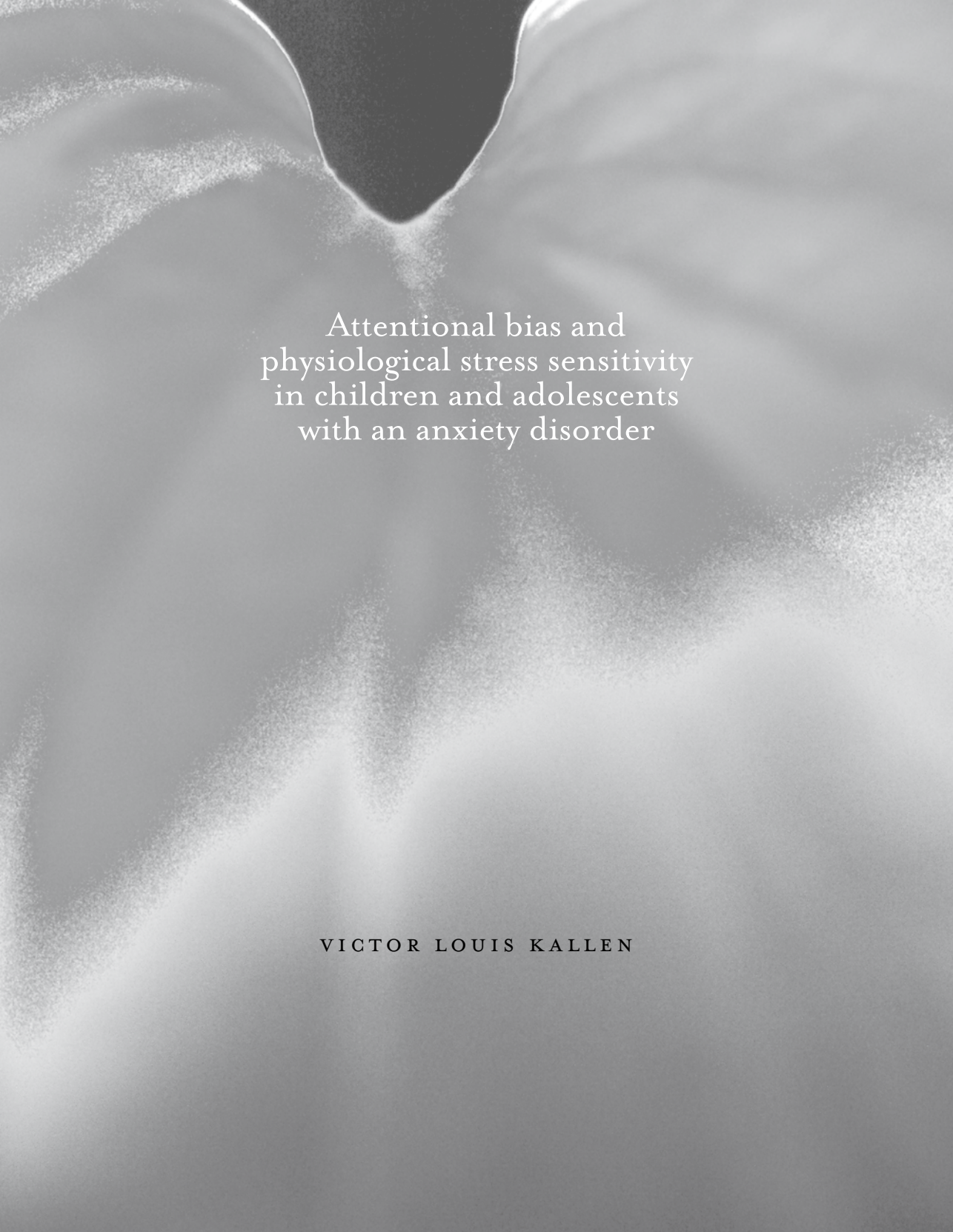

Attentional bias and physiological stress sensitivity in children and adolescents with an anxiety disorder

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The study reported in this thesis was performed at the Department of Child and Adolescent Psychiatry, Erasmus MC, Sophia Children's Hospital Rotterdam, The Netherlands and the Deparment of Child and Adolescent Psychiatry of the Leiden University Medical Center / Curium. This study has been made possible due to financial aid of the Netherlands Foundation for Mental Health, situated in Utrecht, grant no. 2001 5484, and the Sophia Foundation for Scientific Research (SSWO), grant no. 328.

