Stellingen behorende bij het proefschrift

Stressed Out!

Stress physiology in anxious children

1. Low autonomic arousal is a marker for the development of externalizing problems during childhood, while high autonomic arousal is a marker of early and persistent internalizing problems (this thesis).
2. A child with a clinical anxiety disorder experiences persistent stress, as indexed by a basal hypoactivation of the hypothalamic-pituitary-adrenal axis, elevated sympathetic, and lowered parasympathetic autonomic nervous system activity (this thesis).
3. The specific psychophysiological profile that is associated with clinical specific phobia provides evidence that it is a valid taxonomic construct (this thesis).
4. Increase in basal hypothalamic-pituitary-adrenal axis functioning is associated with successful standardized stepped-care cognitive behavioral therapy treatment of children with an anxiety disorder (this thesis).
5. In children with a clinical anxiety disorder a higher pretreatment sympathetic reactivity in response to a stressor is associated with less improvement in anxiety symptoms one year later (this thesis).
6. It is increasingly clear that mind-body dualism is at best an oversimplified way of conceptualizing human illness and at worst the source of serious practical problems that adversely affect patient care (Sharpe & Walker,2010).
7. The decentralization and simultaneous transformation of youth care in the Netherlands cannot be realized successfully as a sharp budget cut in youth care was introduced.

8. The demographic development in the age structure of residents (i.e., fewer children and more elderly) of the Netherlands demands extra investment in education, physical and mental health(care) of children and young people in the Netherlands to bear the societal costs of a large group of elderly residents.
9. Current service configuration of distinct child and adolescent mental health and adult mental health services is considered the weakest link where the care pathway should be most robust (Singh, S. e.a. (2013). *Seventh Framework Programme: "THE MILESTONE PROJECT"*).
10. The increased assessment of metrics and key performance indicators to compare care and research institutions has unintended negative consequences on quality of care and research.
11. "A person's a person, no matter how small." (Dr. Seuss, author).

Stressed Out!

Stress physiology in anxious children

Gwen Dieleman

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Stress physiology in anxious children

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Stress fysiologie in angstige kinderen

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Chapter 1

General introduction

INTRODUCTION

Anxiety is a basic human emotion with expressions falling on a continuum from mild to severe (Pine et al., 2009). The studies presented in this thesis extend the knowledge on the role of stress physiology as a cause, correlate, and predictor of pediatric anxiety.

Of fraidy-cats

Anxiety is not typically pathologic but commonly adaptive when it facilitates anticipation of threat or danger. The organisms' responses to danger and the underlying brain circuitry engaged by threats reflect these adaptive aspects of anxiety (Pine et al., 2009). Pure anxiety problems have a low prevalence at toddler age, but become more prevalent during later childhood (Gilliom, Shaw, 2004; Basten et al., 2016). To some extent, many fears and anxieties in pre-school aged children are age-appropriate and in keeping with normal development (Egger, Angold, 2006). This has made it difficult to discern age-appropriate behavior, reflecting normal development, from persistent anxiety problems and underlines the need to identify early risk factors for deviant developmental pathways.

Maladaptive and pathologic anxiety is characterized by persisting or extensive degrees of anxiety and avoidance associated with subjective distress or impairment (American Psychiatric Association, 2000).

It can be hypothesized that children with an anxiety disorder function under conditions of persistent stress, with an excessive and prolonged stress system activation. Variations in the activity of the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS), two major physiological stress systems, have been implicated as possible biological markers of pathological anxiety in children (Feder et al., 2004; Dietrich et al., 2007). Normally, activation of these stress systems leads to behavioral and physical adaptive changes that improve the ability to survive. However, children with an anxiety disorder may perceive the world as full of stressors that demand endless vigilance and coping,

with no possibility to relax and to regard their living environment as safe (Sapolsky, 2002). The developing stress systems of children and adolescents may be especially vulnerable to stress-induced changes. For instance, permanent HPA-axis dysfunctioning in early life has repeatedly been linked to chronicity and recurrence of affective disorders and affective symptoms (Flory et al., 2009; Nicolson et al., 2010).

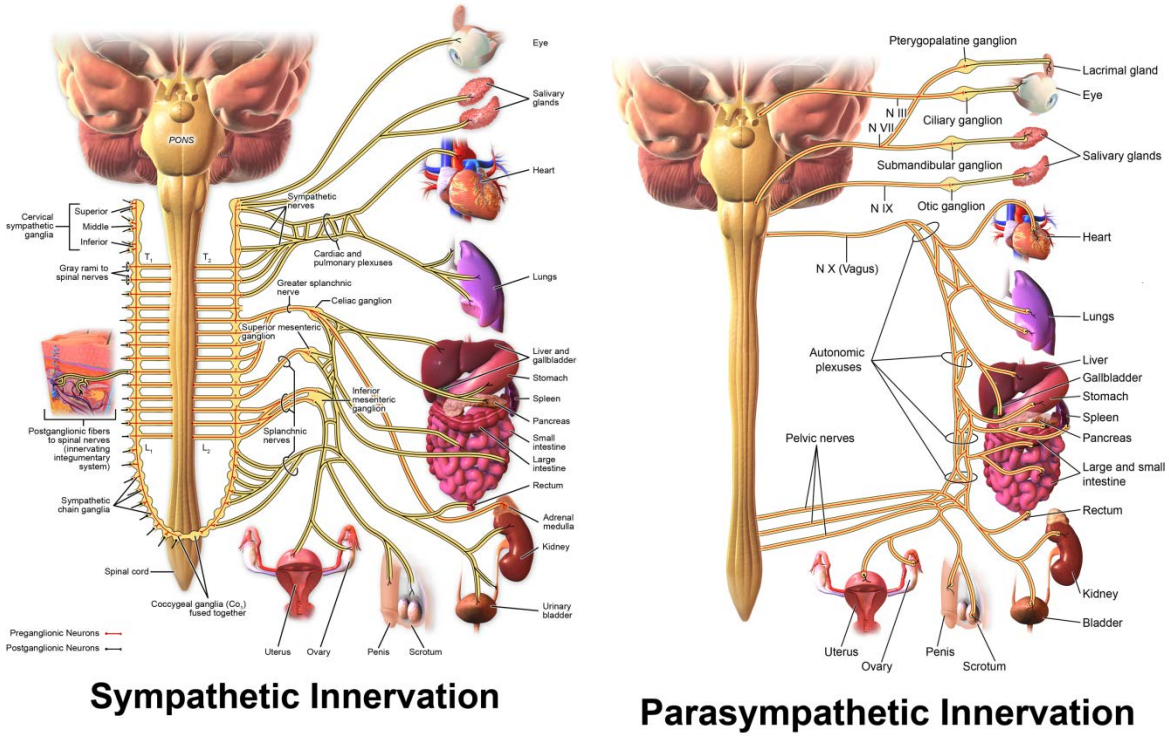

Figure 1. Schematic presentation of the autonomic nervous system. From: Blausen.com staff. "Blausen gallery 2014". Wikiversity Journal of Medicine. DOI:10.15347/wjm/2014.010. ISSN 20018762

The hypothalamic-pituitary-adrenal axis and the autonomic nervous system

Humans have different stress systems, two of which have been mostly studied: the ANS and the HPA-axis. The ANS has two branches: the sympathetic nervous system and the parasympathetic nervous system. The autonomic nervous system regulates critical life functions on a moment-to-moment basis through its sympathetic and parasympathetic branches. The sympathetic branch of the ANS is engaged within seconds of stressor presentation, which ensures an immediate response, which rapidly subsides

as the result of the reflex activation of the parasympathetic branch (McKlveen et al., 2016). To be able to respond to a threatening situation, the body prepares itself for fight or flight. This autonomic activation leads to an increase in heart rate, blood pressure, sweat gland activity, and respiration. Subjectively, the individual feels tense and flushed, has palpitations, shortness of breath and increased perspiration. In many cases, both of these systems have opposite actions where one system activates a physiological response and the other inhibits it. Heart rate is controlled by the sympathetic and parasympathetic branches of the autonomic nervous system, skin conductance is controlled by the sympathetic branches of the ANS, and high frequency variations in heart rate (heart rate variability) is a proxy for the parasympathetic component of autonomic cardiac control.

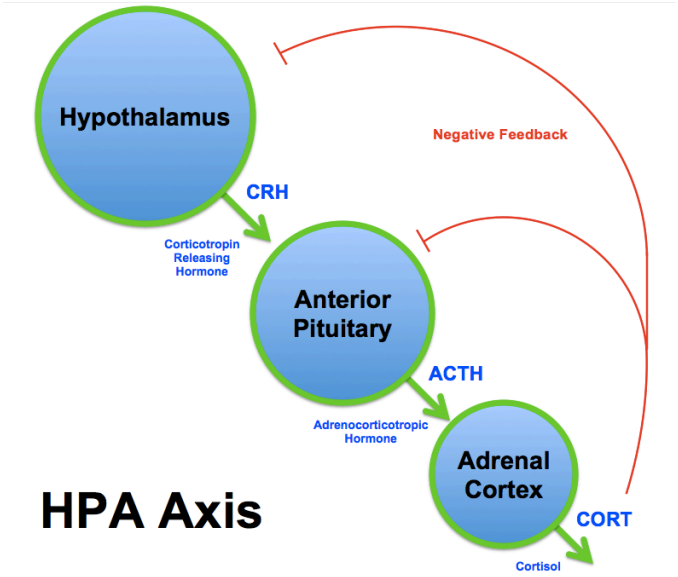

Figure 2. Basic hypothalamic–pituitary–adrenal axis summary (corticotropin-releasing hormone=CRH, adrenocorticotropic hormone=ACTH). Original work from Jessica Malisch and Theodore Garland Feb. 25, 2004.

Besides the ANS, the HPA axis is the major physiological stress response system. Cortisol is the end product of the adrenal axis in humans. During non-stress conditions the HPA-axis shows a diurnal pattern of cortisol secretion, with peak levels approximately 30 minutes after waking up and a

subsequent decline during the day (Wust et al., 2000). The HPA-axis stress response occurs on a slower time scale than the ANS response (Ulrich-Lai, Herman, 2009). Upon stressor initiation, corticotropin-releasing hormone (CRH) is released and travels to the anterior pituitary. In turn, CRH triggers the release of adrenocorticotropic hormone (ACTH). By way of systemic circulation, ACTH acts at the adrenal cortex to induce the release of cortisol. Glucocorticoids are then able to spread via systematic circulation to peripheral targets as well as central targets in the brain. Glucocorticoids can act both to augment and suppress sympathetically mediated changes in e.g. cardiovascular function, metabolism, and immune function. Glucocorticoids exert their effects through binding to mineralocorticoid (MR) and glucocorticoid receptors (GR). The MR is indicated to be important for perceiving resting levels of glucocorticoids for circadian regulation of the HPA-axis, whereas the GR is thought to be important for perceiving stress-induced levels of glucocorticoids. The MR and GR are expressed in key stress-regulatory regions, such as the medial prefrontal cortex, hippocampus, amygdala, hypothalamus, and hindbrain, with MR expression being more limited than that of GR (McKlveen et al., 2016).

Of fraidy-cats, wild tigers and feeling blue

Anxiety disorders are among the most prevalent psychiatric disorders in children and adolescents (Verhulst et al., 1997; Bittner et al., 2007), with separation anxiety disorder, specific phobia, and social phobia being the most frequent childhood anxiety disorders (Beesdo-Baum, Knappe, 2012). High comorbidity rates between anxiety disorders have been reported (e.g. Beesdo, Knappe, Pine, 2009), which will be addressed in *Chapter 2*. The high degree of comorbidity amongst anxiety disorders in children and adolescents seems to point in the direction of one taxonomic construct, instead of a number of separate disorders. However, previous research supports the idea of specific phobia as a distinct taxonomic entity: in a twin study two genetic factors were identified that exclusively predispose to two broad groups of anxiety disorders dichotomized as generalized and panic anxiety plus

agoraphobia versus the specific phobias. Social phobia was influenced by both genetic factors (Hettema et al., 2005). Few studies have compared the endocrine and autonomic profiles between different pediatric anxiety disorders, and if so the focus was on one specific anxiety disorder with a disorder-specific stimulus to elicit stress reactions. At present, it is still unclear as to what extent ANS or HPA-axis activity relates to anxiety in general, or whether they are specific correlates of certain types of anxiety disorders. This will be addressed in *Chapter 5*.

Anxiety and depressive symptoms in children and adolescents are often comorbid, with comorbidity rates ranging from 21 to 54% in population-based studies (e.g. Essau, Conradt, Petermann, 2000; Costello et al., 2003; Ferdinand et al., 2005). Childhood anxiety and depression might be two different disorders that often co-occur, or they could be different manifestations of the same underlying vulnerability. Furthermore, internalizing and externalizing problems in childhood often co-occur (Fanti, Henrich, 2010) and show heterotypic stability, i.e. there is lack of measurement invariance in profiles across ages suggesting that children are very likely to show different patterns of problems across the preschool period (Basten et al., 2016). Given the above, comorbid externalizing problems and depressive symptoms need to be considered when studying risk factors and (bio)markers in anxious children, as described in *Chapters 3, 4 and 5*.

The need to treat

Childhood anxiety has been associated with a range of negative outcomes, including academic underachievement, drug dependency, and an increased risk for developing other psychiatric disorders (Woodward, Fergusson, 2001; Bittner et al., 2007). After the onset of the first anxiety disorder in childhood, a pattern with multiple anxiety disorders often develops by adolescence or early adulthood (Wittchen et al., 2003). The development of these secondary negative outcomes seems to increase with the ‘load’ of anxiety, i.e. the number of anxiety disorders (Woodward, Fergusson, 2001). Given the

differences in outcome, one could argue that the causes and correlates of a high anxiety 'load' may differ from those of a low anxiety 'load' (described in *Chapter 5*).

The chronic and pathological anxiety experienced by children and adolescents in a clinical population is on average more severe than the reported anxiety reported by children and adolescents from the general population, hence it may have a greater impact on the stress systems and influence its future functioning. In addition, children and adolescents in the general population who experience chronic anxiety, but remain untreated, have a significantly poorer prognosis and high persistence (Ferdinand, Verhulst, 1995; Ferdinand, Verhulst, Wiznitzer, 1995).

Cognitive behavioral therapy (CBT) is the treatment of choice for children with an anxiety disorder, with a remission rate of 59% following treatment (James et al., 2013). A 7- to 19-years follow-up study of the long-term outcomes of treated childhood anxiety disorders showed that patients with a poorer response to CBT, had higher rates of panic disorder, substance abuse and dependency in adulthood than the successfully treated patients (Benjamin et al., 2013). It is, therefore, important to identify predictors of symptom improvement in treated children with an anxiety disorder, which will be discussed in *Chapter 7*.

Several studies investigated possible clinical predictors of treatment outcome in children with anxiety disorders. Some studies reported that higher anxiety severity predicts a less favorable outcome (Last, Hansen, Franco, 1998; Liber et al., 2010; Hudson et al., 2013; Compton et al., 2014). A few studies showed that children with comorbid mood disorders are more likely to remit to their primary anxiety disorder following treatment (Liber et al., 2010; Hudson et al., 2013). Various studies examined the role of parental characteristics as predictors of treatment outcome in children, but an inconsistent pattern of findings resulted (Legerstee et al., 2008; Hudson et al., 2013; Compton et al., 2014). Because clinical characteristics are weak or inconsistent indicators of response to CBT, there is an increasing interest in identifying biomarkers to predict differential treatment response (Lester, Eley, 2013). Despite the

evidence of altered pre-treatment HPA axis and autonomic functioning in anxiety-disordered children, studies that have assessed the concomitant changes in stress physiology during treatment or that have investigated stress physiology as a predictor of therapy outcome are lacking. This will be addressed in *Chapter 6*.

AIM OF THIS THESIS

The main aim of the present thesis is to extend the knowledge on the role of stress physiology as a cause, correlate, and predictor of pediatric anxiety disorders with the ultimate goal to improve treatment and prognosis. More specifically, the aim is to examine the specificity of the association of stress physiology with child anxiety problems and its subtypes, given the high co-occurrence with other anxiety disorders, externalizing and depressive problems. In addition, we examine the trajectory of an anxiety disorder and the concomitant change in stress physiology, and stress physiology as a predictor of therapy outcome.

Study samples

The studies described in this thesis were embedded in four study samples.

General population samples

The first study population is the TRacking Adolescents' Individual Lives Survey (TRAILS). **TRAILS** is a prospective cohort study of Dutch early adolescents aged 10-12 years, who are followed biennially. The present study used data from the first (2001-2002; T1 mean age 11.09 years, SD 0.55) and second (2003-2004; T2 mean age 13.56 years, SD 0.53) assessment wave. The TRAILS target sample consisted of young adolescents from five municipalities in the North of the Netherlands, including both urban and rural areas.

The second study population is the **Generation R Study** ("R" for Rotterdam). This study is a longitudinal, population-based cohort in which children are followed up from fetal life forward. The initial cohort comprised 9,778 pregnant women with a delivery date between April 2002 and January 2006, living in Rotterdam, the Netherlands. The aim of Generation R Study is to identify early environmental and genetic determinants of growth, development, and health. Generation R focuses on a wide range of issues relating to physical development, childhood diseases, use of health care, and behavior and cognition. The study in this thesis was conducted within the Focus cohort of the Generation R Study, a population-based prospective cohort from fetal life onwards (Tiemeier et al., 2012). All children were born between February 2003 and August 2005. The cohort consists of Dutch children and their parents and is ethnically homogeneous, to rule out confounding and effect modification by ethnicity. Measurements of infant autonomic indices were added to the protocol of the examination round at age 14 months, while assessment was already ongoing. We obtained physiological measurements for 528 infants.

Patient sample and control group

The third study population is a **clinical sample** of 184 children and adolescents aged 8 to 16 years with a primary diagnosis of generalized anxiety disorder, separation anxiety disorder, social phobia or specific phobia. Eligible for participation were children and adolescents consecutively referred between September 2002 and May 2007 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). All children and adolescents participated in a standardized stepped-care CBT program for childhood anxiety disorders, consisting of two phases (Van der Leeden et al., 2011). In the first phase, children were treated with the FRIENDS program, an

evidence-based treatment program for anxiety disorders (Barrett, Lowry-Webster, Turner, 2000), which encompassed 10 child sessions and 4 separate parent sessions. The FRIENDS program comprised psychoeducation, relaxation and breathing exercises, exposure, problem-solving skills training, social support training and cognitive restructuring exercises (Shortt, Barrett, Fox, 2001; Liber et al., 2008). Parent sessions comprised mainly psychoeducation. All children that were not successfully treated in the first phase, as determined by ADIS-C at three months follow-up, received supplementary CBT. The second phase consisted of 10 manualized sessions, in which parents and child participated together in each session.

The fourth study population, which acts as a control group for children aged 8 to 12 years from the patient sample, is a general population sample drawn from a larger general population sample from the Dutch province of Zuid-Holland (see "2003 sample" in Tick, Van der Ende, Verhulst, 2007), the **Zuid-Holland study**. Of the 2,286 eligible respondents, 1,710 (74.8%) parents of children aged 6-18-year olds participated in this study of Tick, Van der Ende and Verhulst (2007). A subsample of 508 8-12-year-olds living in municipalities relatively close to the city of Rotterdam was selected to participate in a study investigating stress physiology. All 8-12-year-olds with scores above the borderline or the clinical cut-off on the internalizing and/or externalizing problem scales on the Child Behavior Checklist (CBCL; Achenbach, Rescorla, 2001) were invited. This resulted in a selection of 140 children. Furthermore, 156 children aged 8-12 were randomly selected from the remaining 368 children with scores below the borderline cut-off, evenly distributed with regard to degree of urbanization, age and sex. From this subsample three children were excluded because their parents did not speak the Dutch language. Of the remaining 293 eligible respondents, 231 (78.8%) participated.

Methylphenidate treatment in children with ADHD was discontinued the day before and on the day of measurements (clinical sample N=7, general population N=6) because methylphenidate treatment can increase heart rate and blood pressure (Ballard et al., 1976).

Outline

First, given the high degree of comorbidity amongst anxiety disorders in children and adolescents, it is important to extend the knowledge about the taxonomy of anxiety disorders. The main focus of ***Chapter 2*** is the investigation of homotypic and heterotypic longitudinal patterns of symptoms of different anxiety disorders in TRAILS, a large population-based sample of young adolescents.

Second, there is a need to establish the specificity of the association of stress physiology with child anxiety problems, given the high co-occurrence with externalizing and depressive problems. In ***Chapter 3***, we study the tripartite model, in which symptoms of anxiety and depression are viewed along three dimensions. This model groups symptoms of depression and anxiety into three subtypes: negative affectivity, positive affectivity, and physiological hyperarousal. In the Zuid-Holland study, a general population sample of children, we examined whether basal and reactive HPA-axis functioning, as a proxy for physiological hyperarousal, and perceived arousal before, during and after stress differentiate anxious from depressive children. ***Chapter 4*** discusses the longitudinal associations between infant autonomic functioning and early childhood internalizing and externalizing problems simultaneously in the Generation R Study, a large general population sample. Establishing the specificity in a longitudinal design reduceses the risk of reverse causation.

Third, despite a large body of literature detailing an association with stress physiology and anxiety, gaps in our knowledge remain. At present, it is still unclear as to what extent stress physiology relates to anxiety in general, or whether it is a specific correlate of certain types of anxiety disorders. Few studies have compared the stress physiology between different pediatric anxiety disorders, and if

so the focus was on one specific anxiety disorder with a disorder-specific stimulus. ***Chapter 5*** discusses whether HPA-axis, ANS and perceived arousal measures can distinguish children with different primary diagnoses of clinical anxiety disorders (the clinical sample), and from a general population reference group (the Zuid-Holland study). In addition, we explore the association of between stress physiology and the number of clinical disorders.

Fourth, despite the evidence of altered pre-treatment HPA axis and autonomic functioning in anxiety-disordered children, studies that have assessed the trajectory of an anxiety disorders and concomitant change in stress physiology, or stress physiology as a predictor of therapy outcome in childhood anxiety disorders are lacking. In ***Chapter 6***, we study the relation between the trajectory of an anxiety disorder during treatment and the concomitant change in cortisol levels in a clinical sample of children and adolescents with an anxiety disorder. Finally, in ***Chapter 7***, we investigate the longitudinal association of stress physiology at pre-treatment baseline with anxiety and depressive symptoms at one-year follow-up in a clinical sample of children with an anxiety disorder treated with cognitive behavioral therapy. In addition, we explore the longitudinal association of stress physiology with depressive symptoms.

The concluding chapter of this thesis, ***Chapter 8***, discusses the main findings of the studies described in this thesis, including methodological considerations and implications for research and clinical practice.

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Part I:

Clinical perspective

Chapter 2

Homotypic versus heterotypic continuity of anxiety symptoms in young adolescents: evidence for distinctions between DSM-IV subtypes

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ABSTRACT

Objective: to investigate homotypic and heterotypic longitudinal patterns of symptoms of separation anxiety disorder (SAD), generalized anxiety disorder (GAD), social phobia (SoPh), panic disorder (PD), and obsessive compulsive disorder (OCD) in young adolescents from the Dutch general population.

Method: 2,067 individuals (51.4% girls) from a Dutch community sample, who were assessed for the first time when they were aged 10 to 12 years, were followed up across a period of two years. At both assessments, anxiety symptoms were assessed with the RCADS, a self-report questionnaire.

Results: Regression analyses indicated that homotypic continuity was relatively high for SAD, SoPh, and PD (for PD especially in girls), and relatively low for GAD.

Conclusions: In many studies, anxiety disorders are treated as one group of disorders, and some widely used assessment instruments do not even contain scales that tap different anxiety dimensions. In the present study, evidence for homotypic continuity was found for symptoms of separation anxiety and social anxiety, and for panic symptoms in girls, underscoring the usefulness of making distinctions between these different anxiety constructs.

INTRODUCTION

Anxiety disorders are among the most prevalent psychiatric disorders in children and adolescents (Costello et al., 1996; Verhulst et al., 1997; Essau, Conradt, Petermann, 2000), are persistent, and are associated with impaired functioning (McGee, Stanton, 1990; Ferdinand, Verhulst, 1995; Ferdinand, Verhulst, Wiznitzer, 1995; Verhulst et al., 1997; Pine et al., 1998; Canino et al., 2004). The high degree of comorbidity amongst anxiety disorders in children and adolescents seems to point in the direction of one taxonomic construct, instead of a number of separate disorders. High comorbidity rates have been reported by many authors (Newman et al., 1996; Masi et al., 1999; Essau, Conradt, Petermann, 2000; Verduin, Kendall, 2003). Evidence for a higher order factor that explains the presence of different types of anxiety has been found in children (Nauta et al., 2004) and adults (Krueger, 1999; Vollebergh et al., 2001; Hettema et al., 2005). Negative affectivity (NA) (Clark, Watson, 1991; Lonigan et al., 1999; Chorpita, 2002; Lonigan, Phillips, Hooe, 2003; Clark, 2005) may be one of the higher order factors that may explain the finding of heterotypic continuity. NA represents displeasurable engagement with the environment and a sense of high subjective distress (Lonigan, Phillips, Hooe, 2003), and is often considered as a temperament trait that is associated not only with anxiety, but with depression as well (Clark, 2005).

Using data collected at the first assessment wave of a study that was also used to conduct the research that is being described in the present manuscript, support was found for the presence of one single anxiety dimension, instead of a number of separate anxiety concepts (Ferdinand et al., 2006b). The sample of this previous study consisted of 10- to 12-year-olds from the Dutch general population, who completed a self-report questionnaire for anxiety symptoms. Based on item scores on this questionnaire, latent class analysis did not detect classes of individuals with, for instance, high scores on items tapping separation anxiety, and simultaneously low scores on items tapping panic or social

anxiety. Instead, high scores on one anxiety dimension implicated high scores on the other anxiety dimensions as well. However, other studies found evidence for separate anxiety dimensions. By performing factor-analyses, several authors found that DSM-IV anxiety disorders, such as generalized anxiety disorder, separation anxiety disorder, social phobia, and panic disorder represent different problem dimensions in children and adolescents (Spence, 1997; Chorpita, Daleiden, 2000; Muris et al., 2002).

Longitudinal studies can provide valuable information regarding taxonomic constructs. For instance, it was found that symptoms of social phobia in adolescents predicted similar symptoms in adulthood (Pine et al., 1998). The prediction of a disorder by the same disorder is called homotypic continuity. However, social phobia symptoms also predicted simple phobia in adulthood. The prediction of a disorder by another disorder is called heterotypic continuity (Costello et al., 2003). Several mechanisms may explain heterotypic continuity. Heterotypic continuity may occur by chance. In other words, disease A may disappear, and disease B may occur subsequently, as a coincidence. However, in that case, continuity would not be reflected in statistical significance. More likely reasons for heterotypic continuity would be that disease A would be the cause of disease B, or that disease A and B share a common vulnerability factor.

The aim of the present study was to investigate homotypic and heterotypic longitudinal patterns of symptoms of separation anxiety disorder, generalized anxiety disorder, social phobia, panic disorder, and obsessive compulsive disorder in young adolescents from the Dutch general population. For this purpose, individuals from a community sample, who were assessed for the first time when they were aged 10 to 12 years, were followed up across a period of two years. At both assessments, anxiety symptoms were assessed with a self-report questionnaire. Given mixed results of previous studies, we did not formulate specific hypotheses regarding the level of homotypic or heterotypic continuity of different types of anxiety.

METHODS

Sample and procedure

The TRacking Adolescents' Individual Lives Survey (TRAILS) is a prospective cohort study of Dutch early adolescents aged 10-12 years, who are followed biennially. The present study used data from the first (2001-2002) and second (2003-2004) assessment wave. The TRAILS target sample consisted of young adolescents from five municipalities in the North of the Netherlands, including both urban and rural areas. More details about the sample selection have been published elsewhere (De Winter et al., 2005).

Of all subjects who were approached at wave 1 (N=3,145), 6.7% were excluded. The exclusion criteria were (1) adolescent incapable to participate because of mental retardation or a serious physical illness or handicap and (2) Dutch-speaking parent or parent surrogate not available, and not feasible to administer a part of the measurements in parent's own language. Of the remaining 2,935 young adolescents, 24% did not want to cooperate, and 76.0% cooperated with the study at wave 1 (N=2,230, mean age 11.09 years, SD .55, with 50.8% girls). Most frequent reasons for non-response were 'not interested' (33.8%), participation in other research or unfavorable experiences with research (15.4%), too much of a burden on the child (12.2%), lack of time (10.3%), concerns about privacy and confidentiality (8.0%), and the child's refusal to participate because friend(s) did not participate (4.0%). In 34 cases (1.2%) we failed to contact anyone of the household (De Winter et al., 2005). Responders and non-responders did not differ with respect to the proportion of single parent families, or the prevalence of teacher-rated problem behavior. Furthermore, no differences between responders and non-responders were found regarding associations between socio-demographic variables and mental health outcomes (De Winter et al., 2005). To assess anxiety symptoms, the Revised Child Anxiety and Depression Scale (RCADS) (Chorpita, Daleiden, 2000) was used at wave 1, and also at wave 2. For 20

cases, RCADS data were not obtained at wave 1 because respondents were not present during the measurements that were conducted in the classrooms, and could not be reached afterwards. Hence, RCADS data of 2,210 pre-adolescents were available at wave 1.

At the second assessment wave, following similar procedures as at wave 1, RCADS information was obtained from 2,067 individuals. This was 95.5% of those for whom wave 1 RCADS information had been collected (51.4% girls). To examine possible selective attrition, a stepwise logistic regression analysis was performed with 'wave 2 RCADS information available' as a dependent variable, and wave 1 age, sex, and the wave 1 RCADS Total Anxiety score (that was constituted by summing scores on the five anxiety dimensions that were assessed with the RCADS in the present study, see below) as possible predictors. The RCADS Total Anxiety score and sex did not predict attrition. However, younger age predicted attrition significantly (odds ratio = .17, Wald = 93.1, $p < .001$; Model chi-square = 109,551, df = 1, $p < .001$). Cox and Snell R-square of the regression model was .048, which indicated that the effect of age was small. Further, most importantly, the level of anxiety at the initial assessment did not influence cooperation at wave 2.

Measures

The Revised Child Anxiety and Depression Scale (RCADS) (Chorpita, Daleiden, 2000) is a revision of the Spence Children's Anxiety Scale (SCAS) (Spence, 1997). It is a self-report questionnaire with 47 items, that are scored on a 4-point scale (0 = never, 1 = sometimes, 2 = often, 3 = always). The questionnaire covers six scales, corresponding with DSM-IV dimensions of anxiety disorders and depressive disorder. The following five scales were used for the present study: separation anxiety disorder (SAD), generalized anxiety disorder (GAD), social phobia (SoPh), panic disorder (PD), and obsessive compulsive disorder (OCD) (see Table 1). The scale major depressive disorder (MDD) was not used. The internal consistencies of the scales that were used were (respectively at wave 1/wave 2) .66/.59 for SAD, .80/.72 for GAD,

.78/.88 for SoPh, .75/.72 for PD, and .68/.66 for OCD. The factor structure—for all six scales together—that was originally based on data from 1,641 children and adolescents from a community sample from Hawaii (Chorpita, Daleiden, 2000), was confirmed by confirmatory factor analysis in the TRAILS sample at wave 1 (fit indices of NNFI = .96, RMSEA = .05, and SRMR = .05, indicating an adequate fit to the sample data) (Ferdinand et al., 2006b). The association of RCADS dimensions of anxiety with corresponding DSM-IV anxiety disorders was supported by previous research (Nauta et al., 2004).

Table 1. RCADS items

SAD	**SoPh**
Fears being alone at home	Worried when does poorly at things
Scared to sleep alone	Worried when somebody angry
Scared to sleep away from home	Worried will do badly at school
Fears being away from parents	Worried about mistakes
Worried in bed at night	Worried what others think
Trouble going to school	Scared to take a test
Afraid of being in crowded places	Worried might look foolish
	Afraid to talk in front of class
	Afraid to look foolish in front of people
GAD	**PD**
Worried something awful will happen to family	Suddenly trouble breathing without reason
Worried bad things will happen to self	When has a problem, feels shaky
Worried something bad will happen to self	Suddenly trembling, shaking without reason
Thinks about death	Suddenly dizzy, faint without reason
Worried about things	When has a problem, stomach feels funny
Worried about what will happen	When has a problem, heart beats really fast
	Suddenly feeling scared without reason
	Suddenly heart beats too fast without reason
	Worried suddenly get scared without reason

Statistical analyses

First, to obtain information regarding comorbidity between different types of anxiety problems in the study sample, correlations among wave 1 RCADS scale scores were computed for each sex. Then, Pearson correlations were computed between wave 1 and wave 2 RCADS scale scores, separately for

each sex group. Correlations provide insight in the associations between measures. However, by just computing correlations, it cannot be judged if continuity is homotypic or heterotypic. For instance, the magnitude of a correlation between wave 1 SAD and wave 2 SoPh scores depends on the correlation between wave 1 SAD and wave 1 SoPh scores. The higher correlations between wave 1 SAD and wave 1 SoPh are, the higher the correlation between wave 1 SAD and wave 2 SoPh will be. In other words, if assessment of continuity would solely be based on correlations, comorbidity at wave 1 would artificially inflate estimations of the extent of heterotypic continuity between wave 1 and wave 2.

To correct for the effects of wave 1 comorbidity rates, regression analyses were conducted. First, it was assessed which part of continuity in anxiety problems was typically homotypic. For this purpose, for scores on each of the five RCADS scales at wave 2, a set of regression analyses was conducted, with wave 2 RCADS SAD, GAD, SoPh, PD, and OCD scores as dependent variables. These analyses were conducted to investigate how much of the variance in a specific RCADS scale score at wave 2 was not accounted for by an overall elevation in different types of anxiety at wave 1, but instead, specifically by its own counterpart at wave 1. We will now describe the regression analyses that were conducted for wave 2 SAD. Those for GAD, SoPh, PD, and OCD were similar. In the first block of the analyses, wave 1 scores on GAD, SoPh, PD, and OCD were entered simultaneously as predictors. Then, in a second block, wave 1 scores on the SAD scales were added, to see how much of the variance in wave 2 scores was predicted specifically by wave 1 SAD scores, and not by scores on the other RCADS scales at wave 1. This variance reflects specific homotypic continuity. In the third block, sex was added. In the fourth block, an interaction between sex and SAD was added. If this interaction was significant, analyses were conducted for girls and boys separately. For each next block, the variance that was accounted for by the variable in this block was computed (R2).

Second, it was assessed which part of continuity in anxiety problems was specifically heterotypic. For this purpose, for scores on each of the five RCADS scales at wave 2, a set of regression analyses was conducted, with wave 2 RCADS SAD, GAD, SoPh, PD, and OCD scores as dependent variables. These analyses were conducted to investigate how much of the variance in a specific RCADS scale score at wave 2 was not accounted for by its own counterpart at wave 1, but instead, by the other wave 1 anxiety scale scores. We will now describe the regression analyses that were conducted for wave 2 SAD. Those for GAD, SoPh, PD, and OCD were similar. In the first block of the analyses, wave 1 SAD scores were entered as predictor. Then, in the second block, scores on wave 1 GAD, SoPh, PD, and OCD scales were added, to see how much of the variance in wave 2 scores was specifically predicted by other RCADS scales at wave 1. This variance reflects specific heterotypic continuity. In the third block, sex was added.

To judge the magnitude of effects, Cohen's rules for effects sizes can be used (Cohen, 1988). According to Cohen, R2 between 1.0% and 5.9% is small, between 5.9% to 13.8% medium, and above 13.8% large.

RESULTS

Means and standard deviations at wave 1 were calculated for SAD (mean=.375, SD=.356), GAD (mean=.666, SD=.454), SoPh (mean=.779, SD=.427), PD (mean=.428, SD=.363), and OCD (mean=.597, SD=.445). Means and standard deviations were also calculated for wave 2 SAD (mean=.236, SD=.291), GAD (mean=.485, SD=.427), SoPh (mean=.684, SD=.465), PD (mean=.301, SD=.321), and OCD (mean=.339, SD=.348). Means reflect mean item scores for each RCADS scale.

Correlations among wave 1 RCADS scale scores for each sex separately are presented in Table 2. It is shown that all correlations were in a close range, almost similar across sexes, and generally above

.50 which can be regarded as high (Cohen, 1988). For both sexes the highest correlations were found between PD and OCD (r=.61 in boys and girls), and the lowest between SAD and OCD (boys r=.47; girls r=.51).

Table 2. Correlations among wave 1 RCADS scale scores

	Wave 1 RCADS scale			
Wave 1 RCADS scale	**GAD b/g**	**SoPh b/g**	**PD b/g**	**OCD b/g**
SAD	.52/.58	.52/.52	.51/.52	.47/.51
GAD	—	.59/.54	.55/.54	.58/.58
SoPh		—	.56/.55	.54/.53
PD			—	.61/.61
OCD				—

Note. Correlations are presented for boys (b), girls (g) separately.

Correlations between wave 1 and wave 2 RCADS scale scores can be found in Table 3. For instance, in boys, the correlation between wave 1 and wave 2 SAD scores was .30, whereas the correlations between wave 1 GAD, SoPh, and PD scores and wave 2 SAD scores were .27, .29, and .22 respectively. Hence, heterotypic correlations were almost as high as the homotypic correlation. In girls, a similar result was found for wave 2 SAD. Heterotypic correlations ranged between .27 and .29, whereas the homotypic correlation was .38. Similar relatively small discrepancies between homotypic and heterotypic correlations were found for wave 2 GAD, SoPh, and PD scores.

Table 3. Correlations between wave 1 and wave 2 RCADS scale scores

Wave 1 RCADS scale	Wave 2 RCADS scale				
	SAD b/g	GAD b/g	SoPh b/g	PD b/g	OCD b/g
SAD	.30/.38	.23/.32	.35/.29	.28/.27	.21/.25
GAD	.27/.27	.34/.38	.29/.28	.22/.25	.23/.27
SoPh	.29/.29	.35/.29	.42/.41	.29/.28	.25/.25
PD	.22/.29	.25/.31	.24/.27	.32/.42	.25/.29
OCD	.23/.30	.25/.32	.28/.27	.27/.32	.31/.37

Note. Correlations are presented for boys (b), girls (g) separately.

The results of the regression analyses are presented in Tables 4 and 5. Analyses that were aimed at assessing specific homotypic continuity (Table 4) indicated that variances reflecting homotypic continuity were 2.3% for SAD, 3.6% for GAD, 3.7% for SoPh, 3.2% for PD, and .9% for OCD. Analyses aimed as specifically investigating heterotypic continuity revealed variances of 2.0% for SAD, 4.2% for GAD, 1.3% for SoPh, 2.3% for PD, and 2.7% for OCD (Table 5).

