Of Scaredy Cats and Cold Fish

The autonomic nervous system and behaviour in young children

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Colofon
Of Scaredy Cats and Cold Fish.

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Of Scaredy Cats and Cold Fish.
The autonomic nervous system and behaviour in young children.

Over angsthazen en koele kikkers.
Het autonome zenuwstelsel en gedrag in jonge kinderen.

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Chapter 1

General Introduction

Introduction

Psychiatric disorders are diagnosed on the basis of the presence and course of behavioural symptoms according to the classifications of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) or the International Classificiation of Disease (ICD 10). Unfortunately, interpretation of individual diagnostic criteria remains subjective, quantitative criteria are lacking, and many criteria overlap. Moreover, the variability of clinical expression across time introduces further difficulties and can lead to a protracted period of diagnostic uncertainty. In particular in child and adolescent psychiatry, where symptoms are often age-specific and where the course of the disorder during the child's development carries important prognostical information. The relatively high prevalence of both internalising and externalising behaviour in young children has made it difficult to delineate normal age-limited behaviour from pathological externalising behaviour (1).

This diagnostic uncertainty has fuelled the interest in biological markers of psychopathology. Primarily, the aim is to find markers able to facilitate early identification and consistent categorization of patients and thus help to improve treatment approaches. Additionally, biomarkers can facilitate etiological research, especially if they are part of the causal chain leading up to psychopathology.

In search of biomarkers of psychopathology, researchers turned to the autonomic nervous system. It acts largely independent of volition and controls heart rate, respiratory rate, digestion, and perspiration. It is divided into two partially antagonistic systems: the sympathetic nervous system and the parasympathetic or vagal nervous system. In general terms, the vagal system primarily regulates "rest and digest" functions. In contrast, the sympathetic nervous system can elicit the "fight or flight" response with increased arousal and energy generation in response to stress or threat.

Both the sympathetic and the vagal system are responsible for normal heart rate variability. Vagal activity allows for a fast beat-to-beat control. Increased vagal activity lowers heart rate, decreased vagal activity heightens heart rate. Sympathetic control has inverse effects and its control over heart rate works slower than vagal control. Analysing heart rate variability allows for estimating the relative contributions of the sympathetic and vagal systems, as high frequency (i.e. fast) variations are due solely to vagal influence. This makes heart rate and its variability easy to obtain measures of autonomic functioning.

The association between heart rate and behaviour has been studied for decades (2). In particular, the relation between heart rate and externalising behaviour has received a lot of attention. A large number of cross-sectional studies have shown that low heart rate during rest is associated with externalising behaviour (3, 4). Raine et al. showed that heart rate at age 3 predicts behaviour at age 11 (5). A meta-analysis of over 40 studies confirmed the relation between low resting heart rate and externalising behaviour; the relation between low heart rate and externalising behaviour holds throughout childhood, adolescence and beyond (2, 6).

Several theoretical models have been proposed for the observed relation between low heart rate and externalising behaviour. The stimulation seeking theory posits that low heart rate and low physiological arousal in general, is an unpleasant physiological state. Children with low arousal seek stimulation to increase their arousal levels. Externalising behaviour may be viewed as a form of stimulation-seeking for some children (5, 7).

The fearlessness theory argues that low heart rate is a marker of low levels of fear. Children who experience low levels of fear are less likely to head potential negative consequences of

their actions. Furthermore, children with low levels of fear are less likely to be responsive to socializing punishments, which may in turn contribute to poor fear conditioning. (8, 9). Alternatively, low heart rate may not be directly on the causal pathway to externalising behaviour, but instead be a marker for other processes that are implicated in externalising behaviour. For example, research shows that the right hemisphere is dominant for the control of autonomic functions, including heart rate (10, 11). Poor right hemisphere functioning has also been found in antisocial populations and may reflect a weaker withdrawal system that promotes retreat dangerous situations . Genetic factors too could explain the association between low heart rate and externalising behaviour. Heart rate is partially genetically determined (12-14), as is externalising behaviour (15). It is conceivable that a common genetic influence underlies both low heart rate and externalising behaviour.
Finally, heart rate may interact with other, environmental, risk factors associated with externalising behaviour (12). Only a few studies have investigated the interplay of heart rate with environmental risk factors. Farrington reported that the association between low heart rate and aggressive behaviour was stronger when environmental risk factors were present as well (16). Boys with low resting heart rate were more likely to commit acts of violence as an adult if they also had a poor relationship with their parent, and if they came from a large family. Recently, a study investigated the interaction of heart rate and with environmental stressors in a large sample of adolescents. The study showed that adolescents with low heart rate displayed less psychopathology in relation to stressors in life than adolescents with normal or high heart rate (17).
The relationship between heart rate variability and externalising behaviour is less clear than the relationship between heart rate and externalising behaviour (18). Because of the inverse relationship between heart rate and vagal activity, higher vagal activity was expected in children with externalizing problems. While some studies did indeed find an association between high vagal activity in children and aggressive behaviour (4, 19, 20), several other studies suggested lower vagal activity in children with externalizing problems, possibly indicating autonomic dysfunction (21-23). Up until now, this inconsistency in findings is unresolved.

Population

The current study was conducted as part of the Focus Cohort of The Generation R Study. The Generation R Study is a large population-based prospective cohort study from fetal life onwards. The study was designed to identify early environmental as well as genetic causes of abnormal growth, development and health during both the prenatal period and in later life. Research in Generation R focuses on four primary areas of research: (1) growth and physical development; (2) behavioural and cognitive development; (3) diseases in childhood; and (4) health and healthcare for pregnant women and children. In total, 9,778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study. Data collection in mothers, fathers and preschool children included questionnaires, detailed physical and ultrasound examinations, behavioural observations, and biological samples. More detailed assessments of postnatal growth and development were conducted in a randomly selected subgroup, referred to as the Generation R Focus Cohort. The Focus Cohort consists only of Dutch children of Caucasian origin and their parents, to exclude potential confounding or effect modification by ethnicity.

Studies conducted in the Focus Cohort were able to utilise more in-depth assessments that could not be applied in the whole cohort due to time, financial or logistical constraints.

Chapter outline

Despite a large body of literature detailing an intimate relation between the autonomic nervous system and externalising behaviour, gaps in our knowledge remain.

First, establishing whether an association between autonomic nervous system functioning and externalising behaviour already exists at a very young age is important. Childhood-onset externalising psychopathology has a poor prognosis when compared to adolescent-onset psychopathology. The Dunedin Multidisciplinary Health and Development Study, amongst others, showed that childhood-onset externalising behaviour is associated with a greater burden of psychiatric problems, delinquency, aggression and substance abuse in adulthood (24).

Second, while heart rate and its variability are often seen as constitutional factors, they are in fact only partially heritable. Even in infants sleeping mean HR and HRV are determined not only by additive genetic but also by unique environmental factors (13). Research has shown infant autonomic functioning to be influenced by prematurity (25), intra-uterine growth retardation (26), maternal smoking (27), drinking (28), and cocaine use (29) during pregnancy. In Chapters 2 and 3 we investigate how the autonomic nervous system itself is related to other important early environmental factors. Chapter 2 describes the relation between maternal psychiatric symptoms, both pre-and postpartum, and infant heart rate. Of primary interest is whether psychiatric symptoms experienced by the mother during her pregnancy influences the infant's autonomic nervous system after birth. This mirrors ongoing research concerning the hypothalamic-pituitary-adrenal axis, which provided evidence for the hypothesis that prenatal maternal stress exposes the unborn child to high levels of cortisol and may permanently alter the cortisol "set point" (30). In chapter 3 we take a critical look at the association between infant heart rate and the perennial early childhood risk factor: breastfeeding. To address confounding, we compared the relation of breast feeding and heart rate with the relation of another infant dietary factor: fruitpurée consumption.

Third, while gene-environment interplay has received a large amount of attention, we know surprisingly little about how the autonomic nervous system relates to other risk factors in predisposing to certain types of behaviour. In chapter 4, we will investigate the relation between heart rate and behaviour at 18 months. Specifically we will like at the interplay of heart rate with maternal psychopathology in determining infant behaviour.

Fourth, investigating whether low heart rate is a specific marker of certain aspects of externalising behaviour may yield further insight into the aetiology and the development of externalising behaviour and the role of the autonomic nervous system. Children with externalising behaviour are characterised by very different traits. Many young children with these problems are physically aggressive and oppositional, but some are predominantly emotionally (over)reactive; they experience high rates of anxiety and distress, and are often impulsive. Other aggressive children are fearless and plan their rule-breaking behaviour (31). Investigating whether low resting mean rate is a specific marker of certain aspects of externalising behaviour may yield further insight in its aetiology and development.

References

1. Lahey B, Moffit RE, Caspi A. Causes of conduct disorder. New York City: The Guilford Press; 2003.
2. Ortiz J, Raine A. Heart rate level and antisocial behavior in children and adolescents: a meta-analysis. Journal of the American Academy of Child and Adolescent Psychiatry. 2004 Feb;43(2):154-62.
3. van Goozen SH, Matthys W, Cohen-Kettenis PT, Buitelaar JK, van Engeland H. Hypothalamic-pituitary-adrenal axis and autonomic nervous system activity in disruptive children and matched controls. Journal of the American Academy of Child and Adolescent Psychiatry. 2000 Nov;39(11):1438-45.
4. Dietrich A, Riese H, Sondeijker FE, Greaves-Lord K, van Roon AM, Ormel J, et al. Externalizing and internalizing problems in relation to autonomic function: a population-based study in preadolescents. Journal of the American Academy of Child and Adolescent Psychiatry. 2007 Mar;46(3):378-86.
5. Raine A, Venables PH, Mednick SA. Low resting heart rate at age 3 years predisposes to aggression at age 11 years: evidence from the Mauritius Child Health Project. Journal of the American Academy of Child and Adolescent Psychiatry. 1997 Oct;36(10):1457-64.
6. Raine A, Venables PH, Williams M. Relationships between central and autonomic measures of arousal at age 15 years and criminality at age 24 years. Arch Gen Psychiatry. 1990 Nov;47(11):1003-7.
7. Quay HC, Quay LC. Behavior Problems in Early Adolescence. Child development. 1965 Mar;36:215-20.
8. Raine A. The psychopathology of crime: criminal behaviour as a clinical disorder. San Diego: Academic Press; 1993.
9. Venables PH. Psychophysiology of abnormal behaviour. Br Med Bull. 1981 May;37(2):199-203.
10. Zamrini EY, Meador KJ, Loring DW, Nichols FT, Lee GP, Figueroa RE, et al. Unilateral cerebral inactivation produces differential left/right heart rate responses. Neurology. 1990 Sep;40(9):1408-11.
11. Yokoyama K, Jennings R, Ackles P, Hood P, Boller F. Lack of heart rate changes during an attention-demanding task after right hemisphere lesions. Neurology. 1987 Apr;37(4):624-30.
12. Raine A. Biosocial studies of antisocial and violent behavior in children and adults: a review. J Abnorm Child Psychol. 2002 Aug;30(4):311-26.
13. Dubreuil E, Ditto B, Dionne G, Pihl RO, Tremblay RE, Boivin M, et al. Familiality of heart rate and cardiac-related autonomic activity in five-month-old twins: the Quebec newborn twins study. Psychophysiology. 2003 Nov;40(6):849-62.
14. Snieder H, Harshfield GA, Treiber FA. Heritability of blood pressure and hemodynamics in African- and European-American youth. Hypertension. 2003 Jun;41(6):1196-201.
15. Bornovalova MA, Hicks BM, Iacono WG, McGue M. Familial transmission and heritability of childhood disruptive disorders. Am J Psychiatry. 2010 Sep;167(9):1066-74.
16. Farrington DP. Childhood, adolescent, and adult features of violent males. In: Huesmann LR, editor. Aggressive Behavior: Current Perspectives. New York, NY: Plenum Press; 1994. p. 215 –40.
17. Oldehinkel AJ, Verhulst FC, Ormel J. Low heart rate: a marker of stress resilience. The TRAILS study. Biological psychiatry. 2008 Jun 15;63(12):1141-6.
18. Lorber MF. Psychophysiology of aggression, psychopathy, and conduct problems: a meta-analysis. Psychol Bull. 2004 Jul;130(4):531-52.
19. Cole PM, Zahn-Waxler C, Fox NA, Usher BA, Welsh JD. Individual differences in emotion regulation and behavior problems in preschool children. J Abnorm Psychol. 1996 Nov;105(4):518-29.
20. Van Voorhees E, Scarpa A. Psychophysiological variables in childhood proactive and reactive aggression. Psychophysiology. 2002;39:82.
21. Beauchaine TP, Katkin ES, Strassberg Z, Snarr J. Disinhibitory psychopathology in male adolescents: discriminating conduct disorder from attention-deficit/hyperactivity disorder through concurrent assessment of multiple autonomic states. J Abnorm Psychol. 2001 Nov;110(4):610-24.
22. Mezzacappa E, Tremblay RE, Kindlon D, Saul JP, Arseneault L, Seguin J, et al. Anxiety, antisocial behavior, and heart rate regulation in adolescent males. Journal of child psychology and psychiatry, and allied disciplines. 1997 May;38(4):457-69.
23. Pine DS, Wasserman GA, Miller L, Coplan JD, Bagiella E, Kovelenku P, et al. Heart period variability and psychopathology in urban boys at risk for delinquency. Psychophysiology. 1998 Sep;35(5):521-9.
24. Odgers CL, Caspi A, Broadbent JM, Dickson N, Hancox RJ, Harrington H, et al. Prediction of differential adult health burden by conduct problem subtypes in males. Arch Gen Psychiatry. 2007 Apr;64(4):476-84.
25. Longin E, Gerstner T, Schaible T, Lenz T, Konig S. Maturation of the autonomic nervous system: differences in heart rate variability in premature vs. term infants. J Perinat Med. 2006;34(4):303-8.
26. Galland BC, Taylor BJ, Bolton DP, Sayers RM. Heart rate variability and cardiac reflexes in small for gestational age infants. J Appl Physiol. 2006 Mar;100(3):933-9.
27. Browne CA, Colditz PB, Dunster KR. Infant autonomic function is altered by maternal smoking during preg-

nancy. Early Hum Dev. 2000 Sep;59(3):209-18.
28. Haley DW, Handmaker NS, Lowe J. Infant stress reactivity and prenatal alcohol exposure. Alcohol Clin Exp Res. 2006 Dec;30(12):2055-64.
29. John V, Dai H, Talati A, Charnigo RJ, Neuman M, Bada HS. Autonomic alterations in cocaine-exposed neonates following orthostatic stress. Pediatr Res. 2007 Feb;61(2):251-6.
30. Yehuda R, Engel SM, Brand SR, Seckl J, Marcus SM, Berkowitz GS. Transgenerational effects of posttraumatic stress disorder in babies of mothers exposed to the World Trade Center attacks during pregnancy. J Clin Endocrinol Metab. 2005 Jul;90(7):4115-8.
31. Ellis ML, Weiss B, Lochman JE. Executive functions in children: associations with aggressive behavior and appraisal processing. J Abnorm Child Psychol. 2009 Oct;37(7):945-56.

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Chapter 2

Maternal psychopathology influences infant heart rate variability.

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Abstract
The autonomic nervous system as measured by heart rate and heart rate variability, is considered a biological marker of psychopathology in children. However, little is known about determinants of heart rate and heart rate variability in children.
We examined the relationship of maternal psychopathology with infant heart rate and heart rate variability. Heart rate was recorded at 14 months in 528 infants. The high frequency component of heart rate variability was used as an indicator of cardiac vagal modulation. The presence of a lifetime maternal psychiatric diagnosis was assessed with the Composite International Diagnostic Interview. Presence of maternal psychiatric symptoms during pregnancy and two months after birth was assessed using the Brief Symptom Inventory.
A maternal history of a psychiatric disorder was associated with a 0.24 SD higher mean heart rate in the infant (β=0.24, 95%CI: 0.03, 0.4, p=0.025) and a 0.14 SD lower high frequency power (β=-0.14, 95%CI: -0.6, -0.03, p=0.003). Likewise, postnatal maternal anxiety and depression symptoms were associated with infant mean heart rate. A 1 point increase in the mean anxiety symptom score was associated with 0.14 SD higher mean heart rate in the infant (β=0.14, 95%CI: 0.05, 0.2, p=0.004), and a 1 point increase in mean depression score with a 0.11 SD increase (β=0.11, 95%CI: 0.01, 0.2, p=0.025). No significant associations of prenatal maternal affective symptoms with infants autonomic functioning were found.
Maternal lifetime psychiatric diagnosis and postnatal psychiatric symptoms are associated with infant autonomic functioning, namely higher mean HR and lower vagal modulation.

Introduction

The autonomic nervous system (ANS) and its proxies heart rate (HR) and heart rate variability (HRV) are considered biological markers of psychopathology in children (1, 2). HR is controlled by the sympathetic and parasympathetic (vagal) branches of the autonomic nervous system. High frequency (HF) variations of HR are mediated by centrally controlled vagal activity. Determination of HF power by means of spectral analysis can provide a non-invasive estimation of cardiac vagal control (3, 4).

A number of studies suggest an association between higher mean HR and lower vagal modulation with internalising problems. For example, Monk et al. showed that adolescents with anxiety disorders had higher and less fluctuating HR during a baseline condition (1). Similarly, higher resting mean HR has been reported in behaviourally inhibited children (5). Moreover, a recent large study by Dietrich et al. (6) showed that children reporting internalising problems had high resting mean HR and low vagal modulation. Theoretical models postulate that high mean HR and low vagal modulation are causative factors in the development of affect dysregulation and social withdrawal (7) as well as state, trait and pathological anxiety (8). Considering the putative role of mean HR and vagal modulation as markers or causative agents in the development of psychopathology, it is important to elucidate their determinants early in life, some of which may be amendable to prevention strategies.

Mean HR and HRV are partially heritable; Dubreuil et al. (9) showed that individual differences in sleeping mean HR and HRV in infants are largely determined by additive genetic and unique environmental factors. Furthermore, infant ANS is reduced in association with prematurity (10), intra-uterine growth retardation (11), maternal smoking (12), drinking (13), and cocaine use (14) during pregnancy. (14)

Maternal psychopathology has a substantial and wide-ranging influence on the developing infant. Children of mothers suffering from psychopathology not only inherit an unfavourable genetic profile, they are exposed to multiple environmental risk factors associated with maternal psychopathology as well (15). Prenatal maternal psychopathology has a negative impact on infant birth outcome parameters, it is associated with lower birth weight and lower gestational age (16). Also, the presence of maternal psychopathology, both prenatal and postnatal, is associated with a delay in the infant's subsequent cognitive, language and motor development (17-19). Further, maternal psychopathology is associated with difficult and negative reactive infant behaviour and temperament (20-22).

However, little is known about the impact of maternal psychopathology, before or after birth, on infant ANS functioning. Not only are reports on the influence of parental psychopathology on the infant's ANS scarce, they are conflicting and based on small sample sizes. In a recently published study, Kaplan et al. (23) reported no difference in vagal modulation between infants of prenatal depressed women (n=19) and non-depressed women (n=28). On the other hand, Jones et al.(24) showed that infants of mothers with prenatal depressive symptoms (n=35) had a significantly lower vagal modulation than children of non-symptomatic mothers (n=28).

Kaplan et al. also investigated postnatal factors related to maternal psychopathology, they found that high maternal sensitivity toward the infant is associated with a higher infant vagal modulation (23) . This is partially corroborated by Feldman and Eidelman who showed that in premature infants, physical mother-child interaction is important in the development of the infant vagal system (25).

In related research, the association between maternal anxiety and or stress and foetal HR and HRV has been investigated. It has been shown that maternal stress during pregnancy induced by a stress task led to changes in foetal HR (26, 27). Moreover, Monk et al. showed that the rise in mean foetal HR in response to a maternal stress task was higher when the mother was diagnosed with a mood disorder (28). However, whether such effects can lead to lasting changes in infant ANS functioning has not been conclusively shown.
Temperament is an aspect of child behaviour that can be measured early in life. It is seen as the constitutional basis for differences in reactivity and self-regulation, and is influenced by heredity, maturation and experience. Temperament plays a role in the etiology of later psychopathology (29). Several studies have shown a potentially causal relation between maternal psychopathology and infant temperament (20-22). Furthermore, associations between infant temperament and infant autonomic functioning have been reported (30, 31). Hence, infant temperament might mediate an association between maternal psychopathology and infant autonomic functioning.
We assess the relation between maternal psychopathology, especially internalising psychopathology, and child ANS functioning in a population based study. We expect maternal psychopathology to be associated with higher infant mean HR and lower vagal modulation. We measured lifetime prevalence of a maternal psychiatric diagnosis and prevalence of both prenatal and postnatal psychiatric symptoms. We aim to deduce from the measurements at different time points whether maternal psychopathology before and after birth influences the infant's ANS specifically. Finally, we assess infant temperament, to determine whether it mediates any association between maternal psychopathology and infant autonomic functioning.

Methods

Participants

This study was conducted within the Focus cohort of the Generation R Study, a population based prospective cohort study from foetal life until young adulthood (32). All children were born between February 2003 and August 2005. The subgroup for this study consisted of the 884 Dutch pregnant women and their children who attended the Focus Cohort examination at 14 months. Measurements of autonomic indices were added to the protocol of the examination round, while assessment was already ongoing. Hence, we did not obtain physiological measurements for all participants. Physiological measurements were available for 528 infants.
Of these 528 children, prenatal Brief Symptom Inventory (BSI) scores were available for 501 children; BSI scores at two months were available for 409 children. Composite International Diagnostic Interview (CIDI) data were available for 450 participants. Our study population consisted of 514 children, for whom at least one measure of maternal psychopathology was available. Written informed consent was obtained from all participants. Approval for the study was granted by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam.

Measurement of maternal psychopathology

Lifetime maternal psychopathology was assessed by means of the World Health Organisation's (WHO) Composite International Diagnostic Interview (CIDI) Version 2.1. The CIDI is a structured interview based on DSM-IV criteria. Good reliability and validity have been

reported (33). A home interview was conducted 30 weeks after conception by research assistants trained in an official WHO training centre. Lifetime diagnoses were divided into the following groups of disorders: 1) any psychiatric disorder; 2) anxiety disorders, consisting of generalised anxiety disorder, obsessive-compulsive disorder, panic disorder, agoraphobia, social phobia, specific phobia or posttraumatic stress disorder; 3) mood disorders, consisting of a major depressive episode, bipolar disorder or dysthymia.
Information on current maternal psychiatric symptoms at 20 weeks of gestation and at two months after birth was obtained by postal questionnaires. The Brief Symptom Inventory (BSI) is a validated self-report questionnaire consisting of 53 items developed by Derogatis (34). Each item was rated on a 5-point Likert scale, ranging from "0" (not at all), to "4" (extremely). For the current study, we used the anxiety (6 items) and depression (6 items) subscales. Subscale scores were calculated by summing the item scores involved and then dividing by the number of endorsed items.

Measurement of Infant Temperament
Mothers completed the Infant Behaviour Questionnaire (IBQ-R)–Revised when their infants were 6 months old. We chose six out of the original 14 scales of the IBQ-R because the complete instrument of 191 items was not feasible within our multidisciplinary study. The six scales included activity level, distress to limitations, fear, duration of orienting, recovery from distress, and sadness, and were judged of particular importance from a clinical perspective for the most prevalent behaviour disorders in childhood (35).

Psychophysiologic measurements
Mothers and their infants were invited to our laboratory at 14 months after birth. Physiological measurements were performed by trained research assistants. We registered heart rate using a precordial, three pole ECG lead, sampled at 512 Hz. Furthermore, we monitored the breathing pattern using a piëzo-electric transducer, recording expansion and contraction of the thorax. Signals were recorded using a Vitaport 3 digital recording system (Temec Inc, Kerkrade, the Netherlands). We recorded for 8 minutes, while the infant was at ease in its mothers lap. To help the infant relax, we played an episode of the Teletubbies © (BBC/Ragdoll Limited). Recordings did not start until signals had reached a stabilized steady-state.

Power spectral analysis
We analysed the recorded data using custom made software. Irregular, slow breathing distributes the variation attributable to parasympathetic activity across the frequency spectrum. Hence, for each participant, we manually selected 100-180 seconds of the ECG, were breathing was most regular. R-top detection was conducted on the selected data window. Interbeat intervals were examined on the basis of the time between consecutive R-tops in the ECG. Interbeat intervals were used to calculate mean HR for the selected period. We performed spectral analysis using discrete Fourier transformation, based on non equidistant sampling of the R-wave indices (36). As vagal modulation of HR is dependent on respiratory frequency, which is much higher in infants, we adjusted the upper bound of the HF power band according to recommendations from literature (37). We defined our HF power band between 0.15 Hz - 1.04 Hz.

Covariates

We used sex of the infant, gestational age, weight at birth, maternal age, and smoking and drinking behaviour during pregnancy as covariates as they are known to influence mean HR and HRV (10-14). Level of highest completed education by the mother was entered as a measure of socio-economic status. Gestational age at birth and birth weight were obtained from community midwife and hospital registries at birth. Information on maternal smoking and drinking habits during pregnancy as well as highest completed education were obtained by means of a questionnaire completed by the mother. Illicit substance use was evaluated using the CIDI interview, as only two mothers reported substance use during pregnancy, illicit substance use was not entered as a covariate.

Statistical analysis

We compared participants who attended the physiological measurement session and for who at least one measure of maternal psychopathology was available with the other participants of the examination round. For continuous variables approaching a normal distribution we used T-tests, for non-normal distributed continuous variables Mann-Whitney U tests and for dichotomous variables Chi Square tests.

The physiological variables mean HR and vagal modulation were log-transformed to achieve a normal distribution. Physiological variables were divided by their standard deviation to facilitate the interpretation of the regression coefficients. We were not able to normalise the BSI-scores. However, linear regression assumptions where not violated, as entering non-normalised BSI-scores into the regressions yielded normally distributed regression residuals. All reported analyses were adjusted for all the covariates described above.

First, we ran separate analyses: we used maternal lifetime psychopathology and pre-and postnatal maternal psychiatric symptoms as determinants to study whether they are associated with infant autonomic functioning. We assessed the association between lifetime maternal psychopathology and infant mean HR level and vagal modulation with multiple linear regression. We entered the three dichotomous categories: any diagnosis, diagnosis of anxiety disorder, diagnosis of mood disorder into separate regression equations.

To test whether infant temperament is a mediator in the association between maternal psychopathology and infant autonomic functioning, we performed a two-step analysis.

First, we used multiple linear regressions to test for associations between maternal lifetime psychopathology and infant temperament. Any infant temperament scale associated with maternal psychopathology (at $p<0.1$) was retained for further analysis. In the second analysis, these retained scales were entered into multiple linear regressions using infant autonomic functioning as outcome. Only temperamental scales related both to maternal psychopathology and infant autonomic functioning, were considered as mediators of the association between maternal psychopathology and infant autonomic functioning.

We tested for the association between pre-and postnatal psychiatric symptoms and the physiological variables with multiple linear regression.

Additionally, we performed multilevel analyses to assess the influence of maternal psychiatric symptoms, both pre- and postnatal taken together. This analysis not only allows us to evaluate the influence of psychiatric symptoms on infant autonomic functioning regardless of the timing of these symptoms, it will also allow us to assess the influence of change between pre-and postnatal measurements on infant autonomic functioning. These analyses

allowed us to reduce the number of hypotheses tested as well. To further address the issue of multiple testing, results were Bonferroni corrected. We used separate models for anxiety and depression symptoms. The BSI scores were used as the dependent variable in a repeated measure structure, mean HR and vagal modulation were used as predictor. Significance levels are not affected by interchanging dependent and independent variables, hence valid conclusions can be drawn.

To elucidate possible differential effects of pre-and postnatal anxiety symptoms, we dichotomised BSI scores both pre-and postnatal. We used the 25th percentile as a cut off. Based on this dichotomization of maternal psychiatric symptoms, four categories were created. (1) Persistently low anxiety, (2) high prenatal anxiety only, (3) high postnatal anxiety only, (4) persistently high anxiety. Persistently low anxiety was used as a reference group and contrasted against the other three groups for infant mean HR. We followed an identical procedure to elucidate possible differential effects of pre-and postnatal depression symptoms.

To further investigate the effect of maternal psychiatric symptom severity, we defined four categories based on postnatal maternal anxiety symptoms from the BSI anxiety scale. (1) A category of mothers reporting no anxiety symptoms. This category was used as a reference group (65%). (2) A category of mothers reporting some anxiety symptoms, a BSI anxiety score lower then 0.2 (18%). (3) An intermediate category of mothers reporting moderate anxiety symptoms, representing a BSI score between 0.2 and 0.5 (8%). (4) A final category with mothers reporting the most anxiety symptoms, with a BSI score above 0.5 (9%).

Effect sizes for all significant effects of the dichotomous determinants are given as Cohen's d. Cohen's d is calculated by dividing the difference between two means by the pooled standard deviation for those means. By convention, values of d up to 0.2 are defined as a small effect size, values up to 0.5 are defined as medium, values larger than 0.5 are considered large (38).

All statistical analyses were carried out using the Statistical Package for the Social Sciences 13.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Non-response analysis and study characteristics

In a non-response analysis, we compared the characteristics of our study population of 514 children and their mothers to the 370 other children and their mothers who attended this examination round but for whom insufficient data was available. There were no significant differences between the groups.

In table 1, we show the characteristics of the study participants. Of the mothers, 14 % had a lifetime history of an anxiety disorder, 12% had a lifetime history of a mood disorder. There was a high correlation between mean HR level and vagal modulation (Pearson's $\rho = -.634$, $p < 0.01$).

Maternal history of a psychiatric diagnosis

Table 2 shows the relation of a maternal history of a psychiatric diagnosis according to the CIDI with infant mean HR and vagal modulation. Infants of mothers with a lifetime history of a psychiatric diagnosis had a higher mean HR level. Moreover, these infants also had a lower vagal modulation, especially if the mother had a history of an anxiety disorder. The mean HR level of infants whose mothers experienced any psychiatric disorder during their

Table 1. Characteristics of the study population (n=514).

Mother	Age mother at intake, years (SD)	31.8 (3.9)
	Used alcohol during pregnancy %	58
	Smoked during pregnancy %	19
	BSI	
	Depressive symptoms prenatal (SD)	.11 (.2)
	Anxiety symptoms prenatal (SD)	.18 (.3)
	Depressive symptoms postnatal (SD)	.12 (.2)
	Anxiety symptoms postnatal (SD)	.15 (.3)
	CIDI	
	Lifetime psychiatric diagnosis %	25
	Lifetime anxiety disorder diagnosis %	14
	Lifetime mood disorder diagnosis %	12
Child	Female %	50
	Birth weight, g (SD)	3492 (564)
	Gestational age at birth, weeks (SD)	39.9 (1.8)
	Mean heart rate, bpm (SD)	124 (10)
	Vagal Modulation, lg ms^2 (SD)	2.7 (.3)

BSI = Brief Symptom Inventory (range of scores: 0 to 4), CIDI = Composite International Diagnostic Interview

lifetime was 0.24 SD higher. (β=0.24, 95% CI: 0.03; 0.4, p=0.02). Conversely, their vagal modulation was 0.14 SD lower (β=-0.14, 95% CI: -0.6; -0.03, p=0.003). Effect sizes were small to medium, d=0.25 and d=0.34 respectively. Infants of mothers reporting a lifetime presence of an anxiety disorder had a lower vagal modulation, by 0.3 SD (β=-0.30, 95% CI: -0.6;-0.4, p=0.03, d=0.31). There were no significant associations between maternal history of a mood disorder and infant mean HR or vagal modulation.