Attentional bias and physiological stress sensitivity in children and adolescents with an anxiety disorder

Aandachtsvoorkeuren en fysiologische stressgevoeligheid in kinderen en adolescenten met een angststoornis

PROEFSCHRIFT

Ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
Op gezag de
rector magnificus

Prof.dr. S.W.J. Lamberts

en volgens besluit van het College voor Promoties

De openbare verdediging zal plaatsvinden op
Woensdag 31 oktober 2007 om 9.45 uur
door

Victor Louis Kallen
geboren te Boxtel

PROMOTIECOMMISSIE

PROMOTOREN:	Prof.dr. F.C.Verhulst Prof.dr. P.D.A. Treffers
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OPGEDRAGEN AAN

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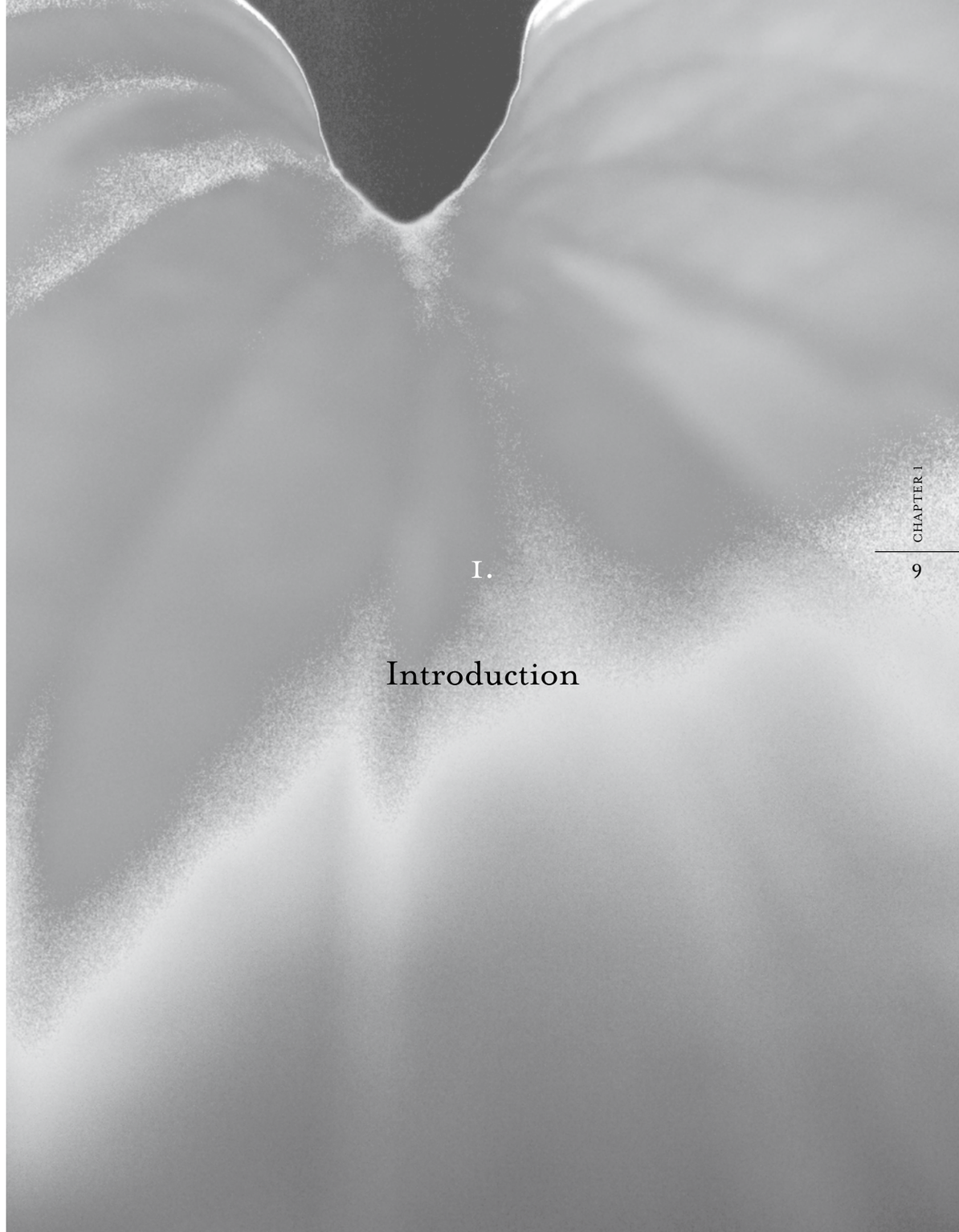

I.

Introduction

1. INTRODUCTION

1.1. *Anxiety disorders in children and adolescents*

Although periods of heightened anxiety are part of normal psychological development (Gullone et al., 2001; Stevenson-Hinde, & Shouldice, 1995; Westenberg et al., 2004), a significant proportion of all children and adolescents report anxiety levels above developmentally appropriate levels and in some of these children and adolescents the anxiety levels may be so severe that they can be diagnosed as an anxiety disorder (Verhulst et al., 1997).