Table 4. Specific homotypic continuity (prediction by target scale). Prediction of wave 2 RCADS scale scores by wave 1 scale scores and sex

	Wave 2 RCADS scale				
	SAD	**GAD**	**SoPh**	**PD**	**OCD**
Wave 1 predictors	**R^2/*F* change/p^1**	**R^2/*F* change/p^1**	**R^2/*F* change/p^1**	**R^2/*F* change/p^1**	**R^2/*F* change/p^1**
Non-target scales (block 1)	.119/69.68/.000	.150/91.19/.000	.132/78.35/.000	.126/74.19/.000	.106/61.22/.000
Target scale (block 2)	.050/123.57/.000	.023/58.06/.000	.069/178.54/.000	.037/90.39/.000	.025/59.20/.000
Sex (block 3)	.023/58.92/.000	.036/93.32/.000	.037/100.65/.000	.032/81.21/.000	.009/21.34/.000
Target-scale*sex (block 4)	.001/2.06/ns	.002/5.12/.024	.000/.81/ns	.009/23.81/.000	.002/5.38/.020
*Models for girls/boys separately in case of interaction target-scale*sex*					
Girls					
Non-target scales (block 1)	—	.145/44.70/.000	—	.128/38.75/.000	.109/32.82/.000
Target scale (block 2)	—	.024/31.06/.000	—	.060/77.54/.000	.038/47.13/.000
Boys					
Non-target scales (block 1)	—	.136/39.32/.000	—	.107/29.72/.000	.089/24.41/.000
Target scale (block 2)	—	.018/20.85/.000	—	.020/22.82/.000	.021/23.05/.000

Note. Target-scale---Wave 1 RCADS scale identical to the wave 2 RCADS outcome scale in the regression model (prediction indicating specific homotypic continuity). Non-target scales---all other wave 1 RCADS scales, not identical to the wave 2 RCADS outcome scale. R^2---explained variance.

In analyses aimed at homotypic continuity, some anxiety*sex interactions were significant. A marked sex difference was found for PD. Table 4 shows that homotypic continuity was much stronger for girls (homotypic R2=6.0%) than for boys (homotypic R2=2.0%).

Table 5. Specific heterotypic continuity (prediction by non-target scales). Prediction of wave 2 RCADS scale scores by wave 1 scale scores and sex

	Wave 2 RCADS scale				
	SAD	**GAD**	**SoPh**	**PD**	**OCD**
Wave 1 predictors	**R^2/F change/p[1]**	**R^2/F change/p[1]**	**R^2/F change/p[1]**	**R^2/F change/p[1]**	**R^2/F char**
Target scales (block 1)	.391/373.39/.000	.375/337.53/.000	.436/483.57/.000	.380/347.98/.000	.337/263.4
Non-target scale (block 2)	.020/83.77/.000	.042/86.59/.000	.013/103.79/.000	.023/80.00/.000	.027/82.20
Sex (block 3)	.027/81.60/.000	.041/90.95/.000	.040/107.45/.000	.028/82.80/.000	.012/55.90

Note. Target-scale---Wave 1 RCADS scale identical to the wave 2 RCADS outcome scale in the regression model. Non-target scales---all other wave 1 RCADS scales, not identical to the wave 2 RCADS outcome scale (prediction indicating specific heterotypic continuity). R^2---explained variance.

DISCUSSION

The present study examined homotypic and heterotypic continuity of symptoms of separation anxiety disorder, generalized anxiety disorder, social phobia, panic disorder, and obsessive compulsive disorder in individuals from a community sample, who were assessed for the first time when they were aged 10 to 12 years, and for the second time two years later. Variances reflecting homotypic continuity were roughly equal to those reflecting heterotypic continuity for OCD (2.5% versus 2.7%). Variances reflecting homotypic continuity were larger than for heterotypic continuity for SAD (5.0% versus 2.0%), SoPh (6.9% versus 1.3%), and PD (3.7% versus 2.3%), for PD especially in girls, and smaller for homotypic than for heterotypic continuity for GAD (2.3% versus 4.2%) (Tables 4 and 5). Applying cross-sectional research designs, previous studies found high comorbidity rates among different types of anxiety problems (Essau, Conradt, Petermann, 1999; Newman et al., 1996; Verduin, Kendall, 2003). The present study extended the knowledge about the taxonomy of anxiety problems in young adolescents with

longitudinal data. In accordance with previous cross-sectional work, SAD, GAD, SoPh, PD, and OCD symptoms appeared to be intertwined in a longitudinal fashion as well. However, considerable homotypic continuity was found as well.

Separation anxiety

For SAD, compared to heterotypic continuity, homotypic continuity was relatively strong. Previous studies indicated considerable comorbidity between SAD and the other anxiety problems, especially with SoPh (Compton, Nelson, March, 2000). In the present study comorbidity rates at wave 1 were high as well, not only with SoPh, but also with other types of anxiety. This suggests that SAD and the other anxiety problems may represent two sides of the same coin. However, homotypic continuity was stronger than heterotypic continuity, which supports the usefulness of SAD as a separate diagnostic construct. The distinction between SAD and other types of anxiety was further supported by another study, in which we conducted latent class analysis to assess the boundaries between SAD and SoPh in referred 8- to 11-year-olds (Ferdinand et al., 2006a). Four different classes of individuals were detected; those with (1) low SAD and SoPh item scores on a self-report questionnaire, (2) high SAD and high SoPh item scores, (3) high SAD and low SoPh scores, and (4) low SAD and high SoPh scores. This also supported the idea that, despite high comorbidity rates, SAD may at least partially represent a separate phenomenon, that, for instance, may be subject to specific etiological influences that differ from influences that affect the course of other anxiety problems.

Generalized anxiety

For GAD, homotypic continuity was relatively low. A previous study (Pine, Cohen, Brook, 2001) in adolescents from the general population indicated that separation anxiety disorder and social phobia in adolescents did not predict future generalized anxiety disorder. This suggested rather strong homotypic

continuity of GAD. However, continuity of GAD was more heterotypic than in the study by Pine, Cohen and Brook (2001), because in our study, heterotypic continuity was also considerable. This contrast may be due to methodological issues such as differences in sample characteristics (Pine, Cohen and Brook investigated older adolescents), assessment procedures (Pine, Cohen and Brook applied standardized interviews instead of self-report questionnaires), or statistical approach (Pine, Cohen and Brook used categorical diagnostic samples whereas the present study used dimensional scale scores). Further, Pine, Cohen and Brook did not use a block design for their regression analyses, but included all predictors in a forward stepwise logistic regression analysis. So, in essence, they did not test if one predictor predicted future GAD, over and above the effect of other predictors.

Social phobia

Compared to heterotypic continuity, homotypic continuity of SoPh symptoms was relatively strong. Pine, Cohen and Brook (2001) found that SoPh, but also GAD, in adolescence predicted future SoPh, independently of other types of anxiety, whereas SAD did not. Remarkably, in their study, GAD was a better predictor of future SoPh than SoPh itself. The aforementioned methodological differences between the Pine et al. study versus the present study may explain differences between findings.

Panic

For PD, homotypic was relatively strong compared to heterotypic continuity. Analyses for boys and girls separately (Table 4) indicated that homotypic continuity was higher in girls than in boys. Previous studies already indicated that panic disorder tends to have a chronic course, in children as well as in adults (Biederman et al., 1997; Bruce et al., 2005). The prevalence of full blown panic disorder, and even of panic attacks, in adolescents is very low (Essau, Conradt, Petermann, 1999). The present study nevertheless suggests that in girls, the disorder may already begin with a chronic homotypic course at a

very young age which is in accordance with the higher prevalence in females versus males (Goodwin et al., 2005), and with studies that retrospectively investigated age at onset, and that often point to childhood or adolescence as a starting point (Sheehan, Sheehan, Minichiello, 1981).

Obsessive compulsive disorder

Comorbidity rates of OCD with other anxiety disorders are generally high (Heyman et al., 2001; Tukel et al., 2002), which was supported by the correlations among the wave 1 RCADS scale scores. The present study showed that homotypic and heterotypic continuity for OCD did not differ much. To our knowledge, previous studies that assessed homotypic continuity of OCD symptoms versus heterotypic continuity with other types of anxiety in young adolescents are not available, so we cannot compare our findings with previous work. Homotypic continuity was somewhat stronger in girls than in boys. This seems to suggest that, longitudinally, OCD symptoms in boys correlate differently with comorbid conditions than OCD symptoms in girls. Again, we were not able to find previous work on this topic. Future studies are needed to investigate if the differences between boys and girls we found can also be found in other samples, countries, and cultures.

Practical implications

In many studies, anxiety disorders are treated as one group of disorders (Barrett et al., 2001; Lipman, MacMillan, Boyle, 2001; MacMillan et al., 2001; Shortt, Barrett, Fox, 2001; Roza et al., 2003), and, even, some widely used assessment instruments do not contain scales that tap different anxiety dimensions (Achenbach, 1991a,b). Previous studies found considerable associations between different types of anxiety symptoms, which suggested the presence of one higher order factor (Nauta et al., 2004). Several studies with adults also found evidence for a higher order factor that explained the presence of different types of anxiety (Krueger, 1999; Vollebergh et al., 2001; Hettema et al., 2005). Given the magnitude of

heterotypic continuity in the present study, a higher order factor is likely to be present. However, the present study also showed that considerable homotypic continuity is present as well, occurring separately from a general propensity for high anxiety levels. This indicates that each type of anxiety problem may, at least partly, represent a distinct taxonomic construct. Homotypic continuity was found specifically for SAD, SoPh, and for PD in girls. This may indicate that SAD, SoPh, and PD represent diagnostic constructs that are at least partially distinct. Hence, in clinical practice, instruments are needed that measure different anxiety dimensions separately. Instruments that just assess on single anxiety dimension may not be sufficient. Further, the distinctions between different anxiety constructs indicate that, despite the evidence that similar treatment methods are generally efficacious for different types of anxiety problems, each type of anxiety might require a slightly different treatment approach, and development of specific treatment modules.

Limitations

The sample consisted of young adolescents only. For older adolescents, different homotypic and heterotypic continuities could apply. Furthermore, questionnaires were used instead of clinical interviews; information about the presence or absence of DSM-IV (American Psychiatric Association, 1994) clinical diagnoses was not obtained. Even though RCADS symptom dimensions have proved to reflect corresponding DSM-IV anxiety disorders (Nauta et al., 2004), still, it may be the case that different results would have been obtained if DSM-IV diagnoses, that take account of the level of functional impairment, would have been used instead of RCADS scale scores. Since different informants may provide different information, this study would have been more valuable if information regarding symptoms of different types of anxiety would also have been gathered from parents or teachers. Unfortunately, such information was not available.

CONCLUSION

In the present study's sample of young adolescents from the Dutch general population, evidence for homotypic continuity was found for symptoms of separation anxiety and social anxiety, and for panic symptoms in girls, underscoring the usefulness of making distinctions between these different anxiety constructs.

Footnote

The erratum ((2008), *Journal of Abnormal Child Psychology*, *36* (3), 457) was integrated in this paper.

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PART II

Physiological stress activity in child general population samples

Chapter 3

Perceived and physiological arousal during a stress task: can they differentiate between anxiety and depression?

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ABSTRACT

Background: Anxiety and depression might be two different valid constructs that often co-occur, or they could be different manifestations of the same underlying vulnerability. A theoretical framework to address this question is the tripartite model, by Clark and Watson, which hypothesizes that physiological hyperarousal (PH) is specific for anxiety. Knowledge about the relationship between PH, psychophysiological measures, perceived arousal, and anxiety would increase our understanding of the validity of the PH construct in this model. Our objective was to assess whether (a) hypothalamic pituitary–adrenocortical (HPA) axis functioning, and (b) perceived arousal before, during and after stress can differentiate anxious from depressive children.

Methods: In a general population sample of 225 children aged 8-12 years, self-reported anxiety and depressive symptoms were assessed using the Multidimensional Anxiety Scale for Children (MASC) and the Children's Depression Inventory (CDI). Perceived arousal was assessed using a self-report questionnaire before, during and after a stress task. Basal and reactive HPA-axis functioning were used as indices for psychophysiological arousal.

Results: Our data showed that the relation between perceived arousal and anxiety problems is stronger than the relation with depressive problems. Reactive HPA-axis functioning is reduced in children with depressive problems.

Conclusions: Some evidence was found in support of the tripartite model. Our findings indicate that perceived arousal to a challenge might be a useful tool to assess the PH component of the tripartite

model. Reactive HPA-axis functioning might be able to differentiate between anxiety and depressive problems in children in a general population sample, but effect sizes are small and replication is needed.

INTRODUCTION

Childhood anxiety and depressive symptoms have been associated with a range of negative outcomes (e.g. Weissman et al., 1999; Van Ameringen, Mancini, Farvolden, 2003; Bastiaansen et al., 2004), persistence is high (e.g. Ferdinand, Verhulst, 1995; Dunn, Goodyer, 2006; Colman et al., 2007), and children with an anxiety or depressive disorder have a greater chance of developing psychopathology later in life (e.g. Pine et al., 1998; Woodward, Fergusson, 2001; Aalto-Setala et al., 2002). Given these negative consequences, a better understanding of the causes and correlates of childhood anxiety and depression is imperative to improve treatment and prognosis for children affected by these disorders.

An essential step forward for research in this field is to better define anxiety and depression in children. Much evidence has shown that anxiety and depressive symptoms in children and adolescents occur frequently and are often comorbid, with comorbidity rates ranging from 21 to 54% in population based studies (e.g. Essau, Conradt, Petermann, 2000; Costello et al., 2003; Ferdinand et al., 2005). Anxiety and depression might be two different valid constructs that often co-occur, or they could be different manifestations of the same underlying vulnerability. There are many models that have tried to disentangle these constructs. An interesting theoretical framework to address this question is the tripartite model, proposed by Clark and Watson (1991) and Watson et al. (1995a,b) in which symptoms of anxiety and depression are viewed along three dimensions. This model groups symptoms of depression and anxiety into 3 subtypes: negative affectivitiy (NA), a measure for general affective distress, positive affectivity (PA), a measure representing pleasurable engagement with the environment, and physiological hyperarousal (PH), a measure representing somatic tension and arousal, e.g. racing heart, sweaty palms and dry mouth. NA is associated with both anxiety and depression. A lack of PA, is hypothesized to be specifically associated with depression, while the third component, PH, is hypothesized to be specific for anxiety (Watson et al., 1995a,b). Research regarding the validity of the

tripartite model in child and adolescent, clinical and general population samples also supports a three-factor structure (e.g. Joiner, Catanzaro, Laurent, 1996; Chorpita, Daleiden, 2002; Turner, Barrett, 2003; Cannon, Weems, 2006).

Nonetheless, earlier studies focusing on the validity of the tripartite model in child and adolescent populations were hampered by methodological problems, such as the use of subsets of items from existing anxiety and depression questionnaires to define the specific constructs. This leads to some difficulty with respect to validation: the most suitable validity criteria, for the three constructs in children and adolescents, were the same anxiety and depression scales from which the tripartite items were chosen (Chorpita, Daleiden, 2002). Additionally, the theory itself describes that PH is only related to anxiety disorders and not to depression. However, recent findings in child literature show moderate correlations between depression and PH measured by questionnaires (Brown, Chorpita, Barlow, 1998; Joiner et al., 1999; Chorpita, Daleiden, 2002).

Another way of measuring PH could be the measurement of psychophysiological measures representing arousal (Laurent, Ettelson, 2001). Knowledge about the relationship between PH, psychophysiological measures, and anxiety would increase our understanding of the validity of the PH construct in the tripartite model. To our knowledge, so far only one study aimed at testing the validity of the PH component of the tripartite model against psychophysiological measures, i.e. heart rate (HR) and respiratory sinus arrythmia (RSA), representing arousal (Greaves-Lord et al., 2007a). Parent-reported anxiety was associated with low RSA in supine posture. This association was also found for self-reported anxiety problems, but only in boys. Self-reported depressive problems were associated with high RSA in supine posture in boys, pointing towards low arousal in depression. Self-reported depressive problems were also associated with high HR in standing posture, suggesting high arousal in depression. Thus, results remain inconclusive.

Besides autonomic nervous system (ANS) functioning, the hypothalamic–pituitary–adrenocortical (HPA) axis is a major physiological stress response system. HPA-axis functioning has important effects on brain stem catecholaminergic input to the cortex through their effects on the locus coeruleus, a brain stem structure essential to the maintenance of arousal. Cortisol is the end product of the adrenal axis in humans. Abercrombie, Kalin and Davidson (2005) found that acute cortisol elevations cause heightened arousal ratings of objectively non-arousing stimuli in humans. Although these findings have to be interpreted carefully, because of the interference with the normal balance of hormones and the difficulties with the time parameters of acute versus long term arousal, they suggest that HPA-axis functioning can be seen as an indicator of PH. According to the tripartite model PH is specific for anxiety and not depression. This results in the hypothesis that HPA-axis functioning as a measure for PH can differentiate anxiety from depression. To our knowledge, no studies investigated the validation of the PH component of the tripartite model against HPA-axis functioning.

Research regarding the association between HPA-axis functioning and depressive and anxiety symptoms in children is inconclusive. Studies that did find an altered HPA-axis functioning in anxious and depressed children, found this association mainly during sleep onset and at nighttime (Dahl et al., 1991; De Bellis et al., 1996; Goodyer et al., 2000; Feder et al., 2004; Forbes et al., 2006). Goodyer, Park and Herbert (2001) examined morning and evening cortisol among clinically depressed 8-16-year-old children followed over 72 weeks. Children with chronic depression had higher evening cortisol levels than children who recovered from their depression. Luby et al. (2003) found a stronger increase in cortisol levels in reaction to a separation stressor between depressed preschoolers and children with no psychiatric disorder. van West et al. (2008) found an elevated cortisol response to a psychosocial stressor in prepubertal subjects with social phobia as compared with healthy controls. In other studies no association was found (e.g. Gerra et al., 2000, Terleph et al., 2006; Greaves-Lord et al., 2007b).

Anxiety and depressive symptoms are highly comorbid, but most previous studies did not adjust for the effect of comorbidity on HPA-axis functioning. Only one study addressed comorbidity in children. Young, Abelson and Cameron (2004) found that only depressed children with a comorbid anxiety disorder showed an exaggerated adrenocorticotropic hormone (ACTH) response and a similar but non-significant effect of a cortisol to a stressor. Subjects with pure mood or anxiety disorders showed normal ACTH and cortisol responses to a stressor.

In sum, findings on the association between cortisol levels and anxiety or depressive problems in children are inconclusive. Further, studies assessing comorbid anxiety and depressive problems in relation to HPA-axis functioning are lacking. This leads to the question whether PH measured by physiological measures is really specific for anxiety.

Another way of measuring arousal is the assessment of perceived arousal before, during and after a stress test. Previous studies suggest that high anxious subjects tend to perceive physiological sensations as more severe than non-anxious subjects (Sturges et al., 1998, Hoehn-Saric, McLeod, 2000; Richards, Bertram, 2000) sometimes even in the absence of an actual difference in physiological measures (Wilhelm, Gerlach, Roth, 2001; Edelmann, Baker, 2002). Our study is the first study to investigate the validation of the PH component of the tripartite model against perceived arousal in stressful (laboratory) situations.

In the present study, we therefore hypothesize that PH measured by physiological measures represents both anxiety and depression. Further, based on the model, we would expect that only high anxious and comorbid children show an elevated perceived arousal to challenge. Thus, in a general population sample of children we examined whether (a) basal and reactive HPA-axis functioning, as a proxy for PH, and (b) perceived arousal before, during and after stress differentiate anxious from depressive children.

METHODS

Participants

This sample was drawn from a larger general population sample from the Dutch province of Zuid Holland (see "2003 sample" in Tick, Van der Ende, Verhulst, 2007). Of the 2,286 eligible respondents, 1,710 (74.8%) parents of children aged 6-18-year olds participated in the study by Tick, Van der Ende and Verhulst (2007). From this sample, a sample of 508 8-12-year-olds living in municipalities relatively close to the city of Rotterdam was selected, to participate in a study investigating stress reactivity. All 8-12-year-olds with scores on the internalizing and/or externalizing problem scales on the Child Behavior Checklist (CBCL; Achenbach, Rescorla, 2001) falling within the borderline or the clinical range were selected. This resulted in the selection of 140 children. Furthermore, 156 children aged 8-12 were randomly selected from the remaining 368 children, evenly distributed with regard to degree of urbanization, age and sex (see Figure 1).

Exclusion criteria were: poor command of Dutch language, serious physical disease (e.g. cardiac, neurologic, respiratory disease), or receiving concurrent pharmacotherapy that could interfere with HPA-axis functioning. From this subsample three children were excluded because their parents did not speak the Dutch language. Of the remaining 293 eligible respondents, 231 (78.8%) participated.

This article focuses on children in this age group because beyond the age of 12 years hormonal changes due to the onset of puberty might have an effect on HPA-axis functioning (Matchock, Dorn, Susman, 2007) and this results in a less homogeneous sample. Furthermore, the stress test we used is not applicable below the age of 8 years. To examine possible selective attrition, a stepwise logistic regression analysis was performed with 'participation yes or no' as a dependent variable, and age, sex, socioeconomic status (SES) and the internalizing and externalizing problem scales on the CBCL as

possible predictors. Sex, age and the externalizing and internalizing problem scales of the CBCL did not predict attrition. However, lower SES predicted attrition significantly (model $\chi 2 = 8.621$, d.f. = 2, $p = .013$). Cox and Snell R-square of the regression model was .029, which indicated that the effect of SES was small. Furthermore the level of psychopathology at the initial assessment, assessed with the externalizing and internalizing problem scales of the CBCL, was not associated with cooperation.

From the 231 participants 6 (2.6%) refused to cooperate with the part of the study regarding physiological measures, 23 (10.0%) only gave permission to collect saliva at home and 202 (87.4%) gave permission for the complete procedure.

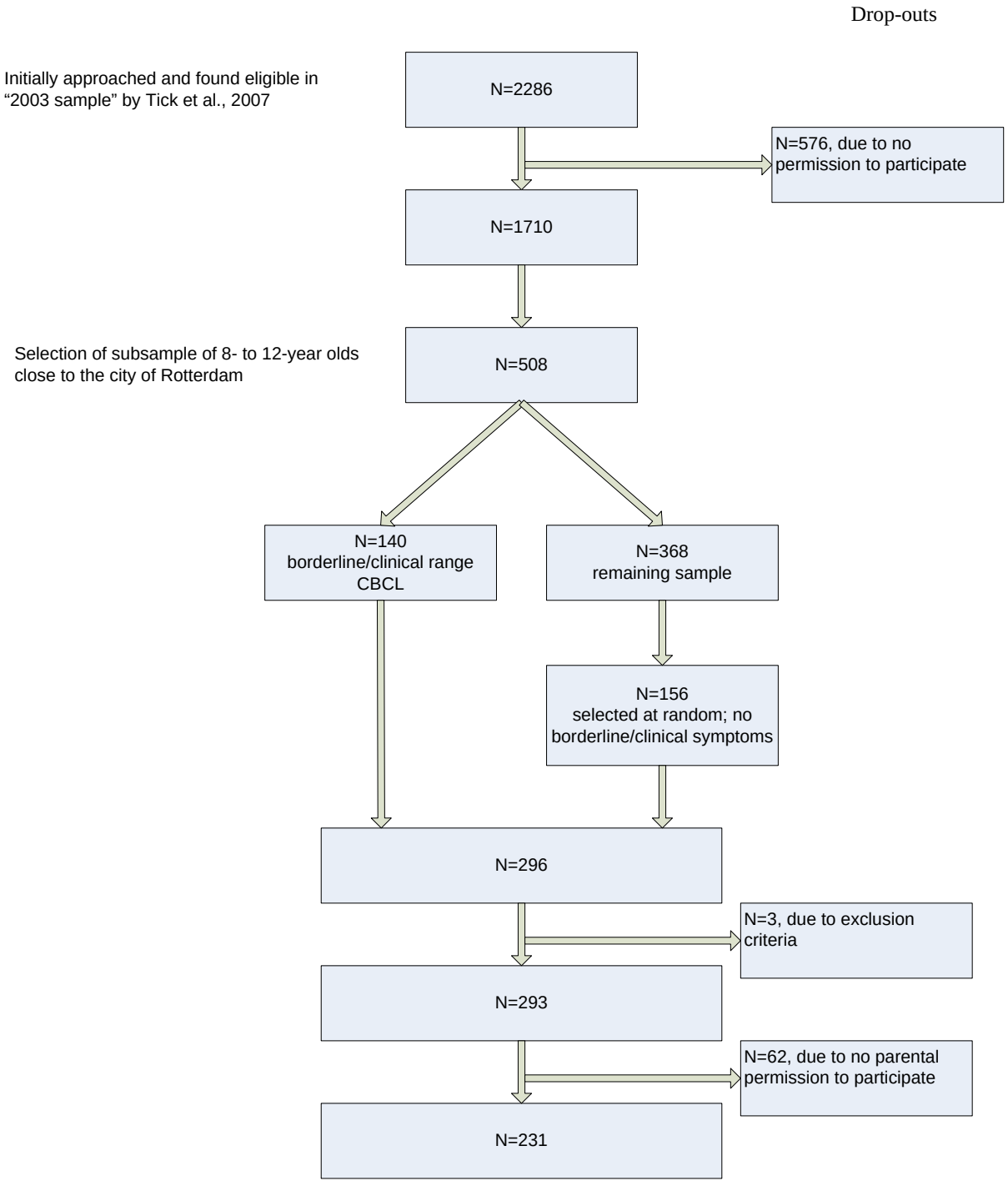

Figure 1. Sample selection

Note. CBCL = Child Behavior Checklist

Procedure

Participants were given written information with details about the objectives of the study, procedures and rights of the participant. A signed informed consent was obtained from each family. Prior to the physiological assessment, children were asked to fill in the Multidimensional Anxiety Scale for Children (MASC; March et al., 1997) and Children's Depression Inventory (CDI; Kovacs, 1992). Activity of the HPA system was assessed using salivary cortisol samples, which reflect the biologically active unbound fraction of serum cortisol (Gozansky et al., 2005), also in children (Shimada et al., 1995). When cortisol samples are examined two things have to be kept in mind; (1) measuring cortisol at a peak time (30 min after awakening- 'cortisol awakening rise' (CAR) (Rosmalen et al., 2005)) can give an indication of cortisol 'reactivity' and (2) cortisol levels have been found to react to stress, typically peaking about 20 30 min following a stressor. Participants were instructed to collect saliva samples at home: (1) a first sample immediately after awakening in the morning (Cort1), when the child was still in bed, (2) a second sample 30 min later (Cort2), (3) a sample at 12:00 h (Cort3), and (4) a final sample at 20:00 h (Cort4).

Participants were instructed to fill the tubes till a marker (at 500 µl). All samples were collected on a regular school day, stored in the freezer at home, and taken to the outpatient department 1 day later, when the physiological assessment took place. Children and parents received a written instruction with drawings and received another instruction by telephone the day before sampling to increase the reliability of cortisol sampling at home. Saliva samples were stored at −20 °C before analysis at the laboratory. Parents were asked to register general physical condition, activity levels (e.g. sports), consumption pattern (e.g. smoking, alcoholic beverages and caffeine intake), and medication use of the participants.

The physiological assessment took place one day after saliva collection at home. Participants were seated comfortably in a light laboratory room where temperature and humidity levels were kept constant. All assessments took part in the afternoon (between 12 p.m. and 6.30 p.m.). Lunch was eaten

before assessment; the gap between lunch and baseline saliva samples was at least 1.5 h. After a rest period of 45 min, the session began with a baseline period of 10 min in which the participant was asked to sit still and relax. Subsequently, a stress test consisting of three subtasks; a mental arithmetic task (MAT) (4 min), a public speaking task (PST) (8 min speech preparation and 6 min public speaking) and a computer task (CT) (5 min) were administered. The public speaking task involved providing the child with a story scenario and then giving him/her 8 min to develop a story (Speech Preparation Period). Then the child had to deliver the speech for 6 min (Speech Period), this was followed by a computer task during 5 min. The story stem used was one in which the child was erroneously accused of stealing and had to describe to a "guard" why they were innocent. During the computer task children had to calculate by heart in how many steps they would be able to order four numbers from lowest through highest. They were told they had to act as quickly as possible and to make as little mistakes as possible. The public speaking task was videotaped and the participant was told that the computer task and the public speaking task were evaluated afterwards. Furthermore, for all tasks there was a time constraint.

Both of these elements and the fact that the test combines a public speaking task with cognitive tasks are associated with the largest cortisol changes and the longest times to recovery in comparison with other stress tests (Dickerson, Kemeny, 2004). After accomplishment of the computer task the participant was asked to watch a movie during 25 min after sitting still and relaxing again for 5 min. This was implemented to allow participants to return to normal after the stressor. At the end of the baseline period, after each subtask of the stress test, and halfway and at the end of the recovery period, a questionnaire concerning physiological arousal was administered and saliva was collected (Cortisol samples 5–10 (Cort5–Cort10)) (see Figure 2). Sampling after each stress task was conducted in order to develop a cortisol profile during stress.

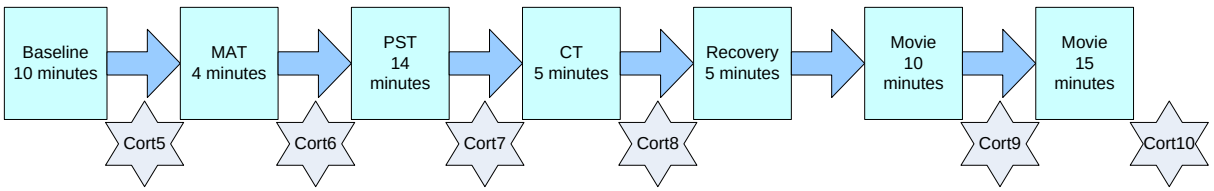

Figure 2. Cortisol sampling

Note. Cort5 = cortisol at the end of the baseline period, Cort6 = cortisol after the mental arithmetic task (MAT), Cort7 = cortisol after the public speaking task (PST), Cort8 = cortisol after the computer task (CT), Cort9 = cortisol 15 minutes after the stress task, Cort10 = cortisol 30 minutes after the stress task

Measures

Child behavior checklist

The CBCL is a parent questionnaire for assessing problems in 4-18-year-olds. It contains 120 items on behavioral or emotional problems in the past 6 months. The response format is 0 = not true, 1 = somewhat or sometimes true, and 2 = very true or often true. The good reliability and validity of the original version of the CBCL (Achenbach, 1991) were confirmed for the Dutch translation (Verhulst, Van der Ende, Koot, 1996). Scores on two broad-band scales were computed. Scores on the Internalizing broad band scale are derived by summing scores on the 'narrow-band' scales 'Withdrawn', 'Somatic Complaints', and 'Anxious/Depressed', whereas scores on the Externalizing broad-band scale are computed by summing scores on the narrow-band scales 'Delinquent Behavior' and 'Aggressive Behavior'. Scores on the Internalizing Problems and Externalizing Problems scales were used to include children.

Multidimensional Anxiety Scale for Children

The MASC is a 39-item self-report questionnaire that assesses anxiety symptoms concerning the last 2 weeks in children and adolescents. Items are scored from 0 to 3 (0 = never true, 1 = rarely true, 2 = sometimes true, 3 = often true). The internal consistency (.90) and 1-month test–retest reliabilities (.87–.90) (March et al., 1997) are very good. Cronbach's α in this study was .87.

Children's Depression Inventory

The CDI is a 27-item self-report questionnaire that assesses depressive symptoms concerning the last 2 weeks in children and adolescents. Items are scored from 0 to 2 (0 = never true, 1 = sometimes true, 2 = always true). The internal consistency (.86) and 1-month test–retest reliabilities (.72) are adequate (Kovacs, 1981). Cronbach's α in this study was .84.

Physiological Arousal Questionnaire (PAQ)

The PAQ (Kallen, 2002) is a 7-item self-report questionnaire developed at our department for assessment of the perceived state of physiological arousal. The child is asked to indicate on a 9-point scale (0–8) to what extent he or she feels aroused: 0 = not at all to 8 = very much. Cronbach's α was .64 (during baseline), .81 (during stress), .84 (during recovery). The specific items are shown in Table 1. The PAQ was administered at the end of the baseline period, after each subtask of the stress test, and after the recovery period.

Table 1. PAQ items

1. Do you have warm or sweaty hands?
2. Are you sweating?
3. Do you feel your heart beating?
4. Are you feeling hot or short of breath?
5. Do you have a dry mouth?
6. Do you have a tingling sensation in your face or hands?
7. Are you nervous?

Cortisol assessment

An Enzyme-Linked Immuno Sorbent Assay (DRG Instruments GmbH, Marburg, Germany) was used to determine cortisol concentrations in 50 µl duplicate samples. The lower limit of detection was 1.12 nmol/l, the intra-assay variation was 6.9% and the inter-assay variation was 8.6%. Of the samples 2.7% is missing due to insufficient saliva or due to intra-assay differences larger than 20%. Cortisol values that were above 3 SD of the mean were excluded (N=22) from the analysis in order to reduce the impact of outliers.

In addition to the separate cortisol variables, we chose to use composite cortisol variables to analyze the diurnal and stress cortisol profile. To characterize the diurnal cortisol profile we calculated three composite measures according to Pruessner et al. (2003): cortisol awakening rise with respect to ground (CAR_g) and with respect to increase (CAR_i) and the area under the curve with respect to ground (AUC_g) (Rosmalen et al., 2005). An area under the curve with respect to ground, such as CAR_g or AUC_g, is a measure that represents the total amount of cortisol that is produced during a specific period and is calculated by computing the area between zero and the curve that is constituted by the respective cortisol levels (Pruessner et al., 2003). In the case of CAR_i, the distance from zero is ignored, thereby emphasizing changes over time (Pruessner et al., 2003). To characterize the cortisol profile during stress the area under the curve with respect to ground and increase (respectively AUC_{sg} and AUC_{si}) were

calculated using cortisol samples representing baseline (Cort5 and Cort6), representing stress (Cort7, Cort8 and Cort9) and representing recovery (Cort10). For formulas see Appendix A. If one of the cortisol samples was missing, an area under the curve could not be calculated; therefore N=19 AUC_{sg} and N=10 AUC_g were missing.

Data analysis

All statistical analyses were performed with SPSS 15.0. Item scores on the MASC, CDI and PAQ were summed to obtain total scores. As shown in Table 2, cortisol levels during stress were at their lowest at Cort6. Cortisol levels across participants reached their peak after the computer task, represented by Cort8, but probably reflect the cumulative response to all three components of the stressor and anticipation of stress due to the lag in cortisol response after stress exposure. Cort10 best reflects recovery values of cortisol.

PAQ Total score, Cort4, Cort6, Cort 8, Cort 10, Body Mass Index (BMI) and CDI Total score were log transformed to normalize their distribution before statistical analyses. All of the not normally distributed measures, except for CDI Total score, Cort4 and PAQ Total score during recovery, were normally distributed after log-transformation.

First, to investigate if the level of anxiety predicted basal HPA-axis functioning nine separate stepwise regression analyses were conducted. CAR_g, CAR_i, AUC_g, AUC_{sg}, AUC_{si}, Cort4, Cort6, Cort8 and Cort10 were entered in nine separate regression analyses as dependent variables. Age, gender and BMI might confound the cortisol-psychopathology relationship (e.g. see Rosmalen et al., 2005 for an overview). To control for possible effects of age, gender and BMI, these variables were first entered as a block into the model as independent variables. MASC Total score was entered in a second block as predictor. Nine similar regression analyses were conducted with the log-transformed CDI Total score as independent variable.

Table 2. Descriptives of anxiety, depression, perceived arousal, and cortisol measures

Measures	**Mean (SD)**	**Measures**	**Mean (SD)**
MASC Total score	41.91 (13.91)	Cort7 (nmol/l)	7.83 (3.21)
CDI Total score	6.14 (5.33)	Cort8 (nmol/l)	8.56 (4.05)
Cort1 (nmol/l)	14.89 (4.69)	Cort9 (nmol/l)	8.08 (3.75)
Cort2 (nmol/l)	17.83 (5.39)	Cort10 (nmol/l)	7.85 (3.94)
Cort3 (nmol/l)	8.59 (2.96)	AUC_{sg}	7.28 (2.55)
Cort4 (nmol/l)	6.50 (3.48)	AUC_{si}	0.05 (1.74)
CAR_g	8.18 (2.03)	PAQ Total score (baseline)	6.78 (5.99)
CAR_i	0.76 (1.47)	PAQ Total score (after MAT)	8.95 (7.95)
AUC_g	116.77 (31.35)	PAQ Total score (after PST)	11.30 (9.47)
Cort5 (nmol/l)	7.88 (3.14)	PAQ Total score (after CT)	8.03 (8.56)
Cort6 (nmol/l)	7.46 (2.55)	PAQ Total score (recovery)	5.96 (7.72)

Note. SD = standard deviation, MASC = Multidimensional Anxiety Scale for Children, CDI = Children's Depression Inventory, Cort1 = cortisol directly after awakening, Cort2 = cortisol half an hour after awakening, Cort3 = cortisol at 12.00 p.m., Cort4 = cortisol at 8.00 p.m., CAR_g = cortisol awakening rise with respect to ground, CAR_i = cortisol awakening rise with respect to increase, AUC_g = area under the curve for Cort2-Cort4 with respect to ground, Cort5 = cortisol at the end of the baseline period, Cort6 = cortisol after the mental arithmetic task (MAT), Cort7 = cortisol after the public speaking task (PST), Cort8 = cortisol after the computer task (CT), Cort9 = cortisol 15 minutes after the stress task, Cort10 = cortisol 30 minutes after the stress task, AUC_{sg}= area under the curve for Cort5-Cort10 with respect to ground, AUC_{si}= area under the curve for Cort5-Cort10 with respect to increase, PAQ = Perceived Arousal Questionnaire.

Second, to investigate if the level of anxiety predicted perceived arousal, three separate stepwise regression analyses were conducted. PAQ Total score before, during and after the stress test were entered as dependent variables and the MASC Total score was entered as predictor. To control for possible effects of age and gender, these variables were first added to the model as independent

variables. Due to overlap between items of the MASC Physical Anxiety Scale and the PAQ, the correlation between the PAQ Total score was calculated. Furthermore the regression analyses were repeated for the MASC Total Score minus the scores on the MASC Phsycical Anxiety Scale. Three similar regression analyses were conducted with the log-transformed CDI Total score as independent variable.

Third, three stepwise regression analyses were conducted with the PAQ Total score before, during and after the stress test as dependent variables. The first block consisted of age and gender, second the MASC Total score was added, third the log-transformed CDI Total score was added and finally the interaction term between the MASC Total score and the log-transformed CDI Total score was added.

Finally, to investigate the relation between perceived and objective arousal, correlations between all cortisol and perceived arousal measures were calculated. For all analyses effect sizes are reported by $R^{2,change}$.

RESULTS

Descriptives

Two hundred and twenty-five children participated in the physiological part of the study (mean age 10.06 years (SD 1.52), with 49.8% boys, and mean Body Mass Index (BMI) 18.11 (SD 3.24)). Mean scores and standard deviations of all the anxiety, depression, perceived arousal, and cortisol measures are presented in Table 2. The PST elicited the largest response in PAQ Total score. Therefore PAQ Total score after the PST was used as a measure of perceived arousal after stress, herein after referred to as PAQ stress. Correlations between MASC Total score and CDI Total score and perceived arousal measures after the different stress tasks were calculated. Perceived arousal elicited by the CT was most strongly correlated with both MASC Total score and CDI Total score (see Table 3).