Infant temperament did not mediate the association between maternal psychopathology and infant autonomic functioning. Two dimensions of infant temperament were associated with maternal psychopathology at p<0.1. Infants of mothers with a lifetime history of any psychiatric diagnosis had more sadness symptoms ((β=0.1, 95% CI: -0.01; 0.2, p=0.08) and recovered slower from stressful situations (β=-0.18, 95% CI: -0.2; -0.03, p=0.006). However, infant sadness was neither associated with infant HR (β=-0.06, 95% CI: -0.7; 0.2, p=0.3), nor with infant vagal modulation (β=-0.01, 95% CI: -0.5; -0.4, p=0.8). Similarly, infant recovery from distress and infant HR (β=-0.01, 95% CI: -0.4; 0.4, p=0.9) and vagal modulation (β=0.05, 95% CI: -0.4; 0.5, p=0.8) were not associated.

Pre-and postnatal maternal psychiatric symptoms

In table 3, we present the associations between pre-and postnatal anxiety and depression symptoms and the autonomic indices. A β of 1 represents a SD increase in mean HR or

Table 2. Association between lifetime history of maternal psychopathology and infant heart rate and heart rate variability. (N = 450)

	Heart Rate (log_{10} bpm / SD)			Vagal Modulation (log_{10} ms^2 / SD)		
Psychiatric lifetime Diagnosis	β	95% CI	p	β	95% CI	p
Any disorder	0.24	0.03; 0.4	0.02	-0.14	-0.6; -0.2	0.003
Any anxiety disorder	0.21	-0.05; 0.5	0.1	-0.30	-0.6; -0.4	0.03
Any mood disorder	0.20	-0.07; 0.5	0.1	-0.26	-0.6; 0.02	0.08

All models are adjusted for maternal age, maternal smoking and alcohol use in the first three months of pregnancy, and gestational age at birth, birth weight, and sex of the infant. A β of 1.0 signifies that the respective physiologic parameter is a standard deviation higher in infants of mothers with a specific diagnosis than in infants of mothers without such diagnosis.

vagal modulation per point increase on mean item score of the relevant BSI subscale. Prenatal maternal psychiatric symptoms were not associated with any of the infant's autonomic indices, although there was a trend for children of mothers with high prenatal anxiety scores to have a higher mean HR level (β=0.08, 95% CI: -0.01; 0.2, p=0.09). In contrast, postnatal maternal psychiatric symptoms were clearly associated with infant mean HR level. Children of mothers with postnatal anxiety symptoms had higher mean HR level (β=0.14, 95% CI: 0.05; 0.2, p=0.004), as did children of mothers with postnatal depression symptoms (β=0.11, 95% CI: 0.01; 0.2, p=0.02).
In infants of mothers who reported postnatal anxiety symptoms, there was a trend towards lower vagal modulation (β=-0.09, 95% CI: -0.2; 0.009 p=0.07). However, associations between postnatal maternal psychopathology and vagal modulation were not significant.
The relationship of maternal anxiety disorders and anxiety symptoms with infant autonomic indices appeared stronger than the relationship between maternal depressive disorder and depressive symptoms with these autonomic indices. However, tables 2 and 3 show that the β values for the associations between maternal anxiety with infant autonomic functioning fall within the 95% CI for the corresponding associations of maternal depression. Hence, the difference between these associations is not statistically significant.

Timing of maternal symptoms
When we studied the combined effect of pre-and postnatal symptoms in a multilevel analysis, maternal anxiety symptoms were consistently associated with a higher infant mean HR level (F=6.91, p=0.001, after Bonferroni correction p=0.004) and lower vagal modulation (F=4.54, p=0.03, after Bonferroni correction p=0.12). Furthermore, depressive symptoms were associated with a higher infant mean HR level(F=4.49, p=0.03, after Bonferroni correction p=0.12). Depressive symptoms were not associated with infant vagal modulation. We found no time trends (all p>0.1), so the change of symptoms over time did not predict mean HR level or vagal modulation.
Finally, we compared the infant's mean HR level and vagal modulation for our previously defined groups based on pre-and postnatal anxiety symptoms. The mean HR level of infants

Table 3. Association between maternal psychiatric symptoms and infant heart rate variability.

		Heart Rate (log_{10} bpm / SD)			Vagal Modulation (log_{10} ms^2 / SD)		
Prenatal maternal symptoms	N	β	95% CI	p	β	95% CI	p
Anxiety symptoms	499	.08	-.01; .2	.09	-.06	-.2; .03	.2
Depression symptoms	499	.03	-.05; .1	.4	-.00	-.09; .09	.9
Postnatal maternal symptoms	N	β	95% CI	p	β	95% CI	p
Anxiety symptoms	409	.1	.05; .2	.004	-.09	-.2; .009	.07
Depression symptoms	410	.1	.01; .2	.02	-.05	-.2; .05	.3

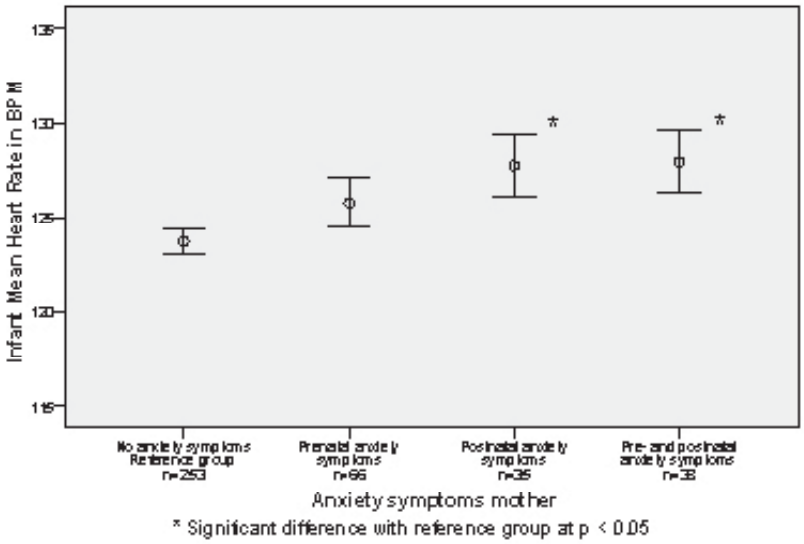
All models are adjusted for maternal age, maternal smoking and alcohol use in the first three months of pregnancy, and gestational age at birth, birth weight, and sex of the infant. A β of 1.0 signifies that the respective physiologic parameter is a standard deviation higher in infants of mothers with a specific diagnosis than in infants of mothers without such diagnosis.

of mothers reporting no anxiety (123.7 bpm, 95% CI: 122.4, 125.1), our reference group, and mothers reporting anxiety prenatal (125.8 bpm, 95% CI: 123.3, 128.4) did not differ (p=0.1). Compared to infants of our reference group, infants of mothers reporting anxiety only postnatal (127.7 bpm, 95% CI: 124.4, 131.0, p=0.02) and infants of mothers who reported anxiety both pre-and postnatal (128.0 bpm, 95% CI: 124.6, 131.3, p=0.01) had a higher mean HR level as shown in figure 1. Effect sizes were d=0.40 and d=0.41 respectively. Additionally, infants of mothers who had anxiety both pre-and postnatal did not differ from infants whose mothers had postnatal anxiety only. We could not distinguish whether this is because the duration of exposure to maternal psychiatric symptoms has no effect on infant autonomic functioning or because prenatal maternal psychiatric symptoms have no such effect. Further analyses for depressive symptoms versus mean HR level yielded no differences between the groups.

Maternal symptom severity

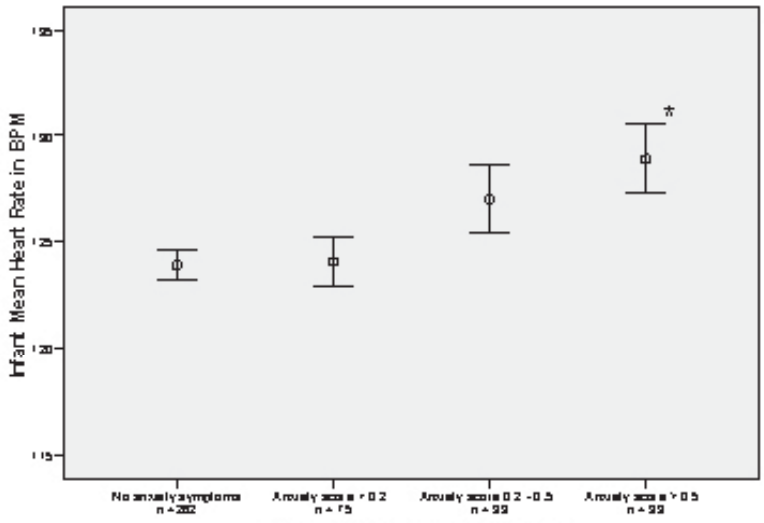
Figure 2 shows that severity of maternal anxiety symptoms was related to infant autonomic functioning. Overall, mothers with more severe postnatal anxiety symptoms had infants with higher mean HR (F=3.657, p=0.01). Posthoc analysis showed that infants of mothers with the highest postnatal had significantly higher HR than infants of mothers from the reference group (p=0.003) and from the low anxiety group (p=0.016).

Figure 1. Infant heart rate as a function of maternal anxiety symptoms. Error bars represent Standard Errors.



* Significant difference with reference group at $p < 0.05$

Figure 2. Infant heart rate as a function of maternal postnatal anxiety symptoms. Error bars represent Standard Errors.



* Significant difference with reference group at $p < 0.05$

Discussion

A maternal history of psychopathology was associated with an increased mean HR level and lower vagal modulation in the infant. Furthermore, postnatal psychiatric symptoms, especially anxiety symptoms, were related to a similar pattern of autonomic functioning in the infant.

Literature on the association between maternal psychopathology and infant ANS functioning is not in agreement. On the one hand, Jones et al. (24) and Pickens and Field (39) showed that infants of mothers with prenatal depressive symptoms had a significantly lower vagal modulation, than infants of non-symptomatic mothers. On the other hand, in a recently published study Kaplan et al. reported no difference in vagal modulation, between infants of prenatal depressed women and non-depressed women (23).

Associations between maternal psychopathology and infant autonomic functioning were not mediated by infant temperament. In literature regarding determinants of infant mean HR and vagal modulation, we come across several other plausible mechanisms for the association between maternal psychopathology and child mean HR and vagal modulation. These mechanisms should not been seen as mutually exclusive, as added effects may account for the observed association between maternal psychiatric symptoms and infant autonomic functioning.

First, the existence of postnatal maternal psychiatric symptoms could be an early stressor for the infant, for example, by a means of an altered mother-child interaction (40). The importance of the mother-child interaction in the development of cardiovascular control has been shown in animals. For instance, separation from the mother induced changes in the circadian regulation of HR in infant monkeys (41) and lead to increased HR in ratpups (42). In humans, Feldman and Eidelman demonstrated that physical mother-child interaction is important for the maturation of the vagal system, at least in premature infants (25). Furthermore, Kaplan et al. showed that maternal sensitivity towards the infant was associated with a higher infant vagal tonus (23). Our analyses clearly show that postnatal psychiatric symptoms, and especially anxiety symptoms, are associated with infant ANS functioning; i.e. a higher mean HR level and a trend toward lower vagal modulation, a measure for cardiac vagal modulation.

A second possible mechanism encompasses genetic factors. There is now substantial evidence that genetic variations are underlying the association found between psychopathology and altered ANS functioning. Our results are compatible with the presence of genetic factors underlying both maternal psychopathology and infant ANS. In a recent behavioural genetic study, Riese et al. examined the nature of the relationship between neuroticism, a marker for internalising psychopathology, and functioning of the ANS in 125 female twin pairs. They reported that shared genetic factors were partly responsible for the association between high neuroticism and an unfavourable ANS profile (43). There is support for an underlying genetic vulnerability from molecular genetic research as well. Studies reported an association between a polymorphism of the angiotensin converting enzyme and lower vagal modulation (44). Variations of the angiotensin converting enzyme were found to be associated with both anxiety (45) and mood disorders (46). Likewise, the alpha2C-adrenoreceptor gene has been implicated both in changes of HRV (47) and in the pathophysiology of mood disorders (48).

Third, according to the fetal programming hypothesis, fetal physiological systems adapt to the characteristics of the intrauterine environment. This adaptation may permanently alter

the set points of these physiological systems (49). Research already showed that women's acute emotional reactivity during pregnancy can influence foetal HR patterns (26) and that this foetal response is dependent on maternal psychiatric status (28, 50). DiPietro et al. subjected pregnant women to a benign cognitive stressor and monitored foetal HRV, which increased in response to this stimulus. They proposed that autonomic development is partially entrained through maternal environmental intrusions (27). However, little is known about the effect of chronic stress on foetal HRV and whether this leads to lasting changes in the autonomic set points. Nonetheless, Jones et al. (16) showed that infants of mothers with prenatal depressive symptoms measured in between week 30 and 35 of the pregnancy had significantly lower vagal modulation than infants of non-symptomatic mothers. In contrast, Kaplan et al. reported no association between prenatal maternal depression measured in the 2nd trimester and infant vagal modulation (23). In our regression analyses, we could not demonstrate an association between prenatal maternal psychiatric symptoms and infant ANS, although there was a trend for infants of woman with higher psychiatric symptoms to have higher mean HR. Furthermore, in a group based analysis, infants of mothers reporting prenatal anxiety symptoms did not differ significantly from our reference group. Taken together our data do not support a lasting effect of prenatal maternal psychiatric status and infant ANS functioning. This may be due to the timing of the exposure of the fetus to maternal psychiatric symptoms. Yehuda et al.(51) have shown that timing of maternal anxiety during pregnancy moderates it's impact on the babies later HPA-axis functioning. This could also explain why, in a study of Jones et al. maternal psychiatric symptoms exerted influence on the infant's autonomic functioning in the 3rd trimester as investigated by Jones et al. (24), but not in other studies that focused on the 2nd trimester, e.g. research by Kaplan et al. (23) and the present study

The relationship between maternal anxiety symptoms and infant autonomic indices appeared stronger than the relationship between maternal depressive symptoms and these indices. However, this marked difference did not reach statistical significance. Therefore, we must interpret these findings very cautiously, but it is tempting to speculate that the genetic factors underlying autonomic functioning are more closely associated with the pathophysiology of anxiety than with the pathophysiology of depression. This would be consistent with the tripartite model by Clark and Watson (52), which states that physiological arousal in anxiety disorders is one of the features which differentiate anxiety from depression. Alternatively, one might speculate that the environment provided by a mother with anxiety symptoms may influence the child's autonomic functioning more than the environment provided by a mother with depressive symptoms. Anxious mothers tend to overprotect their infants (53), which may be more stressful to the infant than the neglectful or distant parenting style associated with maternal depression (54). However, some depressed mothers display a controlling parenting style too (54). Hence, measurement of parenting style is needed to validate the hypothesis that maternal overprotection influences the infants' autonomic functioning.

Strengths and limitations

Our study encompasses a large sample size, especially considering the challenges faced when one works with children this young. The study benefits from a prospective design and measurements and multiple time points. Lifetime history of a psychiatric disorder according to DSM-IV-TR criteria was assessed before birth with the CIDI structured interview. More-

over, we assessed maternal psychiatric symptoms both during pregnancy and after birth. Analyses using both instruments yielded consistent results.
However, not all participants of the Generation R Focus cohort could be included, because we only added physiological measurements to our assessment protocol while the examination round was already ongoing. In combination with some non-participation this may have led to selection effects. Furthermore, the Focus cohort is limited to Dutch participants of Caucasian origin to exclude potential confounding or effect modification by ethnicity. This limits the generalisability of these results to other ethnic groups. Reliable information on antidepressant use during pregnancy was lacking. A Dutch study comprising 15000 women and conducted during the same time frame as our study showed that, although antidepressant use during pregnancy is rising, less than 3% of children were exposed to antidepressant use in utero. Due to this low prevalence, antidepressant use is unlikely to have influenced our results substantially (55). Finally, measuring the infant's autonomic response to a stressor might have supplied additional information on current autonomic functioning and on the risk of future mental health problems.
In conclusion, this study shows that maternal psychopathology and psychiatric symptoms are associated with autonomic functioning in infants, namely increased mean HR and decreased vagal modulation. In older children increased autonomic arousal is a marker for behavioural inhibition, internalising problems and affective disorders(1, 5, 6, 56). Some researchers have even suggested that high autonomic arousal is a causative factor in the development of affective problems (7, 8). Infants of mothers with psychiatric problems already display this vulnerability to psychopathology. In addition to a large body of literature concerning the impact of maternal psychopathology on attachment and infant temperament, the association between maternal psychiatric symptoms and infant autonomic functioning further underscores the importance of early detection and treatment of maternal psychopathology.
Further research is warranted. We suggest investigation of maternal autonomic functioning as a potential mediator for the relation between maternal psychopathology and infant autonomic functioning. If such a mediation effect were to be found, it would support for the notion of genetic factors underlying the association between maternal psychopathology and infant autonomic functioning. Furthermore, studies in a clinical sample could yield additional information for instance on the effect of treatment of maternal psychopathology (e.g. cognitive therapy, vagal stimulation or SSRI) on the reported association.

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References

1. Monk C, Kovelenko P, Ellman LM, Sloan RP, Bagiella E, Gorman JM, et al. Enhanced stress reactivity in paediatric anxiety disorders: implications for future cardiovascular health. Int J Neuropsychopharmacol. 2001 Jun;4(2):199-206.
2. Ortiz J, Raine A. Heart rate level and antisocial behavior in children and adolescents: a meta-analysis. Journal of the American Academy of Child and Adolescent Psychiatry. 2004 Feb;43(2):154-62.
3. Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. Science. 1981 Jul 10;213(4504):220-2.
4. Tulen JH, Man in 't Veld AJ, Van Roon AM, Moleman P, Van Steenis HG, Blankestijn PJ, et al. Spectral analysis of hemodynamics during infusions of epinephrine and norepinephrine in men. J Appl Physiol. 1994 May;76(5):1914-21.
5. Kagan J, Reznick JS, Snidman N. The physiology and psychology of behavioral inhibition in children. Child development. 1987 Dec;58(6):1459-73.
6. Dietrich A, Riese H, Sondeijker FE, Greaves-Lord K, van Roon AM, Ormel J, et al. Externalizing and internalizing problems in relation to autonomic function: a population-based study in preadolescents. Journal of the American Academy of Child and Adolescent Psychiatry. 2007 Mar;46(3):378-86.
7. Porges SW. The polyvagal perspective. Biol Psychol. 2007 Feb;74(2):116-43.
8. Friedman BH. An autonomic flexibility-neurovisceral integration model of anxiety and cardiac vagal tone. Biol Psychol. 2007 Feb;74(2):185-99.
9. Dubreuil E, Ditto B, Dionne G, Pihl RO, Tremblay RE, Boivin M, et al. Familiality of heart rate and cardiac-related autonomic activity in five-month-old twins: the Quebec newborn twins study. Psychophysiology. 2003 Nov;40(6):849-62.
10. Longin E, Gerstner T, Schaible T, Lenz T, Konig S. Maturation of the autonomic nervous system: differences in heart rate variability in premature vs. term infants. J Perinat Med. 2006;34(4):303-8.
11. Galland BC, Taylor BJ, Bolton DP, Sayers RM. Heart rate variability and cardiac reflexes in small for gestational age infants. J Appl Physiol. 2006 Mar;100(3):933-9.
12. Browne CA, Colditz PB, Dunster KR. Infant autonomic function is altered by maternal smoking during pregnancy. Early Hum Dev. 2000 Sep;59(3):209-18.
13. Haley DW, Handmaker NS, Lowe J. Infant stress reactivity and prenatal alcohol exposure. Alcohol Clin Exp Res. 2006 Dec;30(12):2055-64.
14. John V, Dai H, Talati A, Charnigo RJ, Neuman M, Bada HS. Autonomic alterations in cocaine-exposed neonates following orthostatic stress. Pediatr Res. 2007 Feb;61(2):251-6.
15. Kim-Cohen J, Moffitt TE, Taylor A, Pawlby SJ, Caspi A. Maternal depression and children's antisocial behavior: nature and nurture effects. Arch Gen Psychiatry. 2005 Feb;62(2):173-81.
16. Field T, Diego M, Hernandez-Reif M, Figueiredo B, Schanberg S, Kuhn C, et al. Chronic prenatal depression and neonatal outcome. Int J Neurosci. 2008 Jan;118(1):95-103.
17. Buitelaar JK, Huizink AC, Mulder EJ, de Medina PG, Visser GH. Prenatal stress and cognitive development and temperament in infants. Neurobiol Aging. 2003 May-Jun;24 Suppl 1:S53-60; discussion S7-8.
18. Sohr-Preston SL, Scaramella LV. Implications of timing of maternal depressive symptoms for early cognitive and language development. Clin Child Fam Psychol Rev. 2006 Mar;9(1):65-83.
19. Talge NM, Neal C, Glover V. Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? Journal of child psychology and psychiatry, and allied disciplines. 2007 Mar-Apr;48(3-4):245-61.
20. Austin MP, Hadzi-Pavlovic D, Leader L, Saint K, Parker G. Maternal trait anxiety, depression and life event stress in pregnancy: relationships with infant temperament. Early Hum Dev. 2005 Feb;81(2):183-90.
21. Vaughn BE, Bradley CF, Joffe LS, Seifer R, Barglow P. Maternal characteristics measured prenatally are predictive of ratings of temperamental 'difficulty' on the Carey Infant Temperament Questionnaire. . Developmental psychology. 1987;23:152–61.
22. Davis EP, Glynn LM, Schetter CD, Hobel C, Chicz-Demet A, Sandman CA. Prenatal exposure to maternal depression and cortisol influences infant temperament. Journal of the American Academy of Child and Adolescent Psychiatry. 2007 Jun;46(6):737-46.
23. Kaplan LA, Evans L, Monk C. Effects of mothers' prenatal psychiatric status and postnatal caregiving on infant biobehavioral regulation: Can prenatal programming be modified? Early Hum Dev. 2007 Aug 28.
24. Jones NA, Field T, Fox NA, Davalos M, Lundy B, Hart S. Newborns of mothers with depressive symptoms are physiologically less developed. . Inf Beh Dev. 1998;21:537-41.
25. Feldman R, Eidelman AI. Skin-to-skin contact (Kangaroo Care) accelerates autonomic and neurobehavioural maturation in preterm infants. Dev Med Child Neurol. 2003 Apr;45(4):274-81.
26. Monk C, Fifer WP, Myers MM, Sloan RP, Trien L, Hurtado A. Maternal stress responses and anxiety during

pregnancy: effects on fetal heart rate. Dev Psychobiol. 2000 Jan;36(1):67-77.
27. DiPietro JA, Costigan KA, Gurewitsch ED. Fetal response to induced maternal stress. Early Hum Dev. 2003 Nov;74(2):125-38.
28. Monk C, Sloan RP, Myers MM, Ellman L, Werner E, Jeon J, et al. Fetal heart rate reactivity differs by women's psychiatric status: an early marker for developmental risk? Journal of the American Academy of Child and Adolescent Psychiatry. 2004 Mar;43(3):283-90.
29. Muris P, Ollendick TH. The role of temperament in the etiology of child psychopathology. Clin Child Fam Psychol Rev. 2005 Dec;8(4):271-89.
30. Calkins SD. Cardiac vagal tone indices of temperamental reactivity and behavioral regulation in young children. Dev Psychobiol. 1997 Sep;31(2):125-35.
31. Gunnar MR, Porter FL, Wolf CM, Rigatuso J, Larson MC. Neonatal stress reactivity: predictions to later emotional temperament. Child development. 1995 Feb;66(1):1-13.
32. Jaddoe VW, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Verhulst FC, et al. The Generation R Study: Design and cohort profile. Eur J Epidemiol. 2006;21(6):475-84.
33. Andrews G, Peters L. The psychometric properties of the Composite International Diagnostic Interview. Soc Psychiatry Psychiatr Epidemiol. 1998 Feb;33(2):80-8.
34. Derogatis LR, Melisaratos N. The Brief Symptom Inventory: an introductory report. Psychol Med. 1983 Aug;13(3):595-605.
35. Roza SJ, van Lier PA, Jaddoe VW, Steegers EA, Moll HA, Mackenbach JP, et al. Intrauterine growth and infant temperamental difficulties: the Generation R Study. Journal of the American Academy of Child and Adolescent Psychiatry. 2008 Mar;47(3):264-72.
36. van Steenis HG, Tulen JH, Mulder LJ. Heart rate variability spectra based on non-equidistant sampling: the spectrum of counts and the instantaneous heart rate spectrum. Med Eng Phys. 1994 Sep;16(5):355-62.
37. Bar-Haim Y, Marshall PJ, Fox NA. Developmental changes in heart period and high-frequency heart period variability from 4 months to 4 years of age. Dev Psychobiol. 2000 Jul;37(1):44-56.
38. Cohen J. Statistical power analysis for the behavioral sciences (2nd ed.). Hillsdale, NJ: Erlbaum; 1988.
39. Pickens J, Field T. Facial expressions and vagal tone in infants of depressed and non-depressed mothers. Early Dev Par. 1995;4:83-9
40. Nicol-Harper R, Harvey AG, Stein A. Interactions between mothers and infants: impact of maternal anxiety. Infant Behav Dev. 2007 Feb;30(1):161-7.
41. Reite M, Seiler C, Crowley TJ, Hydinger-Macdonald M, Short R. Circadian rhythm changes following maternal separation. Chronobiologia. 1982 Jan-Mar;9(1):1-11.
42. Shear MK, Brunelli SR, Hofer MA. The effects of maternal deprivation and of refeeding on the blood pressure of infant rats. Psychosomatic medicine. 1983 Mar;45(1):3-9.
43. Riese H, Rosmalen JG, Ormel J, Van Roon AM, Oldehinkel AJ, Rijsdijk FV. The genetic relationship between neuroticism and autonomic function in female twins. Psychol Med. 2007 Feb;37(2):257-67.
44. Thayer JF, Merritt MM, Sollers JJ, 3rd, Zonderman AB, Evans MK, Yie S, et al. Effect of angiotensin-converting enzyme insertion/deletion polymorphism DD genotype on high-frequency heart rate variability in African Americans. Am J Cardiol. 2003 Dec 15;92(12):1487-90.
45. Olsson M, Annerbrink K, Westberg L, Melke J, Baghaei F, Rosmond R, et al. Angiotensin-related genes in patients with panic disorder. Am J Med Genet B Neuropsychiatr Genet. 2004 May 15;127(1):81-4.
46. Baghai TC, Binder EB, Schule C, Salyakina D, Eser D, Lucae S, et al. Polymorphisms in the angiotensin-converting enzyme gene are associated with unipolar depression, ACE activity and hypercortisolism. Mol Psychiatry. 2006 Nov;11(11):1003-15.
47. Neumeister A, Charney DS, Belfer I, Geraci M, Holmes C, Sharabi Y, et al. Sympathoneural and adrenomedullary functional effects of alpha2C-adrenoreceptor gene polymorphism in healthy humans. Pharmacogenet Genomics. 2005 Mar;15(3):143-9.
48. Neumeister A, Drevets WC, Belfer I, Luckenbaugh DA, Henry S, Bonne O, et al. Effects of a alpha 2C-adrenoreceptor gene polymorphism on neural responses to facial expressions in depression. Neuropsychopharmacology. 2006 Aug;31(8):1750-6.
49. Barker DJ. Fetal and infant origins of adult disease. London: BMJ Books; 1992.
50. Monk C, Myers MM, Sloan RP, Ellman LM, Fifer WP. Effects of women's stress-elicited physiological activity and chronic anxiety on fetal heart rate. J Dev Behav Pediatr. 2003 Feb;24(1):32-8.
51. Yehuda R, Engel SM, Brand SR, Seckl J, Marcus SM, Berkowitz GS. Transgenerational effects of posttraumatic stress disorder in babies of mothers exposed to the World Trade Center attacks during pregnancy. J Clin Endocrinol Metab. 2005 Jul;90(7):4115-8.
52. Clark LA, Watson D. Tripartite model of anxiety and depression: psychometric evidence and taxonomic implications. J Abnorm Psychol. 1991 Aug;100(3):316-36.

53. Barrett PM, Rapee RM, Dadds MM, Ryan SM. Family enhancement of cognitive style in anxious and aggressive children. J Abnorm Child Psychol. 1996 Apr;24(2):187-203.
54. Lovejoy MC, Graczyk PA, O'Hare E, Neuman G. Maternal depression and parenting behavior: a meta-analytic review. Clin Psychol Rev. 2000 Aug;20(5):561-92.
55. Bakker MK, Kolling P, van den Berg PB, de Walle HE, de Jong van den Berg LT. Increase in use of selective serotonin reuptake inhibitors in pregnancy during the last decade, a population-based cohort study from the Netherlands. Br J Clin Pharmacol. 2008 Apr;65(4):600-6.
56. Scarpa A, Raine A, Venables PH, Mednick SA. Heart rate and skin conductance in behaviorally inhibited Mauritian children. J Abnorm Psychol. 1997 May;106(2):182-90.

Chapter 3

Spot the red herring: breastfeeding, fruitpurée, and infant autonomic functioning.

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Abstract
Several studies have suggested that breastfeeding is related to infant autonomic functioning. The authors investigated whether this is a causal relation.
444 mothers reported breastfeeding practices two months postpartum. Infant autonomic functioning was assessed by heart rate variability at age 14 months, after discontinuation of breastfeeding. The dose-dependent association between breastfeeding and infant autonomic functioning was tested with linear regression models adjusted for multiple confounders. The authors investigated the relation of fruitpurée consumption with infant autonomic functioning. Fruitpurée consumption has similar socio-economic epiphenomena but is not related via the same causal mechanism to autonomic regulation as breastfeeding.
Non-breastfed infants had high sympathetic modulation (7.87 log(ms2)/SD, 95%CI: 7.71, 8.02), partially breastfed infants had intermediate sympathetic modulation (7.75 log(ms2)/SD, 95%CI: 7.51, 7.82), sympathetic modulation of exclusively breastfed infants was low (7.63 log(ms2)/SD, 95%CI: 7.50, 7.77). However, this association could be explained by socio-economic confounders. Furthermore, fruitpurée consumption was similarly associated with reduced infant sympathetic modulation.
The association between breastfeeding practices and infant sympathetic modulation, was accounted for by socio-economic and environmental factors. We found a similar association between fruitpurée consumption and autonomic functioning, further suggesting that the association between breast feeding and infant autonomic functioning is non-causal.

Introduction

Breastfeeding may have several beneficial effects on cardiovascular functioning, including decreased adiposity and blood pressure in later life (1, 2). Likewise, studies suggest that breastfeeding is related to lower total plasma cholesterol and less intima-media thickness in adults (3, 4). Research also indicated that breastfeeding has a beneficial effect on the infant's autonomic cardiovascular regulation. Two decades ago, DiPietro et al. studied autonomic functioning and observed lower heart rate and higher heart rate variability in breastfed neonates compared to formula-fed neonates (5). Similar findings were reported in infants aged 1 and 4 months. Breastfed infants had a lower resting heart rate than infants who were formula fed (6). Long-term benefits of such an autonomic profile have been described, in adults it was shown that lower resting heart rates and higher heart rate variability are associated with a lower risk of cardiovascular mortality (7, 8).

Different aspects of breastfeeding could account for the effect on the infant's cardiovascular regulation. Firstly, research in adults showed that the intake of long chain poly-unsaturated fatty acids is associated with increased vagal modulation and a decreased sympathetic/vagal ratio.(9) The composition of human milk, namely the mixture of fatty acids, meets the nutritional needs of the human infant best.(10) Although formula milk is currently prepared with various concentrations and mixtures of long-chain poly-unsaturated fatty acids, plasma levels of these fatty acids in formula-fed infants do not reach those found in breastfed infants (11, 12).

Secondly, breastfeeding may influence infant cardiovascular regulation because it generates less physiological stress during feeding than bottle feeding. Research in preterm infants has shown that breastfed infant's heart rate, respiratory rate and body temperature increase more during feeding than their bottle fed counterparts (13, 14).