In childhood and adolescence, anxiety disorders are among the most frequently assigned psychiatric diagnoses (Bernstein & Borchardt, 1991). Based on self reports (Diagnostic Interview Schedule for Children: DISC; Shaffer et al., 1993) the prevalence of anxiety disorders in Dutch adolescents (aged 13-18 years) is approximately 10.5 percent (Verhulst et al., 1997). These disorders may be chronic and substantially disrupt the quality of life and development of the children and adolescents involved (Bastiaansen et al., 2006; Keller et al., 1992; Ollendick and King, 1994).

The most frequently diagnosed anxiety disorders during childhood and adolescence are: Separation Anxiety Disorder, Generalized Anxiety Disorder, and Social Phobia. There may be a developmental trajectory underlying the origin of these anxiety disorders, as the average age of being diagnosed with a Separation Anxiety Disorder seems to be significantly lower than, for instance, Generalized Anxiety Disorder, a disorder becoming more prominent during adolescence (Westenberg et al., 1999, 2001).

Scientific interest in factors contributing to the development and maintenance of chronic anxiety and anxiety disorders in youth is developing rapidly. Among others, two factors of interest are: (1) the immediate and unconscious attentional processes when confronted with new and ambiguous and/or emotional stimuli, and (2) stress sensitivity, being the physical vigilance of an individual when experiencing stress.

Deviant but automated attentional strategies and/or deficiencies in the way an individual physically copes with stress are considered to be important moderators in the development of a chronic state of anxiety in children and adolescents. As a consequence, such strategies and deficits may constitute a risk factor for the development of anxiety and stress related psychopathology throughout childhood and adolescence.

However, as yet hypotheses related to these two topics are primarily based on results from studies in adult samples or studies using children and adolescents

without an anxiety disorder, selected from the normal population. The aim of the present thesis is to specifically investigate the role of these two factors in child and adolescent anxiety disorders.

1.2. *Early attentional processes in children and adolescents with an anxiety disorder*

The processing of emotional information is considered to be an important factor in the development and persistence of anxiety symptoms (Mogg et al., 1995; Beck & Clark, 1997; Bradley et al., 1997; Mogg & Bradley, 1998; Keogh et al., 2001). When emotional information is presented the first stages of information processing are aimed at prioritizing the new information obtained. This means that, after an immediate appraisal and based on the extent of threat to, or importance for, the individual, cognitive resources are allocated to process the information further (if necessary) and prepare a response (Williams et al., 1988) (see figure 1.1).
This implies that in the first (unconscious) stages of information processing a decision is made whether or not the new information will be the focus of attention, or will, at present, be neglected. Biases in the early stages of this sequence (like specific attentional preferences) have consequences for the allocation of resources in all following stages. When such biases are present during childhood and adolescence, they may over time develop to automated but deviant cognitive strategies when confronted with ambiguous and/or emotional information (Daleiden & Vasey, 1997; Ehrenreich & Gross, 2002). Not surprisingly, these deviant cognitive schemata are associated with the development of anxiety disorders and may play a prominent role in their persistence (Mogg & Bradley, 1998; Mathews & MacLeod, 1994; Vasey et al., 1996; Williams et al., 1988). As a result, attentional biases during childhood and adolescence are considered to be a risk factor for the development of anxiety disorders (Mogg & Bradley, 1998; Waters et al., 2004). However, as yet only a few studies have been published that compare the attentional preferences of children with an anxiety disorder with children without one (Taghavi et al., 1999; Taghavi et al., 2003; Vasay et al., 1995; Waters et al., 2004). And although to-the-point hypotheses were investigated in these studies, the results are at this moment inconclusive. This calls for additional research including more children, who should be better diagnosed and tested using up-to-date cognitive tasks.

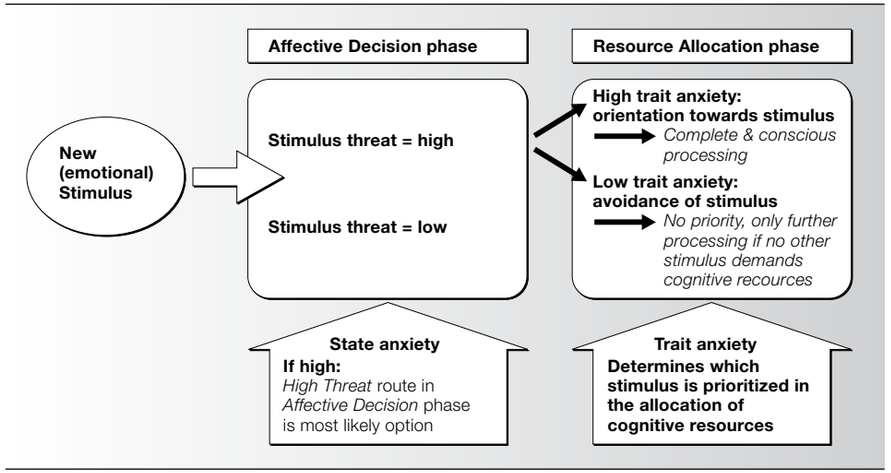

Fig. 1.1. Cognitive mechanisms associated with the initial orientation of attention to threat in anxiety (based on Williams et al., 1988).

