo gemobiliseerd om in te kunnen spelen op gevaren vanuit de omgeving. Het PNS heeft een remmende werking op het SNS en heeft als taak om homeostase te bewaren en herstel te bereiken na stress door onder andere de hartslag en ademhaling te verlagen.

Hoewel regulatie door het ANS gerelateerd lijkt te zijn aan (de ontwikkeling van) agressief gedag, zijn de resultaten van voorgaande onderzoeken complex en inconsistent. Dit heeft waarschijnlijk te maken met een aantal factoren. Ten eerste lijken er verschillen te zijn tussen kinderen uit niet-klinische en klinische populaties (Beauchaine, 2009). In niet-klinische populaties is een sterkere afname in PNS activiteit in reactie op stress stress gerelateerd aan minder externaliserende gedragsproblemen, terwijl een sterkere afname in PNS activiteit ook gevonden wordt bij kinderen met klinisch significante externaliserende gedragsproblemen. Daarnaast suggereren verschillende theoretische modellen dat het effect van individuele verschillen in het functioneren van het ANS op gedrag niet direct zichtbaar zijn, maar in interactie met omgevingsrisicofactoren tot effect leiden (Boyce & Ellis, 2005; El-Sheikh & Erath, 2011). Er zijn echter maar enkele studies die de interactie tussen omgevingsrisicofactoren en het functioneren van het ANS in relatie tot agressief of (externaliserend) probleemgedrag hebben onderzocht in de vroege kindertijd en deze studies hebben zich uitsluitend gericht op de modererende rol van het PNS (Conradt et al., 2016; Conradt, Measelle, & Ablow, 2013; Eisenberg et al., 2012).

De meerderheid van de onderzoeken naar het functioneren van het ANS bij jonge kinderen heeft zich specifiek gericht op PNS activiteit of hartslag als globale maat voor ANS activiteit, zonder metingen van het SNS (Propper & Holochwost, 2013). Echter, een adaptieve ANS respons op een (emotioneel) stressvolle of uitdagende situatie wordt bepaald door het samenspel tussen zowel het PNS als het SNS. Om de link tussen het functioneren van het ANS en gedrag beter te begrijpen, is het daarom belangrijk zowel het PNS als het SNS te onderzoeken (El-Sheikh & Erath, 2011; Quas et al., 2014). Volgens het 'autonomic space model' van Berntson, Cacioppo en Quigley (199) zijn er grofweg vier profielen van PNS en SNS activiteit te onderscheiden. Wederkerige ANS activatie, waarbij het PNS en SNS in tegenovergestelde richting geactiveerd zijn, met verhoogde activiteit in het ene systeem en verlaagde activiteit in het andere system, reprensenteert een gecoordineerde respons op prikkels vanuit de omgeving. Wederkerige ANS activatie wordt als normatief beschouwd (Alkon et al., 2011; Salomon, Matthews, & Allen, 2000) en is gerelateerd aan betere emotieregulatie in jonge kinderen (Stifter, Dollar, & Cipriano, 2011). Niet-wederkerige activatie, met verlaagde of verhoogde activatie van zowel het PNS als het SNS tegelijkertijd, komt echter ook voor (Berntson et al., 1991). Verlaagde PNS en SNS activatie (*coinhibitie*) of verhoogde PNS en SNS activatie (*coactivatie*) geeft aan dat ofwel het PNS ofwel het SNS er niet in slaagt een adaptieve stressreactie te genereren (Porges, 2007). Recent onderzoek in schoolgaande kinderen heeft laten zien dat coinhibitie en coactivatie, in tegenstelling tot wederkerige activatie van het PNS en SNS (*wederkerige SNS activatie* en *wederkerige PNS activatie*), samenhangen met agressief en externaliserend probleemgedrag bij kinderen die blootgesteld zijn aan omgevingsrisicofactoren zoals ouderlijke ruzies (El-Sheikh et al., 2009) en kindermishandeling (Gordis, Feres, Olezeski, Rabkin, & Trickett, 2010).

De relatie tussen het functioneren van het ANS en gedragsuitkomsten in de baby- en vroege kinderleeftijd verschilt mogelijk van latere leeftijden (Beauchaine et al., 2007). Daarnaast ontwikkelen patronen van PNS en SNS activatie zich over de vroege kindertijd (Alkon et al., 2011; Alkon et al., 2003). Dit onderstreept het belang van het onderzoeken van de interactie tussen het PNS en SNS in de babytijd en het onderzoeken van welke patronen van PNS en SNS activatie in deze periode kunnen samengaan met een verhoogde gevoeligheid voor negatieve omgevingsinvloeden.