Thirdly, as Feldman and Eidelman showed, in preterm infants skin-to-skin contact promotes infant autonomic maturation. They documented that infants treated with kangaroo care, which emphasises mother-infant skin-to-skin contact, showed a greater increase in vagal tone than infants who received no such care (15).

However, the evidence for differential autonomic functioning in breastfed infants compared to formula fed infants is not conclusive. The existing studies measured infant autonomic functioning during the breastfeeding period only. Hence, it is unclear whether the effect reported persists after mothers discontinued breastfeeding. Moreover, these studies were relatively small, making them particularly vulnerable to biases.

Further, there are clear socio-economic differences between mothers who chose to breastfeed and those who chose to bottle feed (16). Studies without adequate control for confounders may erroneously infer a causal effect of breastfeeding (17). The US Agency of Healthcare Research and Quality reviewed the current literature on health benefits of breastfeeding and concluded that while breastfeeding is associated with a reduced risk of many diseases in infants, most of the associations were detected in observational studies. They warn not to infer causality based on such findings due to the likelihood of confounding (18).

Indeed, previous studies only controlled for sex, age and weight (5, 6). Other environmental factors may also account for a spurious association between breastfeeding and infant autonomic functioning. Mothers, who breastfeed, display greater sensitivity in interactions with their infants (19). Maternal sensitivity, in particular, is associated with infant autonomic functioning as measured by increased vagal tone (20).

The presence of confounding can be represented by diagrams, known as directed acyclic graphs, as illustrated for birth defects by Hernan et al (21, 22). These graphs depict variables, linked by arrows which represent causal effects. Figure 3a is such a graph and shows that socio-economic factors influence both breastfeeding and autonomic functioning. Socio-economic factors violate the assumption of ignorability; they act as a confounder and need to be controlled for. Socio-economic and educational factors are very important determinants of breastfeeding (23, 24). Hence, as also shown in figure 3a, if breastfeeding is causally related to infant autonomic functioning, it is a potential mediator between socio-economic factors and infant autonomic functioning. However, as shown in figure 3b, even with diligent control for known confounders, residual confounding by socio-economic factors remains a challenge in observational research of breastfeeding (25).
An alternative approach to controlling for confounders is to compare the strength of the association between breastfeeding and infant autonomic functioning with another feeding parameter, which is related to similar socio-economic and behavioural epiphenomena as breastfeeding, but which lacks the specific biological properties of breastfeeding that may influence infant autonomic functioning (26).
In a large, well-defined, population based cohort, we assessed the effect of breastfeeding practices and the effect of fruitpurée consumption on autonomic functioning at 14 months. We hypothesized that breastfeeding has a direct biological effect on infant autonomic functioning, and that the association with autonomic functioning is stronger than the association of a comparator without the postulated physiological effects.

Methods

Participants

This study was conducted within the Focus Cohort of the Generation R Study, a population based prospective cohort study from foetal life until young adulthood (27, 28). Measurements of autonomic indices were added to the protocol of the examination round, while assessment was already ongoing. Physiological measurements were available for 528 infants. Our study population consisted of the 444 children, for whom both data on breastfeeding practices and autonomic indices were available. Written informed consent was obtained from all participants. Approval for the study was granted by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam.

Breastfeeding

Information on breastfeeding practices at 2 months of age was collected by postal questionnaire. Mothers were asked to indicate whether they breastfed their infants exclusively, whether they formula fed their infants exclusively or whether they combined both breastfeeding and formula feeding.

Psychophysiologic measurements

Mothers and their infants were invited to our laboratory 14 months after birth. Trained research assistants performed the physiological measurements. We registered heart rate using a precordial, three pole electrocardiogram (ECG) lead, sampled at 512 Hz. Furthermore, we monitored the breathing pattern using a piëzo-electric transducer. Signals were recorded using a Vitaport 3 digital recorder (Temec Inc, Kerkrade, the Netherlands). We recorded for 8 minutes, while the infant was at ease in its mother's lap. To help the infant relax, we

played an episode of the Teletubbies© (BBC/Ragdoll Limited). Recordings did not start until signals had reached a stabilized steady-state.

Power spectral analysis

We analysed the recorded data using custom made software. Because irregular, slow breathing distributes the variation attributable to vagal modulation across the frequency spectrum, we manually selected 100-180 seconds of the ECG were breathing was most regular. R-top detection was conducted on the selected data window. Interbeat intervals were examined on the basis of the time between consecutive R-tops in the ECG. We performed spectral analysis using discrete Fourier transformation, based on non equidistant sampling of the R-wave indices (29). The low frequency (LF) component of heart rate variability, defined between 0.04 Hz and 0.14 Hz, is dependent on sympathetic modulation and certainly in older individuals partly on vagal modulation as well. However, vagal modulation of heart rate is dependent on respiratory frequency, which is much higher in infants, likely attenuating the vagal component of LF variation. Hence, for reasons of simplicity we will refer to LF variation as a measure of sympathetic modulation. Furthermore, we adjusted the upper bound of the high frequency (HF) band, which is dependent solely on vagal modulation, according to recommendations from literature, to allow for the infant's higher respiratory frequency. We defined our HF band between 0.15 Hz and 1.04 Hz (30).

Covariates

Gender of the infant, gestational age at birth, and weight at time of assessment were used as covariates as they influence mean heart rate and heart rate variability. We obtained gestational age at birth and birth weight from community midwife and hospital registries. To address confounding by socio-economic factors, information on maternal age, maternal parity, maternal age at first birth and maternal level of highest completed education was available as well as information on paternal level of highest completed education and household income (16). Information on socio-economic factors was obtained by a questionnaire completed by the mother and her partner. Confounding by maternal sensitivity and confidence in caretaking was addressed by using the confidence-in-caretaking scale of the Mother and Baby Scale, which was part of the postal questionnaires sent to mothers when their child was 2 months old. The scale contains 13 items, which use a six-point Likert scale. Covariates were assumed to be me missing at random and were imputed by the mean.

Statistical analysis

For the non-response analysis, we compared the 444 mothers and children for whom breastfeeding data were available with the 84 mothers and children, who were not included in the analysis due to missing data on breastfeeding. To describe the study participants, we compared the characteristics of the infants who received no breastfeeding (n=156) to the characteristics of the infants who received partial breastfeeding (n=72) and the infants who received exclusive breastfeeding (n=216). We also investigated the association between the infant's fruitpurée consumption and the family's socio-economic status. For continuous variables we used analysis of variance (ANOVA) and for dichotomous variables Chi Square tests.

We conducted a power analysis with G*power to determine the detectable effect size (31). The physiological variables LF and HF were log-transformed to achieve a normal distribu-

tion. In addition, we divided the physiological variables by their own standard deviation to facilitate interpretation of the estimates.
We introduced sets of variables into successive analysis of covariance (ANCOVA) models to investigate whether the association between breastfeeding and infant autonomic functioning attenuated when confounders were controlled for. Additionally, we tested a dose-response effect with linear regression. Breastfeeding was entered as an ordinal variable with a value of 0, 1 or 2. We used partial breastfeeding as the intermediate category and calculated a p-trend.
First, we studied the univariate relation between breastfeeding and infant autonomic functioning, termed model 1. In model 2 we adjusted for basic confounders comprised of infant gender, infant birth weight and infant weight at 14 months. Finally, in model 3 we adjusted for socio-economic factors and maternal sensitivity.
However, model 3 may not give the definite answer to the study question. Even in large, well-defined studies, which rigorously control for socio-economic and other factors, it is virtually impossible to rule out residual confounding (25). Breastfeeding may be a marker for other factors related to infant autonomic functioning that were not fully captured by the covariables we employed, e.g. differences in rearing style or maternal stress during and after pregnancy (32).
To address this issue, we contrasted the relation between breastfeeding and infant autonomic functioning with the relation between another infant feeding parameter and infant autonomic functioning. We chose infant fruitpurée consumption as the best available contrasting variable. Generally, it is the earliest addition to the infant's diet, complimentary to continued breast or bottle feeding. Fruitpurée, similar to breastfeeding, is seen as healthy nourishment for infants.
Based on our data we know that fruitpurée consumption is related to similar environmental epiphenomena as breastfeeding. Fruitpurée lacks the long-chain unsaturated fatty acids found in breastmilk, it lacks breastfeeding's specific physiologic process and it is not implicitly associated with skin-to-skin contact. In short, it has none of the characteristics which could underlie the putative association between breastfeeding and infant autonomic functioning. Analogous to the analyses of breastfeeding, we performed linear regressions to determine the association between fruitpurée consumption and infant autonomic functioning.
Statistical analyses were carried out with the Statistical Package for the Social Sciences 15.0 (SPSS Inc, Chicago, Il, USA).

Comparator – Fruitpurée consumption
Fruitpurée consumption was assessed at twelve months after birth using a postal questionnaire. Fruitpurée is typically fed from 6 months onwards only. However, a nutritional comparator from the first 6 months of life is not conceivable because many infants are exclusively breastfed in this period. Fruitpurée consumption was assessed on a 7-point scale but collapsed to three categories, because of small numbers and to enable comparison with breastfeeding: 1/ less than one portion of fruitpurée per day, 2/ one portion of fruitpurée a day, 3/ more than one portion of fruitpurée a day.

Non-response and power analysis
Children without information on breastfeeding had similar autonomic functioning, as well

as similar weight at birth and at 14 months. Likewise, their mothers did not differ on age, or education (data not shown).
Power analysis demonstrated our study could detect small effects (f=0.15) at 0.85 power and 0.85 and small to medium effects (f=0.19) at 0.95 power, with α=0.05.

Results
46% of infants were exclusively breastfed at 2 months of age, while 16 % were partly breastfed. The remainder, 38%, received no breastfeeding at 2 months. Table 1 summarises the baseline characteristics for infants according to these categories. Infants, who received no breastfeeding, had a similar boy/girl ratio, birth weight and weight at 14 months compared to those who received partial or exclusive breastfeeding. Furthermore, infants who received no breastfeeding consumed roughly similar amounts of fruitpurée as children receiving partial or exclusive breastfeeding (χ 2 = 4.6, p=0.3). Breastfeeding was positively associated with maternal education (χ 2 = 43.6, p<0.001), paternal education (χ 2 = 29.6, p<0.001) and maternal age at first child birth (F=3.3, p=0.04) but was not associated with current maternal age (F=2.1, p=0.1) or household income (χ 2 = 4.4, p=0.1).

Table 1. Characteristics of the Study Population.

	No breastfeeding (reference) N = 156	Partial breastfeeding N = 72	Exclusive breastfeeding N = 216
Parental indices			
Age mother at intake, years (SD)	31.4 (4.2)	31.9 (3.7)	32.2 (3.3)
Maternal education			
Secondary education or lower (%)	53.9	32.4	23.1
Higher education, phase 1 (%)	23.7	31.0	26.4
Higher education, phase 2 (%)	22.4	36.6	50.5
Infant indices			
Female (%)	49	49	49
Birth weight in g (SD)	3479 (575)	3474 (595)	3590 (541)
Weight at 14 months g (SD)	10579 (1082)	10484 (1033)	10486 (1022)
Fruitpurée consumption			
Less than one a day (%)	13.4	8.3	7.4
One a day (%)	80.9	86.1	84.7
More than one a day (%)	5.7	5.6	7.9
Vagal activity log ms^2 (SD)	2.88 (0.42)	2.90 (0.44)	2.83 (0.42)
Sympathetic activity log ms^2 (SD)	2.79 (0.34)	2.75 (0.35)	2.71 (0.38)

Table 2. Amount of Breastfeeding Received at Age 2 Months and Infant Sympathetic Modulation.

n = 444	No breastfeeding	Partial breastfeeding		Exclusive breastfeeding		Dose response
	Mean Log ms^2 / SD Reference	Mean Log ms^2 / SD	Difference with Reference (95% CI:)	Mean Log ms^2 / SD	Difference with Reference (95% CI:)	*p* for trend
Model 1	7.87	7.75	-0.12 (-0.40; 0.16)	7.63	-0.23 (-0.44; -0.02) *	0.03
Model 2	7.86	7.74	-0.12 (-0.40; 0.17)	7.64	-0.22 (-0.42; -0.01) *	0.04
Model 3	7.86	7.75	-0.11 (-0.40; 0.17)	7.64	-0.22 (-0.45; 0.01)	0.12

* Different from reference group at $p < 0.05$.
Results displayed are from successive Analysis of Covariance models. Model 1 = unadjusted. Model 2 = as model 1, additionally adjusted for infant gender, infant birth weight and infant weight at 14 months. Model 3 as Model 2, additionally adjusted for maternal age, parental education, household income, maternal parity and maternal age at first birth, as well as maternal confidence in caretaking and household income. Dose response effects were investigated with linear regressions.

Table 3. Amount of Fruitpurée Consumed at Age 14 Months and Infant Sympathetic Modulation.

n = 444	< 1 portion a day	1 portion a day		> 1 portion a day		Dose response
	Mean Log ms^2 / SD Reference	Mean Log ms^2 / SD	Difference with Reference (95% CI:)	Mean Log ms^2 / SD	Difference with Reference (95% CI:)	*p* for trend
Model 1	7.99	7.69	-0.30 (-0.61; 0.01)	7.38	-0.62 (-1.05; -0.18)	0.007
Model 2	8.02	7.69	-0.32 (-0.63; -0.02)	7.35	-0.67 (-1.10; -0.23)	0.008
Model 3	8.02	7.69	-0.33 (-0.63; -0.02)	7.36	-0.66 (-1.10; -0.21)	0.008

* Different from reference group at $p < 0.05$.
Results displayed are from successive Analysis of Covariance models. Model 1 = unadjusted. Model 2 = as model 1, additionally adjusted for infant gender, infant birth weight and infant weight at 14 months. Model 3 as Model 2, additionally adjusted for maternal age, parental education, household income, maternal parity and maternal age at first birth, as well as maternal confidence in caretaking and household income. Dose response effects were investigated with linear regressions.

Infants' fruitpurée consumption at 12 months was positively associated with maternal education (χ 2=16.1, p=0.03), as well as current maternal age (F=2.995, p=0.05), maternal age at first childbirth (F=4.28, p=0.01) and household income (χ 2 = 7.17, p=0.03), but was not associated with paternal education (χ 2=4.7, p=0.3), maternal parity (F=0.51, p=0.6) or with household income .

First. we explored the association between breastfeeding and infant HF power, a measure of vagal modulation of the heart rate. The unadjusted model showed that there was no relation between infant breastfeeding and vagal modulation (p for trend = 0.70). We found similar levels of vagal modulation in non-breastfed infants (6.97 log(ms^2)/SD, 95% CI: 6.81, 7.13), partially breastfed (7.02 log(ms^2)/SD, 95% CI: 6.78, 7.26) and fully breastfed infants (6.86 log(ms^2)/SD, 95% CI: 6.72, 7.00) . Adding covariates to subsequent models yielded similar results (data not shown).

Table 2 depicts the results of successive regression models for the association between breastfeeding and LF power, a measure of infant sympathetic modulation. The unadjusted model is pictured in figure 1. Non-breastfed infants had high sympathetic modulation (7.87 log(ms^2)/SD, 95% CI: 7.71, 8.02), sympathetic modulation of partially breastfed infants was intermediate (7.75 log(ms^2)/SD, 95% CI: 7.51, 7.98) whereas sympathetic modulation of exclusively breastfed infants was lowest (7.63 log(ms^2)/SD, 95% CI: 7.50, 7.77). This means that sympathetic modulation in exclusively breastfed infants was 0.23 of a standard deviation lower than in infants who received no breastfeeding. Linear regression analysis showed a dose response effect for breastfeeding.

Next, we examined whether we could identify factors that explained the association between breastfeeding and infant sympathetic functioning. Infant gender and weight characteristics did not change the model substantially (see table 2). In contrast, the introduction of socio-economic factors and maternal sensitivity attenuated the relation between breastfeeding and infant sympathetic modulation. When these confounders were taken in account, non-breastfed infants had similar sympathetic modulation (7.84 log(ms^2)/SD, 95% CI: 7.67, 8.00) compared to partially breastfed infants (7.75 log(ms^2)/SD, 95% CI: 7.52, 7.98) and compared to exclusively breastfed infants (7.65 log(ms^2)/SD, 95% CI: 7.50, 7.79). A dose response effect was no longer present.

Figure 1. Breastfeeding and infant sympathetic modulation.

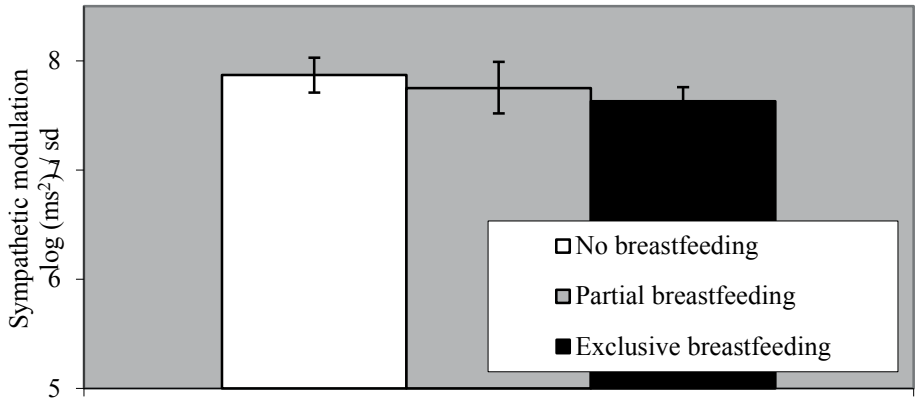


Figure 2. Fruitpurée and infant sympathetic modulation.

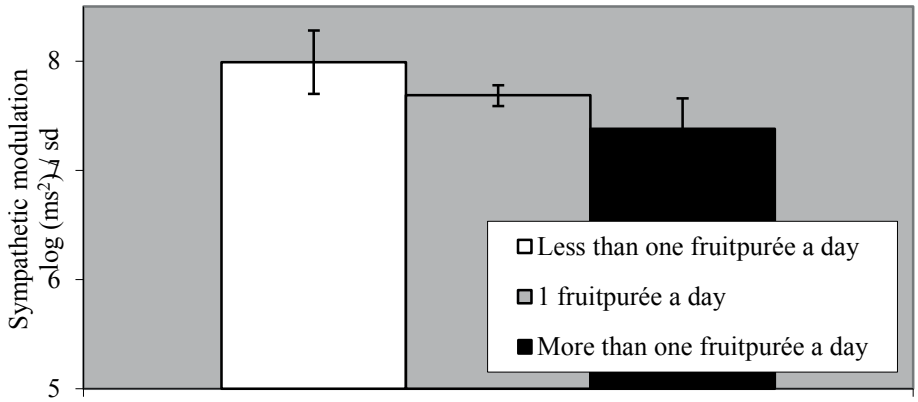


Table 3 displays the results of successive linear regression models for the association between fruitpurée and our measure of infant sympathetic modulation, LF power. The unadjusted linear regression model showed that the more fruitpurée an infant consumed, the lower the infants' sympathetic modulation, as depicted in figure 2. Children eating less than one portion of fruitpurée each day had the highest sympathetic modulation (7.99 log(ms^2)/SD, 95% CI: 7.70, 8.28), followed by children eating on average one portion of fruitpurée a day (7.69 log(ms^2)/SD, 95% CI: 7.60, 7.79), whilst those who ate more than one portion of fruitpurée a day had the lowest sympathetic modulation (7.38 log(ms^2)/SD, 95% CI: 7.10, 7.71) . Even in a fully adjusted model fruitpurée consumption was associated with lower infant sympathetic modulation, probably indicating residual confounding.

Discussion

Infants, who were exclusively breastfed at two months of age, had lower sympathetic modulation than infants not breastfed. However, the observed relation could be accounted for by socio-economic and educational factors. Higher infant fruitpurée consumption was associated with lower infant sympathetic modulation. No association between breastfeeding practises and infant vagal modulation was found.

Before we discuss these findings, some methodological comments have to be made. Our study benefits from a large sample size and a prospective design. Furthermore, we tried to address confounding by socio-economic and educational factors by controlling for several indicators. The Focus cohort is ethnically homogeneous to exclude potential confounding or effect modification by ethnicity. At the same time, this limits the generalizability of the results to other ethnic groups.

Earlier studies reported associations between breastfeeding and infant autonomic functioning (5, 6). However, these studies were small and did not control for socio-economic and educational factors (16, 19).

The association between breastfeeding and infant autonomic functioning disappeared after correction for socio-economic factors. However, the actual degree of attenuation of the association between breastfeeding and autonomic functioning achieved by correcting for socio-economic factors was minor, suggesting little effect of control. In addition, adding

Figure 3a. Confounding by socio-economic factors.

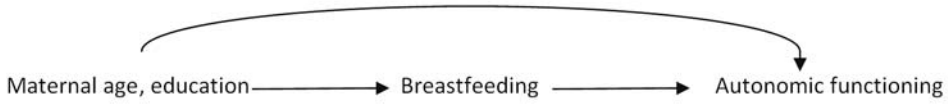
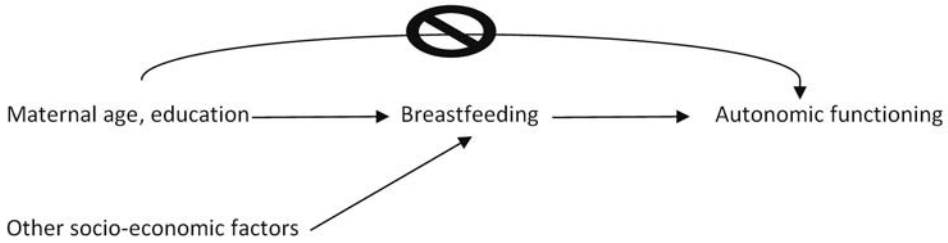


Figure 3b. Residual confounding after controlling for selected socio-economic factors



covariates to a model does not necessarily improve its' validity and hence the causal inferences made on the basis of the model. For example, research has shown that breastfeeding influences maternal sensitivity towards her infant (19). Maternal sensitivity might also be influenced by infant autonomic functioning, because hypothetically autonomic functioning influences the level of interaction an infant displays. In that case controlling for maternal sensitivity would introduce bias to the model.

On the other hand, as shown in figure 3b, even in studies which rigorously control for socio-economic and other factors, it is virtually impossible to rule out residual confounding. Parenting and attachment styles, as well the infant's diet in addition breastfeeding and fruit-purée are examples of potential residual confounders, which we were not able to address in the current study.

Presence of residual confounding can lead to erroneously dismissal of the mediating effect of breastfeeding for socio-economic factors and autonomic functioning (33). This would mean that our model without adjustment for confounders cannot be completely discounted.

Literature recommends several approaches to study causal inferences (26). A randomised controlled trial is seen as the golden standard. However, the established benefits of breastfeeding make randomly withholding breastfeeding from infants unethical. Some research has employed the randomised assignment of mothers to a breastfeeding promotion program (34). Because of the limited influence of breastfeeding promotion programs on breastfeeding practice in a general population setting in the West, the number of participants needed to successfully detect differences in autonomic functioning make this approach unfeasible for our purposes (35).

Another approach is to utilise an instrumental variable, which is related to the exposure variable, in this case breastfeeding, but not to confounding factors or to the outcome, in this case infant autonomic functioning. Such a variable can be genetic, as in mendelian randomisation, or environmental (26). Unfortunately, to our knowledge no instrumental variable, genetic or otherwise, applicable in breastfeeding research has been identified.

A third approach, is to compare the effect of breastfeeding with the effect of a contrasting variable. A contrasting variable should be an indicator of similar feeding or health related parameters, but lacks breastfeeding's proposed causal properties. Thus, if there is indeed a causal relation, the effect of breastfeeding on infant autonomic functioning should be larger.

This approach has been used in the study of intra-uterine exposure, for example, where it is possible, to compare the associations of prenatal maternal smoking and prenatal paternal smoking with infant outcome (26).
Reverse causality also represents a problem. It is conceivable that calmer infants, which are easier to breastfeed (36), already have different autonomic functioning, for example lower sympathetic modulation. This would then account for the relation between breast feeding and sympathetic modulation we observed. Unfortunately, ruling out reverse causality calls for multiple measurements of autonomic functioning and multiple measurements of the infant's agreeableness to be breastfed, which few studies provide.
In our study, frequent infant fruitpurée consumption is the contrasting variable. It is related to the similar environmental epiphenomena as breastfeeding, including maternal socio-economic status, education, and caretaking as well as maternal concepts about health and nourishment. There was no significant association between infant fruitpurée consumption and breastfeeding, thus breastfeeding and fruitpurée consumption did not identify the same mother-infant pairs. Infant fruitpurée does not contain fatty acids, some of which, the long-chain poly-unsaturated fatty acids, have been associated with autonomic functioning. Finally, fruitpurée consumption does not come with skin-to-skin contact. Nonetheless, our data show that, similar to breastfeeding, infants who eat more fruitpurée have lower sympathetic modulation. This supports the notion that the association between breastfeeding and infant autonomic functioning may be similarly non-causal, but instead the result of residual confounding. Though, strictly speaking, we cannot rule out that the relation of breastfeeding and fruitpurée consumption to autonomic functioning is explained via different biological mechanisms. If a common component of the infant's diet, like fruitpurée, is similarly associated with autonomic functioning as breastfeeding but lacks it properties, this certainly casts doubt on the specificity of the postulated mechanisms and the relevance of breastfeeding for low infant autonomic arousal.
Finally, while our study shows no lasting effect of breast feeding on infant autonomic functioning, we cannot rule out a transient effect, limited to the breast feeding period itself. However, it is unlikely that such a transient effect would have substantial future health benefits.
In conclusion, our study does not support a causal relation between breastfeeding and infant autonomic functioning. Instead, it underlines the problem of confounding in observational studies of breastfeeding. Further, we presented a novel way to address the dilemma of confounding and overcorrection in research concerning breastfeeding. It can help to compare the impact of breastfeeding to the impact of another feeding parameter later in life that is not causally related to outcome of interest.

References

1. Buyken AE, Karaolis-Danckert N, Remer T, Bolzenius K, Landsberg B, Kroke A. Effects of breastfeeding on trajectories of body fat and BMI throughout childhood. Obesity (Silver Spring). 2008 Feb;16(2):389-95.
2. Lawlor DA, Smith GD. Early life determinants of adult blood pressure. Curr Opin Nephrol Hypertens. 2005 May;14(3):259-64.
3. Marmot MG, Page CM, Atkins E, Douglas JW. Effect of breast-feeding on plasma cholesterol and weight in young adults. J Epidemiol Community Health. 1980 Sep;34(3):164-7.
4. Martin RM, Ebrahim S, Griffin M, Davey Smith G, Nicolaides AN, Georgiou N, et al. Breastfeeding and atherosclerosis: intima-media thickness and plaques at 65-year follow-up of the Boyd Orr cohort. Arterioscler Thromb Vasc Biol. 2005 Jul;25(7):1482-8.
5. DiPietro JA, Larson SK, Porges SW. Behavioral and heart rate pattern differences between breast-fed and bottle-fed neonates. Developmental Psychology. 1987;23:467-74.
6. Butte NF, Smith EO, Garza C. Heart rates of breast-fed and formula-fed infants. J Pediatr Gastroenterol Nutr.

1991 Nov;13(4):391-6.
7. Kannel WB, Kannel C, Paffenbarger RS, Jr., Cupples LA. Heart rate and cardiovascular mortality: the Framingham Study. American heart journal. 1987 Jun;113(6):1489-94.
8. Tsuji H, Larson MG, Venditti FJ, Jr., Manders ES, Evans JC, Feldman CL, et al. Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. Circulation. 1996 Dec 1;94(11):2850-5.
9. Mozaffarian D, Stein PK, Prineas RJ, Siscovick DS. Dietary fish and omega-3 fatty acid consumption and heart rate variability in US adults. Circulation. 2008 Mar 4;117(9):1130-7.
10. World Health Organization UNCsF. Global Strategy for Infant and Young Child Feeding.: World Health Organization, Geneva; 2003.
11. Chirouze V, Lapillonne A, Putet G, Salle BL. Red blood cell fatty acid composition in low-birth-weight infants fed either human milk or formula during the first months of life. Acta Paediatr Suppl. 1994 Dec;405:70-7.
12. Koletzko B, Lien E, Agostoni C, Bohles H, Campoy C, Cetin I, et al. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. J Perinat Med. 2008;36(1):5-14.
13. Chen CH, Wang TM, Chang HM, Chi CS. The effect of breast- and bottle-feeding on oxygen saturation and body temperature in preterm infants. J Hum Lact. 2000 Feb;16(1):21-7.
14. Meier P, Anderson GC. Responses of small preterm infants to bottle- and breast-feeding. MCN Am J Matern Child Nurs. 1987 Mar-Apr;12(2):97-105.
15. Feldman R, Eidelman AI. Skin-to-skin contact (Kangaroo Care) accelerates autonomic and neurobehavioural maturation in preterm infants. Dev Med Child Neurol. 2003 Apr;45(4):274-81.
16. Simmons D. NIDDM and breastfeeding. Lancet. 1997 Jul 19;350(9072):157-8.
17. Der G, Batty GD, Deary IJ. Effect of breast feeding on intelligence in children: prospective study, sibling pairs analysis, and meta-analysis. Bmj. 2006 Nov 4;333(7575):945.
18. Ip S, Chung M, Raman G, Trikalinos TA, Lau J. A summary of the Agency for Healthcare Research and Quality's evidence report on breastfeeding in developed countries. Breastfeed Med. 2009 Oct;4 Suppl 1:S17-30.
19. Britton JR, Britton HL, Gronwaldt V. Breastfeeding, sensitivity, and attachment. Pediatrics. 2006 Nov;118(5):e1436-43.
20. Kaplan LA, Evans L, Monk C. Effects of mothers' prenatal psychiatric status and postnatal caregiving on infant biobehavioral regulation: Can prenatal programming be modified? Early Hum Dev. 2007 Aug 28.
21. Hernan MA, Hernandez-Diaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. Am J Epidemiol. 2002 Jan 15;155(2):176-84.
22. Pearl J. Causality. Cambridge: Cambridge University Press; 2000.
23. Yngve A, Sjostrom M. Breastfeeding in countries of the European Union and EFTA: current and proposed recommendations, rationale, prevalence, duration and trends. Public Health Nutr. 2001 Apr;4(2B):631-45.
24. van Rossem L, Oenema A, Steegers EA, Moll HA, Jaddoe VW, Hofman A, et al. Are starting and continuing breastfeeding related to educational background? The generation R study. Pediatrics. 2009 Jun;123(6):e1017-27.
25. Martin RM, Goodall SH, Gunnell D, Davey Smith G. Breast feeding in infancy and social mobility: 60-year follow-up of the Boyd Orr cohort. Arch Dis Child. 2007 Apr;92(4):317-21.
26. Smith GD. Assessing intrauterine influences on offspring health outcomes: can epidemiological studies yield robust findings? Basic Clin Pharmacol Toxicol. 2008 Feb;102(2):245-56.
27. Jaddoe VW, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Verhulst FC, et al. The Generation R Study: Design and cohort profile. Eur J Epidemiol. 2006;21(6):475-84.
28. Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol. 2007;22(12):917-23.
29. van Steenis HG, Tulen JH, Mulder LJ. Heart rate variability spectra based on non-equidistant sampling: the spectrum of counts and the instantaneous heart rate spectrum. Med Eng Phys. 1994 Sep;16(5):355-62.
30. Bar-Haim Y, Marshall PJ, Fox NA. Developmental changes in heart period and high-frequency heart period variability from 4 months to 4 years of age. Dev Psychobiol. 2000 Jul;37(1):44-56.
31. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007 May;39(2):175-91.
32. Dierckx B, Tulen JH, van den Berg MP, Tharner A, Jaddoe VW, Moll HA, et al. Maternal psychopathology influences infant heart rate variability: Generation R Study. Psychosomatic medicine. 2009 Apr;71(3):313-21.
33. Herting JR. Evaluating and rejecting true mediation models: a cautionary note. Prev Sci. 2002 Dec;3(4):285-9.
34. Kramer MS, Aboud F, Mironova E, Vanilovich I, Platt RW, Matush L, et al. Breastfeeding and child cognitive development: new evidence from a large randomized trial. Arch Gen Psychiatry. 2008 May;65(5):578-84.
35. Chung M, Raman G, Trikalinos T, Lau J, Ip S. Interventions in primary care to promote breastfeeding: an evidence review for the U.S. Preventive Services Task Force. Ann Intern Med. 2008 Oct 21;149(8):565-82.
36. Niegel S, Ystrom E, Hagtvet KA, Vollrath ME. Difficult temperament, breastfeeding, and their mutual prospective effects: the Norwegian Mother and Child Cohort Study. J Dev Behav Pediatr. 2008 Dec;29(6):458-62.