1.3. *Stress sensitivity in children and adolescents with an anxiety disorder*

Physiological and endocrine responses to stress are generally regarded to be reliable markers of stress sensitivity (Armario et al., 1996; Cacioppo et al., 2000; De Kloet, 2003; Kirschbaum et al., 1993; Nagane, 1980; Schmidt & Zvolensky, 2006; Vogele & Steptoe, 1992). As a result, deviant development of the biological systems involved in stress regulation is considered to be a risk factor for the development and/or the maintenance of anxiety disorders (De Souza, 1995; Garralda et al., 1991; Gunnar & Vazquez, 2001; Kagan and Snidman, 1999; Penza et al., 2003; Pine et al., 1998; Wilhelm et al., 1999).
On the other hand, the development of anxiety related physical deficits in stress regulation is suggested to be a risk factor for future physical health as well, e.g. in relation to an increased risk of negative cardiovascular events later in life (Allen et al., 1997; Jackson et al., 1999; Jemerin & Boyce, 1990; Treiber et al., 2001; Yeragani et al., 2001). The two primary biological systems involved in stress regulation are the Autonomic Nervous System (ANS) and the Hypothalamus-Pituitary Gland-Adrenal gland (HPA)-axis (see figure 1.2).

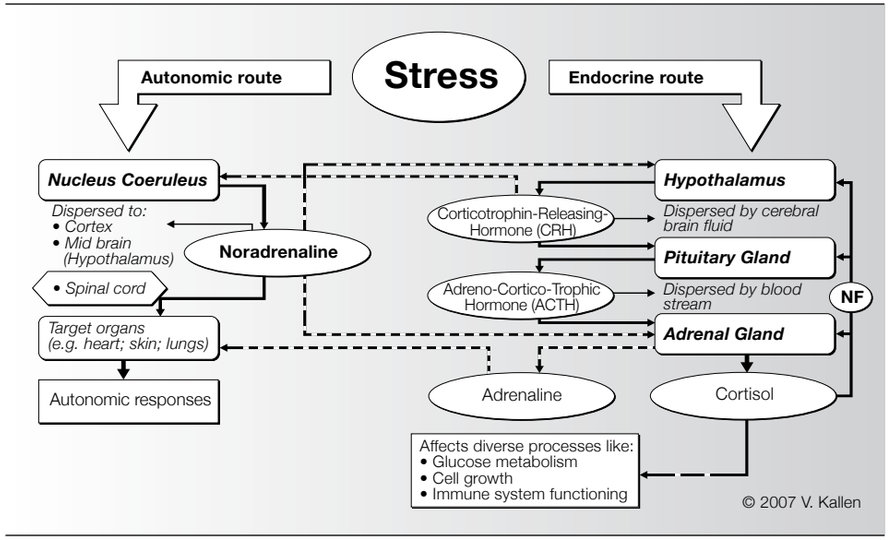

Fig. 1.2. Schematic presentation of the biological stress regulatory systems.
Note: NF = Negative Feedback mechanism.

1.3.1. *HPA-axis functioning in relation to child and adolescent anxiety disorders*

In stressful situations, the hypothalamus secretes corticotrophin-releasing hormone (CRH), which stimulates the pituitary gland to secrete adrenocorticotrophic hormone (ACTH). When ACTH is released in sufficient amounts to significantly increase ACTH concentrations close to the adrenal glands, they produce cortisol. Cortisol is dispersed by the blood stream and changes in cortisol concentrations influence immunity, metabolism, growth, reproduction and other important physiological processes all around the body (Chrousos, 1997; De Kloet, 2003; Sapolsky et al., 2000) (see figure 1.2).

If this system is frequently activated, e.g. due to chronic or regular stress, down regulation of glucocorticoid receptors (GRs) on the organs involved (like the hypothalamus) may be the result (Mizoguchi et al., 2003; Sapolsky et al., 2000). This may cause chronic low basal cortisol concentrations (Mizuguchi et al., 2003; Sapolsky et al., 2000), what is suggested to be associated with (childhood) anxiety (Gunnar & Vazquez, 2001).

HPA-axis functioning has not only been investigated in the context of immediate stress, but has also been associated with specific psychiatric disorders that are related to severe or chronic stress, like anxiety disorders, posttraumatic stress disorder, and depression (Anisman et al., 2001; Bonne et al., 2003; Bremner et al., 2003; Goenjian et al., 2003; Hageman et al., 2001; Tiemeier, 2003; Yehuda et al., 1995). However, although the importance of HPA-axis functioning in relation to (severe) childhood anxiety has been widely recognized (Gunnar & Vazquez, 2001; Rosmalen et al., 2005), up until now only little research has been done investigating the relation between the functioning of the HPA-axis and anxiety disorders in children and adolescents (Coplan et al., 2002; Feder et al., 2004; Gerra et al., 2000; Granger et al., 1994). This is unfortunate, because psychological and physical development go hand in hand in this particular period of rapid individual development. Consequently, deviations in the development of HPA-axis functioning may not only be an indication of emotional disturbances, but may constitute a considerable risk for future physical and psychological health as well (Gunnar and Vazquez, 2001).