In hoofdstuk 4.1 en 4.2 hebben we onderzocht in hoeverre vroege zelfregulatie op fysiologisch niveau, specifiek regulatie van het PNS en SNS, in interactie met prenatale risicofactoren voorspellend is voor agressief gedrag (specifiek fysieke agressie) in de peutertijd. PNS en SNS activiteit werden gemeten op de leeftijd van zes maanden tijdens rust (baseline) en in respons op en tijdens herstel van twee stresstaken; een sociale stresstaak waarbij de moeder na een periode van spel niet meer

op haar kind mag reageren (Still Face Paradigma [SFP]; Tronick, Als, Adamson, Wise, & Brazelton, 1978) en een frustratietaak waarbij het kind gedurende korte tijd vastzit in een autostoeltje zonder enige vorm van afleiding (Car seat [CS]; Goldsmith & Rothbart, 1999a). Fysieke agressie in het dagelijks leven werd gemeten middels een vragenlijst ingevuld door moeder op 20 maanden (hoofdstuk 4.1) en op 30 maanden (hoofdstuk 4.2).

De resultaten beschreven in hoofdstuk 4.1 en 4.2 laten zien dat op de leeftijd van zes maanden, niet-wederkerige activatie van het PNS en SNS, specifiek coinhibitie en coactivatie, gerelateerd was aan een hogere mate van fysieke agressie in de peutertijd, maar alleen bij kinderen die blootgesteld waren aan risicofactoren tijdens de zwangerschap. Blootstelling aan prenatale risicofactoren en fysieke agressie was niet gerelateerd bij kinderen die wederkerige activatie van het PNS en SNS, specifiek wederkerige PNS activatie en wederkerige SNS activatie, lieten zien. Deze resultaten zijn in overeenstemming met eerdere onderzoeken bij schoolgaande kinderen (El-Sheikh et al., 2009; Gordis, et al., 2010) en geven aan dat coinhibitie en coactivatie van het PNS en SNS mogeijk een ambivalente fysiologische respons generen waarin de ene tak van het ANS de fysiologische *arousal* verhoogt terwijl tegelijkertijd de andere tak de fysiologische *arousal* verlaagt (Berntson et al., 1991). Deze ambivalente fysiologische respons is mogelijk van invloed op het vermogen tot (emotionele en cognitieve) zelfregulatie en maakt het voor het kind moeilijker om een adaptieve reactie te geven, met name tijdens stressvolle situaties. Het is mogelijk dat met de tijd deze patronen van coinhibitie en coactivatie consolideren en de neiging tot agressief gedrag vergroten, met name in een hoog-risico omgeving waar dit gedrag vaker uitgelokt wordt.

Hoewel de aanwezigheid van een hoger aantal risicofactoren tijdens de zwangerschap, naast fysieke agressie, tevens voorspellend was voor meer oppositioneel opstandige gedragsproblemen (o.a. niet willen luisteren en koppig zijn), werd deze relatie niet gemodereerd door het PNS, SNS of de interactie tussen beide systemen. Deze resultaten suggereren een mogelijk sterkere biologische oorsprong voor de ontwikkeling van (fysieke) agressie, terwijl de ontwikkeling van oppositioneel opstandige gedragsproblemen mogelijk meer omgevingsbepaald zijn (Burt, 2012). Onze resultaten zijn in overeenstemming met andere onderzoeken. Zo vonden Baker, Shelton, Baibazarova, Hay en van Goozen (2013) dat lage SNS activiteit gemeten door middel van huidgeleiding op éénjarige leeftijd samenhing met een hogere mate van agressief gedrag op driejarige leeftijd, maar vonden zij geen relatie tussen SNS activiteit en niet-agressieve externaliserene gedragsproblemen. Verder vonden Raine, Venables en Mednick (1997) dat een lage hartslag tijdens rust op driejarige leeftijd

voorspellend was voor agressief gedrag op 11-jarige leeftijd, maar niet voor niet-agressieve antisociale gedragsproblemen.