Chapter 4

Low autonomic arousal as vulnerability to externalising behaviour in infants with hostile mothers.

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Abstract
Maternal psychopathology and the child's autonomic nervous system functioning are risk factors for externalising behaviour later in life. While research has shown that maternal psychopathology already affects young children, less is known about the association between autonomic functioning and externalising behaviour in young children. In addition, maternal psychopathology and autonomic nervous system functioning may interact to determine the risk of externalising behaviour.
In a sample of 528 infants and their mothers, maternal psychiatric symptoms were assessed with the Brief Symptom Inventory and toddler externalising behaviour with the Child Behavior Checklist. Infant heart rate was recorded at 14 months.
There was a trend for maternal psychiatric problems to be associated with toddler externalising behaviour (OR=2.4, 95% CI: 0.9; 6.6, p=0.09), whereas, maternal hostility was significantly associated with toddler externalising behaviour (OR=2.0, 95% CI: 1.1; 3.9, p=0.03). Maternal psychiatric problems interacted with mean HR (p=0.01) and vagal tone (p=0.03) in their effect on toddler externalising behaviour.
Mothers with high psychiatric problems, in particular high hostility, were more likely to have toddlers with high externalising behaviour. Moreover, in the presence of maternal risk factors, low autonomic arousal renders children particularly susceptible to externalising behaviour.

Introduction

Maternal psychopathology is a strong predictor of externalising behaviour in young children (1-3). Children of mothers suffering from psychopathology not only inherit an unfavourable genetic profile but are also exposed to multiple environmental risk factors (3). Nonetheless, most children of mothers with psychopathology do not display externalising behaviour later in life. This difference in vulnerability may depend on biological susceptibility. An extensive body of research has investigated the role of genetic susceptibility to environmental factors. Studies in both adoption (4) and twin cohorts (5) have shown the importance of gene-environment interaction in the development of externalising behaviour. Some studies have even been able to identify specific genes responsible for these interactions. In a seminal study, Caspi et al. showed that maltreated children with a genotype conferring high levels of MAOA expression were less likely to develop antisocial problems than other maltreated children (6). Despite such successes, gene-environment interactions are very complex and it has been difficult to identify or replicate candidate genes for gene-environment interactions (7).

Studying the interaction of environmental risk factors with a physiological risk factor for externalising behaviour may alleviate part of this complexity, as physiological risk factors are themselves the product of the interplay of multiple genes and environmental factors. Autonomic nervous system (ANS) functioning, typically measured by mean heart rate (HR), is such a risk factor (8, 9). ANS functioning in infants is primarily determined by the interplay of several genes and to a certain extent environmental factors (10). A large number of cross-sectional studies have shown that low HR during rest is associated with externalising behaviour (11-13). Furthermore, longitudinal studies have shown HR to predict later externalising behaviour (12).

The relationship between vagal tone, another common measure of ANS functioning, and externalising behaviour is less clear (14). Some studies found an association between increased vagal tone in children and externalising behaviour (11, 15-17), other studies reported decreased vagal tone associated with externalising problems (18, 19).

A few studies have previously investigated the interplay of these autonomic indices with other risk factors. Farrington (1997) reported that the association between low HR and externalising behaviour was stronger when environmental risk factors were present as well. Boys with low resting HR were more likely to commit acts of violence as an adult if they also had a poor relationship with their parent, and if they came from a large family. Recently, a study investigated the interaction of HR and with environmental stressors in a large sample of adolescents. The study showed that adolescents with low HR displayed less psychopathology in relation to stressors in life than adolescents with normal or high HR (20). As for vagal tone, Shannon et al. showed that it moderated the association between parental and child externalising behaviour. In their study high vagal tone played a protective role (21). Furthermore, in several studies performed by El-Sheikh et al., vagal tone seemed to play a similar protective role in buffering the influence of marital conflict on child externalising behaviour (22, 23).

In this study we examined the relationship of HR and vagal tone in infants with their behaviour at 18 months. Furthermore, we studied the interaction between maternal psychopathology and infant autonomic indices in predicting toddler behaviour.

Table 1. Characteristics of the study population (n=514).

Mother	Age mother at intake, years (SD)	32.0 (3.6)
	Used alcohol during pregnancy %	59
	Smoked during pregnancy %	18
	Psychiatric symptoms (SD)	0.15 (0.23)
	Hostility symptoms (SD)	0.19 (0.36)
	Depression symptoms (SD)	0.12 (0.29)
Infant	Female %	50
	Birth weight in g (SD)	3519 (542)
	Gestational age at birth, weeks (SD)	39.9 (1.8)
	Mean heart rate Hz (SD)	124 (10)
	HF lg ms^2 (SD)	2.9 (0.4)
	Externalising Behaviour (SD)	9.8 (6.2)

HF = high frequency variability

Methods

Participants

This study was conducted within the Focus cohort of the Generation R Study, a population based cohort study from foetal life until young adulthood (24, 25). All children were born between February 2003 and August 2005. We obtained physiological measurements of 528 infants at age 14 months (26). Child Behaviour Checklist (CBCL) scores at 18 months were available for 474 children, who were included in our primary analyses. In 375 children measurements of maternal psychopathology were available as well.

Psychophysiologic measurements

Infant heart rate at 14 months was registered with a three pole ECG lead. We monitored the breathing pattern using a piëzo-electric transducer. Signals were recorded for 8 minutes using a Vitaport 3 recorder (Temec Inc). Spectral analysis using discrete Fourier transformation was performed on a selected 100-180 seconds of ECG data , were breathing was most regular (27). We used high frequency variation (0.15 Hz - 1.04 Hz) in heart rate variability as a measure for cardiac vagal tone. As vagal tone of HR is dependent on respiratory frequency, which is much higher in infants, we adjusted the upper bound of the high frequency band according to recommendations from literature (28).

Measurement of maternal psychopathology

Information on maternal psychopathology two months after birth was obtained by the Brief Symptom Inventory (BSI), a validated self-report questionnaire consisting of 53 items (Derogatis and Melisaratos, 1993). Each item was rated on a 5-point Likert scale. For the current study, we used the total scale and the hostility, depression and anxiety subscales. Scale scores were calculated by summing the item scores involved and then dividing by the number of endorsed items.

Measurement of toddler behaviour
To assess behavioural problems, we used the Child Behavior Checklist/1½-5 (CBCL), a parent questionnaire for assessing psychopathology in children aged 1½ - 5. The CBCL has good reliability and validity (29). Parents rate the child's emotional and behavioural problems over the preceding 2 months on a 3-point scale. For the current study, we used the externalising scale and its'subscales Attention Problems and Aggressive Behavior.

Covariates
We considered sex, gestational age, weight at birth, maternal age, maternal educational level, and smoking and drinking behaviour during pregnancy as confounders because they can influence autonomic functioning (30-34). Gestational age at birth and birth weight were obtained from community midwife and hospital registries at birth. Information on maternal smoking and drinking habits during pregnancy were obtained by maternal questionnaire.

Statistical analysis
For a non-response analysis, we compared the 474 children with the physiological measurements session and CBCL data with the 54 children who were not included in the analysis due to missing data. We used T-tests, Mann-Whitney U tests and Chi-Square tests where appropriate.
Both maternal BSI scores and toddler externalising behaviour scores were not normally distributed. Positive skewing of maternal psychiatric symptom scores, even in a normal population, is inherent to the BSI. However, CBCL externalising scale scores showed positive skewing as well, which may be indicative of selective non-response. Log transformation of the variables did not correct for the positive skewing. We elected to perform linear regression analyses using untransformed variables. Because of the non-normality of the variables, regression residuals did show some positive skewing and negative kurtosis. Hence, we checked the consistency of our findings with logistic regression models with CBCL scores dichotomised at 1 SD above the mean.
First, we tested whether maternal psychiatric symptoms were associated with toddler externalising behaviour using linear regression models. Then, we tested whether infant mean HR and vagal tone were associated with toddler externalising behaviour again using linear regression models. Recent studies indicate that the relation between autonomic functioning and externalising behaviour may be different for boys and girls, at least at a later age (11, 35). To investigate such a differential relationship in our sample we entered infant mean HR and infant sex together with an interaction tem "infant mean HR*infant sex" into the same linear regression model. We repeated this procedure for infant vagal tone*infant sex.
Our second aim was to investigate the interaction between infant autonomic indices with maternal psychopathology in determining toddler externalising behaviour. We entered infant mean HR and maternal BSI scale score together with an interaction tem "infant mean HR*maternal BSI score" into the same linear regression model. We used the externalising CBCL scale score as dependent variable. We followed an identical procedure for infant vagal tone.
The nature of the interactions was explored in strata. We defined two strata by dichotomisation at 1 SD above the mean. As CBCL scores were normally distributed in each stratum, we examined the relationship of mean infant HR and vagal tone with the CBCL aggression scale by means of linear regression. All analyses were adjusted for all covariates. All statis-

tical analyses were carried out using the Statistical Package for the Social Sciences 13.0 for Windows (SPSS Inc).

Results

Table 1 depicts the characteristics of the study participants. In the non-response analysis we found no significant differences between the children included in the primary analyses and the children excluded due to missing data.

Table 2 demonstrates that toddlers of mothers who reported high psychiatric symptoms tended to have more behavioural problems. High maternal psychiatric symptoms, as measured by the BSI total scale, showed a trend for more externalising and aggressive behaviour in the toddler. Further analysis of subscale scores showed that maternal hostility was significantly associated with both toddler externalising behaviour and toddler aggressive behaviour. We found no association between any BSI scale and toddler attention problems. Table 2 also shows that there was no significant relation between infant ANS indices and toddler behaviour. There was, however, a trend towards an association between low infant HR and high toddler aggressive behaviour.

Table 2. Association of infant ANS indices and maternal psychiatric symptoms with infant aggressive behaviour.

	Heart Rate (log_{10} bpm / SD)		
	β	95% CI	p
Maternal psychiatric symp.	3.15	0.98; 5.32	< 0.01
Maternal hostility	2.61	1.22; 3.98	< 0.01
Maternal depression	1.84	0.12; 3.56	0.04
HR	-0.014	-0.055; 0.027	0.5
HF (log10 ms^2)	0.21	-0.88; 1.3	0.7

HR= heart rate, HF = High frequency variability All linear regressions were corrected for gender of the infant, gestational age, weight at birth, maternal age, maternal educational level, and smoking and drinking behaviour during pregnancy.

In table 3 we show that the association between infant autonomic indices and toddler externalising behaviour depends on maternal psychiatric symptoms. There was an interaction between maternal psychiatric symptoms and infant mean HR in relation to toddler externalising behaviour and in relation to toddler aggressive behaviour. Likewise, we observed an interaction between maternal psychiatric symptoms and infant vagal tone for toddler externalising behaviour. Furthermore there was an interaction between maternal psychiatric symptoms and infant vagal tone in relation to toddler aggressive behaviour, that was of borderline significance. Results using the maternal hostility subscale were similar, including interaction effects. We found no interaction between maternal psychopathology and infant autonomic indices determining the risk of toddler attention problems.

To explore the nature of the interaction effects, we stratified the relation between infant autonomic functioning and toddler aggressive behaviour for maternal psychopathology. Figure 1 shows that in the group with high maternal psychiatric symptoms, infant mean HR was negatively associated with toddler aggressive behaviour (B per SD=-1.7, 95% CI:.-0.2;-3.2, p=0.03). This means that an infant with HR one SD below the mean, had a 1.7 points higher score on the aggressive behaviour scale. The respective β was –0.3, which indicates a medium effect size (36). In contrast, there was no relationship between infant

Table 2. Association of infant ANS indices and maternal psychiatric symptoms with infant aggressive behaviour.

	Heart Rate (log_{10} bpm / SD)		
	β	95% CI	p
HR*Maternal Psy.symp.	-0.36	-0.89; -0.13	< 0.01
HR*Maternal hostility	-0.17	-0.3; -0.04	< 0.01
HR*Maternal depression	-0.20	-0.37; -0.03	0.02
HF*Maternal Psy.symp.	8.07	0.31; 15.8	0.04
HF*Maternal hostility	3.60	-0.5; 7.7	0.08
HF*Maternal depression	3.61	-1.64; 8.86	0.8

HR= heart rate, HF = High frequency variability. BSI scale score and either infant HR or HF were entered into linear regression models together with their interaction term. All linear regressions were corrected for gender of the infant, gestational age, weight at birth, maternal age, maternal educational level, and smoking and drinking behaviour during pregnancy.

mean HR and toddler aggressive behaviour in the stratum with low maternal psychiatric symptoms (B per SD=-0.3, 95% CI:.-0.6; 0.5, p=0.8).
Figure 2 shows the relation of infant vagal tone with toddler aggressive behaviour. In the group with high maternal psychiatric symptoms, infants with high vagal tone displayed more aggressive behaviour, however this association did not reach significance (B per SD=1.3, 95% CI:.-0.5; 3.1, p=0.16). There was an inverse relationship between infant vagal tone and toddler aggressive behaviour in the group with low maternal psychiatric symptoms, which was not significant (B per SD=-0.3, 95% CI:.-0.7; 0.3, p=0.4). The direction of the associations was opposed, which explains why the interaction between vagal tone and maternal psychiatric problems was significant.

Figure 1. Association between infant heart rate and infant behaviour, impact of maternal psychiatric symptoms

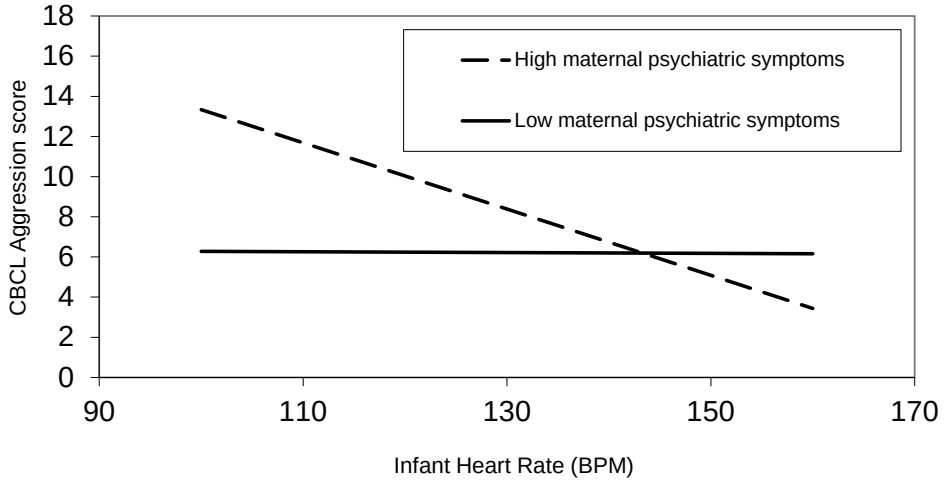
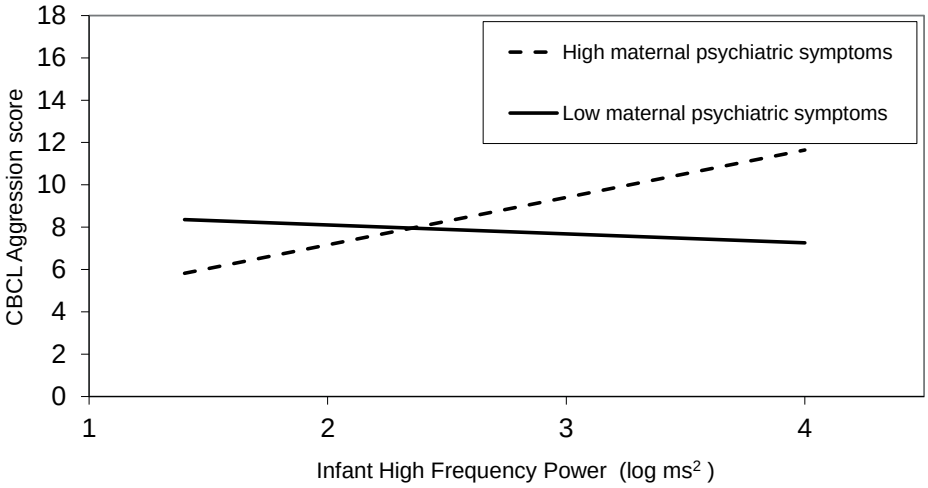


Figure 2. Association between infant high frequency power and infant behaviour, impact of maternal psychiatric symptoms



Discussion

In general, we found a trend that toddlers of mothers with psychiatric symptoms were more likely to show high levels of externalising behaviour. Maternal symptoms of hostility were particularly associated with toddler externalising behaviour. Our study showed that in children of mothers with psychiatric pr oblems low autonomic arousal constitutes an additional risk for externalising behaviour.

Before we discuss these findings, some methodological comments have to be made. Not all participants of the Generation R Focus Cohort could be included, because we added physiological measurements to our assessment protocol while the examination round was already ongoing. Furthermore, the Focus Cohort is ethnically homogeneous which has the advantage that it excludes potential confounding or effect modification by ethnicity but which limits the generalisability of the study. It is important to note that mothers provided the information on one of the predictors and on the outcome of our study. This could lead to shared method variance: hostile mothers may be inclined to rate their children more aggressive. However it is unlikely that such a bias can explain interaction with a biological measure. Strengths of our study include the large sample size, which enabled us to examine the interaction between physiological and maternal factors in determining behaviour, and a focus on an age when externalising psychopathology is still in development.

The intergenerational transmission of maternal externalising behaviour has been well documented. For example, Tremblay et al. documented that toddlers of mothers with a history of antisocial behaviour displayed high levels of aggression (1).

Likewise, the association between low HR and externalising psychopathology has been reported in a large number of studies and even in a meta-analysis (9). A seminal study performed in Mauritius showed that low HR at age three predisposes to aggression at age 11 (12). In the current study, we found no such association in the total study population. However, we observed that in children of mothers with high psychiatric symptoms, low HR and high vagal tone were associated with externalising behaviour.

We discuss two possible explanations. First, the fearlessness theory posits that low autonomic arousal in children is an indicator of fearlessness. Fearless children pay less heed to potential negative consequences of behaviour and thus may be prone to exhibit externalising behaviour (37). We hypothesize that in the presence of maternal psychopathology, with less adequate parenting and maternal guidance in coping with fearless behaviour, these children actually develop externalising/aggressive behaviour. In other words, only within the high-risk group of infants whose mothers had psychiatric symptoms, children with low autonomic arousal were at higher risk of developing externalising behaviour.
Second, it is possible that our stratification delineated a specific group. Toddlers with persistent externalising behaviour are difficult to identify, because oppositional, hyperactive and aggressive behaviour is common at that age and usually time limited (38, 39). The relationship between autonomic indices and externalising behaviour in the unstratified sample may have been diluted by toddlers displaying age appropriate externalising behaviour. Toddlers whose mothers report high maternal psychiatric symptoms may be more likely to exhibit "true" externalising behaviour.
Our results seem to contradict recent findings by Oldehinkel et al., who showed that adolescents with low HR displayed less psychopathology in relation to stressors of daily life than adolescents with normal or high HR (20). However, both age range and type of environmental stressor measured differed greatly between the studies. The fearlessness theory posits that low HR is related to fearless behaviour. It is conceivable that fearless infants who lack maternal guidance are at risk of developing aggressive behaviour, whereas in adolescence fearlessness may indeed buffer environmental stressors such as school work pressure, difficulties with peers and relationship problems. However, it should be noted that a protective effect of low HR as reported by Oldehinkel et al. (20) is at odds with a large body of literature, including studies conducted in low SES samples (12).
Our data indicated that there was no association between infant autonomic functioning and toddler attention problems. This is in line with earlier studies which reported that autonomic functioning predicts the aggressive aspects of externalising behaviour and not attention problems (12).
In conclusion, the association between infant autonomic functioning with toddler externalising behaviour, was moderated by maternal psychiatric symptoms. We could demonstrate this relation for the aggressive component of externalising behaviour, but not for the inattentive component. Within the high-risk group of infants whose mothers have psychiatric symptoms or hostility, children with low autonomic arousal are at an high risk of developing externalising behaviour. We suggest that low autonomic functioning should be seen as a vulnerability factor, which makes children susceptible to additional risk factors.

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References

1. Tremblay RE, Nagin DS, Seguin JR, Zoccolillo M, Zelazo PD, Boivin M, et al. Physical aggression during early childhood: trajectories and predictors. Pediatrics. 2004 Jul;114(1):e43-50.
2. Kim-Cohen J, Caspi A, Rutter M, Tomas MP, Moffitt TE. The caregiving environments provided to children by depressed mothers with or without an antisocial history. The American journal of psychiatry. 2006 Jun;163(6):1009-18.
3. Kim-Cohen J, Moffitt TE, Taylor A, Pawlby SJ, Caspi A. Maternal depression and children's antisocial behavior: nature and nurture effects. Archives of general psychiatry. 2005 Feb;62(2):173-81.
4. Cadoret RJ, Cain CA, Crowe RR. Evidence for gene-environment interaction in the development of adolescent antisocial behavior. Behavior genetics. 1983 May;13(3):301-10.
5. Rowe DC, Almeida DM, Jacobson KC. School Context and Genetic Influences on Aggression in Adolescence. Psychological Science. 1999;10(3):277-80.
6. Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, et al. Role of genotype in the cycle of violence in maltreated children. Science (New York, NY. 2002 Aug 2;297(5582):851-4.
7. Zammit S, Owen MJ. Stressful life events, 5-HTT genotype and risk of depression. Br J Psychiatry. 2006 Mar;188:199-201.
8. Monk C, Kovelenko P, Ellman LM, Sloan RP, Bagiella E, Gorman JM, et al. Enhanced stress reactivity in paediatric anxiety disorders: implications for future cardiovascular health. The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP). 2001 Jun;4(2):199-206.
9. Ortiz J, Raine A. Heart rate level and antisocial behavior in children and adolescents: a meta-analysis. Journal of the American Academy of Child and Adolescent Psychiatry. 2004 Feb;43(2):154-62.
10. Dubreuil E, Ditto B, Dionne G, Pihl RO, Tremblay RE, Boivin M, et al. Familiality of heart rate and cardiac-related autonomic activity in five-month-old twins: the Quebec newborn twins study. Psychophysiology. 2003 Nov;40(6):849-62.
11. Dietrich A, Riese H, Sondeijker FE, Greaves-Lord K, van Roon AM, Ormel J, et al. Externalizing and internalizing problems in relation to autonomic function: a population-based study in preadolescents. Journal of the American Academy of Child and Adolescent Psychiatry. 2007 Mar;46(3):378-86.
12. Raine A, Venables PH, Mednick SA. Low resting heart rate at age 3 years predisposes to aggression at age 11 years: evidence from the Mauritius Child Health Project. Journal of the American Academy of Child and Adolescent Psychiatry. 1997 Oct;36(10):1457-64.
13. van Goozen SH, Matthys W, Cohen-Kettenis PT, Buitelaar JK, van Engeland H. Hypothalamic-pituitary-adrenal axis and autonomic nervous system activity in disruptive children and matched controls. Journal of the American Academy of Child and Adolescent Psychiatry. 2000 Nov;39(11):1438-45.
14. Lorber MF. Psychophysiology of aggression, psychopathy, and conduct problems: a meta-analysis. Psychological bulletin. 2004 Jul;130(4):531-52.
15. Cole PM, Zahn-Waxler C, Fox NA, Usher BA, Welsh JD. Individual differences in emotion regulation and behavior problems in preschool children. Journal of abnormal psychology. 1996 Nov;105(4):518-29.
16. Scarpa A, Ollendick TH. Community violence exposure in a young adult sample: III. Psychophysiology and victimization interact to affect risk for aggression. . Journal of Community Psychology. 2003;31:321-38.
17. Van Voorhees E, Scarpa A. Psychophysiological variables in childhood proactive and reactive aggression. Psychophysiology. 2002;39:82.
18. Beauchaine TP, Katkin ES, Strassberg Z, Snarr J. Disinhibitory psychopathology in male adolescents: discriminating conduct disorder from attention-deficit/hyperactivity disorder through concurrent assessment of multiple autonomic states. Journal of abnormal psychology. 2001 Nov;110(4):610-24.
19. Pine DS, Wasserman GA, Miller L, Coplan JD, Bagiella E, Kovelenku P, et al. Heart period variability and psychopathology in urban boys at risk for delinquency. Psychophysiology. 1998 Sep;35(5):521-9.
20. Oldehinkel AJ, Verhulst FC, Ormel J. Low heart rate: a marker of stress resilience. The TRAILS study. Biological psychiatry. 2008 Jun 15;63(12):1141-6.
21. Shannon KE, Beauchaine TP, Brenner SL, Neuhaus E, Gatzke-Kopp L. Familial and temperamental predictors of resilience in children at risk for conduct disorder and depression. Development and psychopathology. 2007 Summer;19(3):701-27.
22. El-Sheikh M, Harger J, Whitson SM. Exposure to interparental conflict and children's adjustment and physical health: the moderating role of vagal tone. Child development. 2001 Nov-Dec;72(6):1617-36.
23. El-Sheikh M, Whitson SA. Longitudinal relations between marital conflict and child adjustment: vagal regulation as a protective factor. J Fam Psychol. 2006 Mar;20(1):30-9.
24. Jaddoe VW, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Verhulst FC, et al. The Generation R Study: Design and cohort profile. European journal of epidemiology. 2006;21(6):475-84.
25. Jaddoe VW, Bakker R, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. European journal of epidemiology. 2007;22(12):917-23.
26. Dierckx B, Tulen JH, Van den Berg MP, Tharner A, Jaddoe VW, Moll HA, et al. Maternal psychopathology influences infant heart rate variability. The Generation R Study. Psychosomatic medicine. Accepted for publication.
27. van Steenis HG, Tulen JH, Mulder LJ. Heart rate variability spectra based on non-equidistant sampling: the spectrum of counts and the instantaneous heart rate spectrum. Medical engineering & physics. 1994 Sep;16(5):355-62.
28. Bar-Haim Y, Marshall PJ, Fox NA. Developmental changes in heart period and high-frequency heart period variability from 4 months to 4 years of age. Developmental psychobiology. 2000 Jul;37(1):44-56.
29. Achenbach TM, Rescorla LA. Manual for ASEBA Preschool Forms & Profiles. Burlington, VT: University of

Vermont, Research Center for Children, Youth, & Families.; 2000.
30. Browne CA, Colditz PB, Dunster KR. Infant autonomic function is altered by maternal smoking during pregnancy. Early Hum Dev. 2000 Sep;59(3):209-18.
31. Galland BC, Taylor BJ, Bolton DP, Sayers RM. Heart rate variability and cardiac reflexes in small for gestational age infants. J Appl Physiol. 2006 Mar;100(3):933-9.
32. Haley DW, Handmaker NS, Lowe J. Infant stress reactivity and prenatal alcohol exposure. Alcoholism, clinical and experimental research. 2006 Dec;30(12):2055-64.
33. John V, Dai H, Talati A, Charnigo RJ, Neuman M, Bada HS. Autonomic alterations in cocaine-exposed neonates following orthostatic stress. Pediatric research. 2007 Feb;61(2):251-6.
34. Longin E, Gerstner T, Schaible T, Lenz T, Konig S. Maturation of the autonomic nervous system: differences in heart rate variability in premature vs. term infants. Journal of perinatal medicine. 2006;34(4):303-8.
35. Beauchaine TP, Hong J, Marsh P. Sex differences in autonomic correlates of conduct problems and aggression. Journal of the American Academy of Child and Adolescent Psychiatry. 2008 Jul;47(7):788-96.
36. Cohen J. Statistical power analysis for the behavioral sciences (2nd ed.). Hillsdale, NJ: Erlbaum; 1988.
37. Raine A. Biosocial studies of antisocial and violent behavior in children and adults: a review. Journal of abnormal child psychology. 2002 Aug;30(4):311-26.
38. Hay DF, Castle J, Davies L. Toddlers' use of force against familiar peers: a precursor of serious aggression? Child development. 2000 Mar-Apr;71(2):457-67.
39. Dunn J, Munn P. Sibling quarrels and maternal intervention: individual differences in understanding and aggression. Journal of child psychology and psychiatry, and allied disciplines. 1986 Sep;27(5):583-95.

Chapter 5

A prospective study of heart rate and externalising behaviours in young children.

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Abstract
Background. Low heart rate predicts externalising and delinquent behaviour in adults, adolescents and school-age children. In younger children the evidence is less clear. Moreover, the specificity of the relation between the autonomic nervous system and different forms of externalising behaviour is uncertain. We investigated the longitudinal relation between resting mean heart rate and different externalising behaviours.
Methods. In 412 children of the Generation R Study, we measured resting mean heart rate at 14 months. At 3 years, child problem behaviour was assessed by the mother with the Child Behavior Checklist. In a gift delay task, we observed whether children were compliant and whether they lied about their non-compliance. The association of heart rate with behaviour was contrasted to the effect of harsh parenting.
Results. In our main analysis we examined the association between heart rate and reported and observed child behaviour. For comparison, the association of heart rate with behaviour was contrasted to the effect of harsh parenting. Mean heart rate was positively associated with Anxious/Depressed scale scores (β=0.1, 95%CI=0.01;0.2, p=0.04), but not with Aggressive Behaviour (β=0.02;95%CI=-0.1;0.1, p=0.8) nor Attention Problem scale scores (β=0.08, 95%CI=-0.3;0.5, p=0.8). We could not demonstrate an association between mean heart rate and non-compliance during the gift delay task (OR=1.14, 95%CI=0.9;1.1, p=0.2), but lower heart rate predicted higher odds of the child lying (OR=0.56, 95%CI=0.3;0,9, p=0.03). In contrast, harsh parenting was associated with mother-reported Aggressive Behaviour (β=0.7, 95%CI=0.4;0.9, p<0.001) and Attention Problems (β=0.2, 95%CI=0.1;0.3, p<0.001), but not with observed lying (OR=1.03, 95%CI=0.8;1.4, p=0.8).
Conclusions. Lower resting mean heart rate at age 14 months predicts low anxiety symptoms and higher odds of lying at age 3 years. Low resting mean heart rate may be less an indicator of early childhood aggression than of fearless behaviour.

Introduction

Externalising behaviour in early childhood can be a precursor of more severe externalising psychopathology, which may persist into adolescence and adulthood (1, 2). Yet to some extent, oppositional, hyperactive and aggressive behaviour in young children is in keeping with normal development (3). This has made it difficult to delineate normal age-limited behaviour from pathological externalising behaviour (4, 5). This challenge has fuelled interest in identifying early risk factors and markers for more pathological and persistent externalising behaviour.