The few research findings comparing children with and without an anxiety disorder have provided some evidence for lowered early morning cortisol concentrations (Feder et al., 2004), and a stronger increase of cortisol concentrations after a parent-child conflict task (Granger et al., 1994) in children with an anxiety disorder. However, other studies obtained no, or inconclusive results (Coplan et al., 2002; Gerra et al., 2000). This made Gunnar and Vazquez (2001) conclude that in relation to severe anxiety in children and adolescents basal cortisol concentrations and cortisol responses to stressors may be 'blunted'. This indicates a disturbed functioning, although the way in which the functioning is disturbed and how this relates to anxiety is yet unclear. Consequently, their statement underlines the urgency for more research to HPA-axis functioning and childhood anxiety.

1.3.2. *Autonomic stress regulation in children and adolescents with an anxiety disorders*

The second biological system involved in stress regulation is the Autonomic Nervous System (ANS) (see figure 1.2). The reactivity of this system to stress is the result of simultaneous activation and/or inhibition of the sympathetic and the parasympathetic nervous system (Cacioppo et al., 2000). Immediate physiological responses to stress, like increased heart rate, blood pressure and skin conductance, are regarded to be the result of sympathetic activation and parasympathetic inhibition, associated with the 'flight or fight' response (Cannon, 1932). The initial sympathetic response, which demands considerable energetic resources, should be corrected by heightened parasympathetic activity after the stressor has gone, to restore the pre-stress homeostasis (Cacioppo et al., 2000). Chronic or severe stress may, however, disrupt this balance. Persistent distress in youths may even have

severe consequences for ANS functioning throughout development: it may permanently reduce their physiological ability to adequately cope with stress (Musante et al., 2000). The eventual inability to show an appropriate physiological response to stressors may further stimulate already experienced feelings of distress when again confronted with a stressor. As a consequence, the ANS may be moderating the development of anxiety disorders and/or depression (Garralda et al., 1991; Kagan and Snidman, 1999; Penza et al., 2003; Pine et al., 1998).
Only little research has been conducted so far in this field, but the available results show some evidence that childhood anxiety disorders may be associated with heightened activity of the sympathetic nervous system in response to diverse stressors (Gerra et al., 2000; Rogeness et al., 1990) and with a general decrease of parasympathetic control (Boyce et al., 2001; Monk et al., 2001). However, the scientific evidence for sympathetic hyper-responsiveness to stressors and reduction of parasympathetic cardiac control in children and adolescents with an anxiety disorder is not yet very strong and additional scientific results are necessary.

1.3.3. *Combined endocrine and physiological factors in child and adolescent anxiety disorders*

It is likely that the activities of the HPA-axis and ANS interact (Morilak et al., 2005; Nater et al., 2005; Nyklicek et al., 2005). Glucocorticoids (like cortisol) have been suggested to provide feedback to the central nervous system in order to control both the intensity and the duration of the stress responses, not only in the HPA-axis itself, but in other systems as well, like the forebrain, limbic system and the ANS (Boyle et al., 2006). Particular stressors (e.g. public speaking) which induce immediate ANS responses, significantly increase peripheral cortisol concentrations after 15 to 20 minutes as well (e.g. Kirschbaum et al., 1993). This may be explained by the fact that continuing high ANS activity, especially when it is sympathetic in nature, demands extra energetic resources which may be provided by increased glucose metabolism stimulated by cortisol (De Kloet, 2003; Sapolsky et al., 2000).
Although at least some research has been done to the relationships of the independent biological stress regulatory systems with (severe) anxiety in youth, only Gerra et al. (2000) investigated the baseline activity and stress reactivity of both systems simultaneously in 20 boys with an anxiety disorder, compared to 20 boys without overt psychiatric disorder. These authors found increased baseline ACTH concentrations and a significantly stronger sympathetic response to a stressor in the boys with an anxiety disorder. Although this indicates that indeed both systems can be associated with anxiety disorders in at least boys, interactions or combined effects of these two stress regulatory systems in relation to anxiety have not been investigated yet in child and adolescent anxiety disorders.