Table 3. Pearson's correlations between perceived arousal measures after stress and anxiety and depression

PAQ (log-transformed)	MASC Total score	CDI Total score
MAT	.34**	.16*
PST	.32**	.22**
CT	.38**	.25**

*= p<.05, **=p<.01, PAQ = Perceived Arousal Questionnaire, MAT = mental arithmetic task, PST = public speaking task, CT= computer task

54 subjects had a negative value for CAR_i ranging from −.10 to −2.73. 91 subjects had a negative value for AUC_{si} ranging from −.02 to −4.13. We chose to include negative values in our analyses, because we examined the linear relation between physiological arousal and anxiety and depression using regression analyses. Subjects with a negative value of CAR_i had a significant ($T = -2.64$, $p < .01$) lower anxiety score (MASC Total score = 37.5) in comparison to children with positive values (MASC Total score = 43.2). This result was not significant anymore when corrected for age, sex and BMI, using stepwise binary logistic regressions. There was no significant difference in depression scores for CAR_i or AUC_{si}.

Linear regressions

There was no significant relation between any of the cortisol measures and MASC Total score.

Furthermore, there was only one significant relation between log-transformed CDI Total score and any of the cortisol measures; a higher score on the CDI Total score resulted in a lower AUC_{si}. This suggests that children with higher rates of depression show a flattened response to stress. The results of the regression analyses for MASC Total score and log-transformed CDI Total score during baseline, after stress and during recovery are presented in Table 4.

Table 4. Predictive value of anxiety and depression regarding cortisol measures

Predictors	CAR_g	CAR_i	AUC_g	AUC_{sg}
	β /R^2	*β /R^2*	*β /R^2*	*β /R^2*
Gender, Age, BMI (first block)	.23**;.-.02;-.11/.06*	.18*;.13;-.11/.05*	.16*;.12;-.15/.05*	.05;.22**;-.18*/.06*
MASC Total Score (second block)	-.09/.01	.09/.01	-.01/.00	-.03/.00
CDI Total Score (second block)	.02/.00	.12/.01	.06/.01	-.07/.01
Predictors	AUC_{si}	Cort6	Cort8	Cort10
	β /R^2	*β /R^2*	*β /R^2*	*β /R^2*
Gender, Age, BMI (first block)	.20**;.06;-.16*/.06*	-.01;.13;-.10/.02	.15*;.22**;-.20**/.08**	.08;.03;-.12/.02
MASC Total Score (second block)	-.09/.01	-.01/.00	-.08/.01	-.08/.01
CDI Total Score (second block)	-.15/.02*	-.02/.00	-.09/.01	-.12/.01#

Note. β's are standardized betas, uncorrected for age, BMI or gender. $R^{2\ change}$ = explained variance for adding this step, MASC = Multidimensional Anxiety Scale for Children, CDI = Children's Depression Inventory, Cort4 = cortisol at 8.00 p.m., CAR_g = cortisol awakening rise with respect to ground, CAR_i = cortisol awakening rise with respect to increase, AUC_g = area under the curve for Cort2-Cort4 with respect to ground, Cort6 = cortisol after the mental arithmetic task (MAT), Cort8 = cortisol after the computer task (CT), Cort10 = cortisol 30 minutes after the stress task, AUC_{sg}= area under the curve for Cort5-Cort10 with respect to ground, AUC_{si}= area under the curve for Cort5-Cort10 with respect to increase. # = p<.1, *= p<.05, **=p<.01.

Table 5. Predictive value of anxiety and depression regarding perceived arousal

	PAQ (log-transformed)		
Predictors	Baseline *β /R^2*	Stress *β /R^2*	Recovery *β /R^2*
1. Age, gender	-.07;.07/.01	.12;.08/.02	-.16*;.05/.03$^{\#}$
2. MASC Total Score	.35/.12**	.33/.11**	.37/.13**
2. MASC Total Score, without PAS	.28/.07**	.28/.08**	.29/.08**
2. CDI Total Score	.26/.07**	.22/.05*	.22/.05*
	Baseline *β /T*	Stress *β /T*	Recovery *β/T*
1. Age, gender	-.07;.07/-1.03; 1.00	.12;.08/1.65; 1.07	-.16;.05/.-2.21*;.70
2. MASC Total Score	.35/5.07**	.33/4.79**	.37/5.37**
Model statistics	F^{change} = 25.7, $R^{2, change}$ = .12**	F^{change} = 23.0, $R^{2\,change}$ = .11**	F^{change} = 28.84 , $R^{2\,change}$ = .13**
3. MASC Total Score; CDI Total Score,	.29;.13/3.74**; 1.76$^{\#}$	.29; .09/3.71**; 1.22	.33;.08/4.31**;1.04
Model statistics	F^{change} = 3.11, $R^{2\,change}$ = .01$^{\#}$	F^{change} = 1.49, $R^{2\,change}$ = .01	F^{change} = 1.08, $R^{2\,change}$ = .01
4. MASC Total Score; CDI Total Score; MASC Total Score * CDI Total Score	.38;.24;-.17/2.0*; 1.13; -.53	.54;.37;-.46/2.79*;1.78$^{\#}$;-1.43	.54; .31;-.38/2.79*;1.47;-1.17
Model statistics	F^{change} = .28, $R^{2\,change}$ = .00	F^{change} = 2.06, $R^{2\,change}$ = .01	F^{change} = 1.38, $R^{2\,change}$ = .01

Note. β's are standardized betas, uncorrected for age or gender. $R^{2, change}$ = explained variance for adding this step. PAQ = Perceived Arousal Questionnaire, MASC = Multidimensional Anxiety Scale for Children, CDI = Children's Depression Inventory, PAS = Physical Anxiety Scale. $^{\#}$ = p<.1, *= p<.05, **=p<.01

The results of the regression analyses for MASC Total score, the MASC Total Score minus the scores on the MASC Physical Anxiety Scale, and log-transformed CDI Total score and log-transformed PAQ Total score during baseline, after stress and during recovery are presented in Table 5. All three of them predict perceived arousal during baseline, stress and recovery conditions. The regression analyses of MASC Total score and PAQ-score yields the strongest relation according to the effect sizes. When the items of the MASC Physical Anxiety Scale are subtracted from the Total score, the relation remains significant.

Three stepwise regression analyses were conducted with the PAQ Total score before, during and after the stress test as dependent variables. The first block consisted of age and gender, second the MASC Total score was added, third the log-transformed CDI Total score was added and finally the interaction term between the MASC Total score and the log-transformed CDI Total score was added. The results are displayed in Table 5. This table shows that by adding the CDI Total score to the model, the model did not improve. When MASC and CDI Total score were simultaneously added to the model, only MASC Total Score remained significant. The effect size of adding this step is close to zero.

To investigate the relation between perceived and objective arousal, correlations between all cortisol and perceived arousal measures were calculated. None of these correlations were significant, see Table 6.

Table 6. Pearson's correlations between perceived and objective arousal measures

PAQ (log-transformed)	CAR_g	CAR_i	AUC_g	Cort4	AUC_{sg}	AUC_{si}	Cort6	Cort8	Cort10
Baseline	.01	.05	-.02	-.01	-.07	-.01	-.07	-.08	-.12
Stress	-.07	.09	-.01	.02	.08	.08	.07	.06	.02
Recovery	-.04	.06	-.07	-.05	-.06	.02	-.04	-.04	-.08

PAQ = Perceived Arousal Questionnaire, Cort4 = cortisol at 8.00 p.m., CAR_g = cortisol awakening rise with respect to ground, CAR_i = cortisol awakening rise with respect to increase, AUC_g = area under the curve for Cort2-Cort4 with respect to ground, Cort6 = cortisol after the mental arithmetic task (MAT), Cort8 = cortisol after the computer task (CT), Cort10 = cortisol 30 minutes after the stress task, AUC_{sg}= area under the curve for Cort5-Cort10 with respect to ground, AUC_{si}= area under the curve for Cort5-Cort10 with respect to increase. $^{\#}$ = p<.1, *= p<.05, **=p<.01.

We conducted a post hoc analysis to examine whether a group with clinical internalizing problems had the same relationship between anxiety and/or depression measures and perceived or objective arousal. These analyses showed that perceived and objective arousal have the same relation with anxiety and depression measures in children that exceed the borderline cut-off score for internalizing problems on the CBCL (N=54).

DISCUSSION

Principal findings

PH as defined by the model of Clark and Watson is a concept based on anxiety and depression questionnaires. This concept is a rather virtual concept that has no quantifiable substrates; therefore it is difficult to assess the usefulness and significance of this model. Furthermore, the model by Clark and Watson is a model that is frequently cited with regard to the disentanglement of anxiety and depression as different constructs. In our opinion it is interesting, although complicated, to view our results with

respect to the tripartite model by Clark and Watson; it contributes to the discussion of how the model is constructed and why there are so many versions of it. This study attempted to quantify this concept by using two levels of arousal, a subjective and a physiological, in a specific situation (i.e. in basal conditions and during a stresstask) in order to make it more tangible and applicable. We examined whether, in a general population sample of children, basal and reactive HPA-axis functioning, as a proxy for PH, and perceived arousal before, during and after stress could differentiate anxious from depressive children.

Overall, our results provide some evidence in support of the applicability of perceived arousal as a measure of PH, included in the tripartite model, to differentiate between anxiety and depressive problems in children in a general population sample. Perceived arousal is related to both anxiety and depressive problems. However, effect sizes for depressive problems are smaller than the effect sizes during baseline and recovery for anxiety problems. Furthermore, when anxiety and depressive problems are entered simultaneously, only anxiety problems predict perceived arousal.

With respect to cortisol as a measure of perceived arousal, our results suggest that children with higher rates of depression have a flattened response to a stresstask. There was no relation between anxiety problems and any of the other cortisol measures. We can therefore state that our results provide some evidence that in a general population sample, reactive HPA-axis functioning can differentiate between anxiety and depressive problems in children.

HPA-axis

Greaves-Lord et al. (2007a) found some evidence for hyperarousal measured by psychophysiological measures, in both anxiety and depression. We could not replicate these results using indicators of HPA-axis functioning instead of ANS functioning. One reason for the inconsistent findings could be the difference in age groups; the study of Greaves-Lord et al. (2007b) was conducted in a sample of young adolescents, whereas this study was conducted in a sample of school-aged children.

HPA-axis functioning was assessed during the day and in basal as well as in stress conditions. No relation with measures of anxiety problems was found. On the contrary, we found that children with higher rates of depression have a flattened response to stress. This result is in contrast with the findings of Luby et al. (2003) who found a stronger increase in cortisol levels in reaction to a separation stressor between depressed preschoolers and children with no psychiatric disorder. The effect size of our finding is very small, so our results have to be interpreted carefully. Furthermore, this finding could be the result of a type I error. Therefore replication in other samples is needed. An explanation for the lack of significant findings could be that alterations in HPA-axis functioning might only occur in children with a clinical anxiety or depressive disorder. In this general population sample the amount of subjects with clinical anxiety and depression was relatively low. One could hypothesize that only anxiety or depressive problems which interfere with daily life and exist for some time, have an effect on HPA-axis functioning. Or, alternatively, that altered HPA-axis functioning, as a preexisting trait, leads to a clinical anxiety or depressive disorder, but is not related to normal variation in levels of anxiety.

Perceived arousal to a stressor

In our study, perceived arousal to a real stressor was related to both anxiety and depressive problems. Our findings are not consistent with the tripartite model; PH is related to both anxiety and depression, although, PH shows a stronger association with anxiety than with depression. This is in line with recent findings in child and adult literature which show moderate correlations between depression and PH, when PH is measured using questionnaires (Brown, Chorpita, Barlow, 1998; Joiner et al., 1999; Chorpita, Daleiden, 2002).

Limitations and strengths

This study is, to our knowledge, the first study to combine perceived arousal to challenge and HPA-axis functioning, as a measure for PH. Perceived arousal during a challenge is probably a more reliable marker for PH than items in questionnaires tapping arousal in daily life.

The present study tested the validity of the PH component of the tripartite model in a general population sample. Therefore, in comparison to research in clinical samples, results can be generalized due to the lack of selection bias. However, originally, the tripartite model was developed with the use of data from a clinical sample (Clark, Watson, 1991). Clinical and general population samples might differ in impairment and severity and duration of symptoms. Therefore, the findings found in the present study cannot be generalized to clinical samples. This limitation is confirmed by the post-hoc analyses which show that exceeding the clinical cut-off score of internalizing problems on the CBCL moderates the relationship between perceived arousal and anxiety and depressive symptoms. Furthermore, relations that were not found in this study – for instance, the relation between HPA-axis functioning and anxiety problems- might be established in a clinical sample due to reasons mentioned above. Therefore replication in a clinical sample is needed.

One could argue that there is an overlap between items from the PAQ and the MASC. However, the MASC assesses physical anxiety symptoms during the past two weeks and not in relation to an actual stressor. Furthermore, the relation between the MASC remains, even after deleting the items that assess physical anxiety symptoms. The PAQ is used as an instrument to assess the perceived arousal before, during and after a stressor and therefore measures a different construct; it does not measure anxiety symptoms, but it measures actual instantaneous arousal.

In the present study, we used only the child as an informant to assess anxiety and depressive symptoms. One could argue that the use of multiple informants (e.g. parents or teachers) would lead to a more valid assessment of these symptoms (Grills, Ollendick, 2003; Comer, Kendall, 2004). However, multiple informant agreement is higher for externalizing than for internalizing problems; a possible

explanation for this phenomenon can be that internalizing problems tend to be inwardly focused upon the self (Grills, Ollendick, 2003). Therefore, self-report questionnaires are valid tools to assess internalizing problems in children and adolescents.

Other demographic variables such as socioeconomic status, IQ and Tanner stage might have influenced cortisol values. These data are not available for our subjects. This is a limitation of the study.

Previous studies showed inconsistent results regarding the association between cortisol levels and child anxiety or depressive problems. Studies that did find an altered HPA-axis functioning in anxious and depressed children, found this association mainly during sleep onset and at nighttime (Dahl et al., 1991; De Bellis et al., 1996; Goodyer et al., 2000; Feder et al., 2004; Forbes et al., 2006). In our study, we did not assess sleep-onset and nighttime cortisol levels.

We assessed HPA-axis functioning by measuring the end product of this axis, cortisol. However, HPA-axis activity is regulated by multiple hormones and is a very complex system (Sapolsky, Romero, Munck, 2000; De Kloet, 2003; Herman et al., 2005). Hence, the measurement of only the end product is a relatively crude way to measure a possible altered functioning of this system. For instance, Young, Albelson and Cameron (2004), did find alterations in ACTH levels in depressed children with a comorbid anxiety disorder in response to a stressor, but not in cortisol levels. Thus, the lack of significant findings regarding cortisol in our study, does not necessarily mean that HPA-axis functioning is not altered in anxious or depressed subjects. These alterations might occur on different levels in the HPA-axis.

CONCLUSION

This study found some evidence in support of the tripartite model of Clark and Watson (1991). Together, our findings indicate that perceived arousal to a challenge might be a useful tool to assess the PH component of the tripartite model in a general population sample of school-aged children. HPA-axis

functioning measured by cortisol in stress conditions might differentiate between anxiety and depression, but this result is not very strong and needs replication in a general population sample.

Future research is needed to replicate our findings in clinical samples. Findings might be different, when different measures for the assessment of HPA-axis functioning are used, such as evening and nighttime cortisol, or ACTH levels during stress or a Dexamethasone Suppression Test. Furthermore, studies that simultaneously assess HPA-axis and ANS functioning, as well as perceived arousal to a stressor, in different age groups, could contribute to a better differentiation of anxiety and depression in children and adolescents. Further research could also focus on measures of autonomic nervous system activity at rest and during stress, in addition to HPA-axis activity.

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SUPPLEMENTARY MATERIAL

Formulas

The CAR_g and CAR_i were calculated using the following formulas:

$CAR_g = ((Cort1+Cort2) * time_{cort2-cort1})/2$

$CAR_i = CAR_g – (Cort1 * time_{cort2-}cort1)$.

The formula to calculate AUC_g was:

$AUC_g = AUC_{cort2-cort3}+AUC_{cort3-cort4} = ((Cort2+Cort3) * time_{cort3-cort2})/2 + ((Cort3+Cort4) * time_{cort4-cort3})/2$

The AUC_{sg} and AUC_{si} were calculated using the following formulas:

$AUC_{sg} = AUC_{cort6-cort5}+AUC_{cort7-cort6}+AUC_{cort8-cort7}+AUC_{cort9-cort8}+AUC_{cort10-cort9}$

$AUC_{si} = AUC_{sg} -(Cort5 * time_{cort10-cort5})$

The AUCs were calculated in the same manner as the AUCs used in the formula for the AUC_g.

Chapter 4

Of fraidy-cats and wild tigers: a prospective study of infant autonomic functioning and child internalizing and externalizing problems

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ABSTRACT

Background: Variations in the autonomic nervous system and its proxies heart rate and heart rate variability have been implicated as possible biological markers of both internalizing and externalizing pathology in children. Most previous studies of autonomic arousal and problem behavior used a cross-sectional study design or, in case of a longitudinal design, measured autonomic arousal later in life, increasing the possibility of reverse causality. We investigated the longitudinal associations between infant autonomic functioning and early childhood internalizing and externalizing problems.

Methods: In infants of the Generation R Study, we measured resting mean heart rate and heart rate variability at 14 months. At 18 months and 6 years (N=464), child problem behavior was assessed with the Child Behavior Checklist. To investigate the association of infant heart rate and heart rate variability with change in childhood internalizing and externalizing problem behavior, we performed a series of regression analyses.

Results: Autonomic functioning predicted internalizing problems at follow-up independently of externalizing problem behavior at follow-up, and vice versa. Lower infant heart rate was associated with less decrease of childhood externalizing problems, whereas higher infant heart rate was associated with a stronger increase of internalizing problems. Consistently, higher infant heart rate variability was associated with less decrease of childhood externalizing problems, whereas lower infant heart rate variability was associated with a stronger increase of internalizing problems.

Conclusions: Our study shows that both infant heart rate and heart rate variability function as antagonistic predictors of childhood internalizing and externalizing problem behavior.

INTRODUCTION

Mental health problems affect approximately 15% of preschool children aged 2 through 5 years (Egger, Angold, 2006; Skovgaard et al., 2007). Convergent evidence from prospective community studies shows that preschool problem behavior precedes later psychopathological problems (Basten et al., 2016). However, to some extent, many challenging or difficult behaviors in pre-school aged children are age-appropriate and in keeping with normal development (Egger, Angold, 2006). This has made it difficult to discern age-appropriate behavior, reflecting normal development, from persistent pathological behavior and underlines the importance to identify early risk factors for later psychopathology.

Internalizing and externalizing problems in childhood often co-occur (Fanti, Henrich, 2010) and show heterotypic stability (Basten et al., 2016). Prior research indicates that the development of externalizing and internalizing problems each have unique causes as well as shared causes (Mathiesen, Sanson, 2000). Biomarkers that increase one type of problem behavior and decrease the other, might remain undetected when internalizing and externalizing problems are not disentangled (Mathiesen et al., 2009). Therefore, developmental changes in internalizing and externalizing problems, as well as the co-occurrence of internalizing and externalizing problems, need to be considered in studying early risk factors for the continuity of problems among preschool children.

Variations in the autonomic nervous system and its proxies heart rate and heart rate variability have been implicated as possible biological markers of both internalizing and externalizing pathology in children (Ortiz, Raine, 2004; Dietrich et al., 2007; Dierckx et al., 2014; Dieleman et al., 2015). Heart rate is controlled by the sympathetic and parasympathetic branches of the autonomic nervous system, while high frequency variation in heart rate (heart rate variability) is a proxy for the parasympathetic component of autonomic cardiac control. Low autonomic arousal shows an association with externalizing problems and behavioral disorders, whereas high autonomic arousal is associated with

internalizing problems and anxiety disorders (Ortiz, Raine, 2004; Dietrich et al., 2007; Dierckx et al., 2014; Dieleman et al., 2015). Most previous studies that examined the association between autonomic arousal and problem behavior in children used a cross-sectional study design (Posthumus et al., 2009; El-Sheikh, Hinnant, Erath, 2011) or, in case of a longitudinal design, measured autonomic beyond infancy (Hinnant, El-Sheikh, 2013; Hastings, Kahle, Nuselovici, 2014), which increases the possibility of reverse causality. Reverse causation refers to the situation in which the outcome precedes and causes the exposure instead of the other way around. Ideally potential risk factors for the development of serious problem behavior would be measured before the onset of such behaviors.

A few studies investigated the longitudinal associations between infant autonomic functioning and later internalizing problems. Dierckx et al. (2014) showed a positive association between infant heart rate and anxiety symptoms at 3 years, whereas Baker et al. (2012) and Wagner et al. (2016) found no direct association between infant autonomic functioning and later internalizing problems. Studies investigating the association between infant autonomic functioning and externalizing problems showed no association (Van Hulle et al., 2000; Dierckx et al., 2014). However, the study by Dierckx et al. (2014) showed that lower infant resting heart rate predicted higher odds of lying at 3 years. They concluded that lower resting heart rate may be less an indicator of early childhood aggression than of fearless behavior. In these longitudinal studies examining infant autonomic functioning as a predictor for later problem behavior, most studies focused either on internalizing or externalizing problems, but few considered the co-occurrence of internalizing and externalizing problems.

The current study was designed to examine the longitudinal associations between infant autonomic functioning and early childhood internalizing and externalizing problems simultaneously, in a large general population sample. We hypothesized that infant autonomic functioning (at 14 months) would differentially predict internalizing and externalizing problems at the age of 6, adjusting for these

internalizing and externalizing problems at baseline (at 18 months) respectively, reflecting a model of symptom change.

METHODS

Design and study population

This study was conducted within the Focus cohort of the Generation R Study, a population-based prospective cohort from fetal life onwards (Tiemeier et al., 2012). All children were born between February 2003 and August 2005. The cohort consists of Dutch children and their parents and is ethnically homogeneous to rule out confounding and effect modification by ethnicity.

Measurements of infant autonomic indices were added to the protocol of the examination round at age 14 months, while assessment was already ongoing. We obtained physiological measurements for 528 infants, as described previously (Dierckx et al., 2009). When the child was on average 5.9 years old, N=464 mothers rated the child's emotional and behavioral problems (response rate of 87.9%).

Written informed consent was obtained from all participants. 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.

Measures

Psychophysiological 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 (HR) 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 analyzed 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 (Van Steenis, Tulen, Mulder, 1994). We calculated high frequency heart rate variability as a proxy for the parasympathetic component of autonomic cardiac control. As this indicator of autonomic cardiac control is dependent on respiratory frequency, which is much higher in infants, we adjusted the upper bound of the high frequency power band according to recommendations from literature (Bar-Haim, Marshall, Fox, 2000). We defined our high frequency power band between 0.15 Hz - 1.04 Hz. Time segments with more spectral power for respiration in the mid frequency band than in in high frequency band were discarded from heart rate variability analyses. This resulted in the removal of 2 subjects for the respective analyses.

Maternal report of child behavior

We used the Child Behavior Checklist/1½–5 (CBCL), a parent questionnaire for assessing behavioral and emotional problems in children. This version was also chosen at follow-up because of the age range (5.1-8.3 years). The CBCL has good reliability and validity (Achenbach, Rescorla, 2000). At follow-up, the mother rated the child's emotional and behavioral problems over the preceding 2 months on a 3-point rating scale. Higher scores indicate more problems. For most of the children (N=433) CBCL questionnaires at 18 months of age were available to adjust for pre-existing problems at baseline. In the current study, we used the broadband Internalizing symptom score, the internalizing syndrome subscales Anxious/Depressed and Withdrawn, as well as the broadband Externalizing symptom score, and the externalizing syndrome subscales Aggressive Behavior and Attention Problems.

Covariates

Gender of the child, gestational age, weight at birth, age of the infant at physiological measurement, maternal age, smoking and drinking behavior during pregnancy, and level of highest completed education by the mother were studied as covariates, based on previous reports (Dierckx et al., 2014). Gestational age at birth and birth weight were obtained from community midwife and hospital registries at birth. Information on maternal smoking, maternal drinking, and highest completed education by the mother, were obtained during pregnancy by questionnaire. The Dutch Standard Classification of Education was used to define three categories of education: higher education phase 2 (university degree), higher education phase 1 (higher vocational training, Bachelor's degree) and secondary education or lower.

Statistical analyses

The physiological variable heart rate variability was log-transformed to achieve a normal distribution. Box plots were used to identify outliers for heart rate and heart rate variability. This resulted in the

removal of one subject for the heart rate variability analyses. Pearson correlations were calculated to test the relations between predictor and outcome variables. Paired-samples T-tests were used to compare the mean scale and subscale scores of the CBCL at baseline with the mean scale and subscale scores of the CBCL at follow-up.

To investigate the association of heart rate and heart rate variability with emotional or behavioral problems, we performed a series of regression analyses. All regression analyses were adjusted for maternal age, maternal smoking and drinking behavior during pregnancy, maternal highest completed education, child gender, gestational age, weight at birth and age at physiological measurement. In these analyses, we assessed multicollinearity by examining tolerance and the variance inflation factor. None of our analyses showed a tolerance below .2 or a variance inflation factor above 2.5.

First, we performed separate linear regression analyses with heart rate as the independent variable and the Internalizing symptom score, the Anxious/depressed problem score, or the Withdrawn problem score at follow-up as dependent variables. In this model of symptom change, we adjusted the analyses for the baseline Internalizing symptom score.

Second, we repeated these analyses with the Externalizing symptom score, the Aggressive Behavior symptom score, and the Attention Problems score at follow-up as dependent variables corrected for the baseline Externalizing symptom score.

Third, we tested the specificity of infant heart rate and heart rate variability as predictors for the Internalizing symptom score or subscale scores and the Externalizing symptom score or subscale scores at follow-up, respectively, by mutually adjusting the broadband scales.

Fourth, we tested the robustness of significant results of the observed associations using logistic regressions. To this aim, we dichotomized follow-up CBCL scores at the 80^{th} percentile score for each

scale as a cut-off to classify children as having problems, in line with previous publications of this cohort (e.g. Velders et al. (2011)).

For the linear regression analyses effect sizes are reported as delta R squared, with .01 defined as a small effect size, .09 as a medium effect size and .25 as a large effect size (Cohen, 1988). All analyses were performed using SPSS 21.0 for Windows.

Multiple imputation

Multiple imputation was used to account for missing values in covariates and the CBCL at 18 months. Missing values in psychophysiological measures and the CBCL at follow-up were not imputed. Missing values ranged from 0% for gender to 7.3 % for the baseline Internalizing symptom score. Descriptives of original, non-imputed, data are presented in Table 1. To impute the missing values we included all covariates, psychophysiological measures and outcome measures as predictors in the multiple imputation models. The presented data are pooled estimates from 30 imputed datasets.

Non-response analysis

Children with missing data on the CBCL at follow-up (N=64) were compared with children for whom these data were available (N=464). Data was more frequently missing in children with a lower gestational age at birth ($p<.001$), a lower weight at birth ($p<.001$), whose mothers were younger ($p<.01$), and whose mothers were of lower education ($p<.01$).

RESULTS

Sample characteristics

Table 1 presents the baseline characteristics and child behavior scale scores at follow-up. The scores on the CBCL at baseline differed from the scores at 6 years: on average, internalizing scale scores of the CBCL increased, whereas externalizing scale scores decreased. The percentage of children with a CBCL subscale score in the clinical range varied from .5 to 2.3 %, as expected in a population based study. An overview of the correlations between predictor and outcome variables is presented in Supplementary Table 1.

The relation of infant physiology with internalizing problems during follow-up

Table 2 presents the longitudinal associations of infant resting heart rate and heart rate variability with the child CBCL Internalizing symptom score and the subscale scores at follow-up. In a fully adjusted model, we observed a positive association of infant heart rate with the child Internalizing symptom score at follow-up, which given the baseline adjustment, we interpret as change. A higher infant heart rate was associated with a stronger increase in Internalizing symptom and Anxious/depressed problem scores during follow-up. In addition, we observed a negative longitudinal association of infant heart rate variability with the child Anxious/depressed problem score. A lower infant heart rate variability was associated with a stronger increase of Anxious/depressed problem scores during follow-up. There was no longitudinal association between infant heart rate or heart rate variability and the Withdrawn problem score during follow-up.

To facilitate clinical interpretation, we also modelled our outcome dichotomously. The logistic regression analyses were consistent and indicate that our results were not caused by a skewed distribution of the outcome measures.

Table 1. Characteristics of the study population

	Original, non-imputed data	
	N	Mean (SD)[a]
Maternal education	461	3.97 (.99)
Secondary education or lower (%)		33.9
Higher education, phase 1 (%)		27.1
Higher education, phase 2 (%)		39.0
Maternal age at intake, years *(SD)*	464	32.0 (3.7)
Maternal smoking during pregnancy	436	1.32 (.67)
Never smoked during pregnancy (%)		79.8
Smoked until pregnancy was known (%)		8.5
Continued smoking in pregnancy (%)		11.7
Maternal alcohol during pregnancy	432	2.26 (.89)
Never used alcohol in pregnancy (%)		30.3
Used alcohol until pregnancy was known (%)		13.7
Alcohol use continued in pregnancy (%)		56.0
Child gender, male (%)	464	50%
Weight at birth, grams	464	3542 (528)
Gestational age at birth, weeks	464	40.1 (1.7)
Age child at physiological assessment, months	458	14.5 (.6)
Age child at follow-up, months	464	71.2 (3.1)
Heart rate, bpm	464	124.0 (10.7)
High frequency heart rate variability, ms^2	461	2.86 (.42)
CBCL Internalizing symptom score at baseline	430	3.96 (3.3)
CBCL Anxious/depressed problem score at baseline	432	.83 (1.0)
CBCL Withdrawn problem score at baseline	433	.52 (.78)
CBCL Externalizing symptom score at baseline	432	9.81 (6.4)
CBCL Aggressive behavior symptom score at baseline	432	7.89 (5.1)
CBCL Attention Problem score at baseline	433	1.92 (1.8)
CBCL Internalizing symptom score at follow-up	464	4.74 (4.5)***
CBCL Anxious/depressed problem score at follow-up	464	1.11 (1.4)***
CBCL Withdrawn problem score at follow-up	464	.97 (1.2)***
CBCL Externalizing symptom score at follow-up	464	6.55 (5.9)***
CBCL Aggressive behavior symptom score at follow-up	464	5.97 (4.9)***
CBCL Attention Problem score at follow-up	463	1.36 (1.6)***

Note. a=presented are mean and standard deviation, unless otherwise indicated, SD= standard deviation, ***= CBCL score at follow up differed from the same score at baseline with p<.001.

Table 2. Infant mean heart rate and heart rate variability as predictors of internalizing behavior during follow-up reported by the mother

	Internalizing symptom score					Anxious/depressed problem score					Withdrawn problem score				
	Beta	B	95% CI	$R^{2change}$	*p*	Beta	B	*95% CI*	$R^{2\ change}$	*p*	Beta	B	95% CI	$R^{2\ change}$	*p*
Heart rate (*N*=464)	.10	.046	.01, .08	.012	**.011**	.14	.021	.01, .03	.024	**.000**	.04	.005	-.005, .02	.002	.32
Heart rate variability (*N*=461)	-.05	-.55	-1.46, .37	.005	.24	-.10	-.39	-.68, -.11	.017	**.007**	-.03	-.09	-.34, .16	.001	.50

Note. Beta=standardized Beta, B= unstandardized Beta, 95%CI= 95% confidence interval for B, $R^{2\ change}$= R^2 for adding heart rate or heart rate variability to the model. $R^{2\ change}$ is based on original complete cases. All analyses were corrected for internalizing behavior at baseline, gestational age, weight at birth, age at physiological measurement, gender, maternal age at birth, maternal smoking and alcohol consumption during pregnancy, and maternal education.

The relation of infant physiology with externalizing problems during follow-up

Table 3 presents the longitudinal associations of infant resting heart rate and heart rate variability with the child CBCL broadband Externalizing symptom score and the subscale scores. Overall, there were no clear associations between infant heart rate and heart rate variability and change of the externalizing problems during follow-up, although heart rate variability showed a positive longitudinal association with the Attention Problem score during follow-up.

The relation of infant physiology with internalizing and externalizing problems during follow-up, mutually adjusted

We tested the specificity of infant heart rate and heart rate variability as predictors for the Internalizing symptom score or subscale scores and the Externalizing symptom score or subscale scores at follow-up, respectively, by mutually adjusting the broadband scales (Table 4). The addition of the broadband Externalizing symptom score to the fully adjusted model clearly strengthened the longitudinal associations of infant heart rate and heart rate variability with change of child Internalizing symptom and Anxious/depressed problem scores.

Table 3. Infant mean heart rate and heart rate variability as predictors of externalizing behavior during follow-up reported by the mother

	Externalizing symptom score					Aggressive Behavior symptom score					Attention Problem score#				
	Beta	B	95% CI	$R^{2\ change}$	*p*	Beta	B	*95% CI*	$R^{2\ change}$	*p*	Beta	B	95% CI	$R^{2\ change}$	*p*
Heart rate (*N*=464)	-.02	-.011	-.06, .04	.002	.65	-.01	-.004	-.04, .04	.001	.84	-.05	-.007	-.02, 006	.004	.27
Heart rate variability (*N*=461)	.08	1.16	-.03, 2.36	.006	.057	.06	.79	-.21, 1.78	.005	.12	.10	.38	.05, .70	.006	**.024**

Note. Beta=standardized Beta, B= unstandardized Beta, 95%CI= 95% confidence interval for B, $R^{2\ change}$= R^2 for adding heart rate or heart rate variability to the model. $R^{2\ change}$ is based on original complete cases. All analyses were corrected for externalizing behavior at baseline, gestational age, weight at birth, age at physiological measurement, gender, maternal age at birth, maternal smoking and alcohol consumption during pregnancy, and maternal education. #=analyses with attentional problems as outcome variable had *N*=463 for heart rate analyses and *N*=460 for heart rate variability analyses.

Also, clear longitudinal associations of infant heart rate and heart rate variability with the Externalizing symptom and subscale scores emerged when accounting for the concurrent level of internalizing symptoms. Lower infant heart rate was associated with a less strong decrease of the child Externalizing symptom, Aggressive Behavior and Attention Problem scores at follow-up. Similarly, higher infant heart rate variability was associated with a less strong decrease of child Externalizing symptom, Aggressive Behavior and Attention Problem scores at follow-up. The logistic regression analyses showed consistent associations. The results of these analyses are presented in Supplementary Table 2.

Table 4. Infant mean heart rate and heart rate variability as predictors of internalizing and externalizing behavior during follow-up reported by the mother, mutually adjusted

	Internalizing symptom score					Anxious/depressed problem score					Withdrawn pro		
	Beta	B	95% CI	$R^{2\ change}$	*p*	Beta	B	*95% CI*	$R^{2\ change}$	*p*	Beta	B	95% CI
Heart rate (*N*=464)	.12	.053	.03, .08	.019	**.000**	.15	.022	.01, .03	.031	**.000**	.06	.006	-.002, .02
Heart rate variability (*N*=461)	-.09	-1.12	-1.84, -.39	.015	**.003**	-.13	-.51	-.77, -.24	.028	**.000**	-.07	-.20	-.43, .02

	Externalizing symptom score					Aggressive Behavior symptom score					Attention Prob		
	Beta	B	95% CI	$R^{2\ change}$	*p*	Beta	B	*95% CI*	$R^{2\ change}$	*p*	Beta	B	95% CI
Heart rate (*N*=464)	-.09	-.049	-.09, -.01	.011	**.013**	-.08	-.036	-.07, -.004	.009	**.026**	-.09	-.013	-.025, -.001
Heart rate variability (*N*=461)	.11	1.67	.70, 2.64	.016	**.001**	.10	1.22	.42, 2.01	.014	**.003**	.12	.46	.15, .76

Note. Beta=standardized Beta, B= unstandardized Beta, 95%CI= 95% confidence interval for B, $R^{2\ change}$= R^2 for adding heart rate or heart rate variability to the model. $R^{2\ change}$ is based on original complete cases. All analyses were corrected for in-or externalizing behavior at baseline, gestational age, weight at birth, age at physiological measurement, gender, maternal age at birth, maternal smoking and alcohol consumption during pregnancy, and maternal education. The analyses were additionally adjuted for externalizing behavior at 6 years when the broadband Internalizing symptom score and the internalizing subscale scores were entered as outcome, and additionally adjusted for internalizing behavior at 6 years when the broadband Externalizing symptom score and the externalizing subscale scores were entered as outcome. #=analyses with attentional problems as outcome variable had *N*=463 for heart rate analyses and *N*=460 for heart rate variability analyses.

Principal findings

In this large general population cohort, lower infant heart rate was associated with a less strong decrease of childhood externalizing problems, whereas higher infant heart rate was associated with a stronger increase of internalizing problems. Moreover, heart rate variability results were consistent.

Our study emphasizes the importance of using a longitudinal design over a developmental sensitive period when studying early risk factors for the continuity of problems among preschool children. The mutual adjustment for internalizing and externalizing problems in our analyses created statistically homogeneous phenotypes, which enabled us to study the specificity of autonomic arousal as a predictor for change of internalizing or externalizing problems at follow-up in a large non-homogeneous general population sample of children in which co-occurrence of internalizing and externalizing problems was high.

Previous studies suggest that low heart rate may be a static genetically determined marker for later antisocial behavior (Ortiz, Raine, 2004). Our study suggests that both infant resting heart rate and heart rate variability are vulnerability factors for the later development of problem behavior. One could hypothesize that both ends of the extremes, namely physiological under- and overarousal, predisposes individuals to behavior to create homeostasis. Homeostatic mechanisms may lead underaroused individuals to engage in stimulation seeking or risky behaviors, while overaroused individuals avoid situations that are perceived as threatening or fearful as means of bringing their autonomic activity to more desirable optimal levels.

In a previous study in this sample a higher heart rate in infants predicted a higher Anxious/depressed problem score at the age of 3 years, but there was no association with the Aggressive Behavior problem score or the Attention Problem score at 18 months or 3 years (Dierckx et al., 2011; Dierckx et al., 2014). Explanations for the differences in results compared to the current study might lie in the developmental pathways of internalizing and externalizing problems. First, externalizing

problems tend to decrease beyond toddler age, while pure internalizing problems have a low prevalence at toddler age, but become more prevalent with increasing age (Gilliom, Shaw, 2004). Using latent profile analysis, the study by Basten et al. (2016) showed that a profile with predominantly internalizing problems was only discernible at 6 years, while at earlier ages internalizing problems were less prevalent, mild and typically accompanied by at least mild levels of externalizing problems. In contrast, a profile characterized by moderate externalizing problems and emotionally-reactive behavior was visible at all ages, but the prevalence of this profile was higher in infancy than in early childhood. Therefore, higher scores for externalizing problems are less common and arguably more deviant with increasing age. One could hypothesize that low autonomic arousal is a marker for more deviant externalizing problems that become more apparent in childhood. Second, at a younger age, externalizing behavior is most often reactive, triggered on impulse and by strong emotions, while proactive, planned aggression, which is often accompanied by low levels of emotional reactivity, develops later in life (Dierckx et al., 2014). In a cross-sectional study in school-aged children an increased resting heart rate variability was associated with pro-active aggression (Scarpa, Haden, Tanaka, 2010). Possibly, low autonomic arousal is a marker of pro-active, planned aggression which develops later in life and showed no association with the highly prevalent emotional reactive externalizing behavior seen infants and toddlers, while high autonomic arousal is a marker of early and persistent internalizing problems.