Variations in the autonomic nervous system and its proxy heart rate are considered a potential biomarker of externalising problems. While mean heart rate declines substantially between birth and seven years of age, individual differences in heart rate are relatively stable over this age range (6). Several prospective studies have shown that low autonomic arousal, as indexed by low resting mean heart rate, predicts externalising behaviour (7, 8). The relation between low resting mean heart rate and externalising behaviour holds throughout later childhood, adolescence and beyond (8, 9). Several theoretical models have been proposed for the observed relation between low heart rate and externalising behaviour. The stimulation seeking theory posits that low heart rate and low physiological arousal in general, is an unpleasant physiological state. Children with low arousal seek stimulation to increase their arousal levels. Externalising behaviour may be viewed as a form of stimulation-seeking for some children. The fearlessness theory argues that low heart rate is a marker of low levels of fear. Children who experience low levels of fear are less likely to head potential negative consequences of their actions. Furthermore, children with low levels of fear are less likely to be responsive to socializing punishments, which may in turn contribute to poor fear conditioning (Raine, et al., 1997). Alternatively, low heart rate may not be a direct cause of externalising behaviour, but a marker for other processes that are implicated in externalising behaviour, such as poor right hemisphere functioning (10).

Although there is good evidence for an intimate relation between the autonomic nervous system and externalising behaviour, gaps in our knowledge remain. First, it is uncertain if this association is evident in very young children. Early-onset externalising behaviour is of particular interest, because if it persists it has a poor prognosis when compared to adolescent-onset externalising behaviour (11). A cross-sectional study of preschool children showed that children who displayed more externalising behaviour had lower heart rates (12). However, most studies of resting heart rate have targeted older children or adolescents.

Second, the specificity of the relation between the autonomic nervous system and externalising behaviour remains unclear. Children with externalizing behaviour are characterised by very different traits. Many young children with these problems are physically aggressive and oppositional, but some are predominantly emotionally (over)reactive; they experience high rates of anxiety and distress, and are often impulsive. Other aggressive children are fearless and plan their rule-breaking behaviour (13). Together with impaired guilt, these traits are termed callous-unemotional. Children with callous-unemotional traits are capable of premeditated externalising behaviour and aggression and have a heightened risk for developing severe psychopathology as an adult (14). Investigating whether low resting mean rate is a specific marker of certain aspects of externalising behaviour may yield further insight in its aetiology and development.

A relation between low heart rate and emotion regulation has been documented. Previous

studies have established an association between low heart rate and the absence of anxiety in children (15, 16). Furthermore, fearless and emotionally less reactive children with conduct disorder showed little heart rate reactivity in response to a videotaped event involving fear (17). A study of 94 preschoolers found that low resting mean heart rate was not associated with emotional over-reactivity (18).

Our main aim is to investigate the longitudinal relation between resting mean heart rate measured at age 14 months and different aspects of externalising behaviour at age 3 years. We used parental report by means of the Child Behavior Checklist (19) and observed the child's behaviour using a version of the well-known gift delay task. The gift delay task assesses the child's compliance and ability to delay gratification. It is part of a battery aimed at investigating emotional reactivity. (20). In addition, we examined the children's propensity towards lying by asking if they cheated during the gift delay task.

We hypothesize that low resting mean heart rate is indicative of fearless traits. Hence, we expect that low resting mean heart rate predicts low levels of anxiety and high levels of reported externalising behaviour. In addition, we expect low resting mean heart rate to predict higher odds of lying in the gift delay task. There is evidence that different externalizing behaviours are determined to a different extent by constitutional and environmental factors (21, 22). Harsh parenting has been identified as a key environmental risk factor of child and adolescent externalising behaviour (23, 24). Several studies indicate that harsh parenting is specifically associated with childhood aggression and a diminished ability to delay gratification (25, 26). Harsh parenting is a common cause of externalising behaviour with a moderate effect, which makes it a suitable comparator for etiological studies (Brenner & Fox, 1998; Jansen, et al., 2012).

We expect higher levels of harsh parenting to be associated with aggressive behaviour, attention problems as well as an impaired ability to delay gratification during the gift delay task. We will contrast the relation between resting mean heart rate, a biomarker, with the relation of harsh parenting to the various indicators of child externalising behaviour. Of particular interest will be whether mean heart rate, a biomarker, and harsh parenting to will relate differently to various indicators of child externalising behaviour.

Methods

Participants

This study was conducted within the Focus cohort of the Generation R Study, a population-based cohort study from foetal life onwards. All children were born between February 2003 and August 2005 in the city of Rotterdam. The cohort consists of Dutch children and their parents and is ethnically homogeneous to rule out confounding and effect modification by ethnicity. Midwives and obstetricians informed eligible mothers about the study at their first prenatal visit in routine care, handed out the information package and asked these mothers to make an appointment for the first ultrasound examination. The study staff contacted these mothers by phone for additional information about the study and in person at the ultrasound examination to obtain informed consent. The general design, all research aims and the specific measurements in the Generation R Study have been approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam. (27).

As described previously, measurements of autonomic indices were added to the protocol of the examination round, while assessments were already ongoing. We obtained physiological measurements for 528 participants as described previously (28). The current sample con-

sisted of the 412 children for whom physiological measurements at 14 months and Harsh parenting and Child Behavior Checklist scores at 3 years were available.

Psychophysiological measurements
Infant heart rate at 14 months was registered with a three pole ECG lead. We monitored the breathing pattern using a piëzo-electric transducer. Signals were recorded for 8 minutes using a Vitaport 3 recorder (Temec Inc) while the child was resting in its mothers' lap. To calculate the child's mean heart rate level, we used the mean lengths of the interbeat intervals in the 100-180 seconds of time when breathing was most regular.

Harsh parenting
Harsh parenting was assessed with a Dutch version of the Parent-Child Conflict Tactics Scales (29). This instrument was designed to measure psychological and physical maltreatment and neglect of children by parents, as well as nonviolent modes of discipline. The translation was carried out using a standard forward-backward translation method. The scale was completed by the mother when her child was 3 years old. She rated use of discipline types during the past 2 weeks on a 6-point scale ranging from never to five times or more. Several categories were combined because of very low prevalence rates. This resulted in three categories: never (0), once (1), and twice or more (2).
Three items from the Parent-Child Conflict Tactics Scale assessing hitting and spanking were not used. In the Netherlands severe harsh punishment is prohibited. The review board of the Generation R Study decided to exclude items concerning possibly illegal practices. Additionally, one item of the Psychological Aggression scale ("said you would kick child out of the house") was excluded to make the assessment age appropriate (30).

Maternal report of child behaviour
We used the Child Behavior Checklist/1½-5 (CBCL), a parent questionnaire for assessing behavioural and emotional problems in children aged 1½ - 5. The CBCL has good reliability and validity (19). When the child was 3 years old, the mother rated the child's emotional and behavioural problems over the preceding 2 months on a 3-point rating scale. For a subset of the children (n=378) CBCL questionnaires at 18 months of age were available as well. In the current study we used the Anxious/Depressed scale, and the Externalising subscales Aggressive Behaviour and Attention Problems syndrome scales.
Paternal report of child behaviour was available for a subset of the children (n=397). We studied the association of harsh parenting and heart rate with paternally reported behaviour as well.

Gift delay task
We used an adapted version of the well-known gift delay task (Kochanska et al., 2000). This task was originally developed as part of a larger battery of tests aimed at assessing the children's control over their behaviour. It specifically measures situational compliance and the ability to delay gratification. The task was conducted by trained experimenters during a laboratory session. It was framed as a game in which the child was asked to wait for a specific sign by the experimenter to retrieve the gift.
The experimenter brought a paper bag containing a wrapped gift and placed the bag on the table. Then the experimenter asked the child to wait and not to touch the bag or look into

the bag until she brought the card accompanying the gift. Subsequently, the child was left alone in the room for 180 s. The behaviour of the children was monitored with a hidden camera setup. We scored the gift delay task dichotomously, children who did not touch, inspect or open the gift while the experimenter was out of the room scored 0 (compliant), children who touched, inspected or opened the gift scored 1 (non-compliant).
Immediately after the return of the experimenter, children were asked by their attending parent or, in case the attending parent forgot, by the trained experimenter, whether they had cheated or not. Non-compliant children who admitted non-compliance scored 0, non-compliant children who lied scored 1. Compliant children did not receive a score on lying.
369 children performed the gift delay task. Due to procedural errors, 9 children (6.7 %) of the 134 non-compliant children were not asked whether they had cheated and were excluded from the respective analyses.

Covariates
The choice of covariates was based on previous studies (28, 31). Sex of the child, gestational age and weight at birth, maternal age, and smoking and drinking behaviour during pregnancy were available as covariates. Level of highest completed education by the mother was entered as a measure of socio-economic status. The Dutch Standard Classification of Education was used to define 3 categories of education: higher education phase 2 (university degree), higher education phase 1 (higher vocational training, Bachelor's degree) and secondary education or lower. Gestational age at birth and birth weight were obtained from community midwife and hospital registries at birth. Information on maternal smoking and drinking habits during pregnancy as well as highest completed education were obtained by means of a questionnaire completed by the mother.

Statistical analysis
For our main analysis, we performed a series of regression analyses to investigate the association of heart rate with the Anxious/Depressed, Aggressive Behaviour and Attention Problems scores, with observed compliance during the gift delay task and with lying after the gift delay task. First, we performed linear regression analyses with heart rate as the independent variable and child Anxious/Depressed, Aggressive Behaviour or Attention Problems scores as dependent variables. The regression residuals of the analyses between heart rate and anxiety were not normally distributed even after we removed outliers (i.e. the regression residuals deviated more than 3 SD from the mean). In addition, the homoscedacity assumption was violated. Hence, we tested the robustness of significant results of these analyses using logistic regressions. To perform the logistic regressions we dichotomised CBCL scores at 1 SD above the mean in line with earlier reports (Dierckx, Tharner, et al., 2009). Scores above 1 SD should be seen as above average. Again, outliers were excluded from the analysis. Second, we determined whether child heart rate predicted non-compliant behaviour during the gift delay task using logistic regression. Third, we investigated whether child heart rate was associated with lying after the gift delay task using logistic regressions. For comparison, the same set of analyses were run with harsh parenting as independent variable. Heart rate and harsh parenting variables were standardized to facilitate the interpretation of the effect estimates. Heart rate and harsh parenting variables were standardized to facilitate the interpretation of the effect estimates. All analyses were adjusted for gender of the child and maternal education as an indicator of socio-economic

status. Adding gestational age and weight at birth, maternal age, and smoking and drinking during pregnancy to the models did not change the effect estimates. Cases whose regression residuals deviated more than 3 standard deviations from the mean were excluded from the analysis. In exploratory analyses, we used ANOVA to compare compliant children with non-compliant children who admitted non-compliance and non-compliant children who lied. Effect sizes for significant effects are given as Cohen's d. By convention, values of d up to 0.2 are defined as a small effect size, values up to 0.5 are defined as medium, values larger than 0.5 are considered large.
All analyses were performed using the Statistical Package for the Social Sciences 15.0 for Windows (SPSS Inc).

Results
Table 1 displays the baseline characteristics of the participants. Mean maternal age was 31.9±3.6 years. Of the mothers 33.7 percent completed only secondary education or lower, 28% completed higher education, phase 1, while 38.3% completed higher education, phase 2.
The children had a mean resting heart rate of 124.1±10.6 bpm at age 14 months. All heart rate values recored were within a normal, physiologic range for 14 month old infants. About 1-2% of CBCL scale scores were in the clinical range as expected in a population based study. Equal numbers of boys and girls participated.

Child behaviour as reported by the parents
Table 2 displays the associations of mean resting heart rate with child CBCL scores at age 3 years, as well as the associations between harsh parenting and child CBCL scores. The higher a child's heart rate, the higher the child's Anxious/Depressed scores (β=0.1, 95%CI=0.01;0.2, p=0.04). Heart rate explained 10% of the variation in the child Anxious/

Table 1. Characteristics of the study population (n=412).

Age mother at intake, years (SD)	31.9±3.6
Maternal education	
Secondary education or lower (%)	33.7
Higher education, phase 1 (%)	28.0
Higher education, phase 2 (%)	38.3
Child sex, male (%)	49.8
Heart rate, bpm (SD)	124.11±10.6
Aggressive parenting (SD)	2.06±2.3
Non-compliance during Gift Delay Task (n)	134
Lying about compliance during Gift Delay Task (n)	21
CBCL Anxiety symptoms (SD)	0.65±1.1
CBCL Attention problems (SD)	1.28±1.5
CBCL Aggressive behaviour (SD)	6.11±4.5

Table 2. Child heart rate and aggressive parenting as predictors of behaviour symptoms reported by the mother.

	Anxiety symptoms			Attention problems			Aggressive behaviour		
	β	95% CI	p	β	95% CI	p	β	95% CI	p
Heart rate/sd (bpm)	0.1	0.01;0.2	0.04	0.02	-0.1;0.1	0.8	0.08	-0.3;0.5	0.8
Harsh parenting/sd	0.05	-0.02;0.2	0.1	0.2	0.1;0.3	<0.001	0.7	0.4;0.9	<0.001

Results displayed from linear regression models. A β of 1.0 indicates the change in CBCL symptom score when the predictor changes by a standard deviation. All analyses were corrected for child sex and maternal level of education.

Depressed scores. When we corrected for Anxious/Depressed scores at 18 months, the relation was only slightly attenuated (β=0.09, 95%CI=-0.01;0.2, p=0.07). This suggests that part of the relation between heart rate and anxiety was indeed was already present at baseline. In a dichotomous analysis, the resting mean heart rate of the children was positively associated with their odds of having high anxiety symptoms at borderline significance (OR= 1.31, 95%CI=0.99;1.7, p=0.05). We observed no association between child heart rate and child Attention Problems (β=0.02;95%CI=-0.1;0.1, p=0.8) or child Aggressive Behaviour (β=0.08, 95%CI=-0.3;0.5, p=0.8).

In contrast to the pattern of associations of heart rate, there was no association between harsh parenting and child Anxious/Depressed scores (β=0.05, 95%CI=-0.02;0.1, p=0.1), however, harsh parenting was positively associated with both child Attention Problems (β=0.2, 95%CI=0.1;0.3, p<0.001) and with child Aggressive Behaviour (β=0.7, 95%CI=0.4;0.9, p<0.001).). When we corrected for CBCL scale scores at 18 months the relationships where attenuated, but harsh parenting was still clearly associated with both child Attention Problems (β=0.2, 95%CI=0.1;0.3, p=0.002) and with child Aggressive Behaviour (β=0.5, 95%CI=0.1;0.9, p=0.01). Results from dichotomous analysis where similar and showed a significant association between harsh parenting and child Anxiety/Depression (OR= 0.3, 95%CI=1.1;1.75, p=0.008) child Attention problems (OR= 1.48, 95%CI=1.13;1.95, p=0.004) as well as with child Aggressive Behaviour (OR= 1.65, 95%CI=1.2;2.12, p<0.001).
The results from the analyses with paternally reported child behaviour measures did not differ substantially from the results obtained using maternally reported child behaviour measures.

Child behaviour during the gift delay task
Table 3 shows the relations between mean resting heart rate, harsh parenting and child behaviour during the gift delay task. The child's heart rate was not associated with the odds of non-compliance during the gift delay task (OR=1.14, 95%CI=0.9;1.1, p=0.2). Likewise, harsh parenting was not associated with non-compliance (OR=1.13, 95%CI=0.9;1.3, p=0.08). The resting mean heart rate of the children was, however, significantly associated with their odds of lying. A lower heart rate predicted higher risk of lying (OR= 0.56, 95%CI=0.3;0.9, p=0.03);a child with a heart rate at one SD below the mean had a 1.78

Table 3. Child heart rate and aggressive parenting as predictors of behaviour during the gift delay task.

	Odds of non-compliance n=369			Odds of lying n=125		
	OR	95% CI	p	OR	95% CI	p
Heart rate/sd (bpm)	1.14	0.9;1.1	0.2	0.56	0.3;0.9	0.03
Harsh parenting/sd	1.13	0.9;1.3	0.08	1.03	0.8;1.4	0.8

Results displayed from logistic regression models. The OR reflects the change in odds of the child cheating or lying per standard deviation change of the predictor. All analyses were corrected for child sex and maternal level of educationcation.

times higher odds of lying than a child with a heart rate at the mean. Heart rate explained 4 % of the odds of lying. There was no relation between harsh parenting and lying of the child (OR=1.03, 95%CI=0.8;1.4, p=0.8).

The relation between reported behaviour problems and observed compliance and lying
In secondary analyses, we compared the following three groups of children: compliant children, children who were non-compliant but did not lie, and children who were non-compliant and lied after the gift delay task. We found similar levels of mother-reported Anxious/Depressed scores (F=0.41, df=2, p=0.6) and Attention Problems (F=1.66, df=2, p=0.2) across the groups. In contrast, maternal report of child Aggressive Behaviour differed between the groups (F=4.04, df=2, p=0.02). Non-compliant children who lied had lower Aggressive Behaviour scores (3.8±4.4) than compliant children (5.95±4.5, p=0.04, effect size d=0.48) and children who admitted non-compliance (6.80±4.5, p=0.007, effect size d=0.67).

Table 4. Maternally reported behaviour and behaviour during the gift delay task.

	Compliant		Non-compliant				
	n-235		Did not lie n=104		Lied n=21		
	Mean	SD	Mean	SD	Mean	SD	Overall p
Anxiety symptoms	0.67	1.1	0.61	1.1	0.45	0.6	0.6
Attention problems	1.14	1.5	1.44	1.5	1.17	1.0	0.2
Agressive behaviour	5.95	4.5	6.80	4.5	3.8	4.4	0.02

Results displayed from linear regression models. A β of 1.0 indicates the change in CBCL symptom score when the predictor changes by a standard deviation. All analyses were corrected for child sex and maternal level of education.

Discussion

In this longitudinal population-based study we found that a lower heart rate in children aged 14 months positively predicted lying during a gift delay task at age 3 years. Lower heart rate was also positively associated with lower levels of Anxious/Depressed scale scores at age 3 years, but did not predict overt externalising behaviour such as Attention Problems and Aggressive Behaviour.

A few studies have previously reported an association between heart rate and anxiety. For example, Monk et al. showed that adolescents with anxiety disorders had higher and less fluctuating heart rate during a resting condition (32). Similarly, a higher resting mean heart rate has been observed in behaviourally inhibited children (15).

Most psychophysiological research in children or adolescents focused on externalising behaviour. Numerous studies showed an association between low mean resting heart rate and externalising behaviour (9). However, the specificity of the relation between the autonomic nervous system and externalising behaviour remains unclear. In this study we explored several externalising behaviours: aggression, compliance and lying. However, contrary to our hypothesis, heart rate did not predict overt externalising behaviour such as Attention Problems and Aggressive Behaviour.

In the Mauritius study, Raine et al. measured children's heart rate at age 3 and assessed their externalising behaviour at age 11. They suggested that low HR may predispose not so much to externalising behaviour in general, but to aggression in particular (7). We could not demonstrate an association between heart rate at a very young age and aggressive behaviour at age 3 years.

However, the children in our study were considerably younger than those in earlier studies. In young children aggressive behaviour is common, but usually time limited. At this age, aggression is most often reactive, triggered on impulse and by strong emotions (Dunn&-Munn, 1986). Consequently, the relationship between heart rate and aggressive behaviour in our study may have been diluted by the large number of children displaying age appropriate aggressive behaviour. In addition, proactive, planned aggression, which is commonly accompanied by low levels of emotional reactivity, develops later in life than reactive aggression (Dodge, Lochman, Harnish, Bates, &Pettit, 1997).

We found no association between heart rate and compliance during the gift delay task. It is tempting to view compliance during the gift delay task solely as a marker of emotional reactivity (33). However, while the gift delay task was designed to measure situational compliance and the ability to delay gratification, one could argue that the outcome of the task is influenced by such factors as novelty seeking and moral development as well. Unless all these traits are uniformly associated with heart rate, this may have obscured specific associations between heart rate and any of the traits.

In our study, low mean resting heart rate was positively associated with the odds of lying during the gift delay task. This finding should be interpreted with care, as only a small number of children lied (n=21), which may limit the generalisability of the findings. In addition lying is not unequivocally an indicator of externalising behaviour. First, research has shown that the ability to lie depends on the child's cognitive development. Talwar and colleagues used a similar testing paradigm to the gift delay task to probe the association between lying and cognitive development in 150 3 to 8 year old children. They found that the child's odds of lying were positively associated with the child's theory of mind and executive functioning. They suggest that the child needs some understanding of theory of mind

and realise that it is possible to hide non-compliance and escape any negative consequences by denial. In addition, some executive functions, such as working memory and the ability to suppress telling the truth, may need to be developed enough to able to lie (34, 35). Second, lying could be construed as a form of avoidant behaviour. Anxious children may be more likely to lie, because they are keen to avoid the researcher's disapproval. Yet, this cannot easily explain our observations, as the children who lied had similar Anxious/Depressed scores as those who did not lie. Finally, lying can be part of planned, covert, low emotionally reactive externalising behaviour, and is one of the first symptoms of this behaviour to manifest itself in children.

The pattern of low anxiety and a propensity towards lying is in accordance with the fearlessness theory which integrates physiological and psychological perspectives in child development. The fearlessness theory asserts that low resting mean heart rate is indicative of low levels of fear. Children who experience low levels of fear are less likely to head potential negative consequences of their actions (7, 36).

We suggest that low anxiety and a tendency to lie are indicative of low levels of emotional reactivity. Low heart rate may thus delineate a small group of children that are composed and calculating, rather than overly emotionally reactive and overtly aggressive. Indeed, the children who lied during the observational task were less likely to display reactive aggression according to maternal report. It is tempting to speculate that low heart rate at age 14 months may predict future callousness-unemotional traits, which are characterised by a lack of guilt and by shallow affect (Viding&McCrory, 2012). Previous research already demonstrated an association between lower HR reactivity and callous unemotional traits in older, school-age, children (17). While several longitudinal studies suggest that callous–unemotional traits can initially occur in the absence of overt externalising behaviour, however such overt externalising behaviour often develops later on (37, 38). Hence, longer follow-up is needed to ascertain if the children in the current study develop overt externalising behaviour and in particular proactive aggression at an older age.

Harsh parenting was associated with different aspects of externalising behaviour than was heart rate. Harsh parenting was strongly associated with child attention problems and aggressive behaviour. While our results are consistent with previous findings (25, 26), it is important to note that both parenting style and child aggressive behaviour and attention problems were measured with parental report. Lau et al. compared parental report to an independent observation of child behaviour, and concluded that parents with a history of harsh parenting tended to over-report externalizing behaviour in their children (39). This effect is likely to have contributed to the strong association between harsh parenting and child externalising behaviour in the present study. In our study, distinct risk factors were thus associated with different categories of externalising behaviour. If not accounted for by chance, this may point to different pathogenetic pathways in the development of externalising behaviour. Several authors have investigated interaction effects of environmental and biomarkers (10, 40). Some have suggested that environmental factors, such as aggressive parenting, may not affect all children equally. Instead, children with certain innate characteristics such as fearlessness may be more susceptible to environmental influences. Unfortunately, the number of participants in the current study is too low to allow for meaningful statistical analysis of interaction effects. More longitudinal research is needed to further elucidate the complex interplay between biological and environmental risk factors in the developmental trajectory of psychopathology.

The strengths of the present study include the large population based sample, the combination of maternal report and observational measures, the longitudinal design and a focus on an age when aggressive psychopathology is still in development.
Some methodological limitations need to be discussed. Not all participants of the Generation R Focus Cohort could be included, because we added physiological measurements to our assessment protocol while the study was already ongoing. The Focus Cohort is ethnically homogeneous, which has the advantage that it excludes potential confounding or effect modification by ethnicity, but which limits the generalisability of the results. We used a self-report questionnaire to assess smoking and drinking during pregnancy. The limited reliability of such a self-report questionnaire may explain why smoking and drinking behaviour during pregnancy did not influence the effect estimates of our statistical models. Finally, the possibility of false positive results due to the number of statistical analyses performed should be carefully considered.
Low resting mean heart rate level at 14 months was associated with low levels of anxiety and a propensity towards lying at age 3 years. We suggest that even at this young age, low heart rate is an indicator of fearlessness. We recommend longitudinal research to further elucidate the complex interplay between biomarkers and environmental risk factors in the developmental trajectories of externalising behaviour. Future studies may benefit from additional observational measures to assess aggressive behaviour.

References

1. Campbell SB, Ewing LJ. Follow-up of hard-to-manage preschoolers: adjustment at age 9 and predictors of continuing symptoms. Journal of child psychology and psychiatry, and allied disciplines. 1990 Sep;31(6):871-89.
2. Tremblay RE, Nagin DS, Seguin JR, Zoccolillo M, Zelazo PD, Boivin M, et al. Physical aggression during early childhood: trajectories and predictors. Pediatrics. 2004 Jul;114(1):e43-50.
3. Alink LR, Mesman J, van Zeijl J, Stolk MN, Juffer F, Koot HM, et al. The early childhood aggression curve: development of physical aggression in 10- to 50-month-old children. Child development. 2006 Jul-Aug;77(4):954-66.
4. Dunn J, Munn P. Sibling quarrels and maternal intervention: individual differences in understanding and aggression. Journal of child psychology and psychiatry, and allied disciplines. 1986 Sep;27(5):583-95.
5. Hay DF, Castle J, Davies L. Toddlers' use of force against familiar peers: a precursor of serious aggression? Child development. 2000 Mar-Apr;71(2):457-67.
6. Van Hulle CA, Corley R, Zahn-Waxler C, Kagan J, Hewitt JK. An exploration of the genetic and environmental etiology of heart rate in infancy and middle childhood. Twin Res. 2000 Dec;3(4):259-65.
7. Raine A, Venables PH, Mednick SA. Low resting heart rate at age 3 years predisposes to aggression at age 11 years: evidence from the Mauritius Child Health Project. Journal of the American Academy of Child and Adolescent Psychiatry. 1997 Oct;36(10):1457-64.
8. Raine A, Venables PH, Williams M. Relationships between central and autonomic measures of arousal at age 15 years and criminality at age 24 years. Arch Gen Psychiatry. 1990 Nov;47(11):1003-7.
9. Ortiz J, Raine A. Heart rate level and antisocial behavior in children and adolescents: a meta-analysis. Journal of the American Academy of Child and Adolescent Psychiatry. 2004 Feb;43(2):154-62.
10. Raine A. Biosocial studies of antisocial and violent behavior in children and adults: a review. J Abnorm Child Psychol. 2002 Aug;30(4):311-26.
11. Moffitt TE, Caspi A, Harrington H, Milne BJ. Males on the life-course-persistent and adolescence-limited antisocial pathways: follow-up at age 26 years. Development and psychopathology. 2002 Winter;14(1):179-207.
12. El-Sheikh M, Hinnant JB, Erath S. Developmental trajectories of delinquency symptoms in childhood: the role of marital conflict and autonomic nervous system activity. J Abnorm Psychol. 2011 Feb;120(1):16-32.
13. Ellis ML, Weiss B, Lochman JE. Executive functions in children: associations with aggressive behavior and appraisal processing. J Abnorm Child Psychol. 2009 Oct;37(7):945-56.
14. Viding E, Fontaine NM, McCrory EJ. Antisocial behaviour in children with and without callous-unemotional traits. J R Soc Med. 2012 May;105(5):195-200.
15. Kagan J, Reznick JS, Snidman N. The physiology and psychology of behavioral inhibition in children. Child development. 1987 Dec;58(6):1459-73.
16. Gerra G, Zaimovic A, Zambelli U, Timpano M, Reali N, Bernasconi S, et al. Neuroendocrine responses to psychological stress in adolescents with anxiety disorder. Neuropsychobiology. 2000;42(2):82-92.
17. Anastassiou-Hadjicharalambous X, Warden D. Physiologically-indexed and self-perceived affective empathy in Conduct-Disordered children high and low on Callous-Unemotional traits. Child Psychiatry Hum Dev. 2008 Dec;39(4):503-17.
18. Gower AL, Crick NR. Baseline autonomic nervous system arousal and physical and relational aggression in

preschool: the moderating role of effortful control. Int J Psychophysiol. 2011 Sep;81(3):142-51.
19. Achenbach TM, Rescorla LA. Manual for ASEBA Preschool Forms and Profiles. Burlington, VT: University of Vermont, Research Center for Children, Youth and Families; 2000.
20. Kochanska G, Murray KT, Harlan ET. Effortful control in early childhood: continuity and change, antecedents, and implications for social development. Dev Psychol. 2000 Mar;36(2):220-32.
21. Viding E, Blair RJ, Moffitt TE, Plomin R. Evidence for substantial genetic risk for psychopathy in 7-year-olds. Journal of child psychology and psychiatry, and allied disciplines. 2005 Jun;46(6):592-7.
22. Viding E, Jones AP, Frick PJ, Moffitt TE, Plomin R. Heritability of antisocial behaviour at 9: do callous-unemotional traits matter? Dev Sci. 2008 Jan;11(1):17-22.
23. Eddy JM, Chamberlain P. Family management and deviant peer association as mediators of the impact of treatment condition on youth antisocial behavior. J Consult Clin Psychol. 2000 Oct;68(5):857-63.
24. Keiley MK, Lofthouse N, Bates JE, Dodge KA, Pettit GS. Differential risks of covarying and pure components in mother and teacher reports of externalizing and internalizing behavior across ages 5 to 14. J Abnorm Child Psychol. 2003 Jun;31(3):267-83.
25. Chang L, Schwartz D, Dodge KA, McBride-Chang C. Harsh parenting in relation to child emotion regulation and aggression. J Fam Psychol. 2003 Dec;17(4):598-606.
26. Olson SL, Tardif TZ, Miller A, Felt B, Grabell AS, Kessler D, et al. Inhibitory control and harsh discipline as predictors of externalizing problems in young children: a comparative study of U.S., Chinese, and Japanese preschoolers. J Abnorm Child Psychol. 2011 Nov;39(8):1163-75.
27. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, et al. The Generation R Study: design and cohort update 2012. Eur J Epidemiol. 2012 Sep;27(9):739-56.
28. Dierckx B, Tulen JH, van den Berg MP, Tharner A, Jaddoe VW, Moll HA, et al. Maternal psychopathology influences infant heart rate variability: Generation R Study. Psychosomatic medicine. 2009 Apr;71(3):313-21.
29. Straus MA, Hamby SL, Finkelhor D, Moore DW, Runyan D. Identification of child maltreatment with the Parent-Child Conflict Tactics Scales: development and psychometric data for a national sample of American parents. Child Abuse Negl. 1998 Apr;22(4):249-70.
30. Jansen PW, Raat H, Mackenbach JP, Hofman A, Jaddoe VW, Bakermans-Kranenburg MJ, et al. Early determinants of maternal and paternal harsh disciplone: The Generation R Study. Family Relations. 2012;61(2):253-70.
31. Dierckx B, Tharner A, Tulen JH, Jaddoe VW, Hofman A, Verhulst FC, et al. Low autonomic arousal as vulnerability to externalizing behaviour in infants with hostile mothers. The Generation R Study. Psychiatry research. 2009:Accepted.
32. Monk C, Kovelenko P, Ellman LM, Sloan RP, Bagiella E, Gorman JM, et al. Enhanced stress reactivity in paediatric anxiety disorders: implications for future cardiovascular health. Int J Neuropsychopharmacol. 2001 Jun;4(2):199-206.
33. Blair C, Razza RP. Relating effortful control, executive function, and false belief understanding to emerging math and literacy ability in kindergarten. Child development. 2007 Mar-Apr;78(2):647-63.
34. Talwar V, Gordon HM, Lee K. Lying in the elementary school years: verbal deception and its relation to second-order belief understanding. Dev Psychol. 2007 May;43(3):804-10.
35. Talwar V, Lee K. Social and cognitive correlates of children's lying behavior. Child development. 2008 Jul-Aug;79(4):866-81.
36. Raine A. The psychopathology of crime: criminal behaviour as a clinical disorder. San Diego: Academic Press; 1993.
37. Frick PJ, Cornell AH, Bodin SD, Dane HE, Barry CT, Loney BR. Callous-unemotional traits and developmental pathways to severe conduct problems. Dev Psychol. 2003 Mar;39(2):246-60.
38. Rowe R, Maughan B, Moran P, Ford T, Briskman J, Goodman R. The role of callous and unemotional traits in the diagnosis of conduct disorder. Journal of child psychology and psychiatry, and allied disciplines. 2010 Jun;51(6):688-95.
39. Lau AS, Valeri SM, McCarty CA, Weisz JR. Abusive parents' reports of child behavior problems: relationship to observed parent-child interactions. Child Abuse Negl. 2006 Jun;30(6):639-55.
40. Oldehinkel AJ, Verhulst FC, Ormel J. Low heart rate: a marker of stress resilience. The TRAILS study. Biological psychiatry. 2008 Jun 15;63(12):1141-6.