1.4. *Aims of the present thesis*

Although nowadays both the deviant development of early attentional processes and stress sensitivity are recognized as important factors in child and adolescent anxiety disorders, only a few studies so far addressed these issues in relation to anxiety disorders in youth.
In relation to attentional processes and child and adolescent anxiety disorders, some authors hypothesized that findings in adults with heightened levels of anxiety or even anxiety disorders would probably be replicated in children with anxiety disorders (Ehrenreich & Gross, 2002; Vasey et al., 1995, 1996). However, although in boys under quite specific circumstances some evidence has been found (Vasey et al., 1996), generally the studies investigating this matter produced contradicting, and consequently inconclusive results (Kindt et al., 1997; Kind & Brosschot, 1999; Taghavi et al., 1999, 2003; Vasey et al., 1995, 1996; Waters et al., 2004). Differences between the studies in terms of tested age groups, the way the high anxiety group was selected, and the instruments used to assess attentional preferences may at least partially account for the discrepancies in the published findings.
The few published studies on stress sensitivity in youth strengthen the suggestion that in children and adolescents the same physiological systems are associated with stress sensitivity as in adults. However, only a few studies have been conducted to investigate the relation of these systems with stress related psychopathology in children and adolescents (Boyce et al., 2001; Dobkin et al., 2000; Gerra et al., 2000; Granger et al., 1994; Monk et al., 2001; Rogeness et al., 1990; Yeragani et al., 2001). As yet, the available results suggest that child and adolescent anxiety disorders may be related to permanent changes in HPA-axis function and to disturbed autonomic functioning mirrored by increased sympathetic responsiveness to stressors and loss of para-sympathetic cardiac control.
The changes in HPA-axis functioning associated with severe anxiety in youth may be reflected by generally lowered cortisol concentrations (Gunnar & Vazquez, 2001). However, because this hypothesis is primarily based on an extrapolation of findings in the general population (Rosmalen et al., 2005), conclusive evidence in relation to anxiety disorders in children and adolescents is yet lacking (Feder et al., 2004; Gunnar and Vazquez, 2001).
The evidence for a relation of autonomic phenomena with child and adolescent anxiety disorders comes from a few studies only (Boyce et al., 2001; Gerra et al., 2000; Monk et al., 2001; Rogeness et al., 1990), while other studies failed to

confirm either the sympathetic (Boyce et al., 2001; Monk et al., 2001) or the parasympathetic findings (Gerra et al., 2000; Yeragani et al., 2001). There may be good reasons for the discrepancies between these studies, as they differ in the average age of the included sample, the method to classify (non) anxious participants, and especially the parameters selected to describe autonomic processes (Dobkin et al., 2000).

Based on these findings we must conclude that although strong hypotheses have been formulated regarding both deviant attentional processes and heightened stress sensitivity in children and adolescents with an anxiety disorder, only a few studies about these topics have been published. These studies significantly extended our knowledge and helped to formulate new and / or more sophisticated hypotheses, however, there are still issues that need to be clarified to further develop our insight in child and adolescent anxiety disorders. These gaps in our present knowledge are best summarized by:
(1) Whether deviant attentional processes are indeed related to childhood anxiety disorders, as suggested by several studies, but for which conclusive scientific evidence at this moment is lacking.
(2) Whether the stress sensitivity in children and adolescents with an anxiety disorder is related to the severity of their anxiety symptoms. Stress sensitivity may be mirrored by:
A. Basal cortisol concentrations.
B. Sympathetic responsiveness to stressors
C. Parasympathetic cardiac control
D. The combined effect of basal cortisol concentrations and sympathetic arousal to stress on the severity of experienced anxiety symptoms.
The present thesis describes our efforts to clarify these 5 issues and thus to contribute to a better understanding of child and adolescent anxiety disorders.

1.5. *Outline of the present thesis*
After an overview of the current state of scientific progress in the field of child and adolescent anxiety disorders (chapter 1), chapter 2 introduces the concept of attentional bias, which is investigated in a sample of regular primary school children. Using a Probe Detection Task, their initial attentional preferences are related to their self reported anxiety scores. In chapter 3, the association between attentional bias scores and levels of anxiety in children without a psychiatric diagnosis is compared with that in a sample of children diagnosed with an anxiety disorder. Chapter 4 describes our study on the relationship of basal HPA-axis functioning and anxiety levels in children and adolescents with an anxiety disorder. In 99 children and adolescents with an anxiety disorder, multiple saliva samples were collected over a normal school day. In these samples cortisol concentrations were measured. Then, the overall cortisol concentration over that day was estimated for each individual by calculating the Area Under the Curve (AUC). Finally, the AUC was used to investigate its relation with specific anxiety symptoms, reported on the Multidimensional Anxiety Scale for Children (MASC: March et al., 1997).
In chapter 5, we investigated if increased anxiety symptoms in children and adolescents with an anxiety disorder were associated with heightened sympathetic responsiveness to standardized stressors, and / or loss of parasympathetic cardiac control during baseline or in response to a social stressor. If this appeared to be the case, we further investigated whether these physiological phenomena could be associated with specific anxiety symptoms in children and adolescents with an anxiety disorder.
In chapter 6, the combined effects of basal HPA-axis functioning and sympathetic stress reactivity were investigated to find out whether this combined effect may be a stronger predictor of anxiety symptomatology in children and adolescents with an anxiety disorder than each independent system by itself.
Finally, in chapter 7 all findings of the present research are discussed in relation to their relevance for the development and maintenance of child and adolescent anxiety disorders.