Interestingly, although part of the Internalizing problem scale of the CBCL/1½–5, the child Withdrawn problem score was not longitudinally associated with infant resting heart rate or heart rate variability. The Withdrawn problem score predicts not only internalizing problem behavior, but has consistently been associated with autism spectrum problems (Ooi et al., 2014) and consists of items that measure unresponsive and withdrawn behavior (Achenbach, Rescorla, 2000). Possibly, this type of behavior is not specifically predicted by autonomic under- or overarousal.

Strengths and limitations

This study has several methodological advances including the longitudinal design, the large population based sample, and the consideration of the co-occurrence of internalizing and externalizing problems in our statistical analyses. The measurement of autonomic functioning in infancy before the longitudinal assessment of internalizing and externalizing problems correcting for baseline decreases the possibility of reverse causation.

Some methodological issues need to be discussed. First, in the present study we used only maternal report for the assessment of problem behavior. However, a recent study by Ivanova et al. (2010) showed that parents are sufficiently capable of objectively rating their children's problem behavior using the CBCL/1½–5. In addition, although the outcome parameters were assessed with parent-report measures, all predictors were objective biological measures, hence there is no risk of shared method variance bias. Second, the Generation R Focus Cohort is ethnically homogeneous to rule out confounding and effect modification by ethnicity, which as a consequence limits the generalizability of our results to other ethnic populations. Third, although the participation rate in the present study was high (78%), there is a possibility of selection bias as the non-respondents had younger mothers, and mothers with a lower level of education. Finally, although our results show a consistent pattern of associations in the hypothesized directions based on previous literature, which makes the possibility of false positive results less plausible, given the number of performed statistical analyses the possibility of false positive results should be considered.

CONCLUSIONS

The present study suggests that low autonomic arousal is a marker for the development of more deviant, externalizing problems during childhood and shows less association with the highly prevalent

emotional reactive externalizing behavior seen in infants and toddlers, while high autonomic arousal might be a marker of early and persistent internalizing problems. Future studies may benefit from measures of externalizing problems that discern proactive, planned aggression and low emotional reactivity from more impulsive, reactive aggression accompanied by high levels of emotional reactivity.

In conclusion, our study shows that both infant heart rate and heart rate variability function as antagonistic predictors of early childhood internalizing and externalizing problems. In studying early risk factors for the continuity of problems among preschool children, we emphasize the importance of considering developmental changes in and the co-occurrence of internalizing and externalizing problems, because biomarkers that increase one type of problem behavior and decrease the other, such as autonomic functioning, might remain undetected when internalizing and externalizing problems are not disentangled.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Pearson's correlations between predictor and outcome variables

Variables	*N*=464	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.
1.Maternal age at intake																
2.Maternal education		.24**														
3.Maternal smoking during pregnancy		.02	-.18**													
4.Maternal alcohol use during pregnancy		.22**	.28**	.04												
5.Child gender		.04	.03	-.03	-.04											
6. Age child at physiological assessment		.02	.12*	-.05	.14**	-.01										
7.Gestational age at birth		.04	.11*	.03	.10*	-.05	.04									
8.Weight at birth		.01	.04	-.09	-.00	-.09*	-.02	.56**								
9.Heart rate		.06	-.01	.01	.05	.02	-.06	-.08	-.09							
10.Heart rate variability		.00	-.02	.01	-.03	.07	.04	.03	.04	-.66**						
11.CBCL Internalizing baseline		-.05	.00	.04	.06	-.03	.01	-.05	.02	.00	-.03					
12.CBCL Externalizing baseline		-.10*	-.03	.06	.07	-.18**	-.02	-.03	.01	-.03	.00	.56**				
13.CBCL Internalizing follow-up		-.14**	-.04	.06	-.06	-.10*	.02	-.05	-.05	.10*	-.07	.35**	.30**			
14.CBCL Anxious/depressed follow-up		-.15**	-.05	.07	-.07	-.02	.02	-.09	-.06	.15**	-.12**	.36**	.25**	.80**		
15.CBCL Withdrawn follow-up		-.09	-.05	.01	-.04	-.15**	.04	-.08	-.09*	.04	-.05	.26**	.21**	.69**	.47**	
16.CBCL Externalizing follow-up		-.10*	-.08	.06	.00	-.18**	.03	-.07	-.01	-.03	.07	.21**	.34**	.63**	.42**	.48**
17.CBCL Aggressive Behavior follow-up		-.07	-.04	.05	.01	-.14**	.03	-.06	.01	-.02	.06	.21**	.34**	.63**	.42**	.47**
18.CBCL Attention Problems follow-up		-.16**	-.15**	.07	-.02	-.22**	.00	-.09*	-.05	-.06	.09	.12**	.21**	.38**	.26**	.35**

Note. Pearson's correlations are calculated with imputed data,* p<.05, **p<.01 (2-tailed).

Supplementary Table 2. Infant mean heart rate and heart rate variability as predictors of dichotomized 80th percentile scores of internalizing and externalizing behavior during follow-up reported by the mother, mutually adjusted

	Internalizing problems			Anxious/depressed problems			Withdrawn problems		
	OR	95% CI	*p*	OR	*95% CI*	*p*	OR	95% CI	*p*
Heart rate (*N*=464)	1.83	1.37, 2.44	**.000**	1.90	1.42, 2.54	**.000**	.94	.74; 1.19	.61
Heart rate variability (*N*=461)	.52	.39, .70	**.000**	.51	.38, .69	**.000**	.88	.70, 1.11	.27

	Externalizing problems			Aggressive Behavior problems			Attention Problems		
	OR	95% CI	*p*	OR	*95% CI*	*p*	OR	95% CI	*p*
Heart rate (*N*=464)	.65	.48, .89	**.007**	.74	.55, .99	**.046**	.78	.60, 1.01	.065
Heart rate variability (*N*=461)	1.72	1.28, 2.33	**.000**	1.34	1.02, 1.77	**.039**	1.40	1.09, 1.80	**.009**

Note. OR= Odd's ratio, 95%CI= 95% confidence interval for OR. Predictors are standardized to facilitate the interpretation of the odd's ratios. OR's represent the odds of having a broadband In-/Externalizing symptom score or internalizing/externalizing subscale score in the highest 20th percentile when the predictor changes by a standard deviation. An OR of less than one indicates a negative association of the predictor with their odds of having a broadband In-/Externalizing symptom score or internalizing/externalizing subscale score in the highest 20th percentile. An OR of more than one indicates a positive association of the predictor with their odds of having a broadband In-/Externalizing symptom score or internalizing/externalizing subscale score in the highest 20th percentile. To facilitate the interpretation of the odd's ratios, z-scores were calculated for continuous predictors. All analyses were corrected for in-or externalizing behavior at baseline, gestational age, weight at birth, age at physiological measurement, gender, maternal age at birth, maternal smoking and alcohol consumption during pregnancy, and maternal education. The analyses were additionally adjusted for externalizing behavior at 5 years when the broadband Internalizing scale and the subscales were entered as outcome, and additionally adjusted for internalizing behavior at 5 years when the broadband Externalizing scale and the subscales were entered as outcome.

PART III

Physiological stress activity in children with an anxiety disorder

Chapter 5

Alterations in HPA-axis and autonomic nervous system functioning in childhood anxiety disorders point to a chronic stress hypothesis

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ABSTRACT

Background: It is of debate whether or not childhood anxiety disorders (AD) can be captured by one taxonomic construct. This study examined whether perceived arousal (PA), autonomic nervous system (ANS) and hypothalamic-pituitary-adrenal (HPA) axis measures can distinguish children with different primary diagnoses of clinical anxiety disorders (AD) from each other, and from a general population reference group (GP).

Methods: The study sample consisted of 152 AD children (comparing separation anxiety disorder, generalized anxiety disorder, social phobia and specific phobia), aged 8- to 12 years, and 225 same-aged reference children. HPA-axis functioning was measured by a diurnal cortisol profile. ANS functioning was measured by continuous measures of skin conductance level in rest and during a mental arithmetic task and high frequency heart rate variability in rest. PA was assessed by a questionnaire.

Results: The AD sample showed lower high frequency heart rate variability during rest, heightened anticipatory PA, higher basal and reactive skin conductance levels and lower basal HPA-axis functioning compared to the GP sample. The existence of 3 or more clinical disorders, i.e. a high clinical 'load', was associated with lower basal HPA-axis functioning, higher skin conductance level and lower posttest PA. Specific phobia could be discerned from social phobia and separation anxiety disorder on higher skin conductance level.

Conclusions: Our findings indicated that children with AD have specific psychophysiological characteristics, which resemble the psychophysiological characteristics of chronic stress. A high

clinical‘load’ is associated with an altered ANS and HPA-axis functioning. Overall, ANS and HPA-axis functioning relate to AD in general, accept for specific phobia.

INTRODUCTION

Anxiety disorders (AD) are among the most prevalent psychiatric disorders in children and adolescents (e.g. Costello, Egger, Angold, 2005). They are associated with impaired functioning, such as school dropout, social isolation, alcoholism, and suicide attempts, and an increased risk for developing other psychiatric disorders (Bittner et al., 2007). Hence, research into causes and correlates of childhood anxiety disorders is imperative to improve treatment and prognosis for children affected by these disorders.

A complicating issue is whether distinctions between separate anxiety disorders in children can be made. High comorbidity rates between AD have been reported (e.g. Beesdo, Knappe, Pine, 2009). This underlines the hypothesis of one taxonomic construct capturing childhood anxiety disorders. However, substantial heterogeneity exists in the age of onset of specific anxiety disorders (Beesdo et al., 2010), providing, in terms of taxonomy, an indicator to separate different types of anxiety disorders (Beesdo, Knappe, Pine, 2009). Furthermore, age is also related to the clinical 'load'; i.e. the number of anxiety disorders increases with age. Anxiety load is significantly associated with poorer outcome (Woodward, Fergusson, 2001). One could argue that the causes and correlates of a high anxiety 'load' may differ from those of a low anxiety 'load'.

One way to test the taxonomic construct of AD is to study the physiological correlates of childhood anxiety disorders, i.e., the activity of the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS), two major physiological stress systems. During non-stress conditions the HPA-axis shows a diurnal pattern of cortisol secretion, with peak levels approximately 30 minutes after waking up and a subsequent decline during the day (Wust et al., 2000). Glucocorticoids can act both to augment and suppress sympathetically mediated changes in e.g. cardiovascular function, metabolism, and immune function. Normally, activation of these stress systems leads to behavioral and

physical adaptive changes that improve an organism's ability to survive. However, children with an anxiety disorder may perceive the world as full of stressors that demand endless vigilance and coping, with no possibility to relax and to regard things as safe (Sapolsky, 2002). It can be hypothesized that these children function under conditions of persistent stress, with an excessive and prolonged stress system activation. This would result in increased and prolonged production of hypothalamic corticotropin releasing factor (CRF), cortisol and catecholamines (Pervanidou, 2008), which, in turn, could result in hypoactivation of the HPA-axis, as a compensatory down-regulation (Charmandari, Tsigos, Chrousos, 2005).

Studies regarding the association between anxiety disorders and basal HPA-axis functioning in children are scarce and findings are inconclusive. Forbes et al. (2006) found higher peri-sleep-onset cortisol levels in children with an anxiety disorder compared to children with a depression or healthy control children, whereas Feder et al. (2004) found that anxious children exhibited significantly lower nighttime cortisol levels and a delayed rise in cortisol during the nighttime when compared to depressed and healthy children. In other studies no association was found (e.g. Terleph et al., 2006; Greaves-Lord et al., 2007; Dieleman et al., 2010). However, Greaves-Lord et al. (2007) reported that young adolescents with persistent anxiety problems had higher morning cortisol levels and a higher awakening response.

Several problems may have contributed to the inconsistency of these findings. The groups under study were dissimilar in methods of sampling, age, developmental status, and diagnostic status. Some studies used general population samples (e.g. Greaves-Lord et al., 2007), whereas others focused on clinical groups (e.g. Forbes et al., 2006), which are likely to display major differences in symptom severity and comorbidity. Next to that, differences in functioning of the HPA-axis could depend on the stage of the disorder (Pervanidou, 2008).

Overall, research on ANS functioning in children with anxiety problems shows an increased activity of the sympathetic nervous system (e.g. Dietrich et al., 2007; Schmitz et al., 2011; Kossowsky et al., 2012) and a decrease of parasympathetic control (e.g. Schmitz et al., 2011). Furthermore, previous studies suggest that high anxious adults have an increased perception of physiological sensations (e.g. Hoehn-Saric, McLeod, 2000), i.e. increased perceived arousal, sometimes even in the absence of an actual difference in physiological measures (e.g. Edelmann, Baker, 2002).

At present, it is still unclear as to what extent ANS or HPA-axis activity relate to anxiety in general, or whether they are specific correlates of the different types of anxiety disorders. Few studies have compared the endocrine and autonomic profiles between different pediatric anxiety disorders, and if so the focus was on one specific anxiety disorder with a disorder-specific stimulus (i.e. Kossowsky et al., 2012). To our knowledge this is the first study to test whether anxiety load is associated with altered HPA-axis and ANS functioning.

The present study's aim was to test whether HPA-axis (basal), ANS (basal and reactive) and perceived arousal (PA) measures can distinguish children (N=154) aged 8-12 years with different primary diagnoses (separation anxiety disorder (SAD), generalized anxiety disorder (GAD), social phobia (SoPh) and/or specific phobia (SpPh)) of clinical anxiety disorders (AD) from each other, and from a same-aged general population reference group (GP) (N=225). We hypothesized that children in the clinical anxiety sample show 1) elevated sympathetic and lowered parasympathetic activity compared to the general population sample. 2) Furthermore, we expected a pattern of hypoactivation of basal HPA-axis functioning, considering the severity and chronicity of perceived stress in a clinical AD sample, when compared to a general population reference group. 3) Within the AD sample we hypothesized that there would be no difference in activity of both ANS and HPA-axis between different types of anxiety disorders, because of the known high rates of comorbidity in childhood anxiety disorders. 4) Finally, we hypothesized that children with a high anxiety load in the clinical sample show hypoactivation of basal

HPA-axis functioning, have higher sympathetic functioning and lower parasympathetic functioning, and higher perceived arousal in rest and stress conditions when compared to children with a low anxiety load.

METHODS

Participants

Eligible for participation in the clinical anxiety disorder group (AD) were N=152 children aged 8 to 12 years diagnosed with a primary diagnosis of separation anxiety disorder (SAD; N=57), generalized anxiety disorder (GAD; N=47), social phobia (SoPh; N=29) or specific phobia (SpPh; N=19), who had been referred to the outpatient clinic of the department of Child and Adolescent Psychiatry of either Erasmus MC in Rotterdam or Curium-LUMC in Leiden, The Netherlands. All children were diagnosed with the Anxiety Disorders Interview Schedule for DSM-IV (ADIS-C; Silverman, Albano, 1996).

The general population (GP) sample, consisting of 202 participants aged 8 to 12 years, was drawn from a larger general population sample study (Tick, Van der Ende, Verhulst, 2007). Study characteristics of the 2 groups are presented in Tables 1 and 2. All children were diagnosed with the Diagnostic Interview Schedule for Children-Parent version (DISC-P; Shaffer, Fisher, Lucas, 1998).

Exclusion criteria for both the AD and GP group were: IQ < 85, poor command of the Dutch language, serious physical disease, pervasive developmental disorder, selective mutism, schizophrenia or other psychotic disorders, pharmacotherapy that could interfere with HPA-axis or ANS functioning or pharmacotherapy aimed at treating anxiety or depressive symptoms. Methylphenidate treatment in children with ADHD was discontinued the day before and on the day of measurements (AD N=7, GP N=6) because methylphenidate treatment can increase heart rate and blood pressure (Ballard et al., 1976).

The Committees for Medical Ethics of Erasmus MC and LUMC approved the study.

Procedure

Parents and participants signed informed consent before participation. Prior to the physiological assessment, parents and children completed psychological questionnaires. To assess the diurnal cortisol profile, participants collected 4 saliva samples at home by filling tubes till a marker (at 500 μl): (1) a first sample immediately after awakening in the morning (Cort1), when the child was still in bed, (2) 30 minutes later (Cort2), (3) at 12h (Cort3), and (4) at 20h (Cort4). All samples were collected on a single regular school day, stored in the freezer at home, and taken to the assessment at the outpatient department one day later. Parents were asked to register general physical condition, activity levels, consumption pattern, and medication use of their child.

The physiological assessment took place in the hospital between 12h and 1830h. Participants were seated comfortably in a laboratory room where temperature and humidity levels were kept constant. Lunch was eaten before the assessment; the gap between lunch and baseline saliva samples was at least 1.5 h. After an acclimatization period of 45 minutes, the session began with a baseline period of 10 minutes in which the participant was asked to sit still and relax. Subsequently, a mental arithmetic task (MAT) was administered. At the end of the baseline period and after the MAT, a questionnaire concerning physiological arousal (PAQ) was administered. A cortisol sample was collected after the baseline period (Cort5), to have a second basal cortisol measure next to the diurnal profile on a different day.

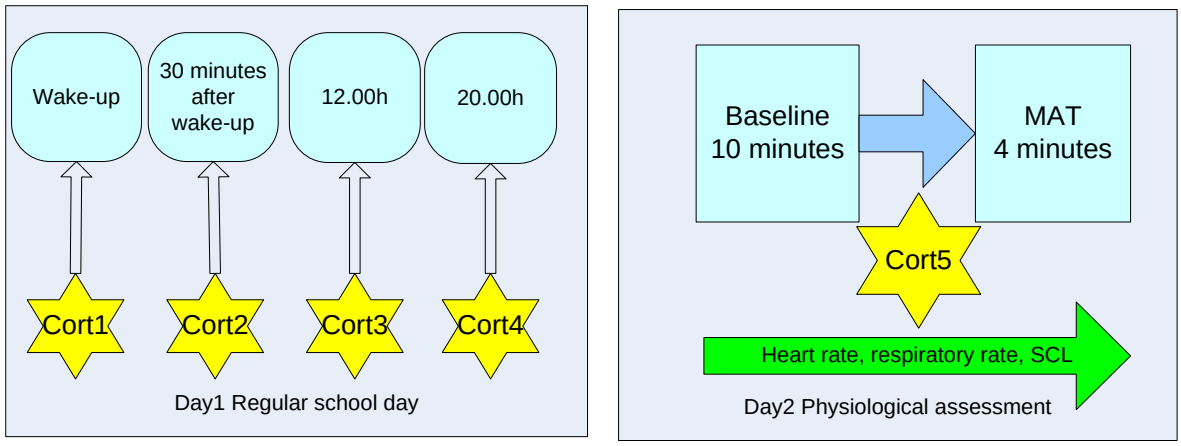

Figure 1 Procedure
Note. Cort1-Cort2-Cort3-Cort4-Cort5 = Cortisol sample 1-5, SCL= Skin conductance level, MAT=Mental arithmetic task.

Measures

Assessment of diagnostic status and symptom severity

Anxiety Disorders Interview Schedule for DSM-IV (AD-group)

ADIS-C (Silverman, Saavedra, Pina, 2001) is a semi-structured interview to assess DSM-IV AD in 7- to 17-year-olds. A trained psychologist conducted the interview with the child and parents separately. To obtain a diagnosis, both a symptom count of DSM-IV symptom criteria, as well as level of impairment according to the parent, child, and interviewer, are taken into account.

Diagnostic Interview Schedule for Children-Parent version (GP group)

The DISC-IV- P/C is a highly structured respondent-based psychiatric interview to assess DSM-IV disorders found in children. The DISC-IV-P showed good inter-rater and test-retest reliability (Shaffer, Fisher, Lucas, 1998; Shaffer et al., 2000). Last year's diagnoses were calculated according to the DISC-IV-P diagnostic algorithm.

Child Behavior Checklist

The CBCL is a parent questionnaire for assessing problems in 4- to 18-year-olds. It contains 120 items on behavioral or emotional problems during the past 6 months. The response format is 0 = not true, 1 = somewhat or sometimes true, and 2 = very true or often true. The good reliability and validity of the original version of the CBCL (Achenbach, Rescorla, 2001) were confirmed for the Dutch translation (Verhulst, Van der Ende, 2013). Scores on two broad-band scales were computed: Internalizing and Externalizing.

Multidimensional Anxiety Scale for Children

The MASC is a 39-item self-report questionnaire that assesses anxiety symptoms concerning the last two weeks in children. Items were scored from 0 to 3 (0 = never true, 1 = rarely true, 2 = sometimes true, 3 = often true). The internal consistency (.93) and test-retest reliabilities (.81) are very good (March et al., 1997; Liber, 2008).

Children's Depression Inventory

The CDI is a 27-item self-report questionnaire that assesses depressive symptoms concerning the last two weeks in children. Items are scored from 0 to 2 (0 = never true, 1 = sometimes true, 2 = always true). The internal consistency (.82) (Liber, 2008) and 1-month test-retest reliabilities (.72) are adequate (Kovacs, 1981).

Stress task

The Mental Arithmetic Task was applied as a standardized laboratory stress task to induce measurable physiological changes (Kirschbaum, Pirke, Hellhammer, 1993). The MAT lasted 4 min, during which the child was asked to subtract numbers as quickly and accurately as possible. If the child made a mistake or

did not respond, he or she was required to start all over again. Dependent on the child's age, the child was asked to subtract 7 from 100 (<12 years), or 23 from 1021 (≥12 years), and so on.

Physiological and hormonal measures

Cortisol assessment

The laboratory that analyzed our cortisol samples switched from solid-phase radioimmunoassay (RIA, Diagnostic Products Corporation, Los Angeles) to Enzyme-Linked Immuno Sorbent Assay (ELISA, DRG-kits, Marburg, Germany) during data collection. Thirty-one cortisol samples were analyzed by both RIA and ELISA. Correlation between both assays was high (R=.994878). The slope was not equal to 1 so the concentrations in the DRG standards were adjusted by the laboratory to guarantee equal results. Cortisol samples of eighty-three children of the AD sample were analyzed by solid-phase radioimmunoassay (Kallen et al., 2008). An Enzyme-Linked Immuno Sorbent Assay (DRG Instruments GmbH, Marburg, Germany) was used to determine cortisol concentrations in 50 µl duplicate samples for the rest of the samples. For technical specifics see (Dieleman et al., 2010). Cortisol values that were above 3 SD of the mean were excluded from the analysis to reduce the impact of outliers.

In addition to the separate cortisol variables, we used composite cortisol variables to analyze the diurnal profile. We calculated three composite measures according to Pruessner et al. (2003): cortisol awakening rise with respect to ground (CAR_g), with respect to increase (CAR_i) and the area under the curve with respect to ground (AUC_g). The CAR_g and CAR_i were calculated using the following formulas: $CAR_g = ((Cort1+Cort2) * time_{cort2-cort1})/2$, $CAR_i = CAR_g – (Cort1 * time_{cort2-cort1})$. The formula to calculate AUC_g was: $AUC_g = AUC_{cort2-cort3}+AUC_{cort3-cort4} = ((Cort2+Cort3) * time_{cort3-cort2})/2 + ((Cort3+Cort4) * time\ time_{cort4-cort3})/2$. If one of the cortisol samples was missing, an area under the curve could not be calculated. Therefore for the GP group, N=13 CAR_g (6.4%), N=13 CAR_i (6.4 %) and N=25 AUC_g (12.4%), for

the AD group, N=17 CAR_g (11.0%), N=21 CAR_i (13.6%) and N=16 AUC_g (10.4%), were missing and excluded for those specific analyses.

Autonomic measures

During the experiment, continuous measurements were made of heart rate (Stratakis, Chrousos, 1995), respiration rate (RESP) and skin conductance level (SCL). HR was recorded using a 3-lead ECG, sampled at 512 Hz. SCL was used as a measure of the sympathetic function of the ANS and was assessed using two adhesive disposable active Ag/AgCl electrodes attached to the volar surfaces of the medial phalanges of the index and ring fingers of the non-dominant hand. SCL was sampled at 8 Hz, and stored in µS. RESP was measured using an inductive plethysmography method (belts containing a magnetic coil, Respitrace™, TEMEC Respiratory Inductive Plethysmography system; TEMEC Instruments B.V., Kerkrade, The Netherlands), sampled at 8 Hz.

Physiological Arousal Questionnaire

The PAQ is a 7-item self-report questionnaire developed at our department for assessment of the perceived state of physiological arousal. The child was asked to indicate on a 9-point scale (0-8) to what extent he or she felt aroused: 0 = not at all to 8 = very much. Cronbach's alpha was .64 (during baseline), .81 (during stress). For a description in detail see Dieleman et al. (2010).

Analysis of physiological data

HRV during rest

A customised software program calculated the interbeat intervals (IBI's) of the ECG using R-top detection, resulting in IBI time series during rest (3 min period of stationary signal; minutes 7-10 of resting period). These time series were inspected and artifacts were removed.

The IBI time series during the 3 min period of rest was further subjected to a discrete Fourier transform, based on non-equidistant sampling of the R-wave incidences (CARSPAN program, Groningen, The Netherlands (Mulder et al., 1988; Van steenis, Tulen, Mulder, 1994), to yield power spectra of the rhythmic oscillations over a frequency range of 0.02–0.50 Hz, with a resolution of 0.01 Hz. For each time segment, the power was calculated for the high-frequency band (HF: 0.14-0.50 Hz), in addition to mean HR and respiration rate. HF HR variability is a commonly used proxy for the vagal component of autonomic cardiac control. Because the HF of HR variability is strongly correlated with respiratory sinus arrhythmia (Kamath, Fallen, 1993), respiratory frequency was monitored separately to control for this effect. Time segments with more spectral power for respiration in the MF band than in the HF band were discarded from HF HR variability analyses. This resulted in the removal of the observations of 3 children from the GP group and 1 from the AD group. HF HR variability was not calculated during the MAT because of breathing irregularities as a consequence of speaking.

Data Analysis

All statistical analyses were performed with SPSS 20.0. For descriptive purposes, means and standard deviations were calculated based on untransformed variables. For further analyses, measures of SCL, Cort1-5, HF HR variability, perceived arousal, CDI Total score and scores on the internalizing and externalizing scales of the CBCL were log-transformed to approach a normal distribution. Subsequently, stress responses in perceived arousal and SCL were calculated as the level during the MAT minus the pretest level for each of these measures. Age, gender and BMI might confound the cortisol-psychopathology relationship (e.g. see Rosmalen et al. (2005) for an overview). To control for possible effects of age, gender and BMI, these variables were entered as covariates. Effect sizes are reported as partial Eta squared, with .01 defined as a small effect size, .06 as a medium effect size and .14 as a large effect size (Cohen, 1988).

First, we investigated whether there was a difference between the AD and GP group in HPA-axis functioning using four two-way between groups ANCOVAs with sex and group as factors and age and BMI as covariates. The dependent variables were CAR_g, CAR_i, AUC_g and Cort5 (after the baseline period). These analyses were repeated with the addition of the externalizing scale of the CBCL and the CDI Total score as covariates to control for the effects of comorbid behavior problems and depressive symptoms. Subsequently, a two-way MANCOVA with group and sex as factors, BMI and age as covariates, and Cort1-4 as dependent variables was conducted.

Further, to analyze if there was a difference between the GP and AD group in autonomic functioning levels during rest, group differences in pretest levels (PAQ, HF HR variability and SCL) and in difference scores between pre- to post/during test levels (PAQ and SCL) corrected for pretest levels, were analyzed using ANCOVAs. All analyses were adjusted for age, gender, and BMI. These analyses were repeated with the addition of the externalizing scale of the CBCL and the CDI Total score as covariates to control for the effects of comorbid behavior problems and depressive symptoms.

Third, to investigate if 'anxiety load' was associated with HPA-axis functioning and autonomic functioning, the AD group was subsequently divided into three groups: a group with one anxiety disorder, a group with two anxiety disorders and a group with three or more anxiety disorders. The analyses as described above for the comparison AD versus GP group were repeated for the comparison between the three groups with a different anxiety load. Posthoc comparisons tests were used to explore which of the three groups were significantly different from each other.

Fourth, to investigate if 'clinical load', which takes comorbidity (Depression, Dysthymic disorder, ADHD, ODD and CD) into account as well, was associated with HPA-axis functioning and autonomic functioning, the AD group was subsequently divided into three groups: a group with one clinical disorder, a group with two clinical disorders and a group with three or more clinical disorders. The analyses as described above for the comparison AD versus GP group were repeated for the comparison

between the three groups with a different clinical load. Posthoc comparisons tests were used to explore which of the three groups were significantly different from each other.

Finally, in order to investigate whether different anxiety disorders have different endocrine or autonomic profiles the AD group was divided into four groups. Groups were based on the anxiety diagnosis with the highest severity score on the ADIS-C according to the clinician. This resulted in groups with, respectively, a main diagnosis of SAD, GAD, SoPh and SpPh. The analyses as described above for the comparison AD versus GP group were repeated for the comparison between the four groups with a different anxiety disorder. Posthoc comparisons tests were used to explore which of the four groups were significantly different from each other.

RESULTS

Table 1. Sample characteristics of AD and GP sample and comorbidity

Measures	**AD** (N=152) Mean (SD)	**GP** (N=200) Mean (SD)
Age	10.2 (1.5)	10.1 (1.5)
Gender (♀, ♂)	46.8%, 53.2%	50%, 50%
BMI	17.9 (4.9)	18.1 (3.2)
$CBCL_{intern}$***	20.6 (9.0)	7.3 (5.8)
$CBCL_{extern}$***	11.4 (7.9)	5.9 (5.9)
MASC Total score***	51.37 (17.72)	42.08 (14.09)
CDI Total score***	9.61 (7.04)	6.41 (5.49)
No medication in last two months#	60.4%	73.8%
Time of awakening	7.15 (0.5)	7.03 (0.85)
Percentage menarche	7.1% of girls	5.9% of girls
Diagnoses	**AD** Main diagnosis ADIS-C /Count anxiety diagnosis ADIS-C N (% of total sample)/N	**GP** Count anxiety diagnosis DISC-P N (% of total sample)
GAD	47 (30.5)/75	0 (0)
SoPh	29 (18.8)/49	2 (1.0)
SAD	57 (37.0)/46	2 (1.0)
SpPh	19 (12.3)/48	25 (12.4)
PD	-	-
OCD	-	-
PTSD	-	-
	Other diagnosis ADIS-C N (% of total sample)	Other diagnosis DISC-P N (%)
ADHD	15 (9.7)	17 (8.4)
ODD	10 (6.5)	15 (7.4)
CD	3 (1.9)	1 (0.5)
DD	2 (1.3)	-
DysD	8 (5.2)	-

Note. GP = general population sample, AD = clinical anxiety disorder sample, SD = standard deviation, BMI = Body Mass Index, $CBCL_{intern}$ = Internalizing scale of Childhood Behavior Checklist, $CBCL_{extern}$ = Externalizing scale of Childhood Behavior Checklist, MASC = Multidimensional Anxiety Scale for Children, CDI = Children's Depression Inventory, ADIS-C= Anxiety Disorders Interview Schedule for DSM-IV, DISC-P= Diagnostic Interview Schedule for Children-Parent version, GAD = generalized anxiety

disorder, SoPh = social phobia, SAD = separation anxiety disorder, SpPh = specific phobia, PD = panic disorder, OCD = obsessive compulsive disorder, PTSD = posttraumatic stress disorder, ADHD = attention deficit hyperkinetic disorder, ODD = oppositional defiant disorder, CD = conduct disorder, DD = depressive disorder, DysD = dysthymic disorder. *= p<.05, **=p<.01, ***=p<.001. # For specification of medication use in the last two months see supplementary Figure 1.

Sample characteristics (see Tables 1-3)

Scores on the internalizing and externalizing scale of the CBCL, the CDI Total score and MASC Total score were significantly higher in the AD group compared to the GP group. Correlations between psychiatric and physiological measures are presented in Tables 2 and 3. MASC Total score is significantly negatively correlated with CAR_i; high levels on the MASC Total score are associated with a low CAR_i in the AD group.

Table 2. Pearson correlations between physiologic and psychiatric measures in the anxiety disorder group

	MASC total Score	CDI total score	CBCL internalizing scale	CBCL externalizing scale	CAR_g	CAR_i	AUC_g	HF HR	SCL baseline	SCL during test
MASC Total Score	1	.419**	.191**	.002	-.01	-.189*	-.025	-.066	-.095	-.036
CDI Total score		1	.297**	.235**	-.026	-.137	-.074	-.103	-.020	.034
CBCL internalizing scale			1	.498**	.086	.016	-.078	-.043	-.004	.041
CBCL externalizing scale				1	.048	-.052	-.160	-.060	.124	.172*
CAR_g					1	.164	.400**	-.012	.064	.071
CAR_i						1	.149	-.032	-.043	.006
AUC_g							1	.071	-.146	-.142
HF HR								1	-.100	-.127
SCL baseline									1	.923**
SCL during test										1

Note. CBCL internalizing = Internalizing scale of Childhood Behavior Checklist, CBCL externalzing = Externalizing scale of Childhood Behavior Checklist, MASC = Multidimensional Anxiety Scale for Children, CDI = Children's Depression Inventory, CAR_g = cortisol awakening rise with respect to ground, CAR_i = cortisol awakening rise with respect to increase, AUC_g = area under the curve for Cort2-Cort4 with respect to ground, SCL = skin conductance level, HF HR= high frequency heart rate variability,*= p<.05, **=p<.01.

Table 3. Pearson correlations between physiologic and psychiatric measures in the general population group

	MASC total Score	CDI total score	CBCL internalizing scale	CBCL externalizing scale	CAR_g	CAR_i	AUC_g	HF HR	SCL baseline	SCL during test
MASC Total Score	1	.468**	.412**	.223**	-.03	-.123	-.015	-.012	.024	-.052
CDI Total score		1	.469**	.429**	-.010	.109	.070	.055	-.012	-.022
CBCL internalizing scale			1	.545**	.053	.097	.118	-.023	-.113	-.096
CBCL externalizing scale				1	-.042	.126	-.038	-.038	.003	.011
CAR_g					1	.096	.574**	-.063	-.056	-.103
CAR_i						1	.207**	.002	.065	-.009
AUC_g							1	.033	-.095	-.031
HF HR								1	-.088	-.058
SCL baseline								¤	1	.836**
SCL during test										1

Note. CBCL internalizing = Internalizing scale of Childhood Behavior Checklist, CBCL externalzing = Externalizing scale of Childhood Behavior Checklist, MASC = Multidimensional Anxiety Scale for Children, CDI = Children's Depression Inventory, CAR_g = cortisol awakening rise with respect to ground, CAR_i = cortisol awakening rise with respect to increase, AUC_g = area under the curve for Cort2-Cort4 with respect to ground, SCL = skin conductance level, HF HR= high frequency heart rate variability,*= p<.05, **=p<.01

Daytime cortisol measures (see Table 4)

Both the AD and GP group included several subjects with negative values for CAR_i (GP: 24.8%, AD 21.4%). There were no differences in cortisol awakening measures between both groups. CAR_g was lower for boys (mean=7.66, SD=2.12) compared to girls (mean=8.46, SD=2.21) ($F=7.31(1, 288)$, $p<.01$, partial Eta squared=.025 (small)).

For AUC_g, there was a highly significant between groups difference (see Figure 2). The difference in AUC_g between the AD group and the GP group was determined by lower values of Cort3 and Cort4 (see Table 4) for the AD group. The same pattern was seen for baseline cortisol levels preceding the stress task. These results remained significant after correcting for externalizing behavior and depressive symptoms. Effect sizes varied between medium to large effects (partial Eta squared .11-.25).

To check that our significant results were not due to medication effects, we did a secondary analysis only for children that did not use any medication. Results indicated that our findings were not influenced by the use of medication.

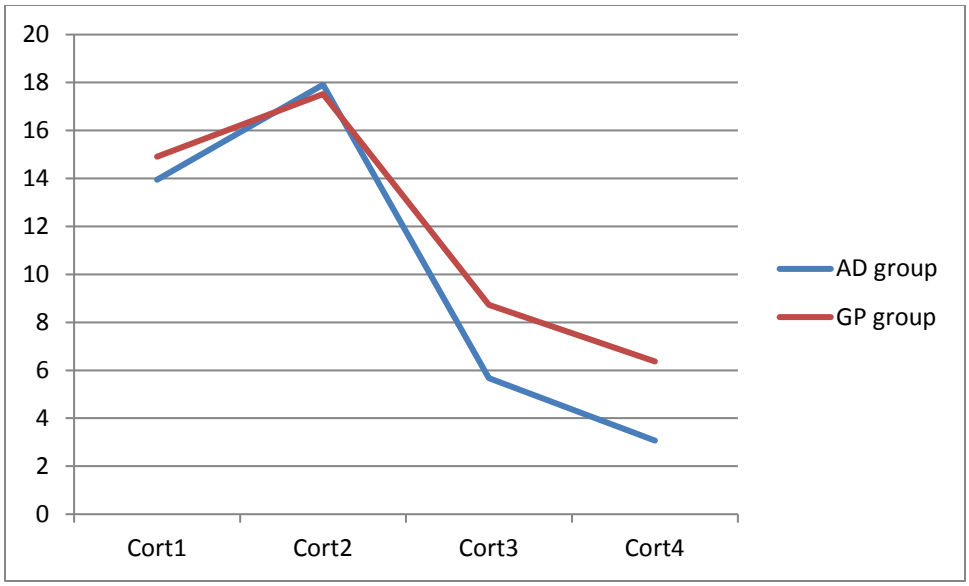

Figure 2. Cortisol diurnal profile

Note. GP = general population group, AD = anxiety disorder group, Cort1 = cortisol directly after awakening, Cort2 = cortisol half an hour after awakening, Cort3 = cortisol at 12.00 p.m., Cort4 = cortisol at 8.00 p.m.

Baseline and stress response measures of perceived arousal and autonomic functioning

The MAT elicited a significant increase in PAQ Total score in the GP group ($T = 5.66$, $p < .001$), thus reflecting a perceived physiological stress response. At pretest, the AD group had significantly higher levels of perceived arousal and SCL, and lower levels of HF HR variability when compared to controls (see Table 4). During the stress task, AD children had a higher difference in SCL when compared to controls. SCL during stress was significantly higher in the AD group (see Figure 3). These results remained significant after correcting for externalizing and depressive symptoms. Effect sizes varied between .01-.16, with medium to large effects for SCL and small effects for perceived arousal and HF HR variability.