Tot slot vonden we geen (directe- en interactie-) effecten van PNS en SNS activiteit tijdens herstel van stress op de fysieke agressie op 20 en 30 maanden (zie discussie in de volgende paragraaf).

Effecten van prenatale risicofactoren op fysiologische zelfregulatie

Fysiologische systemen die onderliggend zijn aan het vermogen tot zelfregulatie zijn met name kwetsbaar voor negatieve invloeden vanuit de omgeving gedurende de perinatale periode (Dawson et al., 2000). Een mogelijke verklaring hiervoor is dat bij blootstelling aan risicofactoren tijdens sensitieve periodes van foetale ontwikkeling, het neuronale systeem van het kind zich aanpast aan (negatieve) omstandigheden in de baarmoeder ('fetal programming'), wat het risico op (gezondheids-) problemen later in de ontwikkeling vergroot (Barker, 1998). Hoewel, zoals beschreven in voorgaande paragraaf, verondersteld wordt dat het functioneren van het ANS op de leeftijd van zes maanden bepalend is voor de effecten van prenatale risicofactoren op de ontwikkeling van agressief gedrag, is het ook van belang om te onderzoeken in welke mate het ANS mogelijk al tijdens de prenatale periode beïnvloed is door blootstelling aan deze risicofactoren (Propper & Holochwost, 2013). Voorgaand onderzoek bij baby's heeft zich bijna uitsluitend gericht op de effecten van prenataal risico op de ontwikkeling van het PNS en er is nog weinig bekend over de effecten op het SNS (Propper & Holochwost. 2013). In hoofdstuk 5 beschrijven we daarom de resultaten van onderzoek naar de relatie tussen prenatale risicofactoren en het functioneren van het ANS (zowel het PNS als het SNS) door te kijken naar verschillen tussen hoog-risico en laag-risico kinderen in respons op en tijdens herstel van een sociale stresstaak (Still Face Paradigma) bij zes maanden oude baby's.

Ten eerste vonden we een sterkere toename in hartslag en sterkere afname in PNS activiteit bij hoog-risico kinderen in vergelijking tot laag-risico kinderen. In overeenstemming met eerder onderzoek (Conradt & Ablow, 2010; Haley & Stansbury, 2003), kwam dit verschil met name naar voren tijdens de herstelfase van de sociale stresstaak. Daarnaast vonden we een sterkere activatie van het SNS tijdens de herstelfase bij hoog-risico kinderen in vergelijking tot laag-risico kinderen en dit effect in de hoog-risicogroep werd sterker naarmate het aantal risicofactoren toenam. Resultaten van aanvullende analyses lieten zien dat de aanwezigheid van psychopathologie bij moeder, en niet middelengebruik en sociaal-demografische risicofactoren, de belangrijkste voorspeller was voor verhoogde SNS activatie tijdens de herstelfase in de hoog-risico groep.

Baby's in de laag-risicogroep lieten het verwachte ANS patroon zien bij sociale stress (zie Mesman, Van IJzendoorn, & Bakermans-Kranenburg, 2009); een afname in PNS activiteit en een milde toename in SNS activiteit in respons op stress en een toename in PNS activiteit en afname in SNS activiteit tijdens herstel van de stress Baby's in de hoog-risicogroep lieten echter een toenemende mate van fysiologische *arousal* zien bij stress. Met andere woorden, bij baby's in de hoog-risico groep bleef ook in de herstelfase activiteit in het PNS geremd en werd het SNS nog verder gemobiliseerd. Hoewel activatie van het SNS tijdens stressvolle gebeurtenissen geassocieerd wordt met een adaptieve fysiologische stressreactie op de korte termijn, vormt frequente en chronische activatie van het SNS een zware belasting voor het lichaam, wat kan leiden tot gezondheidsproblemen en psychopathologie op de langere termijn (McEwen & Gianaros, 2010). Kinderen uit hoog-risico families worden frequenter en chronisch blootgesteld aan risicofactoren tijdens de prenatale en postnatale ontwikkeling. Chronische activatie van het SNS kan met de tijd de werking van systemen onderliggend aan fysiologische regulatie aantasten, wat kan leiden tot afvlakking van ANS reactiviteit (Alkon et al., 2014).