2.

Early attention processes and anxiety in children

Victor L. Kallen
Joke H.M. Tulen
Robert F. Ferdinand

Perceptual and Motor Skills, 2007, 104, 221-235

Summary. - It has been hypothesized that anxiety in children is associated with attentional bias in the early stages of information processing. Bias towards threat indicates the tendency of an individual to direct attention towards threatening information. The aim of the present study was to investigate if high test-anxiety in a sample of non-referred children is associated with attentional bias towards threat pictures, and if low test-anxiety is associated with attentional bias away from threat pictures. A probe detection task was used in 44 10- to 13-year-old children. Our overall analyses indicated the presence of an attentional bias away from threatening pictures in non-referred children. However, in relation to anxiety, our study could not confirm that high anxious children show an attentional bias towards threatening pictures or that low anxious children show an attentional bias away from threatening pictures. Yet, higher anxiety did seem to be associated with longer mean response times. These longer response times might originate from the interpretation of the nature of a stimulus as too threatening, compared to the actual threatening content, in the first stage of information processing. This finding could be useful to improve treatment methods aimed at anxiety symptoms during childhood.

2.1. INTRODUCTION

The way emotional information is processed is considered to be a crucial factor in the etiology, development, and persistence of anxiety symptoms (Mogg et al., 1995; Beck & Clark, 1997; Bradley et al., 1997; Mogg & Bradley, 1998; Mayer & Merckelbach, 1999; Keogh et al., 2001). One aspect of information processing considered to be especially important with regard to anxiety is 'attentional bias' (Mogg & Bradley, 1998). This concept is defined as the preference for, or avoidance of, specific information in the early stages of information processing. When new information is presented it is prioritized and, based on the extent of threat to, or importance for, the individual, cognitive resources are allocated to process the information and prepare a response (Crick & Dodge, 1994). Increased anxiety is associated with alternations in these cognitive processes (Williams et al., 1988; Williams et al., 1996; Beck & Clark, 1997). This can be demonstrated by impaired performance on specific cognitive tasks in anxious individuals (e.g., MacLeod & McLaughlin, 1995; Amir et al., 2003; Spector et al., 2003). Many researchers point out that anxiety-related cognitive strategies originate in (early) childhood (Ehrenreich & Gross, 2002).

With respect to attentional bias in children two topics require further investigation: (1) attentional bias towards threat stimuli in anxious children, and (2) bias away from threatening stimuli in low anxious children. Regarding the first topic, studies in adults using cognitive computer tasks found that, compared to nonanxious individuals, anxious individuals show a shift of attention towards the spatial location of threatening stimuli (Mogg et al., 1995; Mogg & Bradley, 1999; Keogh et al., 2001; Rinck et al., 2003). Several studies reported an attentional bias towards threat stimuli in clinically anxious children as compared to non-referred samples (Vasey et al., 1995; Taghavi et al., 1999; Taghavi et al., 2003). For example, Vasey et al. (1995) compared 12 children (age 9-14 years) with a primary diagnosis of anxiety disorder with 12 children from a primary school. The groups were matched for age, gender, vocabulary, and reading ability. A Probe Detection Task (PDT) was administered to all participants. In this task series of trials were presented. In each trial two words were presented one above the other. Each participant was instructed to read the top word aloud and respond as quickly and accurately as possible to a following probe on either the top or bottom location. Compared to the non-referred group, children with an anxiety disorder responded faster on probes emerging on the location of the threatening words, indicating an attentional bias towards threat.

With respect to the second topic, Yiend and Mathews (2001) compared the response

times on a PDT of 19 university students who scored low, and 21 students who scored high on the Taylor Manifest Anxiety Scale (Bendig, 1956). In their PDT, the authors used pictures instead of words as stimuli. The low anxious students showed a bias away from threatening stimuli. Various authors (e.g., Vasey et al., 1995; Vasey, El-Hag, & Daleiden, 1996; Ehrenreich & Gross, 2002) suggested that low anxious children also show attentional bias away from threatening stimuli. Two studies specifically investigated whether low anxious children avoid threatening information. Vasey et al. (1996) used the same PDT as in their 1995 study to compare the response times of a sample of high anxious children (n = 20) to a group of low anxious children (n = 20). The children (sixth and eighth graders, no information on age provided) were recruited from a suburban school. Allocation to high or low anxious groups was based on the cut-off scores according to Beidel & Turner (1988) on the Test Anxiety Scale for Children (Sarason, Davidson, Lighthall, Waite, & Reubush, 1960). The authors only found attentional bias away from threatening information in low anxious boys. Schippell, Vasey, Cravens-Brown and Bretveld (2003) reported an association between low anxiety and suppressed attention for threat cues, irrespective of gender in a sample of ninety adolescents recruited from public schools (age range= 11.1 to16.5). Other authors failed to find an avoiding tendency in low anxious children (Vasey et al., 1995; Ehrenreich & Gross, 2002). In summary, previous studies found an attentional bias in anxious children, but did not always find a bias away from threatening information in low anxious children.