Table 4. Means, as well as group differences in baseline HPA measures and in baseline and reactive autonomic and perceived arousal measures

Variable	**AD group** ***Mean (SD-N)***	**GP group** ***Mean (SD-N)***	***Group differences#*** *((M)ANCOVA'S//partial Eta squared)*	***Group differences##*** *((M)ANCOVA'S//partial Eta squared)*	***Group differences###*** *((M)ANCOVA'S//partial Eta squared)*
CAR_i	.84 (1.90-117)	.69 (1.44-184)	ns	ns	ns
CAR_g	7.86 (2.44-116)	8.11 (2.04-184)	ns	ns	ns
AUC_g	87.7 (28.9-111)	119.1 (31.7-172)	F(1,277) = 67.95***//.20	F(1,271) = 48.34***//.15	F(1, 268)=57.49***
Cort1-4			F(1,274) = 22.88***//.25	F(1,268) = 15.73***//.19	F(1,265) = 19.06***//.22
Cort1(nmol/l)	13.95 (5.14)	14.91 (4.84)	ns	ns	ns
Cort2(nmol/l)	17.91 (6.58)	17.52 (5.26)	ns	ns	ns
Cort3(nmol/l)	5.67 (2.73)	8.73 (2.95)	F(1,274) = 56.62***//.17	F(1,271) = 36.15***//.12	F(1,268) = 44.31***//.14
Cort4(nmol/l)	3.07 (3.00)	6.37 (2.82)	F(1,274) = 85.95***//.24	F(1,271) = 59.20***//.18	F(1,268) = 72.28***//.21
Cort5(nmol/l)	5.74 (4.24-120)	7.76 (2.56-191)	F(1,305) = 46.02***//.13	F(1,298) = 36.86***//.11	F(1,295) = 37.33***//.11
PAQ pretest	.88 (.34-146)	.76 (.35-198)	F(1,339) = 9.01**//.03	F(1,332) = 6.82*//.02	ns
PAQ posttest	.92 (.33-147)	.87 (.36-199)	ns	ns	ns
PAQ diff	.04 (.23-145)	.11 (.28-198)	ns	ns	ns
SCL (µS) baseline	.54 (.26-121)	.39 (.16-193)	F(1,308) = 37.26***//.11	F(1,301) = 31.40***//.09	F(1,301) = 31.40***//.09
SCL (µS) during test	.67 (.27-120)	.48 (.15-189)	F (1,303)=55.0***//.15	F (1,296)=43.68***//.13	F (1,294)=48.06***//.14
SCL (µS) diff	.13 (.10-119)	.09 (.09-189)	F(1,301)=17.46***//.06	F(1,294)=12.62***//.04	F(1,292)=14.15***//.05
HF HR	3.40 (0.42-119)	3.57 (0.42-192)	F(1,305) = 10.78**//.03	F(1,298) = 7.11**//.02	F(1,298) = 10.24**//.03

Note. SD = standard deviation, GP = general population group, AD = anxiety disorder group, Cort1 = cortisol directly after awakening, Cort2 = cortisol half an hour after awakening, Cort3 = cortisol at 12.00 p.m., Cort4 = cortisol at 8.00 p.m., Cort5 = cortisol during baseline, CAR_g = cortisol awakening rise with respect to ground, CAR_i = cortisol awakening rise with respect to increase, AUC_g = area under the curve for Cort2-Cort4 with respect to ground. SCL = skin conductance level, HF HR= high frequency heart rate variability, PAQ = Perceived Arousal Questionnaire. #covariates: age, sex, bmi. ## covariates: age, sex, bmi, CBCL externalizing behavior, ### covariates: age, sex, bmi, CDI Total score. *= p<.05, **=p<.01, ***=p<.001.

To check that our significant results were not due to medication effects, we did a secondary analysis only for children that did not use any medication. Results indicated that our findings were not influenced by the use of medication.

Comorbidity

As shown in Table 5 comorbidity rates are high; 55.3% had a single clinical anxiety disorder, 30.9% had two clinical anxiety disorders, 11.8% had three clinical anxiety disorders and 2.0% had four clinical anxiety disorders. In the group with a main diagnosis of SAD the proportion of children with three or more clinical anxiety disorders was relatively high. SpPh is more often a comorbid than a main diagnosis, whereas SAD is more often a main than a comorbid diagnosis. One third of children with a main diagnosis of SoPh have a comorbid diagnosis of GAD.

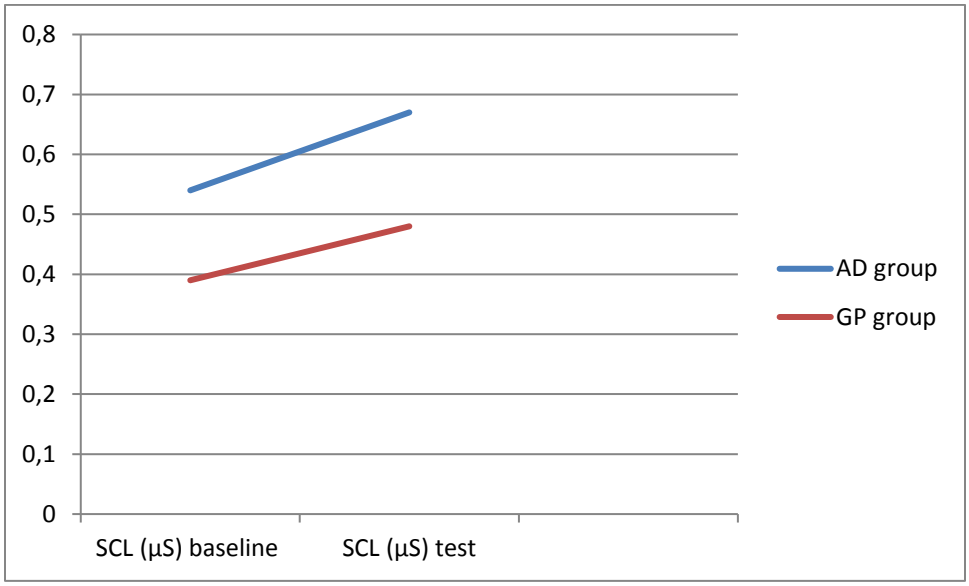

Figure 3. Skin conductance levels, baseline and during test
Note. GP = general population group, AD = anxiety disorder group, Cort1 = cortisol directly after awakening, SCL = skin conductance level.

Anxiety 'load' versus daytime cortisol, autonomic measures and perceived arousal

For AUC_g, there was a non-significant trend between groups. Means show that this trend is a consequence of the difference between the groups with one and two clinical anxiety disorders as compared to the group with three or more clinical anxiety disorders. Further analyses showed that there was no difference in Cort3 and a trend in Cort4. Mean scores in Cort3 and Cort4 showed the same pattern as AUC_g between groups. Results are presented in table 6. There were no significant between groups differences for CAR_g, CAR_i, Cort1, Cort2 and Cort5.

Regarding ANS functioning significant group differences were found in pretest and test levels of SCL. Posthoc analyses revealed that the group with the most clinical anxiety disorders had significantly higher pre- and posttest SCLs than the group with one or two anxiety disorders. These results remained significant after correcting for externalizing and depressive symptoms. There were no significant between group differences for the SCL stress response and HF HR variability.

Pre- and posttest perceived arousal was significantly different between groups. The group with 3 or more clinical anxiety disorders had lower pre- and posttest PAQ-scores in comparison with the groups with one and two anxiety disorders. These results remained significant after correcting for externalizing behavior. When the results were corrected for depressive symptoms, only posttest PAQ remained significantly different between groups. There was no significant between group difference for the PAQ stress response.

For all significant results described above overall effect sizes were medium and varied between .05-.10.

Table 5. Cross tabulation of main diagnosis and comorbid disorders

		Comorbid diagnoses N(% per main anxiety diagnosis)									
		SAD	SoPh	SpPh	GAD	ADHD	ODD	CD	DYS	DD	Total comorbid diagnoses per main anxiety diagnosis
Main diagnosis	SAD=57	-	13(22.8)	15(26.3)	14(24.6)	8(14.0)	5(8.8)	1(1.8)	1(1.8)	2(3.5)	59
	SoPh=29	3 (10.3)	-	2(6.9)	9(31.0)	1(3.4)	1(3.4)	0(0)	3(10.3)	0(0)	19
	SpPh=19	5(26.3)	2(10.5)	-	5(26.3)	2(10.5)	2(10.5)	1(5.3)	0(0)	0(0)	17
	GAD=47	5(10.6)	6(12.8)	12(25.5)	-	4(8.5)	2(4.3)	1(2.1)	4(8.5)	0(0)	34
Count of comorbid diagnosis		13	21	29	28	15	10	3	8	2	129

Note. GAD = generalized anxiety disorder, SoPh = social phobia, SAD = separation anxiety disorder, SpPh = specific phobia

Table 6. Daytime cortisol, ANS measures and perceived arousal versus 'anxiety load'

Variable	**Single clinical anxiety disorder** Mean (SD-n)	**Two clinical anxiety disorders** Mean (SD-n)	**Three or more clinical anxiety disorders** Mean (SD-n)	**Group differences**[#] (ANCOVA'S *//partial Eta squared)*	**Group differences**[##] (ANCOVA'S*//partial Eta squared)*	**Group differences**[###] (ANCOVA'S*//partial Eta squared)*
AUC_g	89.52(31.63-62)	92.17 (24.54-33)	71.45(21.09-16)	ns, trend		
Cort3	.83 (.20-68)	.84 (.15-37)	.72 (.18-17)	ns		
Cort4	.56 (.32-65)	.54 (.31-37)	.38 (.24-17)	ns, trend		
SCL (µS) baseline	.48(.22-62)	.55 (.27-36)	.67(.26-23)	F(2,113)= **4.53***//.07	F(2,108) = **3.27***//.06	F(2,106) = **4.76****//.08
SCL (µS) during test	.61 (.24-61)	.66 (.27-37)	.83 (.29-22)	F (2,112)= **5.07****//.08	F (2,107)= **3.49***//.06	F (2,105)= **4.74***//.08
PAQ pretest	.89 (.31-70)	.93 (.30-36)	.72 (.49-17)	F (2,115)= **4.05***//.07	F (2,110)= **4.90****//.08	ns, trend
PAQ posttest	.96 (.29-72)	.94 (.32-35)	.70 (43-17)	F (2,116)= **5.73****//.07	F (2,111)= **5.80****//.10	F (2,108)= **5.52****//.09

Note. SD = standard deviation, GP = general population group, AD = anxiety disorder group, AUC_g = area under the curve for Cort2-Cort4 with respect to ground, Cort3 = cortisol at 12.00 p.m., Cort4 = cortisol at 8.00 p.m., SCL = skin conductance level, PAQ = Perceived Arousal Questionnaire. All variables, except AUC_g, were log-transformed. *[#]covariates: age, sex, bmi.[##] covariates: age, sex, bmi, CBCL externalizing behavior, [###] covariates: age, sex, bmi, CDI Total score.* *= p<.05, **=p<.01.

Clinical 'load' versus daytime cortisol, autonomic measures and perceived arousal

For AUC_g, there was a significant between groups difference. Post hoc analyses showed that the difference in AUC_g between the groups was determined by the difference between the groups with one and two clinical disorders as compared to the group with three or more clinical disorders. Thus, the group with the most clinical disorders had a significantly lower AUC_g. Further analyses showed that the difference was determined by lower values of Cort3, Cort4 showed a trend. The results remained significant for AUC_g and Cort3 after correcting for externalizing behavior. Only the results for AUC_g remained significant after correction for depressive symptoms. Significant results are presented in table 7. There were no significant between groups differences for CAR_g, CAR_i, Cort1, Cort2 and Cort5.

Regarding ANS functioning significant group differences were found in pretest and test levels of SCL. Posthoc analyses revealed that the group with the most clinical anxiety disorders had significantly higher pre- and posttest SCLs than the group with one or two anxiety disorders. These results remained significant after correcting for externalizing and depressive symptoms. There were no significant between group differences for the SCL stress response and HF HR variability.

Posttest perceived arousal was significantly different between groups. The group with 3 or more clinical anxiety disorders had a lower posttest PAQ-score in comparison with the group with only one anxiety disorder. Although children with two clinical anxiety disorders had rather similar scores on posttest PAQ as the group with one anxiety disorder, there was no significant difference with the group with 3 or more disorders, which may be due to a lack of power. These results remained significant after correcting for externalizing behavior. There was no significant between group difference for pretest PAQ or the PAQ stress response.

For all significant results described above overall effect sizes were medium and varied between .05-.10.

Table 7. Daytime cortisol, ANS measures and perceived arousal versus 'clinical load'

Variable	**Single clinical disorder** Mean (SD-n)	**Two clinical disorders** Mean (SD-n)	**Three or more clinical disorders** Mean (SD-n)	**Group differences[#]** (ANCOVA'S *//partial Eta squared)*	**Group differences[##]** (ANCOVA'S*//partial Eta squared)*	**Group differences[###]** (ANCOVA'S*//partial Eta squared)*
AUC_g	87.82(28.01-54)	97.72 (30.33-35)	71.49(21.70-22)	F (2, 103) = **5.42****//.10	F (2,99) = **5.05****//.09	F (2,99) = **4.37***//.08
Cort3	.82 (.19-59)	.86 (.16-39)	.73 (.18-24)	F (2, 115) = **3.93***//.07	F (2, 110) = **3.53***//.06	ns, trend
Cort4	.55 (.33-57)	.57 (.30-38)	.40 (.24-24)	ns, trend		
SCL (µS) baseline	.48(.22-62)	.55 (.27-36)	.67(.26-23)	F(2,113) = **4.53***//.07	F(2,108) = **3.27***//.06	F(2,106) = **4.76****//.08
SCL (µS) during test	.61 (.24-61)	.66 (.27-37)	.83 (.29-22)	F (2,112) = **5.07****//.08	F (2,107) = **3.49***//.06	F (2,105) = 4.74*//.08
PAQ pretest	.88 (.32-62)	.95 (.30-38)	.76 (.43-23)	ns		
PAQ posttest	.95 (.31-63)	.95 (.32-38)	.76 (.39-23)	F (2,116)= **3.51***//.06	F (2,111)= **3.49***//.06	F (2,108)= 3.32*//.06

Note. SD = standard deviation, GP = general population group, AD = anxiety disorder group, AUC_g = area under the curve for Cort2-Cort4 with respect to ground, Cort3 = cortisol at 12.00 p.m., Cort4 = cortisol at 8.00 p.m., SCL = skin conductance level, PAQ = Perceived Arousal Questionnaire. All variables, except AUC_g, were log-transformed. *[#]covariates: age, sex, bmi. [##] covariates: age, sex, bmi, CBCL externalizing behavior, [###] covariates: age, sex, bmi, CDI Total score.* *= p<.05, **= p<.01.

Type of main anxiety diagnosis versus daytime cortisol, autonomic measures and perceived arousal

As presented in Table 8, significant group differences were found in pretest and test levels of SCL. Posthoc analyses revealed that children with a main diagnosis of SpPh showed higher SCL's compared to the SAD and the SoPh-groups. Effect sizes were medium and varied between .11-.13. These results remained significant after correcting for externalizing and depressive symptoms. GAD was not significantly different from SpPH. There was no significant between-groups difference for any of the cortisol measures, perceived arousal or HF HR variability.

Children in the AD group with a main diagnosis of SpPh differed from children in the GP group with a diagnosis of SpPh (DISC-IV- P/C (N=25)) on several measures. Children with SpPh from the AD group have higher $CBCL_{extern}$ scores (AD mean = 1.06 (sd =.27), GP mean = .78 (sd =.35) T = 3.32, df 54, p<.01), lower AUC_g (AD mean = 82.24 (sd = 28.17), GP mean = 106.17 (sd = 31.8) T = -2.90, df 51, p<.01), higher baseline SCL (AD mean = .69 (sd = .25), GP mean = .42 (sd = .16) T = 4.77, df 52, p<.001) and higher during test SCL (AD mean = .83 (sd = .25), GP mean = .51 (sd = .17) T = 5.45, df 52, p<.001). These results are in line with the general differences between the GP and the AD group. Thus the elevated levels of SCL's of SpPh are specific for clinical SpPh.

Table 8. ANS measures versus type of anxiety disorder

Variable	**Separation anxiety disorder** *Mean (SD-n)*	**Social anxiety disorder** *Mean (SD-n)*	**Generalized anxiety disorder** *Mean (SD-n)*	**Specific phobia** *Mean (SD-n)*	***Group differences[#]*** *(ANCOVA'S//partial eta squared)*	***Group differences[##]*** *(ANCOVA'S//partial eta squared)*	***Group differences[###]*** *(ANCOVA'S//partial eta squared)*
SCL (µS) baseline	.50 (.20-46)	.48 (.21-22)	.53(.29-36)	.75(.26-17)	F(3,111) = **4.36****//.11	F(3,105) = **3.96****//.11	F(3,104) = **4.49****//.12
SCL (µS) during test	.62 (.19-46)	.58 (.24-22)	.68(.31-35)	.89 (.29-17)	F (3,110)= **5.31****//.13	F (3,104)= **5.23****//.13	F(3,103) = **5.91****//.15

Note. SD = standard deviation, SCL = skin conductance level, All variables were log-transformed. *[#]covariates: age, sex, bmi. [##] covariates: age, sex, bmi, CBCL externalizing behavior,* [###] covariates: age, sex, bmi, CDI Total score*.* *= p<.05, **= p<.01.

DISCUSSION

Our results show that children with a clinical anxiety disorder have a pattern of hypoactivation of basal HPA-axis functioning, elevated sympathetic, and lowered parasympathetic activity compared to a general population sample. Anxiety 'load', as a marker of severity, is associated with differences in functioning ANS. A high anxiety 'load' in the clinical sample is associated with higher sympathetic functioning in comparison to children with a low anxiety 'load'. There is no relationship between anxiety 'load' and parasympathetic functioning. Results for children with a high anxiety 'load' show a trend towards hypoactivation of basal HPA-axis functioning. In contrast with our expectations, children with a high anxiety 'load' have lower posttest perceived arousal in comparison with children with a low anxiety 'load'. When adding comorbid disorders to the 'load', the same pattern is seen. Moreover, a high clinical 'load' is associated with hypoactivation of basal HPA-axis functioning in comparison to children with a low clinical 'load' even after correcting for depressive and externalizing symptoms. Although we did not expect a difference in functioning of ANS or HPA-axis between the different types of anxiety disorders, our results show that children with a main diagnosis of specific phobia can be discerned from children with a social phobia and separation anxiety disorder on higher sympathetic functioning during basal and stress conditions, even in the presence of high comorbidity rates, although comorbidity rates were comparable to or even lower than other studies (e.g. Kendall et al., 2010).

Anxiety 'load'

Our results are in support of the hypothesis that a child with a clinical disorder functions under chronic stressful conditions, with concomitant changes in the activity of both the stress systems. This notion is further underlined by the finding that a high clinical 'load' in the clinical sample was associated with lower basal HPA-axis functioning and higher sympathetic functioning in comparison to children with only

one or two clinical anxiety disorders, after correcting for externalizing and depressive symptoms. The analyses for anxiety 'load' show the same pattern, but show only a trend in the results for HPA-axis functioning which may be due to a lack of power. Our findings might indicate that there are already biological underpinnings of the 'load' of anxiety disorders in childhood, which might be predictive of adverse outcomes later in life. The 'load' of anxiety disorders during adolescence has been associated with later risks of anxiety disorders, major depression, substance dependence, suicidal behavior and other adverse outcomes, such as educational underachievement and early parenthood (Woodward, Fergusson, 2001).

Specificity of psychophysiological correlates

Our study underlines the hypothesis that basal ANS and HPA-axis functioning relate to anxiety disorders in general, and only heightened sympathetic (re)activity is a specific correlate for specific phobia. Other lines of research also support the idea of specific phobia as a specific taxonomic entity. In a population-based twin registry, lifetime diagnoses for six anxiety disorders (generalized anxiety disorder, panic disorder, agoraphobia, social phobia, animal phobia, and situational phobia) were obtained during personal interviews (Hettema et al., 2005). The authors concluded that genetic factors predispose to two broad groups of disorders dichotomized as panic-generalized-agoraphobic anxiety versus the specific phobias. Social phobia was regarded as an intermediate as it was influenced by both genetic factors. Further evidence for specific phobia as a specific taxonomic entity comes from longitudinal studies: specific phobia exclusively predicts specific phobia from childhood or adolescence to adulthood (e.g. Pine et al., 1998). In other words: there is strong evidence for longitudinal specificity of specific phobia. The psychophysiological correlates of clinical specific phobia in our study, confirm the existing genetic and longitudinal evidence for specific phobia as a separate taxonomic construct. The lack of

specificity of psychophysiological correlates for the other anxiety disorders is in line with the findings of Hettema et al. (2005).

Daytime cortisol

One of the most robust and striking results of our study is the low diurnal cortisol profile at noon and in the evening in children with AD. The same difference is seen in pretest cortisol levels, with lower levels of cortisol preceding the MAT. Within the AD group the severity of the disorder, or the clinical 'load', resulted in an even lower diurnal cortisol profile at noon and a trend for a lower diurnal cortisol profile in the evening. At least two causal pathways leading to hypocortisolism in clinical anxiety disorders are conceivable.

We propose that chronic and pathological anxiety problems, interfering with daily life and leading to a clinical anxiety disorder, have a different effect on physiological functioning than temporarily heightened, subclinical anxiety symptoms. In subclinical anxiety, the first symptoms of exacerbated worrying, fear and anxiety might lead to an initial increase in adrenocortical activity, i.e. hypercortisolism. When anxiety symptoms become clinical and persist, compensatory mechanisms become activated and gradually result in an attenuation of cortisol secretion, i.e. hypocortisolism. A recent study by Steudte et al. (2011) found evidence in support of this theory. The authors found lower concentrations of cortisol in hair in adults with GAD. Cortisol in hair reflects cortisol secretion over a prolonged period of time. This suggests that under naturalistic conditions GAD in adults is associated with hypocortisolism. Other evidence in support of this theory comes from animal studies. Herman et al. (2005) state that chronic stress leads to two mechanisms in animals. First, facilitation: a new stressor leads to a stronger increase in cortisol in chronically stressed animals compared to non-stressed animals, and second, habituation: a chronic stressor in chronically stressed animals leads to a progressive

decrease in cortisol. Our results are in line with the habituation hypothesis of chronic stress in animal research.

Alternatively, altered physiological functioning, as a vulnerability factor, could influence the expression of clinical anxiety disorders, but is not related to normal variation in levels of anxiety. Following this line of reasoning, moderate changes in HPA-axis functioning might be adaptive, whereas large alterations in physiological functioning may mark dysregulated emotions. Studies in support of this theory showed that cortisol administration reduced fear reports in patients with social phobia (Soravia et al., 2006) and that cortisol responses to psychosocial stress were inversely related to subsequent anxiety ratings (Schlotz et al., 2008).

Autonomic measures

Although effect sizes varied, our study confirms previous findings in studies with children with anxiety symptoms regarding autonomic functioning: AD children show heightened sympathetic functioning in rest and stress (SCL as a proxy for sympathetic ANS) and lower parasympathetic functioning in rest (HF HR variability as a proxy for parasympathetic ANS) compared to controls. With respect to sympathetic ANS functioning there seems to be a dose-response effect; children with a high anxiety 'load' exhibit even higher levels of sympathetic activity in comparison with children with a low anxiety 'load'. Interestingly, in studies finding evidence of low cortisol in association with PTSD or PTSD risk, there was also evidence of greater sympathetic arousal as reflected by catecholamine levels (Radley et al., 2011). Possibly, hypocortisolism in non-stressful conditions due to 'habituation' in children with a clinical anxiety disorder (i.e. a chronic stressor leads to a progressive decrease in cortisol) alters the ability of the HPA-axis to restore homeostasis following exposure to stress, resulting in increased sympathetic activation.

Perceived arousal

Previous studies suggest that high anxious subjects tend to perceive physiological sensations as more severe than non-anxious subjects (Hoehn-Saric, McLeod, 2000), sometimes even in the absence of an actual difference in physiological measures (Edelmann, Baker, 2002). In our study, the perceived arousal during rest in children with an AD compared to controls was higher. Apparently, anticipatory anxiety in children with an AD is high and has reached a plateau even before the stress task begins. This finding is supported by a recent study in healthy adults, relating neuroticism to exaggerated anticipatory anxiety experience (Drabant et al., 2011).

In contrast with these findings is the result that within the AD group children with a high anxiety 'load' experienced less perceived arousal in comparison with children with a low anxiety 'load'. There are several possible explanations: 1) children with a high anxiety 'load' are alexithymic and can not register their own perceived arousal, 2) children actively direct their attention away from threatening bodily sensations and nervous feelings, or 3) children with a high anxiety 'load' already have a continuous plateau level of arousal which they rate as not deviant from normal. This last hypothesis could be in line with the general hypothesis that children with a high anxiety 'load' function under chronic stress conditions.

Limitations

Several limitations of our study need to be taken into consideration. First, the availability of only one cortisol diurnal profile can be seen as a limitation. Rotenberg et al. (2012) show that the cortisol awakening response requires at least 3 weekdays of sampling to yield a stable estimate. However, a review by Golden et al. (2011) states that the use of sampling multiple salivary cortisol measures across the diurnal curve (including awakening cortisol), likely reflects chronic cortisol burden and has the highest reliability of all cortisol measures ($r = 0.63$-0.84).

Second, sampling compliance was based on child-reported time of saliva collection combined with parent-initiated times on a daily log sheet. Use of electronically monitored timing would improve the precision and accuracy of sample timing, as well as the ability to screen for potentially invalid samples. Furthermore, our participants had lunch on the day of the assessment. Although lunch was eaten before the assessment and the gap between lunch and baseline saliva samples was at least 1.5 h, it might still have had an effect on pretest cortisol levels (Rotenberg et al., 2012). Other variables such as socioeconomic status and Tanner stage might have influenced cortisol values, although in a sample of 8 to 12 year olds the influence of Tanner stage will be limited. These data are not available for our subjects. This is a limitation of the study.

Another notable weakness of the current study is the lack of heart rate variability measures and cortisol measures during stress. The MAT is a cognitive task that compared to a public speaking/cognitive task combination is less capable of eliciting a substantial cortisol stress response (Kirschbaum, Pirke, Hellhammer, 1993; Dickerson, Kemeny, 2004). Furthermore, it is a task in which speech limits the possibility to control HF HR variability for respiratory frequency, therefore, HF HR variability was not analyzed during the MAT.

Comorbidity rates and the distribution of main anxiety diagnoses in this sample will exert its effects on the functioning of both stress systems. This is also applicable for the method of sampling and age, although we tried to minimize variation in developmental status through the inclusion of only children below 13 years. Considering these limitations, results regarding our sample cannot be directly extrapolated to other clinical samples. Nonetheless, selecting samples in which no comorbidity exists represent an artificial reality not found in most clinical settings.

CONCLUSION

Our findings indicate that children with an anxiety disorder can be distinguished on several psychophysiological characteristics from healthy children. The results underline the hypothesis that ANS and HPA-axis functioning relate to anxiety disorders in general, and only heightened sympathetic (re)acitivity is a specific correlate for specific phobia, which confirms the existing genetic and longitudinal evidence for specific phobia as separate taxonomic construct. Furthermore, our study found some evidence in support of the hypothesis that a child with a clinical anxiety disorder functions under chronic stressful conditions, with concomitant changes in the activity of both the stress systems. This notion is underlined by the finding that a high clinical 'load' in the clinical sample was associated with an even further deviation of basal HPA-axis functioning and sympathetic functioning. This might indicate that there are already biological underpinnings of the 'load' of anxiety disorders in childhood, which might be predictive of adverse outcomes later in life.

Future research is needed to replicate our findings in other clinical samples. Furthermore, the cross-sectional character of this study makes it difficult to draw conclusions regarding the causality of deviations in HPA-axis and ANS functioning in children with an AD, therefore longitudinal studies in children in which the course and severity of anxiety symptoms and disorders are related to basal and reactive HPA and ANS functioning are needed.

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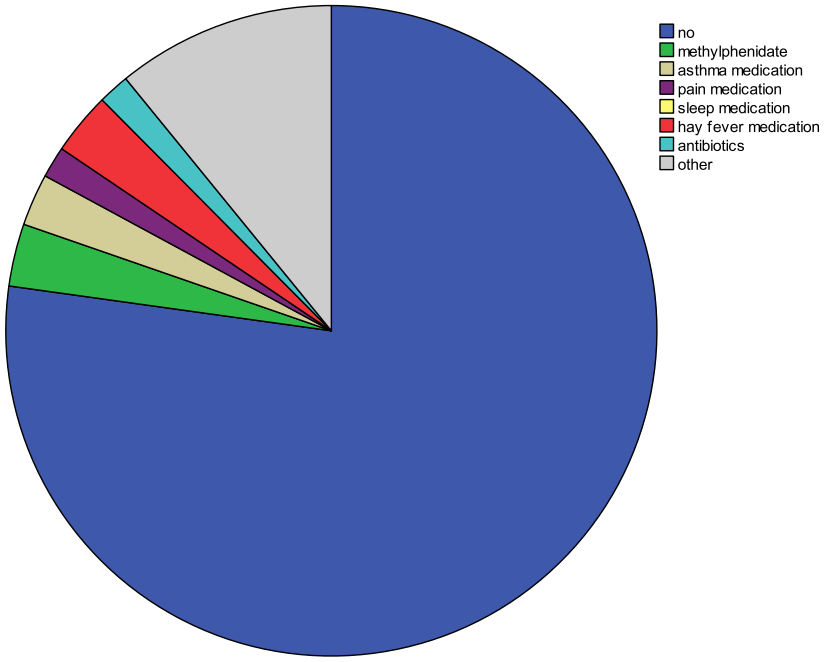
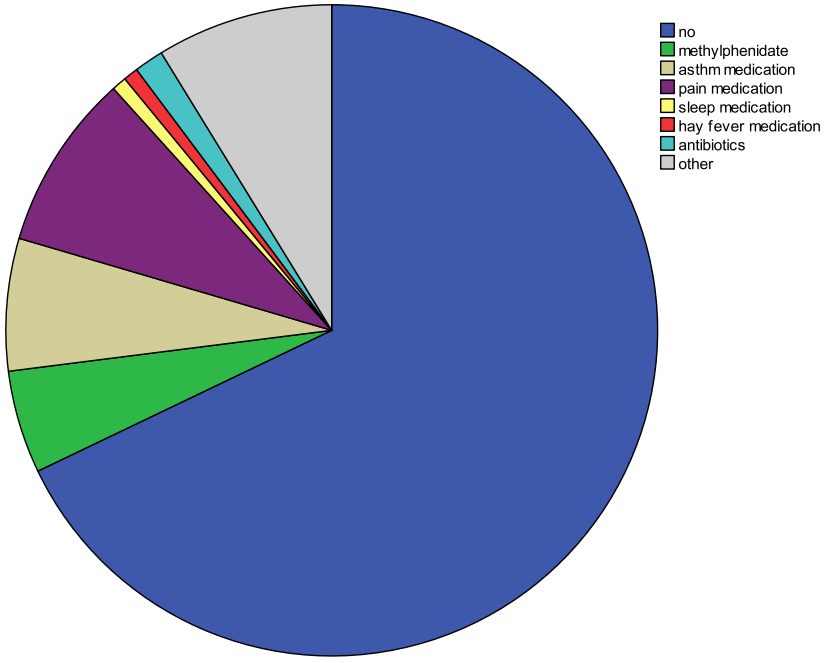
SUPPLEMENTARY FIGURES



General population sample



Anxiety disorder sample

Figure 1. Medication use during last two months

Chapter 6

Persistence of anxiety disorders and concomitant changes in cortisol

Dierckx, B., Dieleman, G., Tulen, J.H., Treffers, P.D., Utens, E.M., Verhulst, F.C., & Tiemeier, H. (2012).

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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 ($F = 3.2$, $p = 0.04$) and a trend towards a decreased cortisol morning rise ($F = 2.4$, $p = 0.09$). 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 (Goenjian et al., 1996; Bonne et al., 2003, Bremner et al., 2003, Goenjian et al., 2003; Burke et al., 2005a; Lopez-Duran, Kovacs, George, 2009). 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 (Wedekind et al., 2000; Vreeburg et al., 2010), whereas others showed normal HPA-axis activity (Gurguis, Mefford, Uhde, 1991). Recently, Mantella et al. (2008) reported increased basal cortisol levels in patients with a generalised anxiety disorder.

Even fewer studies have investigated the association between anxiety problems and cortisol levels in children and adolescents. Kagan, Reznick and Snidman (1987) found that basal cortisol levels were higher in inhibited than in uninhibited young children. Two studies reported similarly increased HPA-activity in anxious children and adolescents. Granger, Weisz and Kauneckis (1994) 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. (2004), 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.

However, several other studies could not demonstrate an association between anxiety problems and cortisol levels. Martel et al. (1999) did not find differences in basal cortisol levels between social phobic adolescent girls and matched controls. A study comparing adolescents with an anxiety disorder

and healthy controls showed no difference in cortisol response induced by a public speaking task (Gerra et al., 2000).

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 (Greaves-Lord et al., 2007). Gunnar and Vazquez (2001) posited that, although stressful events provoke frequent elevations in cortisol at first, these elevations would eventually lead to downregulation of the HPA-axis. On the other hand, elevations in cortisol levels that persist across time could also tune HPA-axis activity to a higher level (Sapolsky, Krey, McEwen, 1986). A recent study by Greaves-Lord et al. (2007) 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. 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 (Flory et al., 2009; Nicolson et al., 2010). 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 hypothesise 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, especially in the non-remitters group.

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 generalised 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 standardised stepped-care cognitive behavioural therapy program for childhood anxiety disorders was used, consisting of two phases. Phase one consisted of the FRIENDS program (Barrett, Lowry-Webster, Holmes, 2000), 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 8–16 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 refrigerator. 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 (Siebelink, Treffers, 2000; Silverman, Saavedra, Pina, 2001).

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 (March et al., 1997).

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 (March et al., 1997). Good reliability (Cronbach's alpha between 0.81 and 0.87) and good test–retest reliability (Intraclass correlation coefficient = 0.80) has been reported (Kovacs, 1985).

To supplement the child report, we used the Child Behaviour 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 minimise collinearity 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) (Achenbach, Dumenci, Rescorla, 2003).

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 summarise 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 Pruessner et al. (2003). 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 (Wust et al., 2000), 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 (Edwards et al., 2001).

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 (Legerstee et al., 2010). 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's 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, and 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 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.).

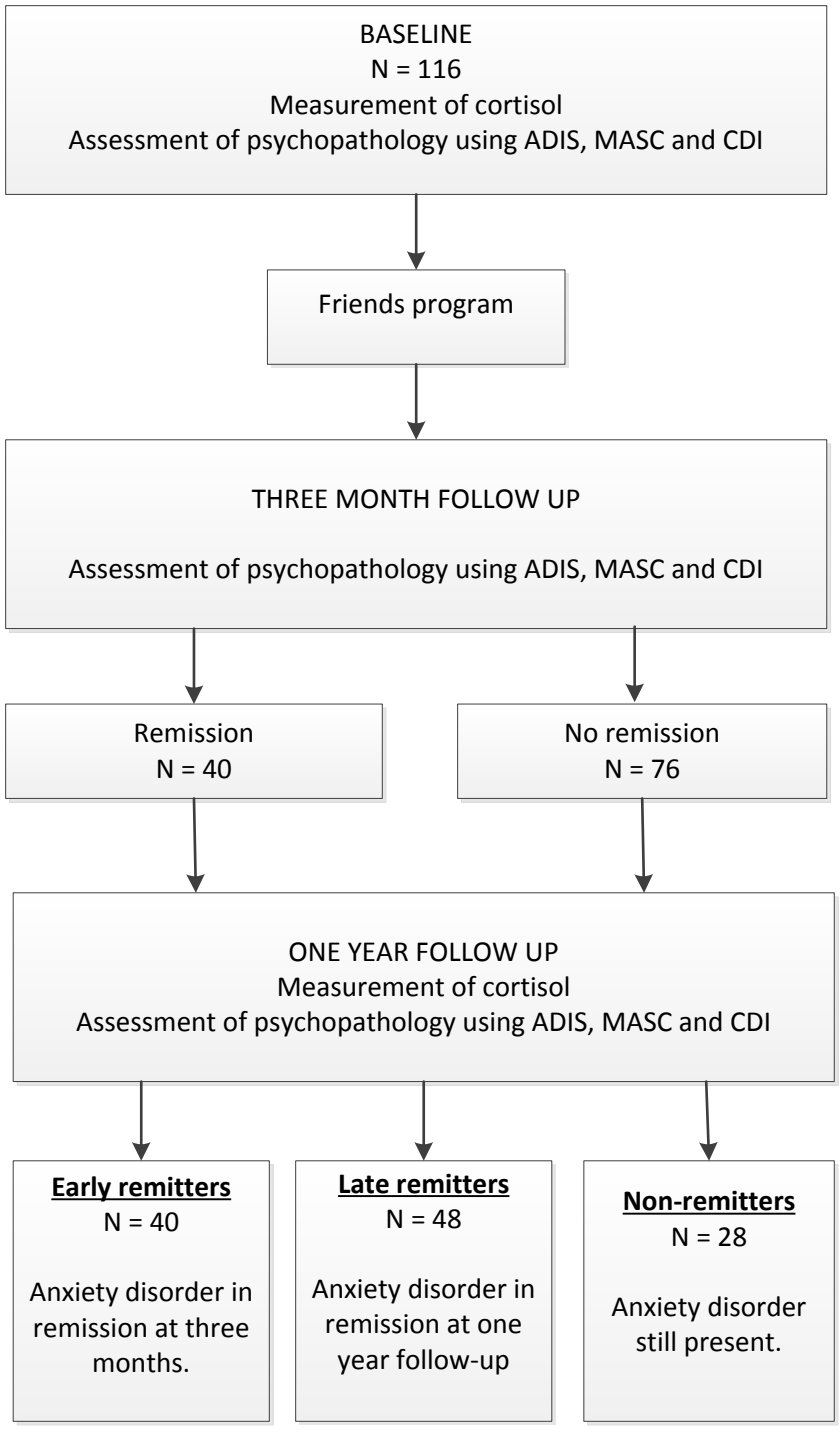


Figure 1. Study overview

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.

In Table 1, we show 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 ($p = 0.001$), however the difference between the early and late remission groups was not significant.

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, $p = 0.3$).

Table 1. Baseline characteristics of the study population

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

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

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 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).

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

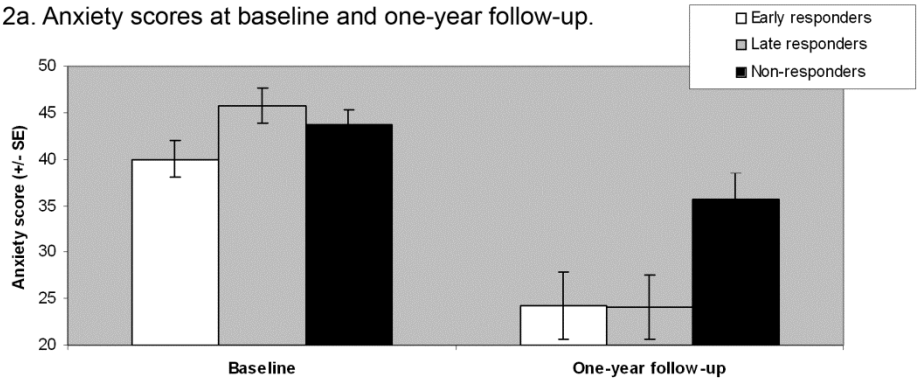


Figure 2b. Cortisol morning rise at baseline and one-year follow-up.