Chapter 6

Persistance of anxiety disorders and concomitant changes in cortisol.

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Abstract
In a clinical sample of 116 children and adolescents we studied the relation between the course of an anxiety disorder during treatment and the concomitant changes in cortisol levels. Assessments at baseline, after three months, and at one-year follow-up were performed with the Anxiety Disorders Interview Schedule.
When we compared cortisol levels at baseline and one-year follow-up, persistence of the anxiety disorder was associated with both increased daytime cortisol production ($F=6.23$, $p=0.01$) and a trend towards a decreased cortisol morning rise ($F=4.19$, $p=0.08$). At one-year follow-up daytime cortisol production was lowest in the early remitters (109.7±29.2 h*mmol/l), higher in the late remitters (121.0±40.0 h*mmol/l) and highest in the non-remitters (131.1±48.9 h*mmol/l). Early remitters had the highest cortisol morning rise (1.1±1.5 h*mmol/l), followed by the late remitters (0.8±1.8 h*mmol/l), the non-remitters had the lowest cortisol morning rise (0.07±1.7 h*mmol/l).
Persistence of an anxiety disorder may thus lead to changes in HPA-axis functioning, underscoring the importance adequate treatment of anxiety disorders.

Introduction

The relation between HPA-axis functioning and internalising problems has been examined repeatedly. In patients with major depression, higher basal cortisol levels, and in patients with posttraumatic stress disorder, lower cortisol levels are consistent findings, although exceptions have also been reported (1-6). In comparison, studies investigating cortisol levels in patients with other anxiety disorders are less common. Research on panic disorder yielded conflicting results. Some authors reported increased HPA-axis activity (7, 8), whereas others showed normal HPA-axis activity (9). Recently, Mantella et al. reported increased basal cortisol levels in patients with a generalised anxiety disorder (10).

Even fewer studies have investigated the association between anxiety problems and cortisol levels in children and adolescents. Kagan et al. found that basal cortisol levels were higher in inhibited than in uninhibited young children(11). Two studies reported similarly increased HPA-activity in anxious children and adolescents. Granger et al. (12) showed that social anxiety in children and adolescents referred to an outpatient clinic was associated with a more pronounced increase in salivary cortisol concentrations after exposure to a mild stressor. In a study conducted by Feder et al., children with an anxiety disorder had lower nighttime cortisol levels and a steeper rise of cortisol concentrations after awakening, compared to depressed and healthy control children (13).

However, several other studies could not demonstrate an association between anxiety problems and cortisol levels. Martel et al. did not find differences in basal cortisol levels between social phobic adolescent girls and matched controls (14). A study comparing adolescents with an anxiety disorder and healthy controls showed no difference in cortisol response induced by a public speaking task (15).

A numbers of problems and caveats may have contributed to the inconsistency of findings. The groups under study where dissimilar. Some studies were conducted in the general population, whereas others focused on clinical groups, which are likely to display important differences in symptom severity. Moreover, most of these studies did not adjust for comorbid symptoms of major depression. As symptoms of major depression are closely associated with anxiety symptoms and have been associated with alterations in HPA-axis activity, they can confound the association under study. Most studies performed were small and hence underpowered to detect subtle differences between groups.

In addition, some authors have suggested that the inconsistent findings may be due to changes in HPA-axis functioning during the course of the disorder (16) . Gunnar and Vazquez posited that, although stressfull events provoke frequent elevations in cortisol at first, these elevations would eventually lead to downregulation of the HPA-axis (17). On the other hand, elevations in cortisol levels that persist across time could also tune HPA-axis activity to a higher level (18). A recent study by Greaves-Lord et al. favoured the second hypothesis. They demonstrated in a large general population sample of young preadolescents that only persistent, and not current anxiety problems were associated with high basal cortisol levels (16). The developing HPA-axis of children and adolescents may be especially vulnerable to such stress-induced changes. This could be indicative of progressing damage or atrophy to elements of the HPA-axis. Permanent HPA-axis dysfunctioning in early life has repeatedly been linked to chronicity and recurrence of affective disorders and affective symptoms (19, 20). Persistence of an anxiety disorder in childhood could have life-long consequences for the neuroendocrine system/health/well-being, underscoring the need for prospective, longitudinal research.

Moreover, the chronic and pathological anxiety experienced by children and adolescents in a clinical population is more severe than the anxiety reported by study participants from the general population, hence it may have a greater impact on the developing HPA-axis and impair its future functioning.
In a clinical sample of 116 children and adolescents aged between 8 and 16 years we studied the relation between the trajectory of an anxiety disorder during treatment and the concomitant change in cortisol levels. Although the findings in literature are inconsistent, most research to date seems to indicate high cortisol levels in individuals with an anxiety disorder, other than PTSD, when compared to normal controls. Hence, we postulate that levels of cortisol in patients in remission are lower than in non-remitted patients. In addition, we hypothesize that persistence of the anxiety disorder is associated with further alteration of HPA-axis functioning. We expect levels of cortisol at follow- up to be higher than at baseline.

Methods

Participants

Participants had been referred to the outpatient clinic of the department of Child and Adolescent Psychiatry of either the Erasmus Medical Center in Rotterdam or Leiden University Medical Center— Curium. All consecutive referrals to these departments were assessed with the Anxiety Disorders Interview Schedule for DSM-IV-Child Version (ADIS-C). Children and adolescents with a primary diagnosis of Generalized Anxiety Disorder, Separation Anxiety Disorder, Social Phobia or Specific Phobia were eligible for inclusion. Exclusion criteria were: current medication for an anxiety disorder or current corticoid medication, co-morbid Pervasive Development Disorder, Obsessive Compulsive Disorder, Post Traumatic Stress Disorder, substance use, and an IQ score below 85 on the Wechsler Intelligence Scale for Children-Revised (WISC-R).
A standardized stepped-care cognitive behavioural therapy program for childhood anxiety disorders was used, consisting of two phases. Phase one consisted of the FRIENDS program (21), which comprises psycho-education, relaxation and breathing exercises, exposure, problem solving skills training, social support training, and cognitive restructuring. FRIENDS encompassed 10 child sessions and 4 separate parent sessions. Children, who were not successfully treated after phase one as determined by ADIS-C at three months follow-up, received a supplementary phase. Phase two consisted of 10 sessions, in which parents and child participated together in each session. Parents were more actively involved in phase two. The skills learned during phase one were elaborated (cognitive restructuring, exposure and long-term relapse control).
In total, 184 children and adolescents aged 8 to 16 years participated in the study. 60 participants were diagnosed with a separation anxiety disorder as the primary diagnosis, 56 with a generalised anxiety disorder, 45 with a social phobia and 23 with a specific phobia. 55 participants had more than one anxiety disorder. Three participants were diagnosed with a co-morbid depression, eight with co-morbid dysthymia. Of the 184 participants, 42 participants did not participate at follow-up due to logistic and practical reasons, cortisol samples for 26 participants where lost due to a failure of the lab storage refrigurator. The Medical Ethical Committees of the Erasmus MC in Rotterdam and the Leiden University Medical Centre in Leiden approved the protocol. All parents and each adolescent provided written consent.

Diagnostic assessment
The Anxiety Disorders Interview Schedule for DSM-IV (ADIS-C) is a semi-structured interview aimed at assessment of DSM-IV anxiety disorders in children and adolescents. The interview was conducted by a trained post-doctoral psychologist and supervised master-level students with the patient and the parents separately. A diagnosis is based both on a symptom count of DSM-IV symptom criteria, and on the level of impairment according to the parent, the patient, and the interviewer. The ADIS-C showed good to excellent interrater reliability, with Kappa for the different diagnoses ranging from 0.63 to 80 for the child interview, and 0.65 to 0.88 for the parent interview. The combined interview showed excellent reliability, with Kappa ranging from 0.80 to 0.92. In addition test-retest reliability was excellent, with intraclass correlation coefficients ranging from 0.78 to 0.95 for the child interview and from 0.81 to 0.96 for the parent interview (22, 23).

Questionnaires
Information on self-reported anxiety was obtained by administering the Dutch version of the Multidimensional Anxiety Scale for Children (MASC). The MASC is a self-report measure of general anxiety in children and includes 39 items. The internal reliability was excellent with internal reliability coefficients ranging from 0.62 to 0.85 for the subscales and to 0.90 for the total scale. Test-retest reliability was excellent as well, with an intraclass correlation coefficient for the total scale of 0.93 with a retest after three months (24).
Information on self-reported depression was obtained by means of the Dutch version of the Children's Depression Inventory (CDI). The CDI is a 27-item scale suited for monitoring changes in a child's mood (24). Good reliability (Cronbachs's alpha between 0 .81 and 0. 87) and good test-retest reliability (Intraclass correlation coefficient = 0.80) has been reported (25).
To supplement the child report, we used the Child Behavior Checklist (CBCL), a parent questionnaire for assessing psychiatric problems in children. At baseline, the mother rated the child's emotional and behavioural problems over the preceding 2 months on a 3-point scale. As a measure of depression severity we used the DSM affective scale to minimize colinearity with anxiety symptoms. This scale was developed by Achenbach in 2003 to represent the DSM category of Major Depressive and Dysthymic disorders. The DSM oriented scales have good reliability (Cronbach's alpha = 0..82) and good test-retest reliability (Intraclass correlation coefficient = 0.88) (26).

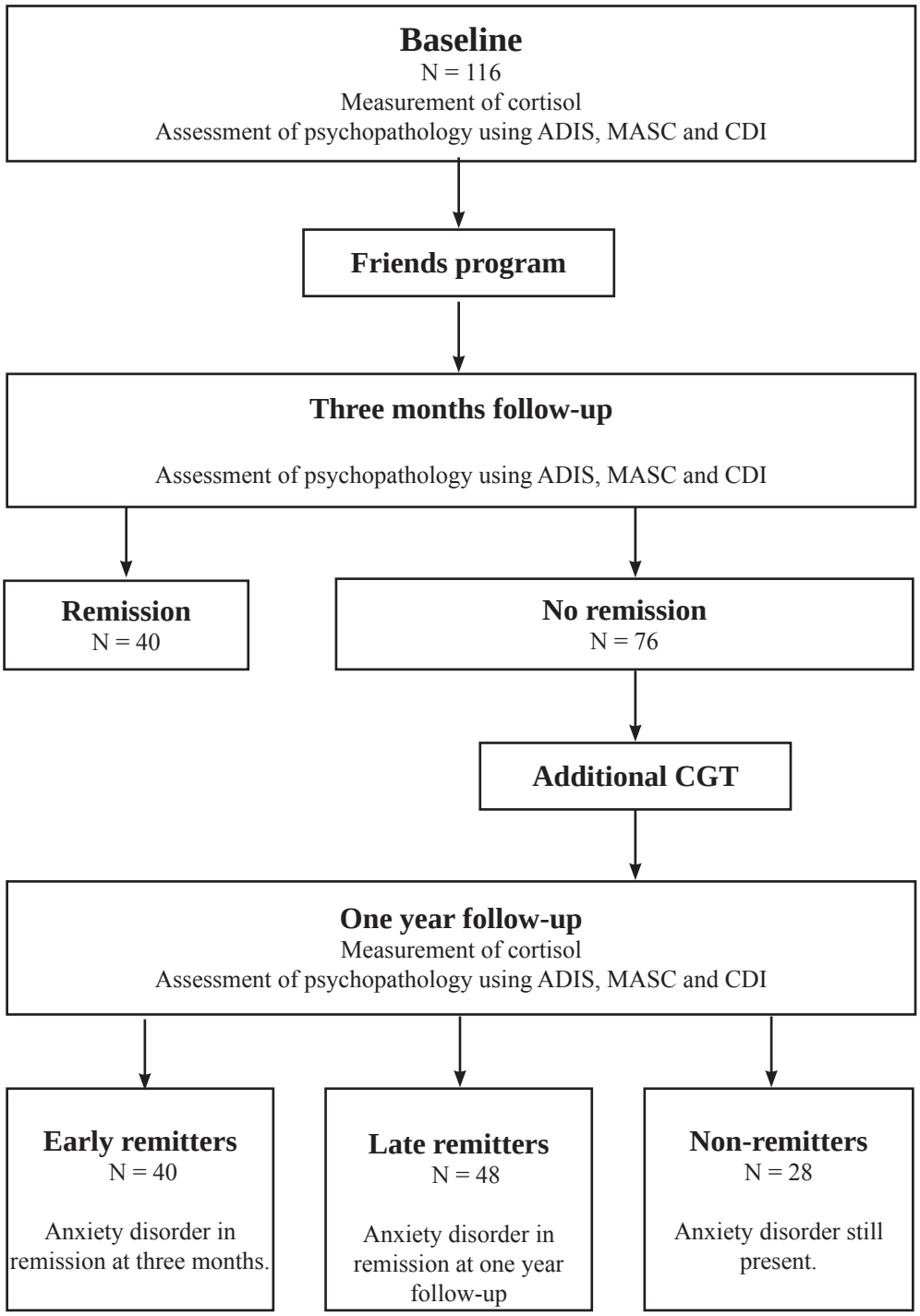
2.4. Cortisol assessment
Participants were asked to collect saliva samples at home. Participants and their parents were briefed on how to sample the saliva by one of the research staff. In addition they were given detailed written instructions. Four samples were collected. (1) Immediately after awakening in the morning, when the child was still in bed, (2) 30 min later, (3) at 12.00 h, and (4) at 20:00 h. The time of awakening was recorded on the first saliva tube. Children were asked not to eat 0.5 h before sampling, and to refrain from consuming dairy products 1 h before sampling. All samples were collected on a regular school day, stored in the freezer at home, and brought along to the clinic a day later. Saliva samples were stored at -20°C. until analysis. Cortisol concentrations
were determined in duplicate 20 ml samples by solid-phase radioimmunoassay with iodinated cortisol (Coat-A-Count, Diagnostic Products Corporation, LA, USA). The lower

limit of detection was 1 nmol/l, the intra-assay variation was 6.9% and the inter-assay variation was 8.6%.
We employed two measures that are commonly used to summarize aspects of the diurnal pattern of cortisol: morning rise and daytime cortisol secretion.
Total daytime cortisol secretion was calculated as the area under the curve (AUC) for samples 1, 3 and 4 with respect to the ground, according to Preussner et al. (27). While cortisol daytime AUC is a reliable index of unstimulated HPA-axis activity is, waking up in the morning is a potent stressor. Hence, cortisol morning rise levels can provide additional information on the reactivity of the HPA-axis (28), which must otherwise be gleaned from stimulation tests with either CRH/ACTH or under influence of a stressor in a laboratory setting. The morning rise was calculated as the area under the curve with regard to the increase for samples 1 and 2 (29).

Procedure
Cortisol saliva samples were collected at baseline and one year later.
The ADIS-C, MASC and CDI were administered to the children and their parents at three time points, namely at baseline, after three months and at one-year follow-up. Interviewers were blind to pre-treatment diagnoses, disease trajectory, and cortisol levels.

Statistical analysis
First we performed a non-response analysis. We compared age, sex, depression and anxiety scores of the 116 participants for whom cortisol samples where available both at baseline and one year later to the 68 participants for whom insufficient data was available. We used T-tests for normally distributed variables, for non-normal distributed continuous variables Mann-Whitney U tests and for dichotomous variables Chi Square tests.
The participants fell into three groups. This categorisation was determined prior to the start of data collection and has been described in an earlier publication based on this sample (30). As shown in figure 1, the groups were based on the trajectories of the anxiety disorders as assessed by ADIS-C: (1) early remitters: those participants who no longer suffered from any anxiety disorder after three months (n=40), (2) late remitters: those participants who had no anxiety disorder at one-year follow up (n=48), (3) non-remitters: those participants who still fulfilled the criteria for an anxiety disorder at one-year follow up(n=28). We compared these three groups on gender, weight and baseline anxiety and depression severity using Chi Square tests and ANOVA with Tukey correction where appropriate.
In a preliminary analysis, we determined whether the severity of the anxiety disorder, as measured by the MASC, was associated with cortisol levels at baseline or follow up. We employed linear regressions, corrected for age and sex.
In our main analysis, we tested the hypothesis, that persistence of an anxiety disorder is associated with changes in cortisol levels between baseline and follow-up. We used mixed model analysis to determine whether levels of cortisol changed between baseline and one-year follow-up. We tested whether any such change was related to the course of the anxiety disorder by means of an interaction term between time and remission status. The analysis was corrected for age, sex, depression severity both at baseline and at one-year follow up.
We employed ANCOVA's to illustrate the findings of the mixed model. We show to what extent cortisol levels at baseline were related to the subsequent course of the anxiety disorder. We corrected for age, sex and depression severity at baseline. Similarly, we show to

Figure 1. Study overview.



what extent early, late or non-remitters had different cortisol levels at one-year follow-up. We corrected for age, sex, baseline cortisol levels, and depression severity at baseline and one-year follow-up. Significant findings were further explored by contrasting the early remitters group against the late and non-remitters.
All statistical analyses were carried out using the Statistical Package for the Social Sciences 13.0 for Windows (SPSS Inc).

Results

The non-response analysis showed that the participants, who were included in the analysis, had a similar boy/girl ratio ($\chi 2=0.65$, df=1, p=0.5) and age range (U=2391.5, p=0.9) , as well as similar baseline depression (U=2290.5 p=0.4 for child report, U=2361.5, p=0.8 for maternal report) and anxiety scores (t=-0.99, df=176, p=0.36) to participants for whom insufficient data was available.
Table 1 summarises the baseline characteristics for the children and adolescents according to the trajectory of their anxiety disorder. There was no difference between the groups in boy/girl ratio ($\chi 2=3.85$, df=2, p=0.14) or age range (F=0.43, df=2,155 p=0.7). Baseline anxiety scores showed a trend for a difference between the groups (F=2.46, df=2,152, p=0.07). However, pair wise comparisons showed no significant differences between the groups. Child reported depressive symptoms differed between the groups (F=8.13, df=2,147, p<0.001).There were lower depressive symptoms in the early remission group versus the late (p<0.001) and non-remission (p<0.065) groups. A similar pattern was found for maternally reported child depressive symptoms (F=6.69, df=2,155, p=0.001. Again, there were lower depressive symptoms in the early remission group when compared to the non-remission group (p=0.001).
Anxiety levels were not associated with concomitant cortisol AUC levels either at baseline (B=0.08, 95% CI -0.16, 0.48, p=0.3) or at follow up (B=0.13, 95% CI -0.21, 0.98, p=0.2). Similarly, anxiety levels were not associated with concomitant cortisol morning rise levels at baseline (B=-0.02, 95% CI -0.02, 0.02, p=0.8) or at follow up (B=-0.01, 95% CI -0.04, 0.01, p=0.3).

Cortisol morning rise

First, we present the results of the multilevel analysis, which focuses on changes in cortisol from baseline to one-year follow up. Overall, cortisol morning rise did not differ between

Table 1. Characteristics of the study population (n=412).

	Early remitters	Late remitters	Non-remitters
N	40	48	28
Age at intake in years (SD)	11.1 (2.1)	10.9 (2.2)	10.5 (2.5)
Female %	42	46	57
Anxiety symptoms (SD)	40.0 (12.8)	45.7 (17.4)	43.7 (17.7)
Depression symptoms (SD)	5.2 (3.7)	10.6 (7.5) *	8.9 (6.5) *

baseline and one-year follow-up (F=0.5, df=247, p=0.5). However, inspection of figure 2b shows that cortisol morning rise increased in early and late remitters, whereas it decreased in non-remitters. Indeed, there was a trend for the interaction between remission category and time (F=2.4, df=247, p=0.09).
We conducted further analyses to illustrate these findings. The trajectory of the anxiety disorder was not associated with baseline cortisol morning rise (F=0.3, df=2,142, p=0.7). Early remitters (mean 1.0, 95% CI 0.5;1.4 h*mmol/l) had similar cortisol morning rise levels at baseline as late remitters (mean 0.8, 95% CI 0.3; 1.2 h*mmol/l) and non-remitters (mean 1.0, 95% CI 0.4;1.6 h*mmol/l).
As shown in figure 2b, at one year follow up, the trajectory of the anxiety disorder was associated with cortisol morning rise (F=4.3,df=2, 100, p=0.01). Early remitters had the highest cortisol morning rise (mean 1.3, 95% CI 0.7;1.8 h*mmol/l, reference group), followed by the late remitters (mean 0.8, 95% CI 0.3; 1.3 h*mmol/l, p=0.2), the non-remitters had the lowest cortisol morning rise (mean -0.2,95% CI -1.0;0.6 h*mmol/l, p=0.004).

Daytime AUC cortisol
Again, we first describe findings from our multilevel analysis. As shown in figure 2c, the overall daytime AUC increased from baseline to one-year follow-up, but this increase did not reach statistical significance (F=2.5, df=249, p=0.08). More importantly however, the increase in cortisol levels was dependent on the trajectory of the anxiety disorder (remission category*time, F=3.2, df=249, p=0.04).
As a next step, we conducted post-hoc analyses to illustrate the significant interaction effect observed in the multilevel analysis. As expected, at baseline, daytime AUC cortisol did not differ (F=0.1,df=2,141, p=0.9) between the early remitters (mean 85.8, 95% CI 76.8;94.8 h*mmol/l), late remitters (mean 88.6, 95 % CI 79.7;97.5 h*mmol/l) and non-remitters (mean 86.4, 95% CI 74.0;98.7 h*mmol/l). In contrast, daytime AUC levels at follow up differed significantly across the groups (F=3.37,df=2, 100, p=0.04), accounting for the interaction effect. It was lowest in the early remitters (mean 108.7, 95% CI=95.6; mean 121.8 h*mmol/l, reference group), higher in the late remitters (mean 120.5, 95% CI 108.6;132.5 h*mmol/l, p=0.2) and highest in the non-remitters (mean 138.5, 95% CI 120.7;156.2 h*mmol/l, p=0.01).

Discussion
The present study investigated associations between the trajectory of an anxiety disorder and the concurrent changes in cortisol levels in a clinical sample of children and adolescents. Daytime AUC cortisol was higher at follow-up than at baseline. This increase was most pronounced for non-remitters, intermediate in late remitters and less obvious in early remitters.
Overall, cortisol morning rise did not differ significantly between baseline and follow-up. However, there was a trend for cortisol morning rise to increase in early and late remitters, whereas it decreased in non-remitters. At one-year follow-up, this corresponded with significant differences between the groups, with lowest cortisol morning rise in non-remitters, followed by late remitters and the highest cortisol morning rise in non-remitters. Again, late remitters formed an intermediate group, consistent with a dose-response effect; the longer participants were the more pronounced the changes in cortisol.

Figure 2a. Anxiety scores at baseline and one year follow-up.

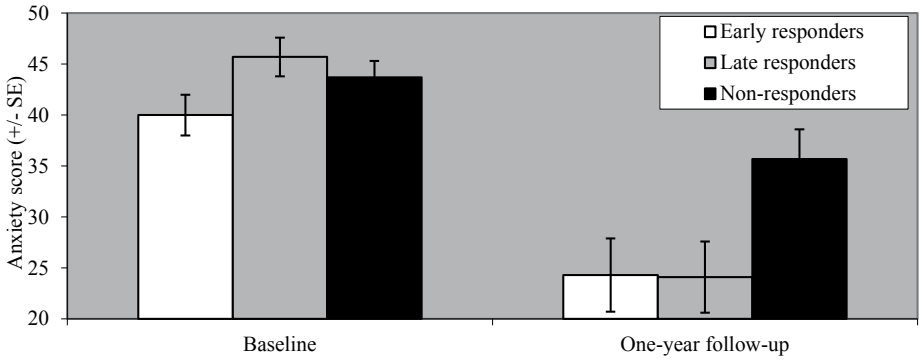


Figure 2b. Cortisol morning rise at baseline and one year follow-up.

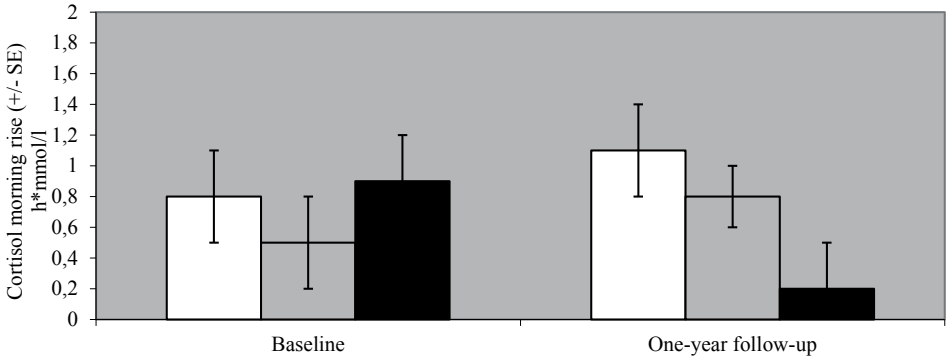
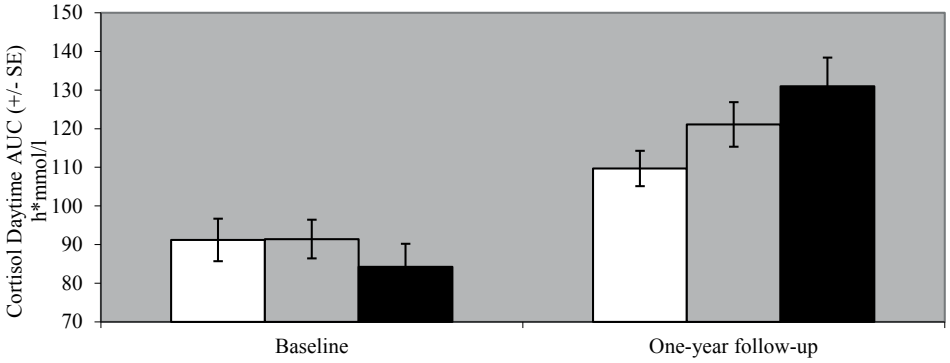


Figure 2c. Cortisol daytime area under the curve at baseline and one year follow-up.



In children and adolescents, research focussing on the relation between anxiety and the HPA-axis has mainly been cross-sectional in nature. For daytime cortisol levels, our findings at one year follow-up, which demonstrate higher cortisol levels in non-remitters versus late and early remitters, are consistent with results from literature which generally describes heightened daytime cortisol levels in relation to anxiety (11).
Studies describing cortisol morning rise in relation to anxiety in children are rare. A number of studies, especially in adults, have shown an increased cortisol moning rise is related to anxiety or stress (31, 32). In contrast, some studies seem to indicate a blunted morning rise. Feder et al. (2004) showed that anxious children exhibited a delayed nocturnal rise before reaching similar peak levels of cortisol near the time of awakening. Some authors have suggested thata blunted cortisol response measure, such as a the cortisol morning rise, can be due to a ceiling effect due to already elevated basal cortisol levels (33, 34) . This is largely in line with our own results.
Although straightforward, the cross-sectional approach has limited our understanding of the temporal interplay between anxiety and HPA-axis functioning, which may have contributed to the lack of agreement among the different studies.
In two recent studies, Greaves-Lord et al. have tried to address this issue In the first study, they investigated a large general population sample of preadolescents. They showed that individuals with high prevalent anxiety levels only showed elevated morning cortisol levels and an elevated cortisol morning rise if they had a history of anxiety problems earlier in life (16). Unfortunately, the history of anxiety problems was determined retrospectively, which may have lead to a recall bias.
In their second study, Greaves-Lord et al. took a prospective approach. They observed that cortisol levels in adolescents from the general population where not associated with anxiety problems at baseline and did not predict anxiety problems two years later. These results seem to contradict earlier research by Smider et al., who found that higher mean daytime cortisol levels at age 4.5 predicted internalizing problems at age 6 (35). Again, this may be due to differences in study population or cortisol measure used.
Prospective studies in a clinical setting have two advantages over studies in a general population sample. First, as anxiety symptoms are bound to be more pronounced in a clinical group, associated changes in HPA-axis functioning may also be more pronounced, and thus easier to detect. Second, one can study the association of treatment induced changes in anxiety with the concomitant pattern of cortisol levels.
Previously, Tafet et al. compared a group of 20 adults who received cognitive therapy for generalised anxiety disorder with 8 adults untreated for their generalised anxiety disorder. They found that in the treated group only, plasma cortisol measured at 16h was lower after treatment than at baseline. Plasma cortisol in the untreated group remained stable (36).
Our results of increased daytime cortisol levels in late and non-responders seem to be at odds with Tafet et al.'s findings. However, differences in population studied i.e. adults versus children and adolescents, cortisol measure i.e. one time point plasma cortisol versus three time point salivary cortisol in our study, and length of the study i.e. 24 weeks versus 1 year make the two studies difficult to compare. Most importantly, Tafet et al. did not differentiate between treatment responders and non-responders, which makes it impossible to determine whether cortisol varies over time depending on treatment success.
Our study differs from previous work in that it has a prospective, longitudinal design and is set in a clinical sample consisting of children and adolescents. In addition to the benefits of

a prospective and longitudinal design, research in children and adolescents is able to study the anxiety disorder while it is still developing. It allowed us to show changes in cortisol functioning during the course of the anxiety disorder. We will now discuss several possible explanations for our results.

First, individuals with higher daytime cortisol and/or lower control morning rise may represent a subgroup that is more difficult to treat successfully and hence takes longer to reach remission. Our results cannot easily be reconciled with this explanation, as we could not demonstrate an association between baseline cortisol levels and subsequent remission status.

Second, our observations may be due to the non-remitter group having the most severe symptoms, either at baseline or at one year follow up. Early remitters showed a non-significant trend towards lower anxiety scores at baseline as compared to late remitters and non-remitters. However, neither at baseline nor at follow up, was anxiety severity associated with cortisol levels.

Third, depression severity was higher in the late and non-remitter groups. This may have confounded the associations. However, we addressed this by correcting for depression severity in all our analyses.

Fourth, our study design did not allow us to contrast the changes in cortisol levels during treatment for anxiety to changes in cortisol levels occurring as part of normal development in a one-year period. Hence, we cannot rule out that normal age-related changes underlie part of the observed changes in cortisol levels. However, the differences between early, late and non-remitters cannot be explained easily by such age-related changes as these are, most likely, very similar in all three groups.

Finally, the changes in cortisol levels could reflect chronic stress associated with the presence of an anxiety disorder. The dose-response pattern, ie the level of change in cortisol was related to the course of the anxiety disorder, strongly supports this interpretation. Indeed, there are biological studies in animals and humans that suggest a potential underlying mechanism for our findings. Prolonged or repeated stress-induced elevations in cortisol levels result in atrophy of hippocampal neurons and loss of synapses (37). Damage to or atrophy of the hippocampus affects the glucocorticoid feedback inhibition of CRH secretion and leads to higher CRH and daytime cortisol concentrations (38). In addition, such atrophy or damage has been associated with a lowered or even absent cortisol morning rise (39, 40).