Maar hoe kan het dat er alleen verschillen waren tussen de laag-risico en de hoog-risico groep tijdens de herstelfase van de sociale stresstaak, terwijl we (ook) verschillen verwachtten tussen de twee groepen in de respons op de taak? Een mogelijke verklaring is dat de sociale stresstaak, naarmate de taak vordert, uitdagender wordt voor baby's die minder goed in staat zijn om zichzelf te reguleren. Goed gereguleerde baby's lieten betere PNS en SNS regulatie zien tijdens herstel en zij konden hun omgeving (moeder) gebruiken om te herstellen van emotionele stress. Dit wordt ondersteund door bevindingen van aanvullende analyses waarin we vonden dat baby's uit de laag-risico groep vaker en meer naar hun moeder keken en minder huilden, een effect dat gemedieerd werd door een toename in PNS activiteit en een afname in SNS activiteit tijdens herstel, het geen wijst op betere regulatie. Baby's in de hoog-risico groep lieten een toename in fysiologische *arousal* zien over de sociale stresstaak heen, zoals te zien was in een afname in PNS activiteit en een toename in SNS activiteit tijdens herstel. Dit hing samen met verminderde aandacht voor moeder en meer huilen tijdens de herstelfase, wat aangeeft dat deze baby's minder goed in staat waren om hun moeder te gebruiken als bron van steun om het niveau van fysiologische *arousal* te laten afnemen.

Hoewel we in hoofdstuk 5 met name effecten van blootstelling aan risicofactoren tijdens de zwangerschap op fysiologische zelfregulatie tijdens het herstel van stress vonden op de leeftijd van zes maanden, kwamen er in hoofdstuk 4.1 en 4.2, tegen verwachting in, geen (directe- en interactie-)effecten van herstel van stress op de ontwikkeling van fysieke agressie naar voren. Een groot aantal onderzoeken heeft zich

tot nu toe gericht op het onderzoeken van ANS activiteit tijdens rust en in respons op stress. Echter, hoewel de mate van herstel in ANS activiteit na stress gezien wordt als een belangrijke indicator van fysiologische zelfregulatie (Porges, 2007), is er nog weinig onderzoek gedaan naar de relatie tussen herstel van het ANS en latere ontwikkelingsuitkomsten (El-Sheikh & Erath, 2011). De afwezigheid van effecten van fysiologische zelfregulatie door het ANS tijdens herstel van stress in hoofdstuk 4.1 en 4.2 is mogelijk toe te schrijven aan een aantal methodologische punten. Zo zijn er verschillen in de manier waarop ANS activiteit in response op en tijdens herstel van stress berekend is. In hoofdstuk 4.1 en 4.2 de gemiddelde ANS activiteit tijdens herstel berekend over de sociale stresstaak en de frustratietaak, terwijl in hoofdstuk 5 alleen de sociale stresstaak is gebruikt. Daarnaast waren er weliswaar significante correlaties tussen herstel van het ANS op de sociale stresstaak en de frustratietaak, echter, de mate van herstel in ANS activiteit tussen de twee taken verschilde. Zo was er gemiddeld genomen sprake van herstel in SNS activiteit, maar niet in PNS activiteit, op de frustratietaak, terwijl er op de sociale stresstaak nauwelijks herstel in PNS en SNS activiteit te zien was.

Conclusie

De resultaten beschreven in dit proefschrift kunnen worden samengevat op drie hoofdlijnen. Ten eerste, agressief gedrag in de voorschoolse leeftijd hangt samen met de interactie tussen zelfregulatie op emotioneel en cognitief niveau (hoofdstuk 2). Kinderen die meer moeite hebben met het reguleren van negatieve emoties zoals boosheid, verdriet en angst, zijn eerder geneigd tot het laten zien van agressief gedrag als zij ook moeite hebben met inhibitie. In tegenstelling tot bij agressief gedrag in algemene zin vonden we voor fysieke agressie dat geslacht en leeftijd van het kind een rol spelen in de mate waarin zelfregulatie op emotioneel en cognitief niveau voorspellend zijn voor fysieke agressie. Een goede inhibitie lijkt met name een belangrijke beschermende factor voor fysieke agressie te zijn voor oudere jongens in de voorschoolse leeftijd met meer problemen in het reguleren van negatieve emoties. Deze bevindingen geven aan dat interventies gericht op het verminderen van agressief gedrag bij peuters en kinderen in de voorschoolse leeftijd zich voornamelijk zouden moeten richten op het verbeteren van inhibitie, met name voor kinderen die veel negatieve emotionaliteit laten zien en voor jongens met een hoge mate van negatieve emotionaliteit en fysieke agressie.