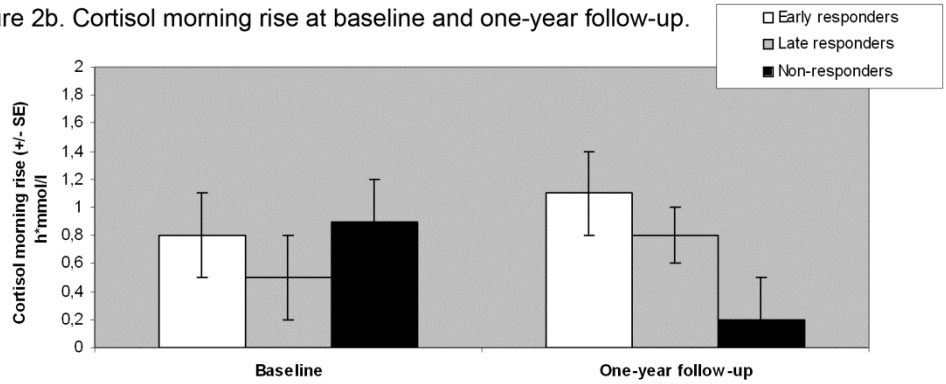
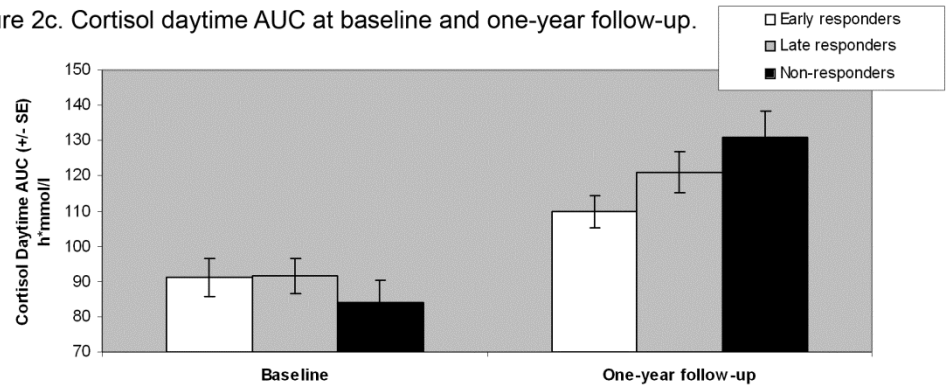


Figure 2c. Cortisol daytime AUC at baseline and one-year follow-up.



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; 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.

There was an increase in daytime AUC cortisol between baseline and follow-up, which was dependent on the trajectory of the anxiety disorder. This increase was most pronounced for non-remitters, intermediate in late remitters and less obvious in early remitters. At one year follow up, AUC cortisol was highest in non-remitters, followed by late remitters and was the lowest 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 anxious the more pronounced the changes in cortisol.

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 (Kagan, Reznick, Snidman, 1987).

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 morning rise is related to anxiety or stress (Kunz-Ebrecht et al., 2004; Vreeburg et al., 2010). 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 that a 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 (Martín del Campo et al., 2000; Burke et al., 2005b). 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. (2007) 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. 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. (2002), who found that higher mean daytime cortisol levels at age 4.5 predicted internalizing problems at age 6. 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. (2005) 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 16 h was lower after treatment than at baseline. Plasma cortisol in the untreated group remained stable (Tafet et al., 2005).

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. (2005) 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, i.e. 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 (Magarinos, McEwen, 1995). Damage to or atrophy of the hippocampus affects the glucocorticoid feedback inhibition of CRH secretion and leads to higher CRH and daytime cortisol concentrations (Schloesser, Manji, Martinowich, 2009). In addition, such atrophy or damage has been associated with a lowered or even absent cortisol morning rise (Buchanan et al., 2004; Pruessner et al., 2007).

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.

CONCLUSIONS

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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Chapter 7

Stress reactivity predicts symptom improvement in children with anxiety disorders

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ABSTRACT

Background: We examined the longitudinal associations of autonomic nervous system (ANS) and hypothalamic-pituitary-adrenal (HPA) axis rest and reactivity measures with anxiety and depressive symptoms at one-year follow-up in children with anxiety disorders.

Methods: In a clinical sample of 152 children with a primary DSM-IV anxiety disorder, aged 8 to 12 years, anxiety and depressive symptoms were assessed with the Multidimensional Anxiety Scale for Children and the Children's Depression Inventory at pre-treatment baseline and one year later, after treatment with cognitive behavioral therapy. At baseline, children participated in a 70 minutes stress task. Salivary cortisol was measured directly prior to and 20 minutes post stress task. Skin conductance level (SCL), heart rate and high frequency heart rate variability (HRV) were continuously measured during rest and the stress task. To investigate if rest or reactivity measures predicted anxiety and depressive symptoms at one-year follow-up, linear regression analyses were conducted for rest and reactivity measures of SCL, heart rate, HRV and cortisol separately.

Results: Higher SCL reactivity predicted less decrease of anxiety symptoms at one-year follow-up. Cortisol reactivity showed a weak association with depressive symptoms at one-year follow-up: lower cortisol reactivity predicted less decrease in depressive symptoms.

Limitations: Only self-reported anxiety and depressive symptoms were used. However, all predictors were objective biological measures, hence there is no risk of shared method variance bias.

Conclusions: These findings suggest that pre-treatment HPA and ANS responsiveness to stress are predictive biomarkers for a lack of symptom improvement in children with a clinical anxiety disorder.

INTRODUCTION

Anxiety disorders are among the most prevalent types of psychiatric disorders experienced by children and adolescents (Verhulst et al., 1997; Bittner et al., 2007), with separation anxiety disorder, specific phobia, social phobia, and generalized anxiety disorder being the most frequent childhood anxiety disorders (Beesdo-Baum, Knappe, 2012). Childhood anxiety has been associated with a range of negative outcomes, including academic underachievement, drug dependency, and an increased risk for developing other psychiatric disorders (Woodward, Fergusson, 2001; Bittner et al., 2007).

Cognitive behavioral therapy (CBT) is the treatment of choice for children with an anxiety disorder, with a remission rate of 59% following treatment (James et al., 2013). A 7 to 19 year follow-up study of the long-term outcomes of treated childhood anxiety disorders showed that patients with a poorer response to CBT, had higher rates of panic disorder, substance abuse and dependency in adulthood than the successfully treated controls (Benjamin et al., 2013). It is, therefore, important to identify predictors of symptom improvement in treated children with an anxiety disorder.

Several studies tried to gain insight into clinical predictors of treatment outcome in children with anxiety disorders. Some studies reported that higher anxiety severity predicts a less favorable outcome (Compton et al., 2014; Hudson et al., 2013; Last, Hansen, Franco, 1998; Liber et al., 2010). A few studies showed that children with comorbid mood disorders are more likely to retain their primary anxiety disorder following treatment (Hudson et al., 2013; Liber et al., 2010). Various studies examined the role of parental characteristics as predictors of treatment outcome in children, but an inconsistent pattern of findings resulted (Legerstee et al., 2008; Hudson et al., 2013; Compton et al., 2014). Because clinical characteristics are weak or inconsistent indicators of response to CBT, there is an increasing interest in identifying biomarkers to predict differential treatment response (Lester, Eley, 2013).

Physiological stress response systems have been implicated as possible important biological state markers for childhood anxiety. It can be hypothesized that children with an anxiety disorder function under conditions of persistent stress, with an excessive and prolonged stress system activation (Dieleman et al., 2015). Alterations in the autonomic nervous system (ANS) have been associated with anxiety disorders in children; children with an anxiety disorder show a pattern of heightened activity of the sympathetic nervous system (Schmitz et al., 2011; Kossowsky et al., 2012; Dieleman et al., 2015) and diminished parasympathetic control (Schmitz et al., 2011; Dieleman et al., 2015), although some studies failed to show this difference (Kossowsky et al., 2012; Kristensen et al., 2014). Another major physiological stress response system is the hypothalamic-pituitary-adrenal (HPA) axis. Glucocorticoids can act both to augment and suppress autonomic mediated changes. Cross-sectional studies related HPA axis functioning to childhood anxiety disorders, but provided inconsistent findings (Feder et al., 2004; Forbes et al., 2006; Krämer et al., 2012; Dietrich et al., 2013; Dieleman et al., 2015). This may reflect the variable methods of sampling, resting state versus stress paradigms, differences in age, developmental status, and diagnostic status of the study populations. Furthermore, differences in functioning of the HPA axis could depend on the chronicity or the severity of the disorder (Pervanidou, 2008; Dieleman et al., 2015).

Despite the evidence of altered pre-treatment HPA axis and autonomic functioning in anxiety-disordered children, studies that have assessed stress physiology as a predictor of therapy outcome in childhood anxiety disorders are lacking. This study aims to investigate the longitudinal association of stress physiology at pre-treatment baseline with anxiety and depressive symptoms at one-year follow-up in a clinical sample of anxiety disordered children treated with CBT. We hypothesize that anxiety-disordered children with a stronger autonomic stress response, i.e. heightened activity of the sympathetic and diminished activity of the parasympathetic nervous system, show persistence of anxiety symptoms one year later. Also, we explore the longitudinal association between cortisol levels

and anxiety symptoms at one-year follow-up, but formulate no specific hypothesis, given the inconsistent results of previous studies. Furthermore, since comorbid depressive symptoms have been associated with a less favorable treatment outcome in children with anxiety disorders (Liber et al., 2010; Hudson et al., 2013) and in previous work we observed that cortisol reactivity was specifically associated with depressive symptoms (Dieleman et al., 2010), we will also explore the longitudinal association of stress physiology with depressive symptoms.

METHODS

Participants

This study included 152 children, aged 8 to 12 years, referred to the outpatient clinic of the Department of Child and Adolescent Psychiatry of Erasmus Medical Center in Rotterdam or the University Medical Center in Leiden, The Netherlands. These hospitals serve as secondary or tertiary referral centers of South-West Netherlands. Children had a primary diagnosis of separation anxiety disorder (N=57), generalized anxiety disorder (N=47), social phobia (N=29) or specific phobia (N=19). All children were diagnosed with the Anxiety Disorders Interview Schedule for DSM-IV (Silverman, Albano, 1996). Exclusion criteria were: IQ < 85, poor command of the Dutch language, serious somatic disease, autism spectrum disorder, selective mutism, psychotic disorders, pharmacotherapy that could interfere with HPA axis or ANS functioning.

Methylphenidate treatment in children with comorbid attention deficit hyperactivity disorder was discontinued the day before physiological measurements (N=7), because methylphenidate treatment can increase heart rate and blood pressure (Ballard et al., 1976). Children on medication for an anxiety disorder were withdrawn from medication, if possible, or otherwise excluded. For children with

comorbid attention deficit hyperactivity disorder, the dosage of medication was kept constant during the study as a constant dosage of medication for attention deficit hyperactivity disorder was considered unlikely to confound treatment effects. The Committees for Medical Ethics of Erasmus Medical Center and Leiden University Medical Center approved the study.

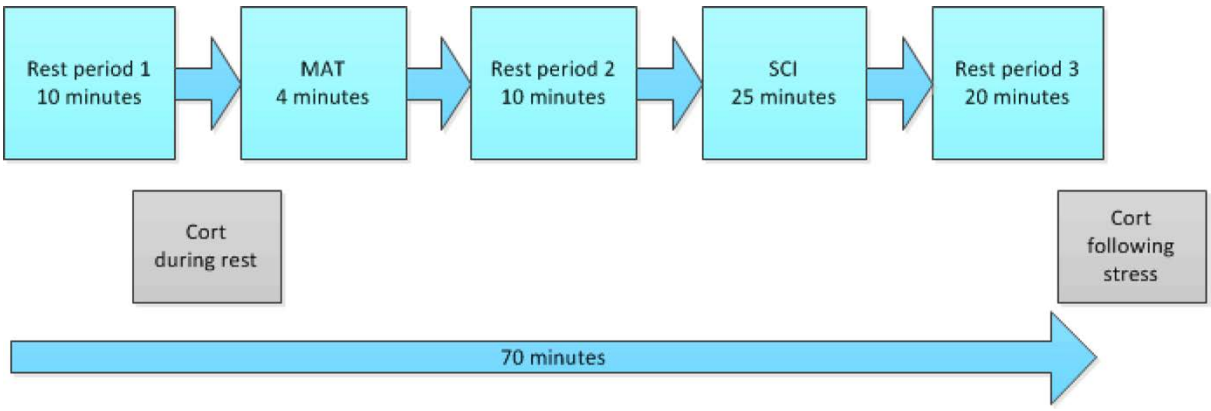

Figure 1. Procedure
Note. MAT= mental arithmetic task, SCI= social competence interview, Cort=cortisol. Cort following stress: cortisol levels in response to stress typically peak about 20 to 30 minutes following a stressor.

Procedure

Parents and participants signed informed consent before participation. Parents and children completed psychological questionnaires and a diagnostic interview before the physiological tests at the pre-treatment baseline and at one-year follow-up.

Physiological assessments took place in the hospital between 12.00h and 18.30h, because variation of cortisol levels is least in the afternoon (Wust et al., 2000). After an acclimatization period of 45 minutes, the session began with a resting period of 10 minutes. Subsequently, a mental arithmetic task and, after another resting period of 10 minutes, a social competence interview were administered. Saliva collection took place after resting period 1 (cortisol during rest) and 20 minutes after the social competence interview (cortisol following stress), because cortisol levels typically peak 20 to 30 minutes

after stress. Figure 1 presents the temporal sequence of measures. Parents were asked to report general physical condition, dietary pattern and medication use of their child. For more information on medication use, see Appendix 1.

Measures

The *Anxiety Disorders Interview Schedule for Children DSM-IV (ADIS-C)* is a semi-structured interview to assess DSM-IV anxiety disorders in 7- to 17-year-olds (Silverman, Saavedra, Pina, 2001). A trained psychologist conducted the interview with the child and parents separately at pre-treatment, post-treatment and one-year follow-up. To obtain a diagnosis, both a count of DSM-IV symptom criteria, as well as the level of impairment according to the parent, child, and interviewer, were taken into account. The parent and the child were asked to indicate on a 9-point scale (i.e., 0-8) to what extent the symptoms interfered with the child's daily life. Subsequently, the interviewer gave an interference rating (Clinician Severity Rating (CSR)), on the same 9-point scale, for the child and parent interview, separately. If the CSR was 4 or higher, a diagnosis was assigned. The anxiety disorder with the highest CSR was regarded as the primary anxiety disorder. Interviewers who administered the ADIS-C at follow-up were blind to pre-treatment diagnoses, disease trajectory, and physiological measures.

The *Multidimensional Anxiety Scale for Children (MASC)* is a 39-item self-report questionnaire (March et al., 1997), administered at pre-treatment, post-treatment and one-year follow-up, assessing anxiety symptoms during the last two weeks in children and adolescents. Items are scored from 0 to 3 (0 = never true, 1 = rarely true, 2 = sometimes true, 3 = often true). The internal consistency (.93) and one-month test-retest reliabilities (.81) of the Dutch translation are good (Liber et al., 2008).

The *Children's Depression Inventory (CDI)* is an age-appropriate 27-item self-report questionnaire (Kovacs, 1992), administered at pre-treatment, post-treatment and one-year follow-up, assessing depressive symptoms in the last two weeks in children and adolescents. Items are scored from 0 to 2 (0 = never true, 1 = sometimes true, 2 = always true). The Dutch translation showed good internal consistency for the total score (.82) (Liber et al., 2008), 1-month test-retest reliabilities (.72) are acceptable (Kovacs, 1981).

Treatment

All children participated in a standardized stepped-care CBT program for childhood anxiety disorders, consisting of two phases (van der Leeden et al., 2011). In the first phase, children were treated with the FRIENDS program, an evidence-based treatment program for anxiety disorders (Barrett, Lowry-Webster, Turner, 2000), which encompassed 10 child sessions and 4 separate parent sessions. The FRIENDS program comprised psychoeducation, relaxation and breathing exercises, exposure, problem-solving skills training, social support training and cognitive restructuring exercises (Shortt, Barrett, Fox, 2001; Liber et al., 2008). Parent sessions comprised mainly psychoeducation. All children that were not successfully treated in the first phase, as determined by ADIS-C at three months follow-up, received supplementary CBT. The second phase consisted of 10 manualized sessions, in which parents and child participated together in each session. See Figure 2 for the study design. Consequently, the change of symptoms predicted by the physiological measures reflect both the variation in natural course of symptoms and the effect of structured and standardized treatment all children received.

The present study is an extension of the data collection described by Liber et al. (2008) with an additional 25 children. Liber et al. (2008) studied the effects of individual versus group CBT in children up to 12 years of age, who attended primary school. The additional 25 children comprised 6 children who attended primary school but could not be randomized, because they refused assignment to group

treatment (N=2), were absent at the start of the group (N=1), or were treated at an affiliated outpatient clinic near to home (N=3). The other 19 children, aged 12-13 years, attended the first grade of secondary school and received individual therapy because of practical reasons (time schedules differed between the different secondary schools). To summarize, all additional 25 children received individual treatment with the FRIENDS program. The efficacy of group CBT did not differ from the efficacy of individual CBT in children up to 12 years of age (Liber et al., 2008).

Adherence was carefully checked as part of the treatment integrity measures. All therapy sessions were videotaped; a random selection of 30% of the tapes of individual sessions and all tapes of the group sessions were checked for adherence. Twenty-two therapists conducted the therapy sessions; seventeen were licensed psychologists and 5 were psychologists in the last year of their training who worked under close supervision of a licensed psychologist. Therapists at each institute met weekly to discuss the treatment and were supervised by two experienced licensed cognitive behavioral therapists. See Liber et al. (2008) and Van der Leeden et al. (2011) for more detailed information on treatment adherence and integrity.

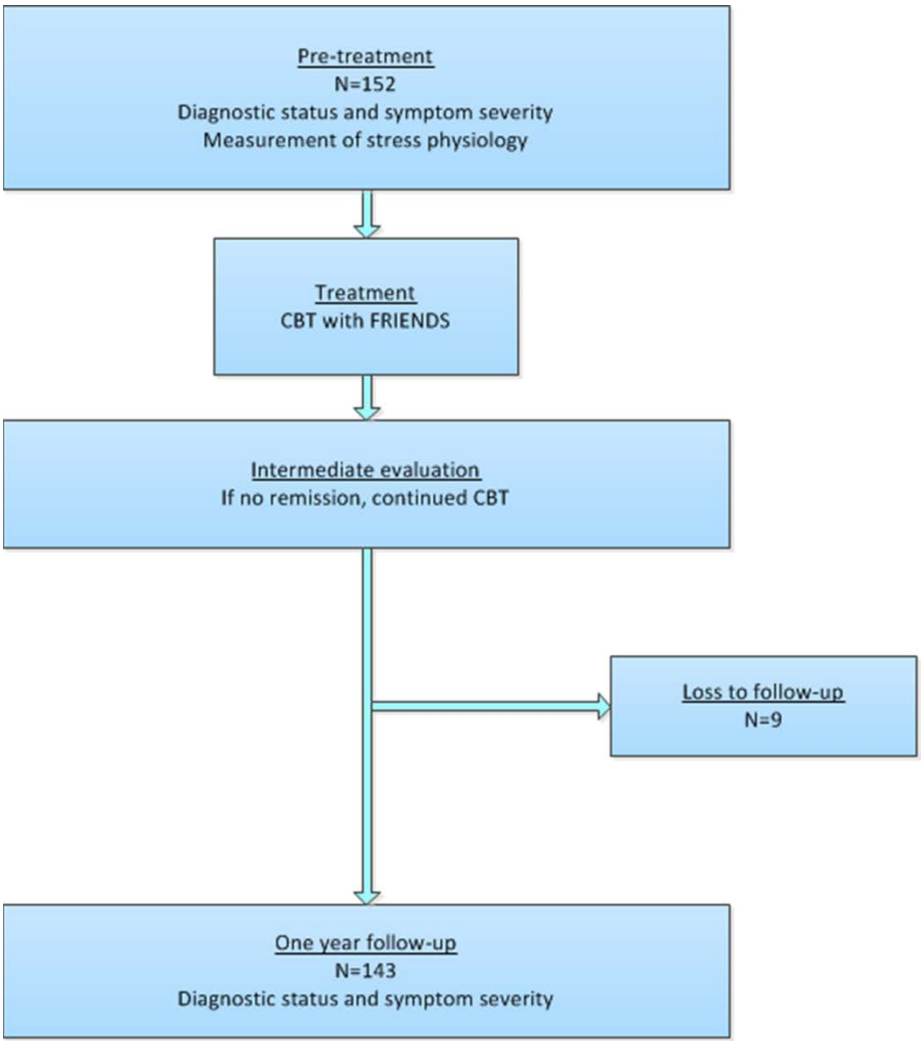

Figure 2. Flowchart

Note. CBT= cognitive behavioural therapy.

Stress tasks

The mental arithmetic task is a standardized laboratory stress task to induce measurable physiological changes (Kirschbaum, Pirke, Hellhammer, 1993). The mental arithmetic task lasted 4 minutes, during which the child was asked to subtract numbers as quickly and accurately as possible. If the child made a mistake or did not respond, he or she was required to start all over again. Dependent on the child's age,

the child was asked to subtract 7 from 100 (<12 years), or 23 from 1021 (≥12 years), and to continue this subtraction till no further subtraction was possible.

The social competence interview (Ewart, Kolodner, 1991) is a standardized stress task that impacts ambulatory blood pressure in adolescents (Ewart, Kolodner, 1993; Ewart, Jorgensen, Kolodner, 1998). For this study, we used a revised version of the social competence interview, in which a stressful situation related to the primary anxiety diagnosis was discussed in detail with the patient. In a pilot study of the original social competence interview (N=8), most participants chose a topic unrelated to their anxiety disorder suggesting avoidance. To elicit a more pronounced physiological reaction the interview topic was adapted. During minutes 14-20 of the social competence interview the participant was asked to imagine, without speaking, that he or she was in that stressful situation again and to re-experience the feelings and thoughts.

Physiological and hormonal measures

Cortisol assessment

Cortisol samples were assessed with solid-phase radioimmunoassay (RIA, Diagnostic Products Corporation, Los Angeles; N=83) and Enzyme-Linked Immuno Sorbent Assay (ELISA, DRG-kits, Marburg, Germany; N=69) as the laboratory changed the standard technique during data collection. To test for possible effects of measurement thirty-one cortisol samples were analyzed by both RIA and ELISA. Correlation between both assays was high (R=.99). The slope was not equal to 1, therefore the concentrations in the DRG standards were adjusted by the laboratory to obtain comparable results. Cortisol values above 3 SD of the mean were excluded from the analysis to reduce the impact of outliers.

Autonomic measures

During the experiment, continuous measurements were made of heart rate, respiration rate and skin conductance level (SCL) in order to assess ANS activity. For technical specifics see Dieleman et al. (2015).

Analysis of physiological data

A customised software program calculated the interbeat intervals of the ECG using R-top detection, resulting in interbeat interval time series during rest (minutes 7-10 of the first resting period; a 3 minutes stationary period) and stress (minutes 17-20 of the re-experiencing part of the social competence interview; a period without speaking and regular breathing). Interbeat interval time series were transformed to heart rate series. We conducted spectral analysis of these intervals using discrete Fourier transformation (Van Steenis, Tulen, Mulder, 1994). For each time segment, we calculated mean heart rate and high frequency heart rate variability (HRV; 0.14-0.50 Hz), the last parameter as a proxy for the parasympathetic component of autonomic cardiac control. Because HRV is strongly correlated with respiratory sinus arrhythmia (Kamath, Fallen, 1993), respiratory frequency was monitored and controlled for. Time segments with more spectral power for respiration in the mid frequency band than in the high frequency band were discarded from HRV analyses. This resulted in the removal of 1 subject for the respective analyses. Mean SCL levels were computed during rest and stress periods as parameters reflecting sympathetic ANS activity.

Data analysis

Measures of depressive symptoms, SCL, cortisol and HRV were log-transformed to approach a normal distribution. Means of heart rate, HRV and SCL were defined for two periods: minutes 7-10 of the first resting period and minutes 17-20 of the re-experiencing part of the social competence interview. Subsequently, stress reactivity in heart rate, HRV and SCL were calculated by subtracting the rest value from the value during the social competence interview. To characterize cortisol reactivity to stress, we

calculated a difference score by subtracting cortisol levels during rest from cortisol levels collected 20 minutes after the social competence interview. Pearson correlation coefficients between autonomic and cortisol measures during baseline and social competence interview were calculated.

The focus of our analyses was on stress reactivity measures. To investigate if reactivity measures of autonomic and HPA axis functioning predicted anxiety symptoms at one-year follow-up, linear regression analyses were conducted for reactivity measures of SCL, HRV, heart rate and cortisol levels, separately. Anxiety symptoms at one-year follow-up were entered as dependent variable. To control for possible effects of age, gender, pre-treatment anxiety symptoms and depressive symptoms at one-year follow-up, all were entered to the first model as independent variables. Subsequently, reactivity measures of SCL, HRV, heart rate and cortisol in response to the social competence interview were additionally entered in the second block as independent variables, all controlled for their respective rest measures. Second, these analyses were repeated separately for rest measures of SCL, HRV, heart rate and cortisol.

Finally, the analyses described above for anxiety symptoms at one-year follow-up were repeated with depressive symptoms at one-year follow-up as the outcome measure. These analyses were controlled for possible effects of pre-treatment depressive symptoms and anxiety symptoms at one-year follow-up.

Effect sizes are reported as R squared, with .01 defined as a small effect size, .09 as a medium effect size and .25 as a large effect size (Cohen, 1988). A post hoc power calculation was performed to compute the achieved power given the final sample size and effect size. The achieved power was 0.71, based on an α-error probability of 0.05. All statistical analyses were performed with SPSS 21.0.

RESULTS

Sample characteristics

Group characteristics, diagnoses and comorbidity at pre-treatment and at one-year follow-up are presented in Table 1. Thirty-seven percent had a primary diagnosis of separation anxiety disorder, 30.5 % a primary diagnosis of generalized anxiety disorder, 18.8% a primary diagnosis of social phobia and 12.3 % a primary diagnosis of specific phobia. Comorbidity rates at pre-treatment were high; 55.3% had a single clinical anxiety disorder only, 30.9% had two clinical anxiety disorders, 11.8% had three clinical anxiety disorders, and 2.0% had four clinical anxiety disorders (Dieleman et al., 2015). A paired samples T-test showed that anxiety and depressive symptoms measured at one-year follow-up were, on average, lower than at pre-treatment (respectively $T=12.81$, $p< .001$; $T=12.8$, $p<.001$). After treatment, 75% of the sample recovered from their anxiety disorder under standardized CBT treatment. The prevalence of comorbid behavioral and mood disorders was reduced by approximately 50% at one-year follow-up.

Table 1. Sample characteristics

Measures	**At baseline** **Mean (SD)** **N=152**	**At one year follow up** **Mean (SD)** **N=143**
Age	10.2 (1.54)	-
Gender	53.3% boys, 46.7% girls	-
Anxiety symptoms***	51.37 (17.72)	27.57 (15.36)
Depressive symptoms***	9.61 (7.04)	3.43 (4.72)
Loss to follow-up	-	9 (5.9)
Anxiety disorders ADIS-C	**At baseline** **Count (% of total sample)**	**At one year follow up** **Count (% of total sample)**
No anxiety diagnosis	-	114 (75.0)
GAD	75 (49.3)	14 (9.2)
SoPh	49 (32.2)	9 (5.9)
SAD	46 (30.3)	13 (8.6)
SpPh	48 (31.6)	14 (9.2)
Comorbid disorders ADIS-C	**At baseline** **Count (% of total sample)**	**At one year follow up** **Count (% of total sample)**
PD	-	1 (.7)
OCD	-	1 (.7)
PTSD	-	-
ADHD	15 (9.7)	7 (4.6)
ODD	10 (6.5)	3 (2.0)
CD	3 (1.9)	-
DD	2 (1.3)	4 (2.7)
DysD	8 (5.2)	1 (.7)

Note. SD = standard deviation, ADIS-C= Anxiety Disorders Interview Schedule for DSM-IV, GAD = generalized anxiety disorder, SoPh = social phobia, SAD = separation anxiety disorder, SpPh = specific phobia, PD = panic disorder, OCD = obsessive compulsive disorder, PTSD = posttraumatic stress disorder, ADHD = attention deficit hyperkinetic disorder, ODD = oppositional defiant disorder, CD = conduct disorder, DD = depressive disorder, DysD = dysthymic disorder, *= p<.05, **=p<.01, ***=p<.001.

Table 2. Pre-treatment descriptives of the autonomic and cortisol parameters during rest and stress, for the whole group

Parameters **Mean (SD)**	**Cortisol during rest^** (nmol/l)	**Cortisol following stress^** (nmol/l)	**Skin conductance level during rest^** (µS)	**Skin conductance level during stress^** (µS)	**Heart rate variability during rest^** (ms^2)	**Heart rate variability during stress^** (ms^2)	**Heart rate during rest** (bpm)	**Hea dur** (bp
Cortisol during rest^ .76 nmol/l (.25)	1	.73**	-.06	-.12	.12	.14	.05	.14
Cortisol following stress^ .71 nmol/l (.23)		1	-.24**	-.28**	.09	.10	.03	.06
Skin conductance level during rest^ .57 µS (.28)			1	.92**	-.10	-.07	.20*	.24
Skin conductance level during stress^ .71 µS (.28)				1	-.11	-.10	.16	.25
HF Heart rate variability during rest^ 3.40 ms^2(.41)					1	.64**	-.41**	-.31
HF Heart rate variability during stress^ 3.45 ms^2 (.32)						1	-.45**	-.44
Heart rate during rest 81.0 bpm (11.0)							1	.92
Heart rate during stress 80.0 bpm (10.5)								1

Note. ^ log transformed , significant correlations are indicated with: *= p<.05, **=p<.01

Descriptives

During the social competence interview, we observed a significant increase in SCL as compared to rest levels (mean difference = .13, standard deviation = .11, paired samples T-test: T=12.41, p< .001). Mean (SD) levels and Pearson correlation coefficients of autonomic and cortisol measures are presented in Table 2. Pearson correlation coefficients showed that all measures during rest were significantly correlated with the equivalent measures during the social competence interview. There were no significant relations between SCL, HRV, heart rate and cortisol measures during rest and anxiety symptoms or depressive symptoms at one-year follow-up.

Longitudinal association of stress physiology with anxiety symptoms at one-year follow-up

The results of the regression analyses for reactivity measures and anxiety symptoms at one-year follow-up are presented in Table 3. As shown, SCL reactivity to stress was positively associated with change of anxiety symptoms at one-year follow-up. The effect size was small to medium with a R^2change of .06. In other words, higher SCL reactivity was associated with less decrease in anxiety symptoms at one-year follow-up. To check that our significant results were not due to medication effects, we did a secondary analysis controlled for medication use. The effect size and p-value were comparable (R^2change=.07, p=.008, N=91), which indicated that our findings were not influenced by the use of medication.

Longitudinal association of stress physiology with depressive symptoms at one-year follow-up

The association of reactivity measures with depressive symptoms at one-year follow-up is presented in Table 4. As shown, there was a weak negative association of cortisol reactivity to stress with change of depressive symptoms at one-year follow-up. The effect size was small with a R^2change of .03. In other words, lower cortisol reactivity was associated with less decrease in depressive symptoms at one-year

Table 3. Longitudinal association of pre-treatment autonomic and cortisol measures with anxiety symptoms at one-year follow-up

	Anxiety symptoms at 1 year follow-up N=107~						
Variable	*B*	*SE B*	*Beta*	*T*	R^2change	*F* for change in R^2	*p* for chang
Age	.3	.9	.03	.3	.17	6.0***	.000
Gender	4.0	2.7	.13	1.5			
Anxiety symptoms pretreatment	.13	.08	.15	1.6			
Depressive symptoms at 1 year follow-up^	13.2	3.5	.3	3.8***			
Reactivity measures							
Cortisol difference adj^ (nmol/l)	7.2	9.5	.09	.8	.01	.6	.45
Skin conductance level (µS) difference adj^ (µS)	33.1	12.3	.26	2.7	.06	**7.3****	**.008**
HF Heart rate variability difference adj ^ (ms^2)	2.3	5.3	.05	.4	.00	.2	.66
Heart rate difference adj (bpm)	-.05	.34	-.01	-.14	.00	.02	.89

Note. HF= high frequency, ^ log transformed, difference= test-rest measure, adj=analysis is adjusted for rest measure. $R^{2\ change}$ = explained variance for adding this step, ~ = number of patients available for cortisol difference analysis (for skin conductance level difference analysis N=100, for HF Heart rate variability difference N=105, for heart rate difference analysis N=105), # = p<.1, *= p<.05, **=p<.01, ***=p<.001.

Table 4. Longitudinal association of pre-treatment autonomic and cortisol measures with depressive symptoms at one-year follow-up

	Depressive symptoms at 1 year follow-up N=105~						
Variable	*B*	*SE B*	*Beta*	*T*	R^2change	*F* for change in R^2	*p* for chang
Age	-.01	.02	-.05	-.6			
Gender	-.04	.06	-.05	-.6			
Depressive symptoms pretreatment	.4	.1	.36	4.4***	.24	9.3***	.000
Anxiety symptoms at 1 year follow-up^	.01	.00	.3	3.8***			
Reactivity measures							
Cortisol difference adj^ (nmol/l)	-.4	.2	-.2	-1.9	.03	**3.7**	**.058**
Skin conductance level (µS) difference adj^ (µS)	.3	.3	.1	1.8	.01	.95	.33
HF Heart rate variability difference adj ^ (ms^2)	.04	.13	.04	.3	.00	.09	.77
Heart rate difference adj (ms)	.01	.01	.09	.94	.01	.88	.35

Note. HF= high frequency, ^ log transformed, difference= test-rest measure, adj=analysis is adjusted for rest measure. $R^{2\ change}$ = explained variance for adding this step, ~ = number of patients available for cortisol difference analysis (for skin conductance level difference analysis N=100, for HF Heart rate variability difference N=105, for heart rate difference analysis N=105), # = p<.1, *= p<.05, **=p<.01, ***=p<.001.

follow-up. To check that our significant results were not due to medication effects, we did a secondary analysis controlled for medication use. The effect size and p-value were comparable (R^2change=.03, p=.063, N=96), which indicated that our findings were not influenced by the use of medication.

DISCUSSION

Principal findings

This clinical study of pediatric anxiety disorders showed that children with higher pre-treatment SCL reactivity to stress, as a proxy of sympathetic reactivity, responded less to treatment. Their anxiety symptoms decreased less over a one-year period as compared to children with a lower pre-treatment SCL reactivity. Furthermore, children with lower pre-treatment cortisol reactivity showed less decrease of depressive symptoms.

Autonomic nervous system

To our knowledge, this is the first longitudinal study that uses ANS functioning as a predictor of treatment outcome in children with an anxiety disorder. Heightened activity of the sympathetic nervous system is associated with anxiety disorders in children (Schmitz et al., 2011; Kossowsky et al., 2012; Dieleman et al., 2015). Our study showed that higher sympathetic reactivity in response to a stressor predicted less improvement in anxiety symptoms one year later. The autonomic nervous system regulates critical life functions on a moment-to-moment basis through its sympathetic and parasympathetic branches. To be able to respond to a threatening situation, the body prepares itself for fight or flight. This autonomic activation leads to an increase in heart rate, blood pressure, sweat gland activity, and respiration. Subjectively, the individual feels tense and flushed, has palpitations, shortness of breath and increased perspiration. We carefully speculate that anxiety disordered children with a

higher reactivity of the sympathetic nervous system, also experience more physiological and subjective arousal in response to daily fear or stress. As a consequence, higher sympathetic reactivity may influence the natural course of anxiety disorders or the efficacy of standardized CBT.

Our study failed to show a longitudinal association of the parasympathetic function of the ANS (HRV) with anxiety or depressive symptoms one year later. Although ANS functioning, with a primary focus on parasympathetic functioning, has been repeatedly studied as a predictor of outcome in adult studies, results are inconsistent (Bornas et al., 2007; Alpers, Sell, 2008; Davies et al., 2015). Studies that have investigated the cross-sectional association between parasympathetic functioning and childhood anxiety disorders have been inconsistent as well (Schmitz et al., 2011; Kossowsky et al., 2012; Kristensen et al., 2014; Dieleman et al., 2015).

HPA-axis

A less responsive HPA-axis to stress predicted less decrease in depressive symptoms one year later. However, the effect size was small. Only a few studies investigated HPA-axis reactivity in response to a psychological stressor in depressed children in comparison with healthy controls (Luby et al., 2003; Hankin et al., 2010; Suzuki et al., 2013). Compared to non-depressed peers, depressed preschoolers show a blunted reactivity (Luby et al., 2003; Suzuki et al., 2013), but higher peak cortisol levels to a psychosocial stressor (Luby et al., 2003). The pattern of blunted reactivity to mild stressors remained at 24-month follow-up in children with a history of major depressive disorder (Suzuki et al., 2013). Also, prepubertal dysphoric children exhibit cortisol hyporeactivity to a psychosocial challenge task (Hankin et al., 2010).

Glucocorticoids can act both to augment and suppress sympathetically mediated changes in cardiovascular function, metabolism, and immune function. Normally, activation of these stress systems leads to behavioral and physical adaptive changes that improve an organism's ability to survive

(Sapolsky, 2002). Possibly, anxiety disordered children with a less responsive HPA-axis to stress might be more susceptible to persistence of depressive symptoms, because a less adaptive HPA-axis leads to more difficulties in mobilizing energy resources to sufficiently cope with stress (Hankin et al., 2010). This might underlie the impaired effects of treatment strategies and, as a result, persistence of depressive symptoms.

Lower cortisol reactivity to stress has been consistently reported in depressive disorders across the age span (Burke et al., 2005; Lopez-Duran, Kovacs, George, 2009; Harkness, Stewart, Wynne-Edwards, 2011; Suzuki et al., 2013). The mechanism underlying lower cortisol reactivity may reflect down-regulation of the HPA axis, because chronic stress repeatedly over-activates and eventually impairs the HPA axis (Gunnar, Vazquez, 2001; Herman et al., 2005; Juster, McEwen, Lupien, 2010; Suzuki et al., 2013). Our results are in line with such a habituation of the stress system. Thus, an alternative explanation is that this low HPA-axis reactivity signals a vulnerability of a subgroup of children with anxiety disorders.

Strengths and limitations

The strengths of our study include the large clinical cohort of pediatric anxiety disorders, the prospective design and the standardized treatment approach. Yet, some important limitations need to be addressed. First, results from our sample cannot be directly extrapolated to other clinical samples with different distributions of age, primary diagnoses, and comorbidity rates. Comorbidity rates and the distribution of primary anxiety diagnoses in this sample will exert its effects on the functioning of both stress systems. Nonetheless, selecting samples without comorbidity does not represent the reality of most clinical settings (Dieleman et al., 2015). Further research is needed to corroborate our findings in other clinical samples of anxiety disordered children. Second, several epidemiological studies have demonstrated that perceived family support is a protective factor for the development of affective symptoms in children

and adolescents (Klasen et al., 2015). In the present study, we did not control for the effect of perceived family support. However, a recent study by Jongerden et al. (2015) showed that most family and parenting variables do not predict referral in a non-referred sample of anxious children. Only child reported parental autonomy granting increased the odds of referral, while child reported overprotection decreased the odds of referral. Further, data on Tanner stage are lacking, but variation in developmental status is minimized through the inclusion of only children below 13 years.

In the present study, we used only self-reported anxiety and depressive symptoms. However, although the outcome parameters were assessed with self-report measures, all predictors were objective biological measures, hence there is no risk of shared method variance bias.