The longitudinal relationship between anxiety disorders and symptoms and HPA-axis functioning should be a focus for further research. Clinical research should follow cases and age and sex matched controls prospectively over time to relate HPA-axis changes during the course of the illness to HPA-axis changes during the course of normal development. In addition, while studies of placebo-treated patient controls are ethically questionable, comparison of different treatment modalities in relation to HPA-axis functioning can shed light on the effect of specific treatments on HPA-axis functioning. Finally, further longitudinal, research in the general population is needed to elucidate the relation between HPA-axis functioning and the development of anxiety symptoms and ultimately the incidence of anxiety disorders.

Several methodological aspects must also be discussed. The absence of a non-treated control group with an anxiety disorder makes it impossible to evaluate the effect of the cognitive behavioural therapy on cortisol levels in this study. Unfortunately, ethical considerations prohibit withholding treatment for a prolonged period of time. Further, the study

was conducted in a university hospital setting, hence referral biases may have limited the generalisability of the study sample. Primarily, our results can most easily be generalised to severely affected, young patients. Because our study was not performed under laboratory conditions, it was not possible to closely monitor adherence to the testing protocol and to assess the participant's sleeping pattern, this may have affected the reliability of the cortisol measurements.

Finally, our study did not assess pubertal status, which is an important determinant of cortisol levels. Should pubertal status be related to remission status as well, this could lead to confounding. However, because we have a broad age range and adjusted for age, this is not very likely.

This is the first prospective study demonstrating specific changes in cortisol levels in relation to the trajectory of an anxiety disorder in a relatively large, clinical sample. Our findings seem to indicate that persistence of an anxiety disorder leads to changes in HPA-axis functioning, underscoring the importance of early detection and adequate treatment of anxiety disorders.

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References

1. Goenjian AK, Pynoos RS, Steinberg AM, Endres D, Abraham K, Geffner ME, et al. Hypothalamic-pituitary-adrenal activity among Armenian adolescents with PTSD symptoms. Journal of traumatic stress. 2003 Aug;16(4):319-23.
2. Goenjian AK, Yehuda R, Pynoos RS, Steinberg AM, Tashjian M, Yang RK, et al. Basal cortisol, dexamethasone suppression of cortisol, and MHPG in adolescents after the 1988 earthquake in Armenia. The American journal of psychiatry. 1996 Jul;153(7):929-34.
3. Lopez-Duran NL, Kovacs M, George CJ. Hypothalamic-pituitary-adrenal axis dysregulation in depressed children and adolescents: a meta-analysis. Psychoneuroendocrinology. 2009 Oct;34(9):1272-83.
4. Burke HM, Davis MC, Otte C, Mohr DC. Depression and cortisol responses to psychological stress: a meta-analysis. Psychoneuroendocrinology. 2005 Oct;30(9):846-56.
5. Bonne O, Brandes D, Segman R, Pitman RK, Yehuda R, Shalev AY. Prospective evaluation of plasma cortisol in recent trauma survivors with posttraumatic stress disorder. Psychiatry research. 2003 Jul 15;119(1-2):171-5.
6. Bremner JD, Vythilingam M, Vermetten E, Adil J, Khan S, Nazeer A, et al. Cortisol response to a cognitive stress challenge in posttraumatic stress disorder (PTSD) related to childhood abuse. Psychoneuroendocrinology. 2003 Aug;28(6):733-50.
7. Vreeburg SA, Zitman FG, van Pelt J, Derijk RH, Verhagen JC, van Dyck R, et al. Salivary cortisol levels in persons with and without different anxiety disorders. Psychosomatic medicine. May;72(4):340-7.
8. Wedekind D, Bandelow B, Broocks A, Hajak G, Ruther E. Salivary, total plasma and plasma free cortisol in panic disorder. J Neural Transm. 2000;107(7):831-7.
9. Gurguis GN, Mefford IN, Uhde TW. Hypothalamic-pituitary-adrenocortical activity in panic disorder: relationship to plasma catecholamine metabolites. Biological psychiatry. 1991 Sep 1;30(5):502-6.
10. Mantella RC, Butters MA, Amico JA, Mazumdar S, Rollman BL, Begley AE, et al. Salivary cortisol is associated with diagnosis and severity of late-life generalized anxiety disorder. Psychoneuroendocrinology. 2008 Jul;33(6):773-81.
11. Kagan J, Reznick JS, Snidman N. The physiology and psychology of behavioral inhibition in children. Child development. 1987 Dec;58(6):1459-73.
12. Granger DA, Weisz JR, Kauneckis D. Neuroendocrine reactivity, internalizing behavior problems, and control-related cognitions in clinic-referred children and adolescents. Journal of abnormal psychology. 1994 May;103(2):267-76.
13. Feder A, Coplan JD, Goetz RR, Mathew SJ, Pine DS, Dahl RE, et al. Twenty-four-hour cortisol secretion patterns in prepubertal children with anxiety or depressive disorders. Biological psychiatry. 2004 Aug 1;56(3):198-204.
14. Martel FL, Hayward C, Lyons DM, Sanborn K, Varady S, Schatzberg AF. Salivary cortisol levels in socially phobic adolescent girls. Depression and anxiety. 1999;10(1):25-7.
15. Gerra G, Zaimovic A, Zambelli U, Timpano M, Reali N, Bernasconi S, et al. Neuroendocrine responses to

psychological stress in adolescents with anxiety disorder. Neuropsychobiology. 2000;42(2):82-92.
16. Greaves-Lord K, Ferdinand RF, Oldehinkel AJ, Sondeijker FE, Ormel J, Verhulst FC. Higher cortisol awakening response in young adolescents with persistent anxiety problems. Acta psychiatrica Scandinavica. 2007 Aug;116(2):137-44.
17. Gunnar MR, Vazquez DM. Low cortisol and a flattening of expected daytime rhythm: potential indices of risk in human development. Development and psychopathology. 2001 Summer;13(3):515-38.
18. Sapolsky RM, Krey LC, McEwen BS. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. Endocrine reviews. 1986 Aug;7(3):284-301.
19. Flory JD, Yehuda R, Grossman R, New AS, Mitropoulou V, Siever LJ. Childhood trauma and basal cortisol in people with personality disorders. Compr Psychiatry. 2009 Jan-Feb;50(1):34-7.
20. Nicolson NA, Davis MC, Kruszewski D, Zautra AJ. Childhood maltreatment and diurnal cortisol patterns in women with chronic pain. Psychosomatic medicine. 2010 Jun;72(5):471-80.
21. Barrett PM, Lowry-Webster H, Holmes J. The Friends Anxiety Prevention Program. Queensland: Australian Academic Press; 2000.
22. Siebelink BM, Treffers PDA. Dutch translation of the AnxietyDisorders Schedule for DSM-IV. Lisse: Swets & Zeitlinger.; 2000.
23. Silverman WK, Saavedra LM, Pina AA. Test-retest reliability of anxiety symptoms and diagnoses with the Anxiety Disorders Interview Schedule for DSM-IV: child and parent versions. Journal of the American Academy of Child and Adolescent Psychiatry. 2001 Aug;40(8):937-44.
24. March JS, Parker JD, Sullivan K, Stallings P, Conners CK. The Multidimensional Anxiety Scale for Children (MASC): factor structure, reliability, and validity. Journal of the American Academy of Child and Adolescent Psychiatry. 1997 Apr;36(4):554-65.
25. Kovacs M. The Children's Depression, Inventory (CDI). Psychopharmacol Bull. 1985;21(4):995-8.
26. Achenbach TM, Dumenci L, Rescorla LA. DSM-oriented and empirically based approaches to constructing scales from the same item pools. J Clin Child Adolesc Psychol. 2003 Sep;32(3):328-40.
27. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology. 2003 Oct;28(7):916-31.
28. Wust S, Wolf J, Hellhammer DH, Federenko I, Schommer N, Kirschbaum C. The cortisol awakening response - normal values and confounds. Noise & health. 2000;2(7):79-88.
29. Edwards S, Clow A, Evans P, Hucklebridge F. Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. Life sciences. 2001 Mar 23;68(18):2093-103.
30. Legerstee JS, Tulen JH, Dierckx B, Treffers PD, Verhulst FC, Utens EM. CBT for childhood anxiety disorders: differential changes in selective attention between treatment responders and non-responders. Journal of child psychology and psychiatry, and allied disciplines. Feb;51(2):162-72.
31. Kunz-Ebrecht SR, Kirschbaum C, Marmot M, Steptoe A. Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. Psychoneuroendocrinology. 2004 May;29(4):516-28.
32. Vreeburg SA, Zitman FG, van Pelt J, Derijk RH, Verhagen JC, van Dyck R, et al. Salivary cortisol levels in persons with and without different anxiety disorders. Psychosomatic medicine. 2010 May;72(4):340-7.
33. Burke HM, Fernald LC, Gertler PJ, Adler NE. Depressive symptoms are associated with blunted cortisol stress responses in very low-income women. Psychosomatic medicine. 2005 Mar-Apr;67(2):211-6.
34. Martin del Campo AF, Dowson JH, Herbert J, Paykel ES. Diurnal variations in endocrine and psychological responses to 0.2 mg/kg naloxone administration in patients with major depressive disorder and matched controls. J Affect Disord. 2000 Jan-Mar;57(1-3):37-47.
35. Smider NA, Essex MJ, Kalin NH, Buss KA, Klein MH, Davidson RJ, et al. Salivary cortisol as a predictor of socioemotional adjustment during kindergarten: a prospective study. Child development. 2002 Jan-Feb;73(1):75-92.
36. Tafet GE, Feder DJ, Abulafia DP, Roffman SS. Regulation of hypothalamic-pituitary-adrenal activity in response to cognitive therapy in patients with generalized anxiety disorder. Cognitive, affective & behavioral neuroscience. 2005 Mar;5(1):37-40.
37. Magarinos AM, McEwen BS. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. Neuroscience. 1995 Nov;69(1):89-98.
38. Schloesser RJ, Manji HK, Martinowich K. Suppression of adult neurogenesis leads to an increased hypothalamo-pituitary-adrenal axis response. Neuroreport. 2009 Apr 22;20(6):553-7.
39. Buchanan TW, Kern S, Allen JS, Tranel D, Kirschbaum C. Circadian regulation of cortisol after hippocampal damage in humans. Biol Psychiatry. 2004 Nov 1;56(9):651-6.
40. Pruessner M, Pruessner JC, Hellhammer DH, Bruce Pike G, Lupien SJ. The associations among hippocampal volume, cortisol reactivity, and memory performance in healthy young men. Psychiatry Res. 2007 May 15;155(1):1-10.

6

Chapter 7

General Discussion

The overall goal of this thesis was to extend the knowledge on the relation between the autonomic nervous system, measured by its proxy heart rate, and externalising behaviour. The following aims were addressed. (1) To investigate whether maternal psychopathology and infant breastfeeding determine infant heart rate. (2) To study the association between heart rate and externalising behaviour at a very young age and to study the specificity of that assocation.

The early environment and heart rate

We investigated two important early environmental factors in their relation to autonomic nervous system functioning, maternal psychopathology during pregnancy and after birth, and breastfeeding.

Maternal psychiatric symptoms

We showed that a maternal history of psychopathology before childbirth was associated with increased heart rate and lower vagal modulation in the infant. Similarly, postnatal maternal symptoms, especially anxiety and depression symptoms, were related to increased heart rate in the infant.

Several explanations for these findings will now be discussed. Both genetic and environmental mechanisms, which are certainly not mutually exclusive, could underlie the association between maternal psychopathology and infant heart rate. .

One. Postnatal environment. The existence of maternal psychiatric symptoms could represent a stressful environment for the infant, for example, by a means of an altered mother-child interaction. The association between postnatal maternal psychiatric symptoms with infant autonomic functioning and the absence of such an association for prenatal maternal symptoms are arguments in favour of this mechanism. Feldman and Eidelman demonstrated that physical mother-child interaction is important for the maturation of the vagal system, at least in premature infants (1). Maternal psychiatric symptoms could be an indicator only of other environmental risk factors, although we corrected our analysis for some environmental factors, including maternal age and education and household income. Finally, postpartum maternal psychiatric symptoms could merely be a proxy for psychiatric symptoms experienced by the mother during pregnancy, which could have influenced the child in utero.

Two. Prenatal environment. According to the fetal programming hypothesis, fetal physiological systems adapt to the characteristics of the intrauterine environment. This adaptation may permanently alter the set points of these physiological systems (2). Research already showed that women's emotional reactivity during pregnancy can influence foetal HR patterns (3) and that the foetal response dependens on maternal psychiatric status (4, 5). However, little is known about the effect of chronic stress on foetal HRV and whether this leads to lasting changes in the autonomic set points. We could not demonstrate an association between prenatal maternal psychiatric symptoms and infant ANS, although there was a trend for infants of women with higher psychiatric symptoms to have higher mean HR. Our data do not support a lasting effect of prenatal maternal psychiatric status and infant ANS functioning. However, we measured maternal psychiatric symptoms early during the pregnancy. It may be that the foetus is more sensitive to maternal psychiatric symptoms at a later stage of the pregnancy. For comparison, Yehuda et al. have shown that the timing of maternal anxiety during pregnancy moderates the impact of maternal anxiety

on the infant's HPA-axis functioning (6).
Three. Genetic mechanisms. There is growing evidence that genetic variations underlie the association between psychopathology and ANS functioning (7-9). Our results are compatible with the presence of genetic factors underlying both maternal psychopathology and infant heart rate, but are insufficient to prove such a relation.
Regardless of the exact mechanisms, the association between maternal psychiatric symptoms and infant autonomic functioning is important because the pattern of low heart rate and low vagal activity is indicative of negative behavioural outcomes in children (10-12). This further underscores the importance of early detection and treatment of maternal psychopathology,

Breastfeeding
We showed that infants, who were exclusively breastfed at two months of age, had lower sympathetic modulation than infants not breastfed. The association between breastfeeding and infant autonomic functioning disappeared after correction for socio-economic factors. However, the actual degree of attenuation of the association between breastfeeding and autonomic functioning achieved by correcting for socio-economic factors was minor, suggesting little effect of control.
To address the issue of residual confounding, we compared the effect of breastfeeding with that of a contrasting variable, infant fruitpurée consumption. It is related to the similar environmental epiphenomena as breastfeeding, including maternal socio-economic status, education, and caretaking as well as maternal concepts about health and nourishment. There was no significant association between infant fruitpurée consumption and breastfeeding, thus breastfeeding and fruitpurée consumption did not identify the same mother-infant pairs.
Infant fruitpurée does not contain fatty acids, some of which, the long-chain poly-unsaturated fatty acids, have been associated with autonomic functioning. Fruitpurée consumption does not come with skin-to-skin contact. Nonetheless, our data show that, similar to breastfeeding, infants who eat more fruitpurée have lower sympathetic modulation. This supports the notion that the association between breastfeeding and infant autonomic functioning may be similarly non-causal. Though, strictly speaking, we cannot rule out that the relation of breastfeeding and fruitpurée consumption to autonomic functioning is explained via different biological mechanisms.

In summary, while we showed that both maternal psychopathology and infant breastfeeding where associated with infant autonomic functioning, association does not imply causation. In fact, in both instances the causal pathways linking the risk factors to autonomic functioning remain unclear. Moreover, in the case of breast feeding, a limited effect size with a similar order of magnitude as the effect of another, seemingly trivial dietary component, call into question the clinical relevance of the association, even if it contains a causal component.

Heart rate and behaviour
While there is good evidence for an intimate relation between the autonomic nervous system and behaviour, gaps in our knowledge remain. First, it is uncertain if this association is evident in very young children. Early-onset externalising behaviour is of particular interest,

because if it persists it has a poor prognosis when compared to adolescent-onset externalising behaviour. Second, the specificity of the relation between the autonomic nervous system and externalising behaviour remains unclear. Is low autonomic arousal specifically associated with aggressive behaviour? Third, only a few studies have investigated the interplay of autonomic functioning with other, environmental, risk factors, and these studies have yielded mixed results.

Age 18 months

We found no association between infant autonomic functioning and behaviour at age 18 months in the total study population. However, we observed that in a subgroup of children whose mothers had high psychiatric symptoms, low heart rate was associated with aggressive behaviour.

We discuss two possible explanations. First, the fearlessness theory posits that low autonomic arousal in children is an indicator of fearlessness. Fearless children pay less heed to potential negative consequences of behaviour and thus may be prone to exhibit aggressive behaviour (13). We hypothesize that in the presence of maternal psychopathology, with less adequate parenting and maternal guidance in coping with fearless behaviour, these children actually develop aggressive behaviour. In other words, only within the high-risk group of infants whose mothers had psychiatric symptoms, children with low autonomic arousal were at higher risk of developing aggressive behaviour.

Second, it is possible that our stratification delineated a specific group. Toddlers with persistent aggressive behaviour are difficult to identify, because oppositional, hyperactive and aggressive behaviour is common at that age and usually time limited (14, 15). The relation between ANS indices and aggressive behaviour in the unstratified sample may have been obscured by toddlers displaying age appropriate aggressive behaviour. Toddlers whose mothers report high maternal psychiatric symptoms may be more likely to exhibit "true" aggressive behaviour.

Our results seem to contradict recent findings by Oldehinkel et al., who showed that adolescents with low heart rate displayed less psychopathology in relation to stressors of daily life than adolescents with normal or high heart rate (16). However, both age range and type of environmental stressor measured differed greatly between the studies. The fearlessness theory posits that low heart rate is related to fearless behaviour. It is conceivable that fearless infants who lack maternal guidance are at risk of developing aggressive behaviour, whereas in adolescence fearlessness may indeed buffer environmental stressors such as school work pressure, difficulties with peers and relationship problems.

Age three years

We showed that a child's heart rate at 14 months predicted the child's behaviour at age 3 years. Low heart rate was specifically and strongly associated with the odds of the child lying during the gift delay task as well as with low levels of anxiety.

We could not demonstrate an association between heart rate and parent rated aggressive behaviour. Moreover, there was no relation between heart rate and maternally reported attention problems and effort-full control as measured in the gift delay task either. Instead, in our study low heart rate was associated with increased odds of lying during the gift delay task. Compared to the other participants, the children who lied actually had lower aggressive behaviour ratings than other children.

A number of studies seem to indicate that heart rate is specifically associated with aggressive behaviour (12, 17). In their seminal Mauritius study, Raine et al. measured children's heart rate at age 3 and assessed their externalising behaviour at age 11. They suggested that low HR may predispose not so much to externalising behaviour in general, but to aggression in particular (12). We could not demonstrate an association between heart rate at a very young age and aggressive behaviour at age 3 years. However, the children in our study were considerably younger than those in earlier studies. In young children aggressive behaviour is common, but usually time limited. At this age, aggression is most often reactive, triggered on impulse and by strong emotions (14, 15). Proactive, planned aggression, which is commonly accompanied by low levels of emotional reactivity, develops later in life than reactive aggression (18).

We found no association between heart rate and compliance during the gift delay task. It is tempting to view compliance during the gift delay task solely as a marker of emotional reactivity (19). However, while the gift delay task was designed to measure situational compliance and the ability to delay gratification, one could argue that the outcome of the task is also influenced by such factors as novelty seeking and moral development. Unless all these traits are uniformly associated with heart rate, this may have obscured specific associations between heart rate and any of the traits.

In our study, low mean resting heart rate was associated with the odds of lying during the gift delay task. This finding should be interpreted with care, as lying is not unequivocally an indicator of externalising behaviour. First, the ability to lie depends on the development of theory of mind. The child has to be able to realise that it is possible to hide non-compliance and escape any negative consequences by denial (20). Second, lying could be construed as a form of avoidant behaviour. Anxious children may be more likely to lie, because they are keen to avoid the researcher's disapproval. This cannot easily explain our observations, as the children who lied had similar Anxious/Depressed scores as those who did not lie. Finally, lying can be part of planned, covert, low emotionally reactive externalising behaviour, and one of the first symptoms of this behaviour to manifest itself in children. The pattern of low anxiety and a propensity towards lying is in accordance with the fearlessness theory, which integrates physiological and psychological perspectives in child development. The fearlessness theory asserts that low resting mean heart rate is indicative of low levels of fear. Children who experience low levels of fear are less likely to head potential negative consequences of their actions (12).

We suggest that low anxiety and a tendency to lie are indicative of low levels of emotional reactivity. Low heart rate may thus delineate a small group of children that are composed and calculating, rather than overly emotionally reactive and overtly aggressive. Indeed, the children who lied during the observational task were less likely to display reactive aggression, according to maternal report. It is tempting to speculate that these traits may be precursors of future callousness-unemotional behaviour, but longer follow-up is needed to demonstrate this prediction.

The relation between autonomic functioning at a young is not clear-cut. Elucidating this association is difficult because oppositional, hyperactive and aggressive behaviour are not necessarily pathological, while other forms of externalizing behaviour have yet to develop. We presented some evidence that low heart rate predicts low levels of emotional reactivity in very young children. However, longer follow up is needed to determine whether the small group of children delineated in our study will indeed develop more severe types of

externalizing behaviour later in life. Finally, interaction effects with environmental factors may be likely, but could not be investigated due to limited power. Even when we demonstrated a statistical interaction effect with child psychophysiology, it was not clear whether is presented a "true" interaction or whether it represented a stratification based on severity of externalising behaviour.

Methodological considerations

Physiological measurements

An association between resting heart rate and externalizing behaviour is observed consistently (17). In contrast, studies on the relation between measures of heart variability and behaviour have yielded largely incongruent results. Relatively small sample sizes and differences in study population and testing conditions may explain part of these inconsistencies. Another problem, however, is the large array of different measures available for quantifying heart variability. Heart rate variability can be evaluated using either time-domain dependent measures or frequency-domain dependent measures. Time domain measures are the easiest to perform. The simplest time domain variables that can be calculated are the mean heart rate and the mean interbeat interval. From a longer series of heart rates or inter-beat intervals, more complex statistical time domain measures can be calculated. There are time domain measures derived directly from heart rate or interbeat intervals, for example the standard deviation of the intervals. This measure reflects all the cyclic components responsible for variability in the recording. Because the total variance of heart rate variability increases with the length of the recording, recordings of different lengths cannot be directly compared. There are also time domain variables derived from interval differences, such as the square root of the mean squared differences of successive interbeat intervals and the number of interval differences of successive intervals greater than 50 ms. These measurements all estimate high-frequency variations and are highly correlated. The series of interbeat intervals also can be converted into a number of geometric patterns. There are multiple approaches to interpret the resulting pattern, such as the sample density distribution of interbeat interval durations. Frequency-domain dependent measures, on the other hand, use mathematical algorithms to estimate how variance is distributed as a function of frequency. While there are various algorithms available, most provide comparable results (21). The frequency distribution is usually partitioned into bands that are thought to reflect various under-lying physiological processes. In short recordings, between 100 to 300 seconds of lengths, three main frequency components are distinguished: the very low frequency, low frequency and high frequency bands. In 24 hours recordings, an ultra low frequency band is present as well. The boundaries between the different bands are fairly well established in adults at rest. However, the boundaries, especially of the high frequency band, are dependent on respiratory frequency. Young children have a higher respiratory rate than adults, this means that changes in heart rate under vagal control occur at a higher frequency in young children. Unfortunately the boundaries of the bands in young children are not so well established in literature. We adjusted the upper bound of the vagal power band in our studies according to previous literature (22), but no real consensus exists in this matter.

In practice, the large selection of available methods of analysis may lead to discrepancies between different studies, even when these studies are examining the same underlying autonomic parameter. For example, because of the inverse relationship between heart rate and vagal functioning, higher vagal functioning would be expected in children with externaliz-

ing problems (23). In practice, some studies did indeed show an association between high vagal functioning and externalizing problems in children, as well as adults (24, 25). However, a number of other studies showed an inverse relationship between vagal functioning and externalising behaviour (26-28). No two of these studies used the exact same method of analysis. Both Pine et al. (1998) and Scarpa and Ollendick (2003) studied time domain measures. While Pine et al. used root mean squared differences in successive interbeat intervals, Scarpa and Ollendick employed just the mean difference in successive interbeat intervals. The other authors employed frequency domain measures using high-frequency variability as a measure for vagal functioning. Dietrich et al. (2007) used a bandwidth from 0.15 to 0.40 Hz. Similarly, both Mezzacappa et al. (1997) and Beauchaine (2001) defined their lower bound at 0.15 Hz, however these authors employed no upper boundary.
In summary, there are a large number of techniques available to analyse heart frequency data. Moreover, results can be further influenced by the length of the recording analysed and by the choice of parameters for the band boundaries. This is compounded by some uncertainty in the interpretation of the variables themselves. For example, the low and high frequency bands vary in relation to the autonomic control of the heart rate. The high frequency band has been linked to vagal activity and is associated with respiratory sinus arrhythmia, which represents vagal modulation of heart rate such that it increases during inspiration and decreases during expiration. However, the changes in the high frequency variation induced by the respiration rate and to a lesser extent by the breathing tidal volume are independent from actual changes in the underlying autonomic cardiac control. Because of this, some researchers have proposed
methods to correct for respiratory variability (29). Previously, the low frequency band was linked solely with sympathetic activity, more recently however, it is supposed that it reflects a mixture of both parasympathetic and vagal inputs. The physiological explanation of the very low frequency band is not clear, while the ultra low frequency band may be indicative of the circadian rhythm.

Selection effects
The initial response rate of the Generation R Study was 61% (30). Non-response was not random. The percentage of participating mothers with a lower socio-economic status was less than expected from the population figures in Rotterdam (31). The Focus Cohort in which this study was performed consists of a randomly selected subgroup of Dutch children and their parents. It is ethnically homogeneous, which has the advantage that it excludes potential confounding or effect modification by ethnicity at the same time this design limits the generalisability of the study to other ethnicities. Moreover, this selection further diminished the percentage of participants with a lower socio-economic status. In addition, as reported by Roza et al., there was selective loss to follow-up within the Focus cohort. Analysis of missing data showed continued attrition of lower educated, younger mothers with higher psychopathology scores, and of children with lower birth weights and shorter gestation (32). Finally, not all participants of the Generation R Focus cohort could be included in our studies, because we added the physiological measurements to our assessment protocol while the examination round was already ongoing. In summary, the participants of the studies in chapter 1 to 4 are not very representative of the general population in Rotterdam; they are characterized by more favourable socio-economic characteristics.
A recent Generation R study showed that, in line with a large body of literature, poor

socio-economic status and low maternal education were associated with more externalising problems at age 3 (33). This may have led to an underrepresentation of children with problem behaviour, our main outcome in chapters 4 and 5.This, in turn, may have diminished the power of these studies to find significant differences between children with and without externalising behaviour. The social push hypothesis as put forward by Mednick and Raine may offer some solace though. It states that where a child with externalising behaviour lacks social factors that "pushed" its development towards externalising behaviour, then biological factors may more likely explain any observed externalizing behaviour (13). In short, while there may be less children with externalising behaviour in our studies, those children who do display externalizing behaviour might be more likely to carry biological risk factors. The small group of children who lied during the gift delay task in study 4, may thus be of particular interest. Indeed these children had a lower resting heart rate than children who did not lie during the task.
However, if autonomic functioning and socio-economic factors interact in determining behavioural outcome, then non-participation based on environmental factors will lead to selection bias. This is because the relation between exposure and outcome will be different in those who participate and those who do not participate in the study. This would be the case, for example, if low heart rate leads to externalizing behaviour only in the presence of certain negative environmental factors; this is possible as we have suggested in chapter 4. Under these circumstances, non-participation related to socio-economic factors could lead to underestimations of the effect of heart rate on behaviour. However, other authors have posited the inverse, that low heart rate is actually protective in the presence of negative environmental factors (16). In that case selective non-participation as occurring in the Generation R Focus cohort will have lead to an overestimation of the total effect size.

Shared method variance
Shared method variance is variance due to the measurement method, and not due the underlying construct one is trying to measure. Shared method variance may be one of the most relevant contributors to systematic measurement error in behavioural research (34), although some authors consider it of lesser importance (35). Systematic measurement errors are particularly problematic because they can lead to biased results. Podsakoff et al. distinguish four categories of shared method biases. (1) Bias due to exposure and outcome variables being obtained from the same source. (2) Bias due to the measurement items. (3) Bias due to the context of the items within the measurement. (4) Bias due to the context in which the measures are obtained (36).
In the studies presented in chapters 4 and 5, mothers provided the information on one of the predictors and on the outcome of the study. This could lead to shared method variance. Mothers with psychiatric problems, may be more inclined to rate their children with similar, high levels of behavioural problems. Hence, relying solely on parental report may introduce significant biases. In chapter 4 we were mainly interested in the relation of autonomic functioning with toddler behaviour, and the interaction of autonomic functioning with maternal psychopathology in determining toddler behaviour. It is unlikely that a bias stemming from shared method variance can explain the interaction we demonstrated. When we explored the interaction, we showed that infant mean heart rate was associated with high toddler aggressive behaviour only in the group whose mothers had high maternal psychiatric symptoms. In contrast, there was no relationship between infant mean heart rate and toddler

aggressive behaviour in the stratum with low maternal psychiatric symptoms. This interaction effect cannot easily be explained by shared method variance. The level of maternal psychiatric symptoms was used to define to strata only, in which objectively measured heart rate was associated with child behaviour.
However, it is possible that shared method variance has contributed to the strong association between our contrasting variable aggressive parenting and child externalising behaviour reported in chapter 5. This is in line with findings from literature. For example, Lau et al. compared parental report to independent observation of child behaviour. They concluded that parents with a history of aggressive parenting, tended to over-report externalising behaviour in their children (37).

Adjustment for confounding
Even in large, well-defined studies, which rigorously control for socio-economic and other factors, it is virtually impossible to rule out residual confounding. This can occur when the variable used to adjust is an imperfect match for the underlying confounding variable, when a confounder remains unaccounted for in study design and analysis, or when there are unknown confounders.
On the other hand, blindly adding variables to the model may decrease precision and may even increase bias. Several definitions of overadjusment exist. Schisterman et al. define overadjustment as control for an intermediate variable, or a descending proxy thereof, on a causal path from exposure to outcome. Overadjustment defined this way typically causes a bias towards the null hypothesis. Unnecessary adjustment as opposed to overadjustment then, is control for a variable that does not increase or decrease bias of the causal relation between exposure and outcome but may affect its precision (38). In a special case of unnecessary adjustment, the variable being adjusted for is only related to the outcome by means of the exposure and could be considered as an instrumental variable. When the variable has a weak association with the outcome, independent of the exposure, it is a near-instrumental variable (39).
Szklo and Javier Nieto define overadjustment in a different way. In line with Schisterman et al. they posit that overadjustment occurs when controlling for a variable, on a causal path from exposure to outcome. They add however, that overadjusment can also occur when the variable being controlled for is strongly related to the exposure or to the outcome. Adding such a variable to the model leads to reduced precision. An example would be adjusting for education levels when income is the exposure of interest (40). When encountered in the context of a linear regression model, there would be collinearity present.
This problem was prevalent in chapter 3, where we investigated the relation between breastfeeding and infant autonomic functioning. Socio-economic and educational factors are important determinants of breastfeeding. Because of the high correlation between breast feeding and socio-economic factors, some collinearity was present as well as overadjustment as defined by Szklo et al. (40). Moreover, the association between socio-economic factors and breastfeeding was much stronger than the association between socio-economic factors and infant autonomic functioning, which means socio-economic factors may have functioned as a near-instrumental variable. Thus, we argued that controlling the relation between breastfeeding and autonomic functioning for these factors can lead to a loss of precision. This is especially problematic when investigating causality and led us to employ a contrasting variable. Recent statistical modeling however, seems to caution against exclud-

ing near-instrumental or even probable instrumental variables as confounders. Simulations showed that increases in error due to the inclusion of such variables were relatively small and must be weighed against introduction of possible bias due to confounding when they were excluded (39).