Ten tweede, de impact van prenatale risicofactoren op de ontwikkeling van fysieke agressie in de peuterleeftijd is afhankelijk van fysiologische zelfregulatie in de babytijd, en dan specifiek de coördinatie tussen het PNS en SNS (hoofdstuk 4.1 en 4.2). Kinderen met een lage (re)activiteit van zowel het PNS als het SNS (coinhibitie)

of hoge (re)activiteit van zowel het PNS als SNS (coactivatie), zijn kwetsbaarder voor de negatieve effecten van prenatale risicofactoren (laten een hogere mate van fysieke agressie zien), in vergelijking tot kinderen waarbij het PNS en SNS wederkerig aan elkaar samenwerken. Kinderen waarbij beide takken van het ANS wederkerig aan elkaar samenwerken, laten opmerkelijk genoeg hetzelfde niveau van fysieke agressie zien, ongeacht of ze blootgesteld zijn aan risicofactoren tijdens de zwangerschap. Het is belangrijk om op te merken dat fysiologische zelfregulatie alleen voorspellend was voor fysieke agressie in combinatie met blootstelling aan prenatale risicofactoren. Dit geeft aan dat de kwetsbaarheid voor de ontwikkeling van agressie ligt in de combinatie van het specifieke ANS profiel in de babytijd en de invloed van (prenatale) risicofactoren (Moore, 2009), en dat deze fysiologische maten daarom samen met de context waarin kinderen opgroeien onderzocht moeten worden.

Dit is het eerste onderzoek dat de coördinatie tussen het PNS en SNS in de babytijd in relatie tot de ontwikkeling van agressief gedrag heeft onderzocht. De resultaten van dit onderzoek benadrukken het belang van het onderzoeken van zowel het PNS als het SNS in interactie met de (opvoedings-)omgeving om beter zicht te krijgen op processen die leiden tot agressief gedrag in de (vroege) kindertijd. Echter, we dienen voorzichtig te zijn met het aandragen van praktische implicaties van onze bevindingen. Vervolg onderzoek is nodig waarin onze resultaten gerepliceerd worden. Vooralsnog laten onze bevindingen zien dat kinderen met een verhoogd risico op het ontwikkelen van agressief gedrag in de peutertijd mogelijk al kunnen worden geïdentificeerd op basis van de evaluatie van prenatale blootstelling aan risicofactoren en het ANS profiel in de babytijd. In de babytijd wordt door middel van de rijping van het ANS de basis gelegd voor gezonde emotionele en cognitieve zelfregulatie later in de ontwikkeling (Porges & Furman, 2011). Bovendien ontwikkelt het ANS zich zeer snel in het eerste levensjaar (Porges, 2003). Dit wordt in verband gebracht met verhoogde gevoeligheid voor omgevingsinvloeden en brengt kansen met zich mee voor vroege interventieprogramma's gericht op het aanpakken van negatieve omgevingsinvloeden (Beauchaine, Neuhaus, Brenner, & Gatzke-Kopp, 2008). Een aanzienlijk aantal studies heeft laten zien dat de ontwikkeling van het ANS gevoelig is voor invloeden vanuit de vroege opvoedingscontext. Zo hangt een hogere mate van sensitiviteit van moeder samen met betere parasympatische stressregulatie bij baby's (Conradt & Ablow, 2010; Moore et al., 2009). Kinderen met minder goede fysiologische zelfregulatie zouden daarom kunnen profiteren van vroege (preventieve) interventieprogramma's gericht op het verbeteren van sensitiviteit bij moeder.

Ten derde, fysiologische zelfregulatie in de babytijd hangt samen met de aanwezigheid van risicofactoren tijdens de prenatale periode (hoofdstuk 5). Kinderen die blootgesteld zijn aan risicofactoren tijdens de zwangerschap, zoals