Another aspect is that the stress task did not elicit a significant increase in mean cortisol compared to baseline, or significant alterations in mean heart rate and HRV, although we observed a significant increase in SCL as compared to rest levels, which showed that the social competence interview is capable of eliciting an autonomic stress response. A possible explanation might be that coming into the laboratory itself may have served as a significant stressor for some of these anxiety disordered children, suggestive of anticipatory anxiety, with already high cortisol levels after the first resting period. To address this phenomenon, all reactivity values were corrected for their corresponding values during rest.

Despite these limitations, the present findings may have several implications for further research and clinical practice. If replicated, our results suggest that pre-treatment HPA and ANS reactivity to stress are candidate predictors of a lack of symptom improvement in children with a clinical anxiety disorder. Given the small to medium effect sizes of HPA and ANS reactivity to stress as predictors of anxiety and depressive symptom improvement in this study, we emphasize the importance of combining multiple predictors, such as clinical and demographic factors, polygenic risk scores, and neuroimaging measures, to enhance the predictive power. In conclusion, our study shows that pre-

treatment HPA and ANS reactivity to stress are longitudinally associated with a change in anxiety and depressive symptoms at one-year follow-up in a clinical sample of pediatric anxiety disorders.

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SUPPLEMENTARY MATERIAL

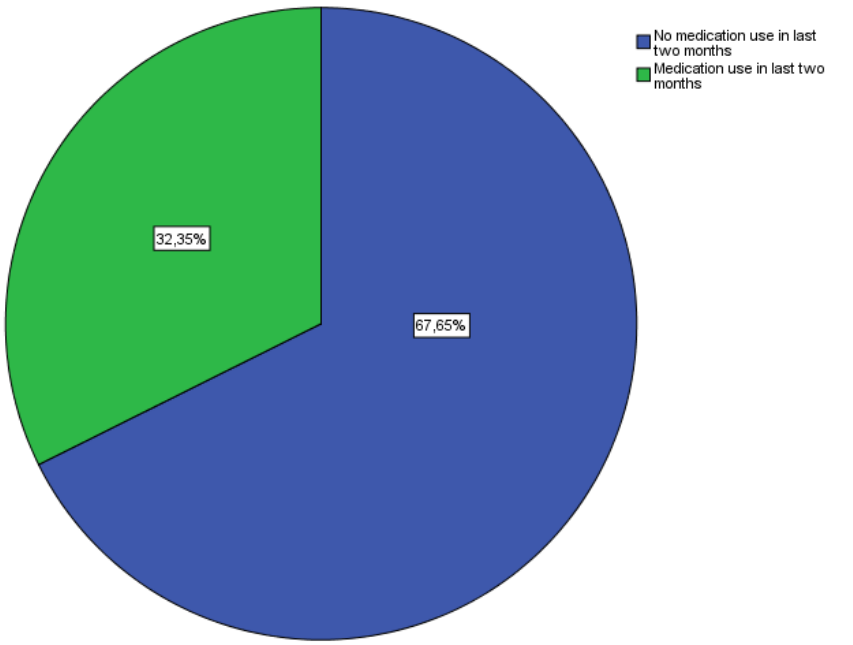

Figure 1. Medication use during last two months

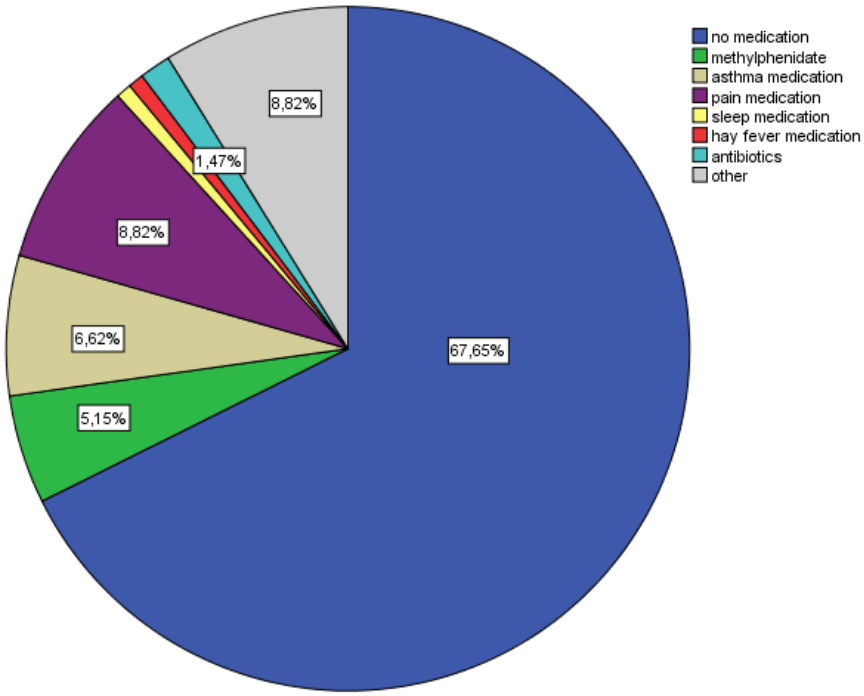

Figure 2. Type of medication use in last two months

PART IV

Chapter 8

General discussion

GENERAL DISCUSSION

Anxiety is a basic emotion, not typically pathologic but commonly adaptive when it facilitates anticipation to a threat or danger. However, when children perceive the world as full of threats and dangers, with no possibility to relax and to regard their living environment as safe, anxiety becomes pathologic (Sapolsky, 2002). Variations in the activity of the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS), two major physiological stress systems, have been implicated as possible biological markers of pathological anxiety in children (Feder et al., 2004; Dietrich et al., 2007). Normally, activation of these stress systems leads to behavioral and physical adaptive changes that improve an organism's ability to survive. In children with an anxiety disorder, the persistent stress they experience might lead to an excessive and prolonged stress system activation. In the current thesis, we focused on the role of stress physiology as a cause, correlate, and predictor of pediatric anxiety disorders with the ultimate goal to improve treatment and prognosis. More specifically, the series of studies described in this thesis examined the specificity of the association of stress physiology with child anxiety problems, given the high co-occurrence with other anxiety, externalizing and depressive problems. In addition, the studies examined the trajectory of an anxiety disorder and the concomitant changes in stress physiology, and stress physiology as a predictor of therapy outcome. In this chapter the main findings are summarized and discussed in a broader context. The chapter concludes with implications for research and clinical practice together with considerations for future studies.

MAIN FINDINGS

Autonomic nervous system

Of fraidy-cats and wild tigers

Pure anxiety problems have a low prevalence at toddler age, but become more prevalent with increasing age (Gilliom, Shaw, 2004; Basten et al., 2016). To some extent, many fears and anxieties in pre-school aged children are age-appropriate and reflect normal development (Egger, Angold, 2006). There is a need to discern persistent anxiety problems from normal development and to identify early risk factors for deviant developmental pathways. This is complicated by the fact that internalizing and externalizing problems in childhood often co-occur (Fanti, Henrich, 2010), show heterotypic stability (Basten et al., 2016), and have unique causes as well as shared causes (Mathiesen, Sanson, 2000). The study described in *Chapter 4* was designed to disentangle the longitudinal associations between infant autonomic functioning and early childhood internalizing and externalizing problems, in a large general population sample. We showed that infant autonomic functioning (at 14 months) differentially predicts internalizing and externalizing problems at the age of 6, but only after mutually adjusting for the broadband scales. In a fully adjusted model, a higher infant heart rate and lower infant heart rate variability were associated with a stronger increase in internalizing symptoms during follow-up. In addition, lower infant heart rate and higher infant heart rate variability were associated with a less strong decrease of the child externalizing symptoms during follow-up.

Ideally potential risk factors for the development of serious problem behavior would be measured before the onset of such behaviors. Most previous studies that examined the association between autonomic arousal and problem behavior in children used a cross-sectional study design (Posthumus et al., 2009; El-Sheikh, Hinnant, Erath, 2011) or, in case of a longitudinal design, measured autonomic arousal beyond infancy (Hinnant, El-Sheikh, 2013; Hastings, Kahle, Nuselovici, 2014). This increases the possibility of reverse causality, a situation in which the outcome precedes and causes the exposure instead of the other way around. This study stresses the importance of using a longitudinal

design over a developmental sensitive period when studying early risk factors for the continuity of problems among preschool children.

In a previous study in this sample, a higher heart rate in infants predicted more internalizing problems at the age of 3 years, but there was no association with externalizing problems at 18 months or 3 years (Dierckx et al., 2011, 2014). Results with diurnal cortisol measures in this sample were consistent with this finding (Saridjan et al., 2014). Explanations for the differences in results compared to the current study might lie in the developmental pathways of internalizing and externalizing problems. Externalizing problems tend to decrease beyond toddler age, while pure internalizing problems have a low prevalence at toddler age, but become more prevalent with increasing age (Gilliom, Shaw, 2004). Using latent profile analysis, the study by Basten et al. (2016) showed that a profile with predominantly internalizing problems was only discernible at 6 years, while at earlier ages internalizing problems were less prevalent, mild and typically accompanied by at least mild levels of externalizing problems. In contrast, a profile characterized by moderate externalizing problems and emotionally-reactive behavior was visible at all ages, but the prevalence of this profile was higher in infancy than in early childhood. Therefore, higher scores for externalizing problems are less common and arguably more deviant with increasing age. Our data suggest that low autonomic arousal is a marker for the development of more deviant, externalizing problems during childhood and shows less association with the highly prevalent emotional reactive externalizing behavior seen in infants and toddlers, while high autonomic arousal might be a marker of early and persistent internalizing problems.

Of fraidy-cats

Maladaptive and pathologic anxiety is characterized by persisting or extensive degrees of anxiety and avoidance associated with subjective distress or impairment (American Psychiatric Association, 2000). The study in *Chapter 5* confirms previous findings in studies with children with anxiety symptoms

regarding autonomic functioning: children with a clinical anxiety disorder have a pattern of elevated sympathetic (re)activity, and lowered parasympathetic activity compared to same-aged children from a general population sample. With respect to sympathetic ANS functioning, there was a dose-response effect; children with a high anxiety 'load' exhibited higher levels of sympathetic activity in comparison with children with a low anxiety 'load'. The results were consistent after correcting for externalizing and depressive symptoms. Our results are in support of the hypothesis that a child with a clinical anxiety disorder functions under chronic stressful conditions, with concomitant changes in the activity of the ANS. This notion is underlined by the finding that there is a dose-response effect between clinical 'load' and sympathetic functioning. This might indicate that there are already biological underpinnings of the 'load' of anxiety disorders in childhood, which could be predictive of adverse outcomes later in life.

Applying cross-sectional research designs, previous studies found high comorbidity rates among different types of anxiety problems (Newman et al., 1996; Masi et al., 1999; Essau, Conradt, Petermann, 2000; Verduin, Kendall, 2003). The high degree of comorbidity amongst anxiety disorders in children and adolescents seems to point in the direction of one taxonomic construct, instead of separate disorders. The results of the clinical study of pediatric anxiety disorders in *Chapter 5* underline the hypothesis that basal parasympathetic activity and sympathetic (re)activity relate to anxiety disorders in general. In addition, this study shows that only a clinical main diagnosis of specific phobia can be discerned from social phobia and separation anxiety disorder on higher sympathetic functioning during basal and stress conditions, even in the presence of high comorbidity rates and after correcting for externalizing and depressive symptoms. Other lines of research also support the idea of specific phobia as a distinct taxonomic entity. In a population-based twin registry, lifetime diagnoses for six anxiety disorders (generalized anxiety disorder, panic disorder, agoraphobia, social phobia, animal phobia, and situational phobia) were obtained during personal interviews (Hettema et al., 2005). The authors concluded that genetic factors predispose to two broad groups of disorders dichotomized as panic-generalized-

agoraphobic anxiety versus the specific phobias. Social phobia was regarded as an intermediate as it was influenced by both genetic factors. Further evidence for specific phobia as a specific taxonomic entity comes from longitudinal studies: specific phobia exclusively predicts specific phobia from childhood or adolescence to adulthood (e.g. Pine et al., 1998). The psychophysiological correlates of clinical specific phobia in our study confirm the existing genetic and longitudinal evidence for specific phobia as a separate taxonomic construct.

The need to treat

As shown in *Chapters 4 and 5*, heightened autonomic arousal is longitudinally associated with early and persistent internalizing behavior and is cross-sectionally associated with anxiety disorders in children. The results of the clinical study of pediatric anxiety disorders in *Chapter 7* show that children with higher pretreatment sympathetic reactivity in response to a stressor have less improvement in anxiety symptoms one year later. Their anxiety symptoms decreased less over a one-year period as compared to children with a lower pre-treatment sympathetic reactivity. We carefully speculate that anxiety disordered children with a higher reactivity of the sympathetic nervous system, also experience more physiological and subjective arousal in response to daily fear or stress. As a consequence, individuals with a more reactive sympathetic nervous system might be less capable of transferring treatment strategies from the therapy room into daily life, because they experience more physiological en subjective arousal when exposing themselves to anxious situations in daily life. Accordingly, higher sympathetic reactivity may influence the natural course of anxiety disorders or the efficacy of standardized cognitive behavioral therapy. This study failed to show a longitudinal association of the parasympathetic function of the ANS with anxiety or depressive symptoms one year later. Although ANS functioning, with a primary focus on parasympathetic functioning, has been repeatedly studied as a predictor of outcome in adult studies, results are inconsistent (Bornas et al., 2007; Alpers, Sell, 2008;

Davies et al., 2015). Studies that have investigated the cross-sectional association between parasympathetic functioning and childhood anxiety disorders have been inconsistent as well (Schmitz et al., 2011; Kossowsky et al., 2012; Kristensen et al., 2014; Dieleman et al., 2015). Based on the current and previous studies, I hypothesize that in children with a clinical anxiety disorder pretreatment sympathetic functioning as compared to parasympathetic functioning is a more informative predictor of posttreatment anxiety symptoms.

Hypothalamic-pituitary-adrenal axis

Of fraidy-cats and feeling blue

A substantial body of evidence has shown that anxiety and depressive symptoms in children and adolescents are often comorbid (e.g. Essau, Conradt, Petermann, 2000, Costello et al., 2003; Ferdinand et al., 2005). Anxiety and depression can be two different valid constructs that often co-occur, or they could be different manifestations of the same underlying vulnerability. The tripartite model, by Clark and Watson (1991), hypothesizes that physiological hyperarousal is specific for anxiety. Physiological hyperarousal as defined by the model of Clark and Watson is a concept based on anxiety and depression questionnaires. The study described in *Chapter 3* attempts to quantify the concept of physiological hyperarousal by using cortisol (re)activity as a proxy for physiological hyperarousal in order to make it more tangible and applicable. In a general population sample of children higher rates of depressive symptoms were associated with less reactive HPA-axis functioning in response to a stress task. There was no relation between anxiety symptoms and any of the cortisol measures. Our results provided some evidence that in a general population sample, reactive HPA-axis functioning can differentiate between anxiety and depressive problems in children. An explanation for the lack of significant findings for anxiety symptoms in relation to cortisol (re)activity could be that alterations in HPA-axis functioning only occurs in children with a clinical anxiety disorder. In this general population sample the number of

subjects with clinical anxiety and depression was relatively low. An alternative explanation for our findings could be that altered HPA-axis functioning, as a preexisting trait, leads to a clinical anxiety or depressive disorder, but is not related to normal variation in levels of anxiety. The results of the study described in *Chapter 7* are in line with the results of the study described in *Chapter 3*, as this clinical study of pediatric anxiety disorders showed that children with lower pre-treatment cortisol reactivity showed less decrease of depressive symptoms. A less responsive HPA-axis to stress predicted less decrease in depressive symptoms one year later. This study is discussed in more detail later on in this discussion.

Of fraidy-cats

The chronic and pathological anxiety experienced by children and adolescents in a clinical population may have a great impact on the developing HPA-axis and impair its future functioning. The study in *Chapter 5* focused on whether basal HPA-axis functioning could distinguish children (N=154) aged 8-12 years with different primary clinical anxiety disorders (separation anxiety disorder, generalized anxiety disorder, social phobia and/or specific phobia) from a same-aged general population reference group (N=225) and from each other. This study showed that children with a clinical anxiety disorder have a pattern of hypoactivation of basal HPA-axis functioning compared to same-aged children from a general population sample. These results remained the same after correcting for externalizing and depressive symptoms. One of the most robust and striking results of this study is the low diurnal cortisol levels at noon and in the evening in children with a clinical anxiety disorder. Within the clinical anxiety group, the severity of the disorder, or the clinical 'load', resulted in an even lower diurnal cortisol level at noon and a trend for a lower diurnal cortisol profile in the evening.

At least two causal pathways leading to hypocortisolism in clinical anxiety disorders are conceivable. First, altered physiological functioning, as a vulnerability factor, could influence the

expression of clinical anxiety disorders. Following this line of reasoning, moderate changes in stress physiology might predispose to increased internalizing problems. The findings of Saridjan et al. (2014) in the Generation R study support this theory. Basal HPA-axis functioning in predicted an increase of internalizing problems in pre-schoolers. These results suggest that variations in diurnal cortisol patterns early in life precede internalizing problems in a general population sample. Second, chronic and pathological anxiety problems, interfering with daily life and leading to a clinical anxiety disorder, might have a different effect on physiological functioning than temporarily heightened, subclinical anxiety symptoms. In subclinical anxiety, the first symptoms of exacerbated worrying, fear and anxiety might lead to an initial increase in adrenocortical activity, i.e. hypercortisolism. When anxiety symptoms become clinical and persist, compensatory mechanisms become activated and gradually result in an attenuation of cortisol secretion, i.e. hypocortisolism. Evidence in support of this theory comes from animal studies. Herman et al. (2005) state that chronic stress leads to two mechanisms in animals. First, facilitation: a new stressor leads to a stronger increase in cortisol in chronically stressed animals compared to non-stressed animals, and second, habituation: a chronic stressor in chronically stressed animals leads to a progressive decrease in cortisol. Our results are in line with the habituation hypothesis of chronic stress in animal research.

The need to treat

Persistence of an anxiety disorder in childhood could have life-long consequences for the neuroendocrine system, general health, and well-being, underscoring the need for prospective, longitudinal research. The study in *Chapter 6* investigated associations between the trajectory of an anxiety disorder and the concurrent changes in cortisol levels in a clinical sample of children and adolescents during one year. All children and adolescents received a standardized stepped-care CBT-program for childhood anxiety disorders. Overall, there was an increase in diurnal cortisol between pre-

treatment baseline and one-year follow-up, which was dependent on the trajectory of the anxiety disorder. The mean increase in diurnal cortisol after treatment seems comparable to the mean diurnal cortisol of the reference group described in the study in *Chapter 5*. However we did find differences in cortisol levels at baseline related to the subsequent course of the anxiety disorder; the increase in diurnal cortisol was most pronounced for non-remitters, intermediate in late remitters and less obvious in early remitters. Depression severity was higher in the late and non-remitter groups. In a meta-analysis, Burke et al. (2005) showed that cortisol levels are generally elevated in depressive adults during recovery. Possibly, the relation between the trajectory of an anxiety disorder and concurrent changes in diurnal cortisol is confounded by comorbid depressive symptoms. However, we addressed this by correcting for depression severity in all our analyses. Another explanation for the differences between these trajectories might be that a stronger increase in diurnal cortisol signals a vulnerability of a subgroup of children with anxiety disorders that do not respond well to treatment. Overall, in this study 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. These results are consistent with a dose–response effect, the longer participants were anxious the more pronounced the changes in cortisol.

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 (Magarinos, McEwen, 1995). Damage to or atrophy of the hippocampus affects the glucocorticoid feedback inhibition of CRH secretion and leads to higher CRH and daytime cortisol concentrations (Schloesser, Manji, Martinowich, 2009). In addition,

such atrophy or damage has been associated with a lowered or even absent cortisol morning rise (Buchanan et al., 2004; Pruessner et al., 2007).

The study design did not allow us to compare the changes in cortisol levels during treatment for anxiety with changes in cortisol levels occurring as part of normal development in a one-year period. For that, a control group that did not receive any treatment during this period would have been necessary. However, it would have been unethical to deny children with a clinical aniety disorder treatment for the duration of one year. 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.

In children and adolescents, research focusing on the relation between anxiety and the HPA-axis has mainly been cross-sectional in nature. 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 cross-sectional studies. 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. 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.

The study in *Chapter 6* investigated associations between the trajectory of an anxiety disorder and the concurrent changes in cortisol levels in a clinical sample of children and adolescents during one year.This study gave insight into changes in cortisol functioning during the course of the anxiety disorder. Our findings seem to indicate that after receiving a standardized stepped-care CBT-program,

children with an anxiety disorder show changes in HPA-axis functioning which depend on the trajectory of the anxiety disorder. Persistence of an anxiety disorder in childhood could have life-long consequences for the neuroendocrine system, which has repeatedly been linked to chronicity and recurrence of affective disorders and affective symptoms (Flory et al., 2009; Nicolson et al., 2010), underscoring the importance of early detection and adequate treatment of anxiety disorders.

Despite the evidence of altered pre-treatment HPA axis and autonomic functioning in anxiety-disordered children, studies that have assessed stress physiology as a predictor of therapy outcome in childhood anxiety disorders are lacking. The study in *Chapter 7* aims to investigate the longitudinal association of stress physiology at pre-treatment baseline with anxiety and depressive symptoms at one-year follow-up in a clinical sample of anxiety disordered children treated with CBT. This clinical study of pediatric anxiety disorders showed that children with lower pre-treatment cortisol reactivity showed less decrease of depressive symptoms. A less responsive HPA-axis to stress predicted less decrease in depressive symptoms one year later. However, the effect size was small.

Lower cortisol reactivity to stress has been consistently reported in depressive disorders across the age span (Burke et al., 2005; Lopez-Duran, Kovacs, George, 2009; Harkness, Stewart, Wynne-Edwards, 2011; Suzuki et al., 2013). Only a few studies investigated HPA-axis reactivity in response to a psychological stressor in depressed children in comparison with healthy controls (Luby et al., 2003; Hankin et al., 2010; Suzuki et al., 2013). Compared to non-depressed peers, depressed preschoolers show a blunted reactivity (Luby et al., 2003; Suzuki et al., 2013).

Glucocorticoids can act both to augment and suppress sympathetically mediated changes in cardiovascular function, metabolism, and immune function. Normally, activation of these stress systems leads to behavioral and physical adaptive changes that improve an organism's ability to survive (Sapolsky, 2002). Possibly, anxiety disordered children with a less responsive HPA-axis to stress aremore susceptible to persistence of depressive symptoms, because a less adaptive HPA-axis leads to more

difficulties in mobilizing energy resources to sufficiently cope with stress (Hankin et al., 2010). This might underlie the impaired effects of treatment strategies and, as a result, persistence of depressive symptoms. Another explantion is that the mechanism underlying lower cortisol reactivity may reflect down-regulation of the HPA axis, because chronic stress repeatedly over-activates and eventually impairs the HPA axis (Gunnar, Vazquez, 2001; Herman et al., 2005; Juster, McEwen, Lupien, 2010; Suzuki et al., 2013). Our results are in line with such a habituation of the stress system. Thus, an alternative explanation is that this low HPA-axis reactivity signals a vulnerability of a subgroup of children with anxiety disorders that appear to be less responsive to treatment.

Physiological hyperarousal

Of fraidy-cats and feeling blue

The tripartite model by Clark and Watson (1991) is a model that is frequently cited with regard to the disentanglement of anxiety and depression as different constructs.This model hypothesizes that physiological hyperarousal is specific for anxiety.The study described in *Chapter 3* attempts to quantify the concept of physiological hyperarousal by using perceived arousal to a real stressor as a proxy for physiological hyperarousal. Previous studies suggest that high anxious subjects tend to perceive physiological sensations as more severe than non-anxious subjects (Hoehn-Saric, McLeod, 2000), sometimes even in the absence of an actual difference in physiological measures (Edelmann, Baker, 2002).

Overall, the results from the study in *Chapter 3* provide some evidence in support of the applicability of perceived arousal as a measure of physiological hyperarousal to differentiate anxiety from depressive symptoms in a general population sample of children. Although perceived arousal to a real stressor was related to both anxiety and depressive problems, perceived arousal showed a stronger association with anxiety than with depressive symptoms. Furthermore, when anxiety and depressive

problems were entered simultaneously in our statistical model, only anxiety problems predicted perceived arousal. These findings are in line with findings in child and adult literature (Brown, Chorpita, Barlow, 1998; Joiner et al., 1999; Chorpita, Daleiden, 2002). Together, the findings indicate that anxious children indeed perceive more arousal when exposed to daily fear or stress. This increased perceived arousal might be a vulnerability factor for the development of pathologic anxiety, because when children perceive the world as full of threats and dangers, with no possibility to relax and to regard their living environment as safe, anxiety becomes pathologic (Sapolsky, 2002), underscoring the importance of early detection and offering preventive strategies.

Of fraidy-cats

The study in *Chapter 5* shows that perceived arousal during rest is higher in children with a clinical anxiety disorder compared to their non-anxious peers. However, after a stress-task perceived arousal is comparable between children with a clinical anxiety disorder compared to healthy controls. Apparently, anticipatory anxiety in children with a clinical anxiety disorder is high and has reached a plateau even before the stress task begins. This finding is supported by a study in healthy adults, relating neuroticism to exaggerated anticipatory anxiety experience (Drabant et al., 2011).

Strikingly, within the group children with a clinical anxiety disorder, children with three or more anxiety disorders experienced less perceived arousal both in rest and after a stress-task in comparison with children with one or two anxiety disorders. There are several possible explanations: children with a high anxiety load 1) are alexithymic and cannot register their own perceived arousal, 2) actively direct their attention away from threatening bodily sensations and nervous feelings, or 3) already have a continuous plateau level of arousal which they rate as not deviant from normal. This last hypothesis could be in line with the general hypothesis that children with a high anxiety ‘load’ function under chronic stress conditions.

Methodological considerations

Endophenotypes and biomarkers

The terms endophenotype and biomarker are often used interchangeably in the psychiatric literature, yielding conceptual confusion (Lenzenweger, 2013a). The overall liability to mental illness consists of a heritable substrate in interaction with other genetic liabilities as well as environmental and epigenetic inputs. According to Gottesman and Gould (2003, 2006) “an endophenotype is a measurable component, unseen by the unaided naked eye, that lies along (i.e., within) the pathway between disease (i.e., observable phenotype) and distal genotype”. An endophenotype is a manifestation of the underlying disease liability that is not visible to common observation, exists in the person, and predates observable signs and symptoms of the illness (Lenzenweger, 2013b).

Endophenotypes are heritable, biomarkers are not necessary subject to genetic influences. “The biomarker term captures the domain of *any* biologically influenced factor or deviation in relation to psychopathology (including endophenotypes)” (Lenzenweger, 2013b). Thus, a biomarker could be correlated with psychopathological symptoms, but not fall within the genotype to phenotype pathway. It may be useful to discern biological factors that develop secondary to an illness, but fall outside the the genotype to phenotype pathway, i.e. state markers (Lenzenweger, 2013b). To summarize: alle endophenotypes are biomarkers, but not all biomarkers are endophenotypes.

In order to define whether a candidate measurement is reflective of a biomarker or an endophenotype Lenzenweger (2013b) gives clear guidance to the questions we have to ask ourselves:

1. Is this candidate measurement/process heritable?
2. Is this candidate measurement/process reflective of the origin of the illness or the effect of the illness?

3. Does a deviation on the candidate measurement/process predate the onset of the illness, and can it be detected earlier in development, well before the onset of clinical symptomatology and signs of illness?
4. Is the candidate measurement/disease process merely a variable that speaks to elevated risk for a disorder but, as a process, lies outside of the core pathological process(es) in the disorder/condition?

In other words, is there a correlational or causal relationship between a candidate measurement and the illness? Cross-sectional studies merely allow us to identify correlates and to generate initial hypotheses about potential risk factors. Prospective longitudinal studies are necessary to show that a factor precedes the outcome and can therefore be considered a risk factor or a prospective non-causal biomarker. The study in *Chapter 4*, which measured stress physiology in infancy before the longitudinal assessment of internalizing and externalizing problems in order to decrease the possibility of reverse causation, shows that alterations in stress physiology precede problem behavior and may therefore be regarded as a biomarker. Although it is tempting to define it as a causal risk factor, even with this design we are not able to definitively pin down causality as we cannot rule out other factors that have influenced infant stress physiology from conception till infancy. For instance, during the first three months of life even small variations in caregiving are reflected in HPA-axis activity (e.g. Albers et al., 2008).

The other cross-sectional and longitudinal studies described in this thesis are conducted in both general population and clinical samples. Clinical and general population samples might differ in impairment, severity, and duration of symptoms and therefore different conclusions can be inferred on the role of stress physiology in anxiety. The studies in *Chapters 3, 5 and 6* suggest that stress physiology is a biological state marker for the severity and course of the disease. The study in *Chapter 7* implies

stress physiology as a predictor for treatment outcome. Altered stress reactivity in response to daily stressors might underlie the impaired effects of treatment strategies and result in persistence of symptoms.

In conclusion, the results of the studies described in this thesis suggest that stress physiology is a biological marker for the onset, severity, and course of anxiety problems in childhood. Based on this thesis, there is not enough scientific evidence to state that stress physiology can be seen as an endophenotype for childhood anxiety.

I propose a bidirectional relationship between stress physiology and childhood anxiety. Altered physiological functioning of the stress systems, as a vulnerability factor, could influence the expression of clinical anxiety disorders. In addition, chronic and pathological anxiety problems, interfering with daily life and leading to a clinical anxiety disorder, have a different effect on physiological functioning than temporarily heightened, subclinical anxiety symptoms.

Structure of psychopathology

All studies in this thesis show that in anxious children there is most often both homotypic (other anxiety symptoms/disorders) and heterotypic (i.e. depressive and externalizing symptoms/disorders) comorbidity. When using diagnostic criteria according to DSM-5 (American Psychiatric Association, 2013) in clinical practice, many children meet the criteria for multiple diagnoses; comorbidity is the rule rather than the exception across common psychiatric disorders and development. For parents and children seeking consultation for their problems, this comorbidity might result in the unjust implication of increased severity, just because the symptoms of the child do not neatly fall into one diagnostic category multiple diagnoses are given by a clinician.

In recent years, concurrently with the development of the DSM-5, there is increasing notion that neither psychiatric disorders nor their etiologies may be as specific as assumed. Both the observed

psychiatric phenotypic manifestations and the underlying etiological factors show great transdiagnostic overlap and within-diagnostic heterogeneity. This increased notion does justice to the complexity of psychopathology, especially to the field of developmental psychopathology, because developmental changes and differences are per definitionincompatible with static diagnostic categories. This view on developmental psychopathology is actually not new and was already described in the eighties by Achenbach (Achenbach, Edelbrock, 1983). The renewed interest is fueled by recent advances in neuroimaging and genetic techniques, advances in statistical and machine learning data analytic approaches that make it possible to extract information from complex and high-dimensional data, and increasing emphasis on using biological data to tailor therapy to the needs of the individual patient (Marquand et al., 2016).

Recently, Lahey et al. (2014) showed in adults that mental disorders are not fixed and independent entities; each diagnosis is robustly related to other diagnoses in a correlational structure that is manifested both concurrently and in patterns of heterotypic continuity over time. This co-variation cuts across not only specific diagnostic categories, but also higher-order dimensions of psychopathology such as internalizing and externalizing dimensions, with correlations >0.5 (Murray, Eisner, Ribeaud, 2016). Furthermore, there is both homotypical and heterotypical continuity of childhood psychopathology into adulthood (Lahey, 2015).

Hierarchical dimensional models are an alternative to diagnostic categories, generating dimensional scores in relation to norms for the child's age and gender, type of informant, and appropriate multicultural norms. Correlations between particular subsets of symptoms can be embodied in higher-order dimensions, such as externalizing, internalizing and general psychopathology (Achenbach, 2015). The results of the study in *Chapter 2* are in line with the existence of a higher-order internalizing dimension. Hierarchical-dimensional conceptualizations of psychopathology hypothesize that each form of psychopathology has both unique and broadly shared etiologies and mechanisms.

Interestingly, recent studies have suggested that the structure of psychopathology may be usefully represented in terms of a general factor of psychopathology (p-factor) capturing variance common to a broad range of symptoms transcending higher-order dimensions of psychopathology in addition to specific factors capturing variance common to smaller subsets of more closely related symptoms (Caspi et al., 2014; Murray, Eisner, Ribeaud, 2016). Using multi-informant testing the Generation R study recently showed that in addition to the existence of a general psychopathology factor, both an externalizing and internalizing factor exist independently of the general factor and of each other (Neumann et al., 2016). This model with a general p-factor results in a *still strong, but negative* correlation between the internalizing and externalizing factor. This suggests the presence of a single internalizing/externalizing dimension in young children, once accounted for the general psychopathology factor. Another important finding is that the genetic correlation between an externalizing and internalizing factor defined in a second model is 0.8, suggesting that very much the same factors contribute to the two symptom domains.

Because internalizing and externalizing factors are correlated in many samples, we may find that covarying one type of score out of the other type of score changes associations with other variables, risk factors, endophenotypes or biomarkers (Madigan et al., 2013; Achenbach et al., 2016). These results are strengthened by the conclusion of the study described in *Chapter 4*. In conclusion, this study showed that infant stress physiology functions as an antagonistic predictor of early childhood internalizing and externalizing problems. In studying early risk factors for the continuity of problems among preschool children, we emphasize the importance of considering developmental changes in and the co-occurrence of internalizing and externalizing problems, because biomarkers that increase one type of problem behavior and decrease the other, such as stress physiology, might remain undetected when internalizing and externalizing problems are not disentangled.The mutual adjustment for internalizing and externalizing problems in our analyses created statistically homogeneous phenotypes, a theoretical

construct, which is scientifically relevant in understanding mechanisms of the development of problem behavior.

However, there are also some critical notes when using only hierarchical-dimensional conceptualizations of psychopathology, also called 'lumping'. Lumping might obscure possible etiologic substrates for specific phenotypic dimensions of psychopathology. As a result, we might miss some opportunities for treatment and cure.

For the future it will continue to be important to use both approaches: on the one hand careful clinical examinations, detailed history taking, and phenotypic specification as essential elements of clinical practice and clinical research, while on the other hand examining large, representative (clinical) epidemiologic cohorts. With an increased understanding of the substrates for the heterogeneous presentation of psychopathology, we will be able to make better informed decisions about whether and when we should be taking a more general or specific approach to psychopathology in daily clinical practice (Leventhal, 2012).

Theoretical perspectives on stress and development

The past decades our understanding of the systems that are involved in the stress response has increased, along with the recognition that the stress system reacts uniquely to different types of stressors, and whose patterning of responses changes over time and development (Joëls, Baram, 2009; Doom, Gunnar, 2013). For instance, we have to acknowledge that elevations in cortisol, once almost synonymous with stress, may be only one way in which the HPA-axis responds to stressors. Chronic activation of the stress system may lead to both low or high levels of cortisol depending on development, life-events and the type of affective psychopathology (Doom, Gunnar, 2013). Several theoretical models have been developed to capture the complexity of the stress response.

Allostasis is a theoretical concept which refers to the physiological short-term adaptations that maintain homeostasis through change via allostatic mediators. Allostasis is a fundamental process which enables organisms to actively adjust to both predictable and unpredictable events. Allostatic load refers to the cumulative cost of allostasis to the body. The wear-and-tear on the body and the brain, resulting from chronic dysregulation of physiological systems that are normally in place to adapt to environmental challenge, is referred to as allostatic overload (McEwen, Wingfield, 2003). Based on Sterling and Eyer's (1988) allostasis concept, McEwen and Wingfield (2003) proposed that the allostatic load model could provide a framework for understanding how an animal copes with unpredictable challenges. McEwen and Gianaros (2010) state that a state of prolonged anxiety and anticipation can theoretically result in allostatic load, because anticipation can drive the output of allostatic biomediators (McEwen, Gianaros, 2010). This model could be applied to the study in *Chapter 5*, which shows the impact of experienced persistent stress in children with a clinical anxiety disorder, as indexed by the activity of both the stress systems.

Diathesis-stress models postulate that a predispositional vulnerability, when present in individuals, is activated in poor environments to produce worse outcomes in comparison to individuals without that vulnerability. The term diathesis derives from the Greek term (διάθεσις) for a predisposition, or vulnerability. A diathesis can take the form of genetic, psychological, biological, or situational factors (Ingram, Luxton, 2005). Diathesis-stress models are often not sufficient for studying factors that may lead to differential effects depending on the environment, because the sensitivity to positive effects of a positive environment is not included in the model. Belsky theorized from an evolutionary perspective that children should vary in their susceptibility to both adverse and beneficial effects of rearing styles within a family. The differential susceptibility hypothesis extends the diathesis-stress model to include a sensitivity to positive environments as well as negative environments or stress (Belsky, Pluess, 2009). Individuals are differentially susceptible to environmental influences due to

plasticity factors that promote maladaptation in some contexts and enhance adaptation in others. An example of this hypothesis is, for instance, a study by Kochanska et al. (2007) which shows that highly fearful infants experiencing high levels of power-assertive paternal discipline had the highest chance to cheat in a game as toddlers, however when cared for in a supportive those same highly fearful infants manifested the most rule-compatible conduct as toddlers. The biological sensitivity to context theory argues that all ranges of (re)activity are evolutionary adaptive to (future) contexts (Boyce, Ellis, 2005).

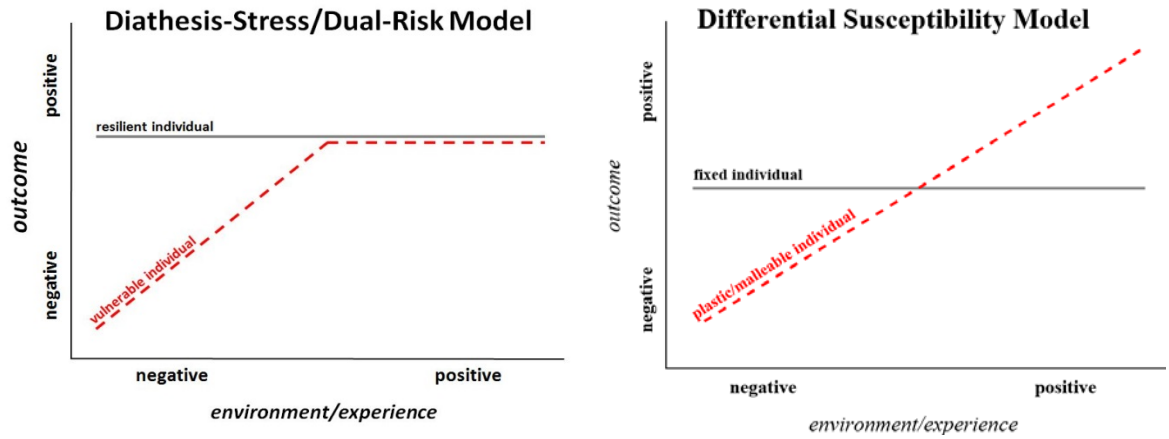

Figure 1. Graphical display of the diathesis-stress and differential susceptibility model. By Mpluess - own work, public domain

The above described well-known models of stress address changes over time, but are not explicitly sensitive to developmental changes. Extensive research has shown that stressors have differential impact depending on when in development they are experienced. Furthermore, animal research has shown that part of the shaping of the stress system takes place very early in life, possibly even prenatally, with consequences for disorders in adult life (Barker, 2007; Doom, Gunnar, 2013).