Inferring causality in epidemiological research

The process of causal inference is complex and partially subjective. Bradford Hill put forward his famous criteria for inferring causality in 1965. Initially these criteria were seen as set of minimal conditions necessary to prove a causal relationship. More recently, this view has become more nuanced. For instance, according to Rothman, the only criterion that is truly necessary is 'temporality' (41). The cause has to precede the effect. However, even this may be difficult to ascertain. In epidemiology, a randomised controlled trial is seen as the golden standard. However, this is not always possible, and epidemiologists have developed several alternatives. In practice, literature recommends several approaches to study causal inferences.

One approach is to utilise an instrumental variable, which is related to the exposure variable, in this case breastfeeding, but not to confounding factors or to the outcome, in this case infant autonomic functioning. Such a variable can be genetic, as in mendelian randomisation, or environmental. Mendelian randomisation can be seen as a specific form of the instrumental variable approach. Mendelian randomisation uses common genetic polymorphisms that are known to influence exposure patterns (such as propensity to drink alcohol) or have effects equivalent to those produced by modifiable exposures (such as raised blood cholesterol concentration) ((42, 43).

Another approach is to compare the effect of the exposure with the effect of a contrasting variable. A contrasting variable should be an indicator of similar parameters as the exposure under study, but lack the exposure's proposed causal properties. Thus, if there is indeed a casual relation, the effect of the exposure under study should be larger. This approach has been used in the study of intra-uterine exposure, for example, where it is possible, to compare the associations of prenatal maternal smoking and prenatal paternal smoking with infant outcome (44).

In chapter 3, we studied breastfeeding. The proven benefits of breastfeeding make randomly withholding breastfeeding from infants unethical. Some research has employed the randomised assignment of mothers to a breastfeeding promotion program (45). Because of the limited influence of breastfeeding promotion programs on breastfeeding practice in a general population setting in the West, the number of participants needed to successfully detect differences in autonomic functioning made this approach unfeasible for our purposes (46).

We decided to compare the effect of breastfeeding with the effect of a contrasting variable. A contrasting variable should be an indicator of similar feeding or health related parameters, but lack the exposure's proposed causal properties. Thus, if there is indeed a causal relation, the effect of the exposure should be larger. Our data showed that infants who eat more fruitpurée have lower sympathetic modulation and this association was of similar strength as the association of breastfeeding and sympathetic modulation. This led us to doubt whether the relation between breastfeeding and infant autonomic functioning is causal.

In chapter 2 we investigated the relation between maternal psychopathology and infant heart rate. We focused mainly on the temporal aspects of the association. We had multiple

measurements at our disposal, including prenatal maternal psychopathology rates, which allowed us to rule out mechanisms such as reverse causality. However, even with such a data set we were not able to definitively pin down causality in observed associations.

Clinical implications

The main clinical implications of this thesis are threefold.

One, we showed that infant autonomic functioning was associated with maternal psychopathology in the postnatal period. In older children autonomic nervous system dysfunction is a marker for both internalizing and externalizing problems. Some researchers have even suggested that autonomic nervous system dysfunctioning is a causative factor in the development of these problems. Infants of mothers with psychiatric problems display this vulnerability early in life. While the exact causal pathway behind this association remains unclear, it remains likely that the influence of maternal psychopathology on the infant is important. In addition to a large body of literature concerning the impact of maternal psychopathology on attachment and infant temperament further underscores the importance of early detection and treatment of maternal psychopathology.

Two, the association between infant ANS functioning with toddler externalising behaviour, was moderated by maternal psychiatric symptoms. Within the high-risk group of infants whose mothers have psychiatric symptoms or hostility, children with low autonomic arousal are at a particularly high risk of developing externalising behaviour.

Three, low heart at 14 months was associated with low levels of anxiety and a propensity towards lying at age 3 years. These might be early signs of impaired affective control and callous-unemotionallity. This is particularly worrying because callousness is indicative of stable and severe externalising behaviour (47).

Future research

This thesis joins an growing number of publications which highlight the interplay of biological and environmental factors. Heart rate is both partially dependent on environmental factors and may interact with environmental factors in determining behavioural outcome. Most studies about the autonomic nervous system and heart rate, however, do not address this interplay with environmental factors. This may be because such a research design calls for large population sizes. Subsequent studies of the autonomic nervous system and its impact on behaviour and psychopathology should focus on the interaction with environmental factors such as socio-economic status, parenting, early adversity and life events. In addition, more research should be aimed at the developmental aspects of the relation between the autonomic nervous system and behaviour. Of particular interest is whether heart rate in young infants is indeed indicative of externalizing behaviour at a much later age.

In summary we would like to recommend larger prospective studies, spanning longer time periods and encompassing a great number of measurement points. However, the costs of such undertakings have to be weighed against the benefits. Even within an interactional framework, or by focusing on high-risk groups, it is unlikely that heart rate will be a sufficiently sensitive or specific marker of problem behaviour to be employed for diagnosis or prediction in clinical practice. Instead, the future utility of heart rate research may lie in further elucidating the etiological pathways to externalizing behaviour.

References

1. Feldman R, Eidelman AI. Skin-to-skin contact (Kangaroo Care) accelerates autonomic and neurobehavioural maturation in preterm infants. Dev Med Child Neurol. 2003 Apr;45(4):274-81.
2. Barker DJ. Fetal and infant origins of adult disease. London: BMJ Books; 1992.
3. Monk C, Fifer WP, Myers MM, Sloan RP, Trien L, Hurtado A. Maternal stress responses and anxiety during pregnancy: effects on fetal heart rate. Dev Psychobiol. 2000 Jan;36(1):67-77.
4. Monk C, Myers MM, Sloan RP, Ellman LM, Fifer WP. Effects of women's stress-elicited physiological activity and chronic anxiety on fetal heart rate. J Dev Behav Pediatr. 2003 Feb;24(1):32-8.
5. Monk C, Sloan RP, Myers MM, Ellman L, Werner E, Jeon J, et al. Fetal heart rate reactivity differs by women's psychiatric status: an early marker for developmental risk? Journal of the American Academy of Child and Adolescent Psychiatry. 2004 Mar;43(3):283-90.
6. Yehuda R, Engel SM, Brand SR, Seckl J, Marcus SM, Berkowitz GS. Transgenerational effects of posttraumatic stress disorder in babies of mothers exposed to the World Trade Center attacks during pregnancy. J Clin Endocrinol Metab. 2005 Jul;90(7):4115-8.
7. Riese H, Rosmalen JG, Ormel J, Van Roon AM, Oldehinkel AJ, Rijsdijk FV. The genetic relationship between neuroticism and autonomic function in female twins. Psychol Med. 2007 Feb;37(2):257-67.
8. Thayer JF, Merritt MM, Sollers JJ, 3rd, Zonderman AB, Evans MK, Yie S, et al. Effect of angiotensin-converting enzyme insertion/deletion polymorphism DD genotype on high-frequency heart rate variability in African Americans. Am J Cardiol. 2003 Dec 15;92(12):1487-90.
9. Olsson M, Annerbrink K, Westberg L, Melke J, Baghaei F, Rosmond R, et al. Angiotensin-related genes in patients with panic disorder. Am J Med Genet B Neuropsychiatr Genet. 2004 May 15;127(1):81-4.
10. Kagan J, Reznick JS, Snidman N. The physiology and psychology of behavioral inhibition in children. Child development. 1987 Dec;58(6):1459-73.
11. Monk C, Kovelenko P, Ellman LM, Sloan RP, Bagiella E, Gorman JM, et al. Enhanced stress reactivity in paediatric anxiety disorders: implications for future cardiovascular health. Int J Neuropsychopharmacol. 2001 Jun;4(2):199-206.
12. Raine A, Venables PH, Mednick SA. Low resting heart rate at age 3 years predisposes to aggression at age 11 years: evidence from the Mauritius Child Health Project. Journal of the American Academy of Child and Adolescent Psychiatry. 1997 Oct;36(10):1457-64.
13. Raine A. Biosocial studies of antisocial and violent behavior in children and adults: a review. J Abnorm Child Psychol. 2002 Aug;30(4):311-26.
14. Dunn J, Munn P. Sibling quarrels and maternal intervention: individual differences in understanding and aggression. Journal of child psychology and psychiatry, and allied disciplines. 1986 Sep;27(5):583-95.
15. Hay DF, Castle J, Davies L. Toddlers' use of force against familiar peers: a precursor of serious aggression? Child development. 2000 Mar-Apr;71(2):457-67.
16. Oldehinkel AJ, Verhulst FC, Ormel J. Low heart rate: a marker of stress resilience. The TRAILS study. Biological psychiatry. 2008 Jun 15;63(12):1141-6.
17. Ortiz J, Raine A. Heart rate level and antisocial behavior in children and adolescents: a meta-analysis. Journal of the American Academy of Child and Adolescent Psychiatry. 2004 Feb;43(2):154-62.
18. Dodge KA, Lochman JE, Harnish JD, Bates JE, Pettit GS. Reactive and proactive aggression in school children and psychiatrically impaired chronically assaultive youth. J Abnorm Psychol. 1997 Feb;106(1):37-51.
19. Blair C, Razza RP. Relating effortful control, executive function, and false belief understanding to emerging math and literacy ability in kindergarten. Child development. 2007 Mar-Apr;78(2):647-63.
20. Talwar V, Gordon HM, Lee K. Lying in the elementary school years: verbal deception and its relation to second-order belief understanding. Dev Psychol. 2007 May;43(3):804-10.
21. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation. 1996 Mar 1;93(5):1043-65.
22. Bar-Haim Y, Marshall PJ, Fox NA. Developmental changes in heart period and high-frequency heart period variability from 4 months to 4 years of age. Dev Psychobiol. 2000 Jul;37(1):44-56.
23. Scarpa A, Raine A, Venables PH, Mednick SA. Heart rate and skin conductance in behaviorally inhibited Mauritian children. J Abnorm Psychol. 1997 May;106(2):182-90.
24. Scarpa A, Ollendick TH. Community violence exposure in a young adult sample: III. Psychophysiology and victimization interact to affect risk for aggression. . Journal of Community Psychology. 2003;31:321-38.
25. Dietrich A, Riese H, Sondeijker FE, Greaves-Lord K, van Roon AM, Ormel J, et al. Externalizing and internalizing problems in relation to autonomic function: a population-based study in preadolescents. Journal of the American Academy of Child and Adolescent Psychiatry. 2007 Mar;46(3):378-86.
26. Beauchaine TP, Katkin ES, Strassberg Z, Snarr J. Disinhibitory psychopathology in male adolescents: discriminating conduct disorder from attention-deficit/hyperactivity disorder through concurrent assessment of multiple autonomic states. J Abnorm Psychol. 2001 Nov;110(4):610-24.
27. Pine DS, Wasserman GA, Miller L, Coplan JD, Bagiella E, Kovelenku P, et al. Heart period variability and psychopathology in urban boys at risk for delinquency. Psychophysiology. 1998 Sep;35(5):521-9.
28. Mezzacappa E, Tremblay RE, Kindlon D, Saul JP, Arseneault L, Seguin J, et al. Anxiety, antisocial behavior, and heart rate regulation in adolescent males. Journal of child psychology and psychiatry, and allied disciplines. 1997 May;38(4):457-69.
29. Ritz T, Dahme B. Implementation and interpretation of respiratory sinus arrhythmia measures in psychosomatic medicine: practice against better evidence? Psychosomatic medicine. 2006 Jul-Aug;68(4):617-27.
30. Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update until the age of 4 years. Eur J Epidemiol. 2008;23(12):801-11.

31. van Lith H. Demografische gegevens Rotterdam. Rotterdam2004.
32. Roza SJ, van Lier PA, Jaddoe VW, Steegers EA, Moll HA, Mackenbach JP, et al. Intrauterine growth and infant temperamental difficulties: the Generation R Study. Journal of the American Academy of Child and Adolescent Psychiatry. 2008 Mar;47(3):264-72.
33. Rijlaarsdam J, Tiemeier H, Hofman A, Jaddoe VW, Mackenbach JP, Verhulst FC, et al. Home environments of infants: relations with child development through age 3. J Epidemiol Community Health. 2013 Jan;67(1):14-20.
34. Bagozzi RP, Yi Y. Multitrait-multimethod matrices in consumer research. . Journal of Consumer Research. 1991(17):426-39.
35. Spector PE. Method variance in organizational research: truth or urban legend? Organizational Research Methods. 2006;9(2):221-32.
36. Podsakoff PM, MacKenzie SB, Lee JY, Podsakoff NP. Common method biases in behavioral research: a critical review of the literature and recommended remedies. J Appl Psychol. 2003 Oct;88(5):879-903.
37. Lau AS, Valeri SM, McCarty CA, Weisz JR. Abusive parents' reports of child behavior problems: relationship to observed parent-child interactions. Child Abuse Negl. 2006 Jun;30(6):639-55.
38. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology. 2009 Jul;20(4):488-95.
39. Myers JA, Rassen JA, Gagne JJ, Huybrechts KF, Schneeweiss S, Rothman KJ, et al. Effects of adjusting for instrumental variables on bias and precision of effect estimates. Am J Epidemiol. 2011 Dec 1;174(11):1213-22.
40. Szklo C, Javier Nieto F. Epidemiology. Beyond the basics. Sudbury: Jones and Bartlett Publishers; 2007.
41. Rothman KJ. Epidemiology: An Introduction. USA: Oxford University Press; 2002.
42. Smith DJ. Insulin resistance and depression: difference in patient groups could have been explained by other factors. BMJ. 2005 Apr 23;330(7497):965; author reply -6.
43. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol. 2003 Feb;32(1):1-22.
44. Roza SJ, Verhulst FC, Jaddoe VW, Steegers EA, Mackenbach JP, Hofman A, et al. Maternal smoking during pregnancy and child behaviour problems: the Generation R Study. Int J Epidemiol. 2009 Jun;38(3):680-9.
45. Kramer MS, Aboud F, Mironova E, Vanilovich I, Platt RW, Matush L, et al. Breastfeeding and child cognitive development: new evidence from a large randomized trial. Arch Gen Psychiatry. 2008 May;65(5):578-84.
46. Chung M, Raman G, Trikalinos T, Lau J, Ip S. Interventions in primary care to promote breastfeeding: an evidence review for the U.S. Preventive Services Task Force. Ann Intern Med. 2008 Oct 21;149(8):565-82.
47. Frick PJ, White SF. Research review: the importance of callous-unemotional traits for developmental models of aggressive and antisocial behavior. Journal of child psychology and psychiatry, and allied disciplines. 2008 Apr;49(4):359-75.

Chapter 8

Summary/samenvatting

Dankwoord

Phd Portfolio

Summary
The autonomic nervous system regulates the body's internal functions. The goal of this regulation is to maintain bodily homeostasis in a changing external environment. The autonomic nervous system acts largely independent of volition and controls heart rate, respiratory rate, digestion, and perspiration. It is divided into two partially antagonistic systems: the sympathetic nervous system and the parasympathetic or vagal nervous system. In general, the vagal system primarily regulates "rest and digest" functions. In contrast, the sympathetic nervous system can elicit the "fight or flight" response with increased arousal and energy generation in response to stress or threat. Together with the hypothalamic-pituitary adrenal (HPA) axis, the autonomic nervous system mediates the body's response to stress. Because of this central role in stress response and the close relation of stress with the onset and recurrence of psychiatric disorders, both systems are potential biomarkers for psychiatric disorders. The current thesis investigates possible determinants of stress regulation, as well as the associations of stress regulation with emotional and behavioural symptoms very young age. The majority of the studies in this thesis were performed within the Generation R study. The Generation R Study is a large population-based prospective cohort study from fetal life onwards. It was designed to identify early environmental as well as genetic causes of abnormal growth, development and health from fetal life onwards. In total, 9,778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study. The studies presented here concern children who participated in the Focus Cohort. The Focus Cohort consists of a randomly selected subgroup of Dutch children of Caucasian origin and their parents. Studies conducted in the Focus Cohort were able to utilise more in-depth assessments. The research discussed in chapter 6 was performed within the "Beren van de Weg" study. This is a clinical, outpatient study, conducted by the departments of Child and Adolescent Psychiatry of either the Erasmus Medical Center in Rotterdam and Leiden University Medical Center— Curium. All consecutive referrals with a primary diagnosis of Generalized Anxiety Disorder, Separation Anxiety Disorder, Social Phobia or Specific Phobia were eligible for inclusion .
In chapter 2 we examined the potential impact of maternal psychopathology on infant heart rate and heart rate variability. We showed that a maternal history of psychopathology before childbirth was associated with increased heart rate and lower vagal modulation in the infant. Similarly, postnatal maternal symptoms, especially anxiety and depression symptoms, were related to increased heart rate in the infant. Both genetic and environmental mechanisms, which are certainly not mutually exclusive, could underlie the association between maternal psychopathology and infant heart rate.
In chapter 3 we assessed the putative beneficial effect of breastfeeding on child autonomic functioning. To address the issue of residual confounding, we compared the effect of breastfeeding with that of a contrasting variable, infant fruitpurée consumption. It is related to similar environmental epiphenomena as breastfeeding. We found that infants, who were exclusively breastfed at two months of age, had lower sympathetic modulation than infants not breastfed. However, our data showed that, similar to breastfeeding, infants who ate more fruitpurée had lower sympathetic modulation as well. Association does not imply causation. In fact, in both instances the causal pathways linking the risk factors to autonomic functioning remain unclear. Moreover, in the case of breast feeding, a limited effect size with a similar order of magnitude as the effect of another, seemingly trivial dietary component, calls into question the clinical relevance of the association,

even if it contains a causal component.
In chapter 4 we addressed the relation between infant autonomic functioning at 14 months and behaviour at age 18 months. We found that the association between autonomic functioning and infant externalising behaviour, was moderated by maternal psychiatric symptoms. We observed that low heart rate was associated with aggressive behaviour, only in a subgroup of children whose mothers had high psychiatric symptoms. This suggests that in the presence of maternal risk factors, low autonomic arousal renders children particularly susceptible to externalising behaviour. This lends support to the fearlessness theory, which posits that low autonomic arousal in children is an indicator of fearlessness. We hypothesize that in the presence of maternal psychopathology, with less adequate parenting and maternal guidance in coping with fearless behaviour, these children actually develop aggressive behaviour.
In chapter 5 we examined the association between a child's heart rate at 14 months and behavior at 3 years. Low heart rate was specifically and strongly associated with the odds of the child lying during the gift delay task as well as with low levels of anxiety. We suggest that low anxiety and a tendency to lie are indicative of low levels of emotional reactivity. Low heart rate may thus delineate a small group of children that are composed and calculating, rather than overly emotionally reactive and overtly aggressive. However, we could not demonstrate an association between heart rate and parent rated aggressive behaviour. This could be because the children in our study were considerably younger than those in earlier studies. In young children aggressive behaviour is common, but usually time limited. Proactive, planned aggression, which is commonly accompanied by low levels of emotional reactivity, develops later in life than reactive aggression.
Chapter 6 details the course of an anxiety disorder during treatment and the concomitant changes in cortisol levels in a clinical sample of 116 children and adolescents. When we compared cortisol levels at baseline and one-year follow-up, persistence of the anxiety disorder was associated with both increased daytime cortisol production and a trend towards a decreased cortisol morning rise. Persistence of an anxiety disorder may change HPA-axis functioning, underscoring the importance adequate treatment of anxiety disorders.
Chapter 7 highlights the main results of the previous chapters and places them in a broader context. The main body of the chapter details the different methodological issues encountered during this research. Clinicial implications and recommendations for future research are discussed as well.

Samenvatting
Het autonome zenuwstelsel regelt de interne functies van het lichaam . Het beoogt de lichamelijke homeostase te behouden in een veranderende externe omgeving . Het autonome zenuwstelsel handelt grotendeels onafhankelijk van de wil en controleert hartslag, ademhaling, spijsvertering en transpiratie. Het autonome zenuwstelsel is verdeeld in twee gedeeltelijk antagonistische systemen : het sympathische zenuwstelsel en het parasympatische of vagale zenuwstelsel . Het vagale systeem regelt voornamelijk de "rust en verteren" functies. In tegenstelling daarmee, kan het sympathische zenuwstelsel de "vecht of vlucht" reactie met een verhoogde opwinding en energie-opwekking in reactie op stress of dreiging initiëren. Samen met de hypothalamus - hypofyse- bijnier -as , medieert het autonome zenuwstelsel de reactie van het lichaam op stress . Vanwege deze centrale rol in stress-respons en de nauwe relatie van stress met het ontstaan en de herhaling van psychiatrische stoornissen ,zijn beide systemen potentiële biomarkers voor psychiatrische stoornissen.
Het huidige proefschrift onderzoekt mogelijke determinanten van deze stress response systemen regelgeving, evenals de associatie van deze systemen met gedrags-en emotionele symptomen op zeer jonge leeftijd. Het merendeel van de studies in dit proefschrift werden uitgevoerd binnen de Generation R studie. De Generation R Study is een grote populatie-gebaseerde prospectieve cohortstudie, vanaf het foetaal leven en verder. In totaal werden 9778 moeders met een leverdatum van april 2002 tot januari 2006 deelnamen aan de studie . De hier gepresenteerde studies betreffen kinderen die deelnamen aan het Focus Cohort. Het Focus Cohort bestaat uit een willekeurig gekozen subgroep van Nederlandse kinderen van Kaukasische afkomst en hun ouders. Studies uitgevoerd in het Focus Cohort konden meer diepgaande evaluaties gebruiken. Het onderzoek beschreven in hoofdstuk 6 werd uitgevoerd binnen de " Beren van de Weg " studie. Dit is een klinisch studie, uitgevoerd door de afdeling Kinder-en Jeugdpsychiatrie van ofwel het Erasmus Medisch Centrum in Rotterdam en het Leids Universitair Medisch Centrum - Curium. Alle opeenvolgende verwijzingen met een primaire diagnose van gegeneraliseerde angststoornis, separatie angststoornis, sociale fobie of specifieke fobie kwamen in aanmerking voor inclusie.
In hoofdstuk 2 onderzochten we de mogelijke invloed van maternale psychopathologie op de hartslag en hartslagvariabiliteit in het zeer jonge kind. We hebben laten zien dat een maternale geschiedenis van psychopathologie geassocieerd was met een verhoogde hartslag en lagere vagale modulatie bij de zuigeling. Evenzo, waren maternale postnatale symptomen, vooral angst en depressie symptomen, gerelateerd aan een verhoogde hartslag bij het kind . Zowel genetische als omgevingsfactoren mechanismen, welke niet noodzakelijk mutueel exclusief dienen te zijn, zouden aan de associatie tussen maternale psychopathologie en hartslag van het kind ten grondslag kunnen liggen.
In hoofdstuk 3 onderzochten we het vermeende gunstige effect van borstvoeding op het functioneren van het autonome zenuwstelsel bij het kind. Om het probleem van de residuele confounding te pakken, vergeleken we het effect van borstvoeding met die van een contrasterende variabele, consumptie van fruithapjes door het kind. Deze variable is gerelateerd aan vergelijkbare omgevingsfactoren als borstvoeding. We vonden dat de kinderen, die uitsluitend borstvoeding kregen op twee maanden oud ,op 14 maanden een lagere sympathische modulatie hadden dan kinderen die geen borstvoeding kregen. Echter bleek dat , vergelijkbaar met borstvoeding, kinderen die meer fruithapjes aten ook lagere sympathische modulatie vertoonden. Vereniging impliceert geen veroorzaken. In feite, in beide gevallen blijven de causale paden die een eventueel verband tussen de voedingsfactoren en

autonoom functioneren verklaren onduidelijk. Bovendien, in het geval van borstvoeding, is er sprake van een beperkte effect grootte, vergelijkbaar met het effect van een schijnbaar triviale dieetcomponent. Dit zet vraagtekens bij de klinische relevantie van de gevonden associatie, zelfs als er een onderliggende causale component is.
In hoofdstuk 4 bestudeerden we de relatie tussen kind autonome werking op 14 maanden en het gedrag op de leeftijd van 18 maanden. We vonden dat de associatie tussen autonome werking en externaliserend gedrag van het kind, werd gemodereerd door de psychiatrische symptomen van moeder. We zagen dat lage hartslag alleen was geassocieerd met agressief gedrag in een subgroep van kinderen van wie de moeder hoge psychiatrische symptomen had. Dit suggereert dat in de aanwezigheid van maternale risicofactoren autonome arousal kinderen bijzonder gevoelig maakt voor externaliserend gedrag. Dit spoort met de fearlessness theorie die stelt dat autonome opwinding bij kinderen een indicator van is onbevreesdheid . Onze hypothese is dat in aanwezigheid van maternale psychopathologie, met mogelijk minder adequate opvoeding, kinderen minder leren omgaan met hun eigen onbevreesheid en vervolgens externaliserend gedrag ontwikkelen.
In hoofdstuk 5 keken we naar het verband tussen hartslag van een kind op 14 maanden en gedrag op 3 jaar . Lage hartslag was sterk geassocieerd met de kansen van het kind liegt tijdens de gift-delay taak, en met lage niveaus van angst. Wij stellen dat angst en een neiging te liegen indicatief zijn voor lage niveaus van emotionele reactiviteit . Lage hartslag kan dus een kleine groep kinderen omlijnen die berekend zijn, in plaats van emotioneel reactief en openlijk agressief. We konden echter geen verband tussen hartslag en agressief gedrag aantonen . Dit kan zijn omdat de kinderen in onze studie aanzienlijk jonger waren dan die in eerdere studies . Bij jonge kinderen komt agressief gedrag vaak voor, maar is meestal in de tijd beperkt. Proactieve, geplande agressie, die gewoonlijk gepaard gaat met een laag niveau van emotionele reactiviteit, ontwikkelt later in het leven dan reactieve agressie.
Hoofdstuk 6 beschrijft de loop van een angststoornis tijdens de behandeling en de daarmee gepaard gaande veranderingen in cortisol niveaus in een klinische steekproef van 116 kinderen en adolescenten. We vergeleken cortisol spiegels bij aanvang voor behandeling en na een jaar follow-up, persistentie van de angststoornis was geassocieerd met zowel verhoogde cortisol productie overdag, als met een trend naar een lagere cortisol ochtend piek. Voortbestaan van een angststoornis kan dus mogelijk HPA - as functioneren veranderen , dit benadrukt het belang van een adequate behandeling van angststoornissen .
In hoofdstuk 7 worden de belangrijkste resultaten van de voorgaande hoofdstukken overlopen en in een bredere context geplaatst. Daarnaast wordt uitgebreid stilgestaan bij de verschillende methodologische kwesties die tijdens dit onderzoek aan bod kwamen. Tevens worden de klinische implicaties en adviezen voor toekomstig onderzoek beschreven.

Dankwoord
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PhD Portfolio Summary
Summary of PhD training and teaching activities

1. PhD training	Year	ECTS
Research Skills		
- Course Clinical Psychiatric Research, BKOP, Corsendonck	2010	3
- Modern Statistical Methods, NIHES, Rotterdam	2011	4
- Basic Course Clinical Research, BROK, Rotterdam	2012	2
Conferences attended		
- 33th congress of the Dutch Association of Psychiatry. Den Haag	2005	0.3
- 34th congress of the Dutch Association of Psychiatry. Groningen	2006	0.3
- 17th IACAPAP, Melbourne, Australia	2006	0.5
- 35th congress of the Dutch Association of Psychiatry. Maastricht	2007	0.3
- 6th World Congress on Stress. Vienna, Austria	2007	0.5
- 20th World Congress Psychiatric Genetics, Hamburg, Germany	2013	0.5
- 12th World Congress of Biological Psychiatry, Kyoto, Japan	2013	0.5
- 21st IACAP, Durban, South Africa	2014	0.5
- 17th World Congress of Psychophysiology, Hiroshima, Japan	2014	0.5
National Presentations		
- 33th congress of the Dutch Association of Psychiatry. Den Haag	2005	0.08
- 34th congress of the Dutch Association of Psychiatry. Groningen	2006	0.08
- 35th congress of the Dutch Association of Psychiatry. Maastricht	2007	0.08
International presentations		
- 17th IACAPAP, Melbourne, Australia	2006	0.16
- 6th World Congress on Stress. Vienna, Austria	2007	0.08
- 17th World Congress of Psychophysiology, Hiroshima, Japan	2014	0.08

2. Teaching activities	Year	ECTS
Lecturing		
- Course, medical students about observation skills and childhood psychiatric disorders. Erasmus Medical University, Rotterdam	2005-2014	0.5
- Course, medical students about how to interact with patients on the topic of alternative treatment methods. Erasmus Medical University, Rotterdam	2013-2014	0.2
Supervising master theses		
- Alexandra Bijdevaate, Master Psychology, Leiden University		2
Title: De hartslagfrequentie voor, tijdens en na het uitvoeren van een stresstaak bij kinderen met een angststoornis en bij hun ouders.		
- Cigdem Tunca, Master Psychology, Erasmus University, Rotterdam		2
Title: De relatie tussen hartslagniveau tijdens stress en externaliserend gedrag bij kinderen.		
- Emily van Kampen, Master Psychology, Leiden University		2
Title: Symptoms of anxiety and depression in children with a somatoform disorder.		

	ECTS
- Hans Neyndorff, Master Medicine, Erasmus University, Rotterdam	2
Title: Stressing out. Functioning of the autonomic nervous system and hypothalamic-pituitary-adrenal-axis in children a year after CBT treatment for anxiety disorders.	
- Jolanda Zijderlaan, Master Psychology, University of Utrecht	2
Title: Anxious and Depressive Symptomatology in Children and Adolescents with Somatoform Disorders (DSM-IV); A Comparison with Anxious, Depressive, and Matched Controls.	2
- Lizann Tjon, Master Psychology, Leiden University	2
Title: Relation between ANS functioning of child and parents with a history of anxiety disorder.	
- Natasha Hogenkamp, Master Psychology	2
Title: De Invloed van Opvoedingsstijl op de Stressreactie van Kinderen.	
- Nesibe Peker, Master Medicine, Erasmus University, Rotterdam	2
Title: Cognitieve gedragstherapie bij kinderen met een angststoornis: psychologie en fysiologie.	
- Ruth van Holst, Master Psychology	2
Title: Changes in autonomic reactions of children with anxiety disorders after CBT.	
- Sander Bodmer, Master Medicine, Erasmus University, Rotterdam	2
Title: The Influence of Parental Rearing Style on HPA mediated Cortisol Response in Children Performing a Stress Task.	
- Sarah Prins, Master Psychology, Leiden University	2
Title: HPA-axis function in antisocial children; do they experience stress?	
- Saskia Nyst, Master Psychology, University of Amsterdam	2
Title: HPA-axis function in antisocial children; do they experience stress?	
- Suzan Kaya, Master Psychology, Erasmus University, Rotterdam	2
Title: De relatie tussen cardiovasculaire parameters en angst- en depressieve klachten bij kinderen uit de algemene bevolking.	

3. Publications not listed in thesis

- CBT for childhood anxiety disorders: differential changes in selective attention between treatment responders and non-responders.
Legerstee J.S., Tulen J.H., Dierckx B., Treffers P.D., Verhulst F.C., Utens E.M.
J Child Psychol Psychiatry. 2010 Feb;51(2):162-72.
- The efficacy of electroconvulsive therapy in bipolar versus unipolar major depression: a meta-analysis.
Dierckx B., Heijnen W.T., van den Broek W.W., Birkenhager T.T.
Bipolar Disorders. 2012 Mar;14(2):146-50.
- Attachment disorganization moderates the effect of maternal postnatal depressive symptoms on infant autonomic functioning.
Tharner A., Dierckx B., Luijk M.P., van Ijzendoorn M.H., Bakermans-Kranenburg M.J., van Ginkel J.R., Moll H.A., Jaddoe V.W., Hofman A., Hudziak J.J., Verhulst F.C., Tiemeier H.
Psychophysiology. 2013 Feb;50(2):195-203.
- Psychofarmaca in de KJP.
Editors: Dieleman G.C., Dierckx B., Hofstra M.
Uitgeverij van Gorcum. 2011