psychopathologie bij moeder, middelen gebruik en sociaal-demografische risicofactoren, laten deficiënties zien in fysiologische zelfregulatie. Dit bleek uit een sterkere afname in PNS activiteit en toename in SNS activiteit tijdens herstel van emotionele stress. Deficiënties in fysiologische zelfregulatie dragen mogelijk bij aan problemen in de emotionele zelfregulatie. Deze bevindingen onderstrepen het belang van het identificeren van vrouwen met een hoog-risicoprofiel tijdens de zwangerschap om zo tijdig (preventieve) interventieprogramma's in te kunnen zetten gericht op het verbeteren van prenatale en vroege postnatale omstandigheden. Intensieve *home-visiting* programma's hebben positieve invloeden gevonden op de ontwikkeling van zowel moeder als kind (Avellar & Supplee, 2013; Mejdoubi et al., 2015; Olds, Sadler, & Kitzman, 2007; Ordway et al., 2014; Peacock, Konrad, Watson, Nickel, & Muhajarine, 2013; Sweet & Appelbaum, 2004). Het is echter nog onbekend in hoeverre deze programma's de fysiologische zelfregulatie van het kind beïnvloeden. Het opnemen van metingen van het ANS als onderdeel van onderzoek naar interventie effecten zou zowel inzicht kunnen geven in hoeverre deze programma's van invloed zijn op dit basale niveau van zelfregulatie, als in de mate waarin dit de latere ontwikkeling van emotionele en cognitieve zelfregulatie beïnvloedt.

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CURRICULUM VITAE

Jill Suurland werd geboren op 13 februari 1985 te Vlaardingen. In 2003 behaalde zij haar VWO diploma aan het Lyceum Schravenlant in Schiedam. In het jaar erna behaalde zij haar propedeuse Bedrijfskunde aan de Erasmus Universiteit in Rotterdam. In 2004 startte Jill met de bachelor Pedagogische Wetenschappen aan de Universiteit Leiden. Aansluitend begon zij bij de afdeling Orthopedagogiek van de Universiteit Leiden aan de research master 'Educational Sciences: Normal and Deviant Patterns of Attachment and Self Regulated Learning' (track: Clinical Practice and Research), die ze in 2009 cum laude afrondde. Voor haar research master scriptie ontving Jill de Emile Scriptieprijs (Pedagogische Wetenschappen, Universiteit Leiden) en de NVO aanmoedigingsprijs. Tijdens haar studie heeft zij voor een half jaar gestudeerd aan de University of Sydney (Australië). Ook heeft zij gedurende een jaar een klinische stage gelopen op het Ambulatorium (Neurospreekuur) verbonden van de Universiteit Leiden, waar zij haar Basisaantekening Diagnostiek haalde. Naast haar studie is Jill werkzaam geweest als student-assistent op diverse onderzoeksprojecten (waaronder 'complexe informatieverwerking bij autisme spectrum stoornissen' en 'slaap en geheugen') aan de afdeling Orthopedagogiek. In september 2010 is Jill gestart als promovenda op het project 'Een goed begin: Vroege preventie van antisociaal gedrag', waar zij onderzoek deed naar de rol van zelfregulatie in de ontwikkeling van agressie bij jonge kinderen. De resultaten van dit onderzoek staan beschreven in dit proefschrift. Naast haar aanstelling als promovenda werkte zij als docent binnen het bachelor- en masteronderwijs aan de afdeling Orthopedagogiek en verzorgde zij met name klinisch onderwijs. Ook behaalde zij haar Basiskwalificatie onderwijs (BKO) en Basiskwalificatie Engels (BKE). Momenteel werkt Jill als universitair docent op de afdeling Orthopedagogiek van de Universiteit Leiden.

LIST OF PUBLICATIONS

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Suurland, J., Van der Heijden, K.B., Smaling, H.J.A., Huijbregts, S.C.J., Van Goozen, S.H.M., & Swaab, H. (2016). Infant autonomic nervous system response and recovery: Associations with maternal risk status and infant emotion regulation. *Development and Psychopathology, x,* xx-xx. Epub ahead of print.

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* Hanneke Smaling and Jill Suurland share first authorship.

Suurland, J., Van der Heijden, K. B., Huijbregts, S. C. J., Van Goozen, S. H. M., & Swaab, H.. Interaction between prenatal risk and infant parasympathetic and sympathetic stress reactivity predicts early aggression. Manuscript invited to revise and resubmit.

Suurland, J., Van der Heijden, K. B., Huijbregts, S. C. J., Van Goozen, S. H. M., & Swaab, H.. Infant parasympathetic and sympathetic activity during baseline, stress and recovery: interactions with prenatal adversity predict aggressive behavior in toddlerhood. Manuscript invited to revise and resubmit.

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