Recently, Hankin et al. (2016) have developed a developmental sensitive heuristic conceptual model aimed at the comorbidity, continuity and discontinuity of internalizing psychopathology across development, based on a modern latent dimensional structural model of psychopathology. In this model

risk traits interact with environmental stressors to confer risk for broad latent psychopathology dimensions or specific symptom manifestations. The model uses five levels of analysis: 1) neural and endocrine systems implicated in internalizing psychopathology, 2) latent vulnerability traits, which are fairly stable individual differences in cognitive and affective vulnerabilities, 3) latent psychopathology liabilities, which are broad psychopathology liability dimensions that span disorders, 4) symptom specific syndromes, which are specific constellations of internalizing symptoms that systematically and characteristically group together as part of a coherent pattern, and 5) stressors, from different domains and types of events, transpiring across the lifespan that may trigger symptom specific syndrome manifestations at particular points during development. A similar approach has recently been applied to externalizing psychopathology (Beauchaine, McNulty, 2013). Given the accumulating evidence from studies into stress physiology and studies into the developmental structure of psychopathology (see previous sections in the discussion of this thesis) in my opinion a heuristic conceptual model which incorporates the general psychopathology, the externalizing and the internalizing latent factor would be more appropriate.This view is strengthened by the conclusion of the study described in *Chapter 4*, which showed that infant stress physiology functions as an antagonistic predictor of early childhood internalizing and externalizing problems. We showed that stress physiology can increase one type of problem behavior and decrease the other. Stress physiology might therefore remain undetected as a relevant biomarker for certain behaviors in models that do not enter internalizing and externalizing latent factors simultaneously.

The studies conducted in this thesis underline the complexity and plasticity of the stress system. Therefore, a careful, modest approach to studying normative and atypical patterns of the stress system in relation to psychopathology is needed.

POTENTIAL LIMITATIONS

Several other issues must also be considered when interpreting the findings of the studies in this thesis.

Confounders in stress physiology

Many of the possible confounding variables in pediatric stress physiology research find their origin in adult research (thesis Saridjan, 2015). Although useful, not all of these possible confounding factors are relevant for stress physiology research in early life. In the relationship between stress physiology and psychiatric disorders, most often effect sizes are small. In large general population samples it is possible to take into account several confounding factors, however in smaller clinical samples correcting for too many potential confounding variables could lead to type II errors due to insufficient power to detect small effects. This is reflected in the number of potential confounding variables taken into account in the studies described in this thesis.

In the study described in *Chapter 4* gender of the child, gestational age, weight at birth, age of the infant at physiological measurement, maternal age, smoking and drinking behavior during pregnancy, and level of highest completed education by the mother were studied as covariates, based on previous reports (Dierckx et al., 2014). All the studies described in this thesis corrected the associations of interest for age and gender (for a review of gender and age differences during childhood, see Van der Voorn et al., 2017), most studies corrected for body mass index or used gestational age at birth and birth weight as a proxy for body constitution. All studies described in this thesis were also ethnically homogeneous,ruling out confounding and effect modification by ethnicity, which as a consequence limits the generalizability of our results to other ethnic populations. Pubertal status is an important determinant of cortisol levels (Shirtcliff et al., 2015). Data on Tanner stage, a proxy for pubertal status, are lacking in the described studies, but variation in pubertal status is minimized through the inclusion of only children below 13 years in most studies. Only the study described in

Chapter 6 included adolescents up to 16 years. Should pubertal status be related to remission status as well, this could have led to confounding. However, because we have a broad age range and adjusted for age, this is not very likely.

Measures of stress physiology

We assessed HPA-axis functioning by measuring the end product of this axis, cortisol. However, the HPA-axis is a very complex system and for instance, its activity is regulated by multiple hormones (Sapolsky, Romero, Munck, 2000; De Kloet, 2003; Herman et al., 2005). Hence, the measurement of only the end product is a relatively crude way to measure a possible altered functioning of this system. For instance, Young, Abelson and Cameron (2004) did find alterations in ACTH levels in depressed children with a comorbid anxiety disorder in response to a stressor, but not in cortisol levels. Thus, the lack of significant findings regarding cortisol in some of the studies described in this thesis, does not necessarily mean that HPA-axis functioning is not altered in these subjects. These alterations might occur on different levels in the HPA-axis.

Furthermore, the availability of only one cortisol diurnal profile can be seen as a limitation. Rotenberg et al. (2012) show that the cortisol awakening response requires at least 3 weekdays of sampling to yield a stable estimate. However, a review by Golden et al. (2011) states that the use of sampling multiple salivary cortisol measures across the diurnal curve (including awakening cortisol), as we did in most of the described studies, likely reflects chronic cortisol burden and has the highest between-visit reliability of all cortisol measures ($r = 0.63$-0.84).

Sampling compliance was based on child-reported time of saliva collection combined with parent-initiated times on a daily log sheet. Use of electronically monitored timing would improve the precision and accuracy of sample timing, as well as the ability to screen for potentially invalid samples.

Another notable weakness of the current studies is the lack of consistency in the available stress measures during rest, stress, and recovery between the different studies. For instance for the study in *Chapter 5* heart rate variability measures and cortisol measures during stress were not available due to differences in the applied stress task between cases and controls. The stress task that was applied in both groups consisted of the mental arithmetic task, a cognitive task that compared to a public speaking/cognitive task combination is less capable of eliciting a substantial cortisol stress response (Kirschbaum et al., 1993; Dickerson, Kemeny, 2004). Furthermore, it is a task in which speech limits the possibility to control HF HR variability for respiratory frequency, therefore, HF HR variability was not analyzed during the MAT.

Informants

The studies described in *Chapters 2, 3 and 4* used only one informant to assess psychiatric symptoms. One could argue that the use of multiple informants (e.g. parent- and self-report) would lead to a more valid assessment of these symptoms (Grills, Ollendick, 2003; Comer, Kendall, 2004). Multiple informant agreement is higher for externalizing than for internalizing problems; a possible explanation for this phenomenon can be that internalizing problems tend to be inwardly focused upon the self (Grills, Ollendick, 2003). Therefore, the choice for self-report questionnaires in *Chapters 2 and 3* instead of parent-report is defendable given the focus on internalizing problems in children and adolescents.

The study described in *Chapter 4* included children that were not able to fill in self-report questionnaires, given their age. A study by Ivanova et al. (2010) showed that parents are sufficiently capable of objectively rating their children's problem behavior using the CBCL/1½–5. In addition, although the outcome parameters in *Chapters 2, 3, 4, 6 and 7* were assessed with parent-report or self-report measures, all predictors were objective biological measures, hence there is no risk of shared method variance bias.

IMPLICATIONS FOR RESEARCH AND PRACTICE

First, this thesis showed that infant stress physiology functions as an antagonistic predictor of early childhood internalizing and externalizing problems. In particular, our data suggest that low autonomic arousal is a marker for the development of more deviant, externalizing problems during childhood and shows less association with the highly prevalent emotional reactive externalizing behavior seen in infants and toddlers, while high autonomic arousal might be a marker of early and persistent internalizing problems. In studying early biomarkers for the continuity of problems among preschool children, we emphasize the importance of considering developmental changes in and the co-occurrence of internalizing and externalizing problems. Because biomarkers that are associated with a heightened risk for one type of problem behavior and decrease of the other, such as stress physiology, might remain undetected when internalizing and externalizing problems are not disentangled. Future research should extend the follow-up of internalizing and externalizing problems across the lifespan, with repeated measurements of basal and reactive stress physiology through experience sampling methods, and measurement of relevant environmental factors. This will elucidate the relation between stress physiology and the development of psychiatric symptoms and ultimately the incidence of psychiatric disorders. Further, it would be interesting to conduct the same measurements and analyses over time in a sample at-risk for internalizing and externalizing disorders, and an unselected clinical sample.

Second, this thesis showed that children with an anxiety disorder can be distinguished on several psychophysiological characteristics from healthy children. Children with a clinical anxiety disorder have a pattern of elevated sympathetic (re)activity, and lowered parasympathetic activity compared to same-aged children from a general population sample. We report that children with a clinical anxiety disorder have a pattern of hypoactivation of basal HPA-axis functioning compared to

same-aged children from a general population sample. These results are in support of the hypothesis that a child with a clinical anxiety disorder experiences persistent stress, indexed by the activity of both the stress systems. This notion is underlined by the finding that a high clinical load in the clinical sample was associated with an even further deviation of perceived arousal, basal HPA-axis functioning and sympathetic functioning. The load of anxiety disorders during adolescence has been associated with later risks of anxiety disorders, major depression, substance dependence, suicidal behavior and other adverse outcomes, such as educational underachievement and early parenthood (Woodward, Fergusson, 2001). These findings underscore the importance of early detection and adequate treatment of anxiety disorders. Interestingly, in studies finding evidence of low cortisol in association with posttraumatic stress disorder or posttraumatic stress disorder risk, there was also evidence of greater sympathetic arousal as reflected by catecholamine levels (Radley et al., 2011). Possibly, hypocortisolism in non-stressful conditions due to 'habituation' in children with a clinical anxiety disorder (i.e. a chronic stressor leads to a progressive decrease in cortisol) alters the ability of the HPA-axis to restore homeostasis following exposure to stress, resulting in increased sympathetic activation. This might indicate that there are already biological underpinnings of the severity of anxiety disorders in childhood, which might be predictive of adverse outcomes later in life. Future longitudinal studies are needed to study the predictive value of stress physiology as a marker for severity and predictor of adverse outcomes of childhood anxiety disorders later in life.

Third, only a clinical main diagnosis of specific phobia can be discerned from social phobia and separation anxiety disorder on higher sympathetic functioning during basal and stress conditions. The psychophysiological correlates of clinical specific phobia in our study, confirm the existing genetic and longitudinal evidence for specific phobia as separate taxonomic construct.

Finally, if replicated, our results suggest that pre-treatment HPA and ANS reactivity to stress are candidate predictors of a lack of symptom improvement in children with a clinical anxiety disorder. We

carefully speculate that anxiety disordered children with a higher reactivity of the sympathetic nervous system, also experience more physiological and subjective arousal in response to daily fear or stress. As a consequence, higher sympathetic reactivity may influence the natural course of anxiety disorders or the efficacy of standardized cognitive behavioral therapy. Further, anxiety disordered children with a less responsive HPA-axis to stress might be more susceptible to persistence of depressive symptoms, because a less adaptive HPA-axis leads to more difficulties in mobilizing energy resources to sufficiently cope with stress (Hankin et al., 2010). This might underlie the impaired effects of treatment strategies and, as a result, persistence of depressive symptoms. However, our findings seem to indicate that after receiving a standardized stepped-care CBT-program children with an anxiety disorder show restoration of HPA-axis functioning, underscoring the importance of early detection and adequate treatment of anxiety disorders. Current treatments for childhood anxiety disorders are aimed at reducing anxiety symptoms through cognitive behavioral therapy and sometimes pharmacotherapy, which also has an influence on the stress system. It might be interesting to study the effects of a more direct approach to influence the stress system by biofeedback aimed at both the sympathetic and parasympathetic nervous system.

In summary, this thesis focused on the role of stress physiology as a cause, correlate, and predictor of pediatric anxiety disorders with the ultimate goal to improve treatment and prognosis. The results of the studies described in this thesis suggest that stress physiology is a biological marker for the onset, severity, and course of anxiety problems in childhood. Altered physiological functioning of the stress systems, as a vulnerability factor, could influence the expression of clinical anxiety disorders. In addition, chronic and pathological anxiety problems, interfering with daily life and leading to a clinical anxiety disorder, have a different effect on physiological functioning than temporarily heightened, subclinical anxiety symptoms. Given the small magnitude of the associations in this thesis, it is unlikely that stress physiology will be sufficiently sensitive and specific to serve as a biomarker for diagnosis,

treatment outcome, or prognosis. Our studies provide a small but important contribution to the overall knowledge concerning stress physiology in anxious children.

Future research is needed to replicate our findings in other clinical samples. Furthermore, the cross-sectional character of the case-control study makes it difficult to draw conclusions regarding the causality of deviations in stress physiology in children with an anxiety disorder, therefore longitudinal case-control studies in children, matched for age and gender, in which the course and severity of anxiety symptoms and disorders are related to basal and reactive stress physiology are needed. Changes in stress physiology during the course of normal development can be controlled for in a case-control design over time. Moreover, in such a design the bidirectional relationship between psychiatric symptoms and stress physiology over time can be investigated. This will help to gain insight in the etiological pathways of childhood anxiety.

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Summary

SUMMARY

Anxiety is a basic emotion, not typically pathologic but commonly adaptive when it facilitates anticipation to a threat or danger. However, when children perceive the world as full of threats and dangers, with no possibility to relax and to regard their living environment as safe, anxiety becomes pathologic. Variations in the activity of the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS), two major physiological stress systems, have been implicated as possible biological markers of pathological anxiety in children. Normally, activation of these stress systems leads to behavioral and physical adaptive changes that improve an organism's ability to survive. In children with an anxiety disorder, the persistent stress they experience might lead to an excessive and prolonged stress system activation. In the current thesis, we focused on the role of stress physiology as a cause, correlate, and predictor of pediatric anxiety disorders with the ultimate goal to improve treatment and prognosis. More specifically, the series of studies described in this thesis examined the specificity of the association of stress physiology with child anxiety problems, given the high co-occurrence with other anxiety, externalizing and depressive problems. In addition, the studies examined the trajectory of an anxiety disorder and the concomitant changes in stress physiology, and stress physiology as a predictor of therapy outcome.

The high degree of comorbidity amongst anxiety disorders in children and adolescents seems to point in the direction of one taxonomic construct, instead of a number of separate disorders. The study in *Chapter 2* assessed homotypic and heterotypic continuity of symptoms of separation anxiety disorder, generalized anxiety disorder, social phobia, panic disorder, and obsessive compulsive disorder in individuals from a community sample, who were assessed for the first time when they were aged 10 to 12 years, and for the second time two years later. In conclusion, this study extended the existing knowledge about the taxonomy of anxiety problems in young adolescents with longitudinal data. Given

the magnitude of heterotypic continuity in this study, a higher order factor is likely to be present. However, this study also showed that considerable homotypic continuity is present as well, occurring separately from a general propensity for high anxiety levels. This indicates that each type of anxiety problem may, at least partly, represent a distinct taxonomic construct.

Much evidence has shown that anxiety and depressive symptoms in children and adolescents occur frequently and are often comorbid. Childhood anxiety and depression might be two different disorders that often co-occur, or they could be different manifestations of the same underlying vulnerability. There are many models that have tried to disentangle these constructs. An interesting theoretical framework to address this question is the tripartite model, in which symptoms of anxiety and depression are viewed along three dimensions. This model groups symptoms of depression and anxiety into 3 subtypes: negative affectivity, a measure for general affective distress, positive affectivity (PA), a measure representing pleasurable engagement with the environment, and physiological hyperarousal (PH), a measure representing somatic tension and arousal. In *Chapter 3* we examined in a general population sample of 231 children whether (a) basal and reactive HPA-axis functioning, as a proxy for physiological hyperarousal, and (b) perceived arousal before, during and after stress differentiate anxious from depressive children. Together, our findings indicate that perceived arousal to a challenge might be a useful tool to assess the PH component of the tripartite model in a general population sample of school-aged children.

The study described in *Chapter 4* was designed to examine the longitudinal associations between infant autonomic functioning and early childhood internalizing and externalizing problems simultaneously, in a large general population sample (N=464). Lower infant heart rate was associated with less decrease of childhood externalizing problems, whereas higher infant heart rate was associated with a stronger increase of internalizing problems. Consistently, higher infant heart rate variability was associated with less decrease of childhood externalizing problems, whereas lower infant heart rate

variability was associated with a stronger increase of internalizing problems. In conclusion, this study showed that both infant heart rate and heart rate variability function as antagonistic predictors of early childhood internalizing and externalizing problems. Our study emphasizes the importance of using a longitudinal design over a developmental sensitive period when studying early risk factors for the continuity of problems among preschool children. The mutual adjustment for internalizing and externalizing problems in our analyses created statistically homogeneous phenotypes, which enabled us to study the specificity of autonomic arousal as a predictor for change of internalizing or externalizing problems at follow-up in a large non-homogeneous general population sample of children in which co-occurrence of internalizing and externalizing problems was high.

The study in *Chapter 5* focused on whether HPA-axis (basal), ANS (basal and reactive) and perceived arousal measures can distinguish children (N=154) aged 8-12 years with different primary diagnoses (separation anxiety disorder, generalized anxiety disorder, social phobia and/or specific phobia) of clinical anxiety disorders from each other, and from a same-aged general population reference group (N=225). Our findings indicate that children with an anxiety disorder can be distinguished on several psychophysiological characteristics from healthy children. The results underline the hypothesis that ANS and HPA-axis functioning relate to anxiety disorders in general, and only heightened sympathetic (re)activity is a specific correlate for specific phobia, which confirms the existing genetic and longitudinal evidence for specific phobia as separate taxonomic construct. Furthermore, our study found some evidence in support of the hypothesis that a child with a clinical anxiety disorder functions under chronic stressful conditions, with concomitant changes in the activity of both the stress systems. This notion is underlined by the finding that a high clinical 'load' in the clinical sample was associated with an even further deviation of basal HPA-axis functioning and sympathetic functioning. This might indicate that there are already biological underpinnings of the 'load' of anxiety disorders in childhood, which might be predictive of adverse outcomes later in life.

The study in *Chapter 6* investigated associations between the trajectory of an anxiety disorder and the concurrent changes in cortisol levels in a clinical sample of children and adolescents. All children and adolescents received a standardized stepped-care cognitive behavioral program for childhood anxiety disorders. Overall, there was an increase in daytime AUC cortisol between pre-treatment baseline and one-year follow-up, which was dependent on the trajectory of the anxiety disorder. This increase was most pronounced for non-remitters, intermediate in late remitters and less obvious in early remitters. Depression severity was higher in the late and non-remitter groups. An explanation for the differences between these trajectories might be that a stronger increase in diurnal cortisol signals a vulnerability of a subgroup of children with anxiety disorders. Overall, cortisol morning rise did not differ significantly between baseline and follow-up.

Chapter 7 explores the longitudinal association of stress physiology at pre-treatment baseline with anxiety and depressive symptoms at one-year follow-up in a clinical sample of anxiety 152 disordered children treated with cognitive behavioral therapy. This clinical study of pediatric anxiety disorders showed that children with higher pre-treatment skin conductance reactivity to stress, as a proxy of sympathetic reactivity, responded less to treatment. Their anxiety symptoms decreased less over a one-year period as compared to children with a lower pre-treatment sympathetic reactivity. Furthermore, children with lower pre-treatment cortisol reactivity showed less decrease of depressive symptoms. If replicated, our results suggest that pre-treatment HPA and ANS reactivity to stress are candidate predictors of a lack of symptom improvement in children with a clinical anxiety disorder.

SAMENVATTING

Voor kinderen met een angststoornis kan de wereld er uit zien als een plek vol met stressvolle situaties die voortdurende waakzaamheid en coping vereisen, zonder de mogelijkheid om te ontspannen en de omgeving als veilig te beschouwen. Variaties in de activiteit van de hypothalamus-hypofyse-bijnier (HPA) -as en het autonome zenuwstelsel (ANS), twee belangrijke fysiologische stresssystemen, worden gezien als mogelijke biologische markers voor angstklachten bij kinderen. In dit proefschrift hebben we ons gericht op de rol van stressfysiologie bij angstklachten op de kinderleeftijd, met als uiteindelijk doel de behandeling en prognose van angststoornissen op de kinderleeftijd te verbeteren. We onderzoeken de specificiteit van de samenhang van stressfysiologie met pediatrische angststoornissen, gezien de hoge mate waarin angststoornissen op deze leeftijd samen voorkomen met gedragsproblemen en depressieve klachten. Daarnaast onderzoeken we in hoeverre stressfysiologie bij kinderen betrekking heeft op angststoornissen in het algemeen, of dat er een specifiek verband is tussen stressfysiologie en de verschillende soorten angststoornissen op de kinderleeftijd. Verder onderzoeken we het traject van een angststoornis op de kinderleeftijd en de daarmee gepaard gaande verandering in stressfysiologie, en stressfysiologie als een voorspeller van de uitkomst van de therapie.

De hoge mate van comorbiditeit tussen angststoornissen bij kinderen en adolescenten lijkt te wijzen in de richting van één taxonomisch construct, in plaats van een aantal afzonderlijke angststoornissen. De studie in *Hoofdstuk 2* beoordeelde homotypische en heterotypische continuïteit van symptomen van separatieangststoornis, gegeneraliseerde angststoornis, sociale fobie, paniekstoornis en obsessieve compulsieve stoornis bij individuen uit de algemene bevolking, die voor de eerste keer werden onderzocht toen ze tien tot twaalf jaar oud waren, en voor de tweede keer twee jaar later. Concluderend breidde dit onderzoek de bestaande kennis over de taxonomie van angstproblemen bij jonge adolescenten met longitudinale gegevens uit. Gezien de omvang van

heterotypische continuïteit in dit onderzoek, is waarschijnlijk een hogere ordefactor aanwezig. Echter, deze studie toonde ook aan dat ook een aanzienlijke homotypische continuïteit aanwezig is, los van een algemene neiging tot hoge angstniveaus. Dit geeft aan dat elk type angstprobleem, ten minste gedeeltelijk, een duidelijk taxonomisch construct kan vertegenwoordigen.

Eerder onderzoek heeft aangetoond dat angst- en depressieve symptomen bij kinderen en adolescenten vaak voorkomen en vaak comorbide zijn. Angst en depressie kunnen twee verschillende stoornissen zijn die vaak samen voorkomen, of ze kunnen verschillende manifestaties zijn van dezelfde onderliggende kwetsbaarheid. Er zijn veel modellen die hebben geprobeerd om deze stoornissen te onderscheiden. Een interessant theoretisch kader om deze vraag te beantwoorden is het tripartite model, waarin symptomen van angst en depressie worden bekeken langs drie dimensies. Dit model groepeert symptomen van depressie en angst in 3 subtypes: negatieve affectiviteit, een maat voor negatieve emotionaliteit, positieve affectiviteit (PA), een maat die weergeeft hoe enthousiast, energiek en alert iemand met zijn omgeving omgaat, en fysiologische hyperarousal (PH), een maat voor lichamelijke spanning, zoals bijvoorbeeld hartkloppingen, zweethanden en droge mond. In *Hoofdstuk 3* onderzochten we in een algemene populatiesteekproef bij 231 kinderen of (a) basale en reactieve HPA-as werking, als een maat voor fysiologische hyperarousal, en (b) waargenomen lichamelijke spanning vóór, tijdens en na stress angstige kinderen van depressieve kinderen konden onderscheiden. Deze studie vond enig bewijs ter ondersteuning van het tripartite model. Samen geven onze bevindingen aan dat ervaren lichamelijke spanning een nuttige maat kan zijn om de PH-component van het tripartiete model te meten in een algemene populatiesample van schoolgaande kinderen. De reactiviteit van de HPA-as, gemeten door cortisolwaardes tijdens een stresstaak, kan onderscheid maken tussen angst en depressie.

De studie beschreven in *Hoofdstuk 4* onderzocht de longitudinale associaties tussen autonoom functioneren bij zeer jonge kinderen en internaliserende en externaliserende problemen tijdens de

kleutertijd, in een grote algemene populatiesample (N=464). Lagere hartfrequentie bij het zeer jonge kind was geassocieerd met minder afname van externaliserende problemen bij kleuters, terwijl een hogere hartfrequentie werd geassocieerd met een sterkere toename van internaliserende problemen. De resultaten voor hartritmevariabiliteit, een maat voor parasympathisch functioneren, lieten vergelijkbare resultaten zien. Een hogere hartritmevariabiliteit van het zeer jonge kind was geassocieerd met minder afname van externaliserende problemen bij kleuters, terwijl lagere hartritmevariabiliteit geassocieerd waren met een sterkere toename van internaliserinde problemen op de kleuterleeftijd. Concluderend toonde deze studie aan dat zowel de hartfrequentie als de hartritmevariabiliteit van zeer jonge kinderen fungeren als antagonistische voorspellers van internaliserende en externaliserende problemen op de kleuterleeftijd. Onze studie benadrukt het belang van het gebruik van een longitudinaal studiedesign gedurende een voor de ontwikkeling gevoelige periode bij het bestuderen van vroege risicofactoren voor de continuïteit van problemen bij kleuters. De onderlinge correctie voor internaliserende en externaliserende problemen in onze analyses creëerde statistisch homogene fenotypen. Dit stelde ons in staat om de specificiteit van autonoom functioneren op zeer jonge leeftijd als voorspeller voor verandering van internaliserende of externaliserende problemen inde ontwikkeling tot kleuter te onderzoeken in een grote niet-homogene algemene bevolkingssample van kinderen, waarin co-incidentie van internaliserende en externaliserende problemen hoog was.

De studie in *Hoofdstuk 5* concentreerde zich op de vraag of het functioneren van de HPA-as (basaal), het AZS (basaal en reactief), en waargenomen lichamelijke spanning kinderen (N=154) van 8-12 jaar met verschillende primaire klinische angststoornissen (separatieangststoornis, gegeneraliseerde angststoornis sociale fobie en / of specifieke fobie) kunnen onderscheiden van elkaar en van een populatie-referentiegroep van dezelfde leeftijd (N=225). Onze bevindingen laten zien dat kinderen met een angststoornis kunnen worden onderscheiden op verschillende psychofysiologische kenmerken van gezonde kinderen. De resultaten onderschrijven de hypothese dat AZS en HPA-as functioneren in

verband staan met angststoornissen in het algemeen, en laten zien dat alleen verhoogde sympathische (re) activiteit een specifiek correlaat is voor specifieke fobie, hetgeen het bestaande genetische en longitudinale bewijs voor specifieke fobie als afzonderlijke taxonomische entiteit bevestigt. Bovendien heeft onze studie enig bewijs gevonden voor de hypothese dat een kind met een klinische angststoornis functioneert onder chronische stressvolle omstandigheden, met bijbehorende veranderingen in de activiteit van beide stresssystemen. Deze hypothese wordt versterkt door de bevinding dat een hoge klinische 'belasting' in de groep van kinderen met een angststoornis geassocieerd was met een nog verdere afwijking van het functioneren van beide stresssystemen. Dit zou erop kunnen wijzen dat er al een biologisch fundament bestaat voor de klinische 'belasting' van angststoornissen in de kindertijd, wat zou kunnen leiden tot nadelige effecten op latere leeftijd.

In *Hoofdstuk 6* werd de associatie tussen het traject van een angststoornis en de gelijktijdige veranderingen in cortisolspiegels in een klinische groep van kinderen en adolescenten met een angststoornis onderzocht. Alle kinderen en adolescenten kregen een gestandaardiseerd stepped-care cognitieve gedragstherapie programma voor angststoornissen bij kinderen en adolescenten. Over het algemeen was er een toename van diurnaal afgegeven cortisol tussen baseline, voorafgaand aan de behandeling, en follow-up één jaar na aanvang van het behandelprogramma. De mate van toename was afhankelijk van het traject van de angststoornis. Deze toename was het meest uitgesproken voor kinderen wiens angststoornis niet was verdwenen, intermediair bij kinderen bij wie er sprake was van een laat herstel en het minst uitgesproken bij kinderen die een vroeg herstel lieten zien. De depressieve klachten waren ernstiger in de groep kinderen bij wie de angststoornis niet was verdwenen en de groep kinderen die een laat herstel lieten zien. Een verklaring voor de verschillen tussen deze trajecten zou kunnen zijn dat een sterkere toename van het diurnaal cortisol de kwetsbaarheid van een subgroep van kinderen met angststoornissen aangeeft. Over het algemeen was de ochtendstijging van cortisol niet significant verschillend tussen baseline en follow-up.

Hoofdstuk 7 onderzoekt de longitudinale associatie van stressfysiologie bij baseline, voorafgaand aan de behandeling, met angst- en depressieve symptomen na één jaar follow-up in een klinische groep van 152 kinderen met een angststoornis die werden behandeld met cognitieve gedragstherapie. Deze klinische studie van pediatrische angststoornissen toonde aan dat kinderen met een hogere reactiviteit van huidgeleiding in reactie op stress, als proxy van sympathische reactiviteit, minder reageerden op de behandeling. Hun angstsymptomen daalden minder gedurende een periode van een jaar in vergelijking met kinderen met een lagere sympathische reactiviteit vóór de behandeling. Bovendien vertoonden kinderen met een lagere cortisolreactiviteit vóór de behandeling minder vermindering van depressieve symptomen. Indien gerepliceerd, suggereren onze resultaten dat HPA-as en ANS stressreactiviteit voorafgaand aan de behandeling kandidaat-voorspellers zijn van verminderde symptoomverbetering bij kinderen met een klinische angststoornis.

Addendum

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MANUSCRIPTS AND PUBLICATIONS

Ayer, L., Greaves-Lord, K., Althoff, R.R., Hudziak, J.J., **Dieleman, G.C.**, Verhulst, F.C., & Van der Ende, J. (2013). Blunted HPA axis response to stress is related to a persistent Dysregulation Profile in youth. *Biological Psychology*, *93* (3), 343-351.

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Dieleman, G.C., Dierckx, B., Jonker, C.C.T., Tulen, J.H.M., Verhulst, F.C., Jaddoe, V.W.V., & Tiemeier, H. Of fraidy-cats and wild tigers: a prospective study of infant autonomic functioning and child internalizing and externalizing problem behavior. *Submitted for publication*.

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Velders, F.P., **Dieleman, G.**, Cents, R.A., Bakermans-Kranenburg, M.J., Jaddoe, V.W., Hofman, A., Van IJzendoorn, M.H., Verhulst, F.C., & Tiemeier, H. (2012). Variation in the glucocorticoid receptor gene at rs41423247 moderates the effect of prenatal maternal psychological symptoms on child cortisol reactivity and behavior. *Neuropsychopharmacology*, *37* (11), 2541-2549.

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Verhulst, F.C., **Dieleman, G.C.**, Hofstra, M., & Demmendaal, I.M. (Red.) (2014)**.** *Veertig jaar kinder- en jeugdpsychiatrie; Terugblikken en vooruitzien*. Assen: Van Gorcum.

Wesseldijk, L.W., **Dieleman, G.C.**, Lindauer, R.J., Bartels, M., Willemsen, G., Hudziak, J.J., Boomsma, D.I., & Middeldorp, C.M. (2016). Spousal resemblance in psychopathology: A comparison of parents of children with and without psychopathology. *European Psychiatry*, *34*, 49-55.

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ABOUT THE AUTHOR

Gwen Dieleman was born on June the 14th 1976, in Axel, the Netherlands. After obtaining her VWO diploma at the Petrus Hondius in Terneuzen in the summer of 1994, she moved to Delft to study Industrial Design at the Technical University Delft. She completed the first two years of Industrial Design. After these two years, she drew a place by lot and started her medical education at the Erasmus University in Rotterdam (1997). She obtained her bachelor (1998) and clinical internship both cum laude (2003). After graduating from medical school in 2003, Gwen started with her first clinical job as a medical doctor in child and adolescent psychiatry at the Sophia Children's Hospital, in Rotterdam. In 2004, she obtained a grant to fund two years of her PhD and started the work described in this thesis at the Department of Child and Adolescent Psychiatry/Psychology. During her PhD, she also worked as a resident in training in child and adolescent psychiatry (2005-2007; 2011-2012) and psychiatry (2008-2011). Between 2007 and 2012 she became mother of a boy, Noah (11), and two girls, Julie (9) and Cato (6). As of 2012, she is working as a child and adolescent psychiatrist specializing in eating disorders and has been head of the outpatient department of Child and Adolescent Psychiatry/Psychology from 2012 to 2019. In this capacity she was responsible for the management, strategy and organization of the outpatient department. In her clinical and scientific work she has a special interest and expertise in children who have a disorder at the interface between somatics and psychiatry. Since 2012 she is clinical research coordinator for the department of Child and Adolescent Psychiatry/Psychology. She set up and shaped the clinical research line of the department and obtained several grants. Her research interests are in the field of transition psychiatry, clinical (genetic) epidemiology, neurobiology and stress physiology.

PHD PORTFOLIO

Summary of PhD training and teaching activities

Name PhD student:	G.C. Dieleman
Erasmus MC Department:	Child and Adolescent Psychiatry/Psychology
PhD period:	2005-2018
Promotors:	Prof.dr. A.C. Huizink, Prof.dr. H. Tiemeier

	Year	Workload (ECTS)
1. PhD training		
Research skills		
Erasmus Summer programme	2004	
Principles of genetic epidemiology		0.7
Searching genes of complex disorders		0.7
Classical Methods for Data-analysis	2005	4
Basic Course Clinical Research, BROK, Rotterdam	2016	2
Relevant presentations for this PhD		
- 33th Congress of the Dutch Association of Psychiatry, Den Haag.	2005	0.5
- Studiedagen Angst en Autisme, Rotterdam.	2006	0.5
- 34th Congress of the Dutch Association of Psychiatry, Groningen.	2006	1.5
- 17th IACAPAP, Melbourne, Australia.	2006	1
- 35th Congress of the Dutch Association of Psychiatry, Maastricht.	2007	1
- 6th World Congress on stress, Vienna, Austria.	2007	0.5
- 36th Congress of the Dutch Association of Psychiatry, Maastricht.	2008	0.1
- Lustrumsymposium Department Child and Adolescent Psychiatry of the Dutch Association of Psychiatry.	2008	0.5
- XVIII World Congress on Psychiatric Genetics, Athens, Greece	2010	0.5
- VU Wetenschapsdag , Amsterdam.	2012	0.1
- 40th Congress of the Dutch Association of Psychiatry, Maastricht.	2013	0.7
- Jubilee Congress 65 years Department Child and Adolescent Psychiatry of the Dutch Association of Psychiatry	2013	0.5
- ROMCKAP expertmeeting Wetenschap meets praktijk, Amsterdam.	2013	0.5
- 41th Congress of the Dutch Association of Psychiatry, Maastricht.	2014	0.2
- 17th World Congress of Psychophysiology, Hiroshima, Japan.	2014	0.5
Relevant (Inter)national conferences for this PhD		
- 33th Congress of the Dutch Association of Psychiatry, Den Haag.	2005	0.3
- Studiedagen Angst en Autisme, Rotterdam.	2006	0.6
- 34th Congress of the Dutch Association of Psychiatry, Groningen.	2006	0.3
- 17th IACAPAP, Melbourne, Australia.	2006	0.6
- 35th Congress of the Dutch Association of Psychiatry, Maastricht.	2007	0.3

- 6th World Congress on stress, Vienna, Austria.	2007	0.6
- 36th Congress of the Dutch Association of Psychiatry, Maastricht.	2008	0.3
- Lustrumsymposium Department Child and Adolescent Psychiatry of the Dutch Association of Psychiatry.	2008	0.3
- XVIIIth World Congress on Psychiatric Genetics, Athens, Greece	2010	0.85
- 40th Congress of the Dutch Association of Psychiatry, Maastricht.	2013	0.3
- Jubilee Congress 65 years Department Child and Adolescent Psychiatry of the Dutch Association of Psychiatry.	2013	0.3
- XXth World Congress Psychiatric Genetics, Hamburg, Germany.	2013	0.5
- 41th Congress of the Dutch Association of Psychiatry, Maastricht.	2014	0.3
- 17th World Congress of Psychophysiology, Hiroshima, Japan	2014	0.5
- XXIIth World Congress Psychiatric Genetics, Copenhagen, Denmark.	2015	0.85

Other

- Attending seminars of the Departments of Psychiatry and Child and Adolescent Psychiatry/Psychology	2004-2016	5
- Attending research meetings	2004-2008	2
- Writing medical ethical protocols for several projects	2004-2016	1.4
- Obtaining grants for several research projects	2005-2018	5.6
- Organization congress: Studiedagen Angst en Autisme, Rotterdam.	2006	0.5
- Editor of 'Psychofarmaca in de KJP', Van Gorcum.	2011	2
- Organization symposium: 'Sophia 150 years: Children of the future', Erasmus Medical Center, Rotterdam.	2013	0.7
- Writing book chapters in Dutch textbooks for child and adolescent Psychiatry	2007-2017	4
- Editor of 'Veertig jaar Kinder- en Jeugdpsychiatrie: terugblikken en vooruit kijken.', Van Gorcum.	2014	1
- Development of JGZ-richtlijn Angst, Trimbos-instituut	2014-2016	1

2. Teaching

Practicals, workshops and lectures

- Training students and research assistants in psychophysiological Assessments	2005-2008	3
- Course, medical students about observation skills and childhood psychiatric disorders, Erasmus Medical Center, Rotterdam	2005-2016	0.5
- Annual lecture for medical students 'An anxious child'	2012-2018	1.8
- Refresher course for general practitioners	2014	0.3
- Development Masterclass psychofarmacologie bij kinderen en adolescenten, in collaboration with Prelum, Houten	2014-2016	0.6

Supervising master theses

- Clara Elbers, Thesis title: 'Autonomous nervous system activity in	2004	2
- Dennis den Uijl, Thesis title: 'Fysiologische reacties op een stresstaak bij kinderen met Attention-deficit/hyperactivity disorder'	2004	2
- children with anxiety disorders and children with externalizing disorders'		
- Lisette Beets, Thesis title: 'Stress reacties bij kinderen met een	2005	2

- angststoornis in vergelijking met de algemene bevolking’		
- Hanan el Marroun, Thesis title: ’ANS Reactivity to stress in externalizing children compared to healthy controls’	2005	2
- Dana Kuil, Thesis title: ‘Do anxious parents see more anxiety in their children?’	2006	2
- Marieke Sikkes, Thesis title: ‘Stresskip of ijskonijn?’	2006	2
- Floor Sherman, Thesis title: ‘Het verband tussen het functioneren van het autonome zenuwstelsel en de mate van agressief en delinquent gedrag bij kinderen met externaliserende stoornissen’	2006	2
- Simone Voortman, Thesis title: ‘HPA-as acitiviteit Als indicator voor de ontwikkeling van gedragsproblemen bij kinderen’	2006	2
- Janneke Horseling, Thesis title: ‘Waar vind je klavertjes vier?’	2006	2
- Marieke de Vries, Thesis title: ‘Fysiologische reacties op stress en subjectieve beleving hiervan bij kinderen met en zonder een angststoornis’	2006	2
- Marian Hoek, Thesis title: ’Het functioneren van de	2006	2
- hypofyse-bijnier-as bij kinderen met een angststoornis’		
- Fayola Cairo, Thesis title: ‘De invloed van stress op cortisol bij kinderen met een angststoornis’	2006	2
- Melissa Spoor, Thesis title: ‘Angst bij ouder en kroost, reactie op stress het grootst?	2007	2
- Dorien van Amsterdam, Thesis title:’Physiological reactivity in anxious children who experienced stressful life events’	2008	2
- Sytske Altena, Thesis title: ‘Differences in Autonomic Response in Children with ‘pure’ Attention-Deficit Hyperactivity Disorder versus Oppositional Defiant Disorder and Conduct Disorder’	2008	2
- Ivrian Lautenslager, Thesis title: ‘HPA-axis reactivity in children with externalizing disorders’	2009	2

Note: 1 ECTS (European Credit Transfer System) is equal to a workload of 28 hours. For supervising articles, see publication list.

Stressed Out! Stress physiology in anxious children

PhD thesis, Erasmus University Rotterdam, The Netherlands

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