Part of the discrepancies observed may be related to the choice of stimuli. To investigate attentional bias the studies used a wide variety of tasks that have in common that words were used as stimuli. In at least three studies these words had to be read aloud (Vasey et al., 1995; Vasey et al., 1996; Taghavi et al., 2003). Using words has important drawbacks that should be taken into account, especially when investigating attentional processes in children. Firstly, several authors argued that high anxious individuals may be more familiar with threatening words, because they use them more often and are more aware of them (Bradley et al., 1997; Mogg & Bradley, 1998; Yiend & Mathews, 2001; Ehrenreich & Gross, 2002). Due to familiarity to certain words, anxious individuals will identify such words faster than low anxious individuals, which may influence their response times. Secondly, the cognitive processing of words followed by reading the word aloud or mentioning the color of the word in some experiments, appeals on cognitive domains, like reading ability and verbal processes. The capacities of an individual in these domains may influence the performance of an individual in tasks that use words. Schippell et al. (2003) tried to correct for inter-individual differences in reading ability by adjusting the presentation intervals between word pairs, based on the performance on a pretest reading-speed test. However, a significant effect for intertrial interval was found, which suggested that this approach was "not entirely successful" (Schippell et al., 2003).

In research with adults, tasks have been developed in which pictures or faces are used as stimuli (Bradley et al., 1997; Yiend & Mathews, 2001). These tasks do not, or to a lesser extent, suffer from the drawbacks that are associated with the use of words: the stimuli are richer in contextual cues, have higher ecological validity, and might be processed in a more self-referent way than abstract stimuli like words (Schippell et al., 2003). Another advantage is that, as a result of the use of more basic threat stimuli that do not require reading efforts, more trials can be presented, covering different emotional domains during one session.

Based on the above, we sought to clarify the issue of attentional bias to threatening stimuli in children by using emotional pictures as stimuli in a sample of non-referred 10- to 13-year-old school children. Response times to three threat conditions (Neutral, Mild, Severe pictures), as well as aspects of spatial attention (Engagement or Disengagement; e.g., Posner, 1988) were related to scores on the Multidimensional Anxiety Scale for Children (MASC; March, 1997). In this group, we expected high anxiety according to MASC scores to be associated with attentional bias towards threat pictures and low anxiety according to MASC scores to be associated with attentional bias away from threat pictures. Although in earlier studies gender generally appeared not to be a factor, Vasey et al. (1996) reported significant differences in attentional strategy between low anxious boys and girls. For this reason we included gender as a covariate in our analyses.

2.2. METHOD

2.2.1. *Participants*

Two classes of two primary schools in the Rotterdam area participated. The teacher gave all children a letter with information about the study procedures, with a request for approval from the parents. All parents but one consented to participation of their child in the study, which left 44 children (ages 10 to13 years, M age = 11.6; 16 boys, 28 girls) who participated.

2.2.2. *Materials and measurements*

The survey was completed in the classroom in the week prior to the PDT.

Multidimensional Anxiety Scale for Children (MASC; March, 1997). This is

a 39-item self-report questionnaire for the assessment of anxiety symptoms in children and adolescents. Scores on 5 scales can be derived: Total Anxiety (39 items), Physical Symptoms (12 items, example: "I have pains in my chest"), Harm Avoidance (9 items, example: "I check to make sure things are safe"), Social Anxiety (9 items, example: "I worry about other people laughing at me"), and Separation Anxiety (9 items, example: "I get scared when my parents go away") (March, Parker, Sullivan, Stallings, & Conners, 1997). All items are scored on a 4-point scale with 0 = never true about me, 1 = rarely true about me, 2 = sometimes true about me, and 3 = often true about me, resulting in a scoring range for Total Anxiety from 0 to 117. A MASC Total Anxiety score of larger than 47 has been found to be indicative for the presence of a generalized anxiety disorder diagnosis in children (Rynn et al., 2006).

The Probe Detection Task (PDT) was based on the task of Yiend and Mathews (2001). Two modifications were made: pictures more suitable for children replaced the original pictures (see below) and the responses had to be given on a computer keyboard instead of a response box. The task consisted of a series of randomized neutral/severely threatening, neutral/mildly threatening and neutral/neutral picture pairs. These picture combinations were presented horizontally during 500 msec. on a computer screen. Before presenting the picture pair, a white cross was presented in the middle of the screen during 500 msec. Immediately after the picture pair disappeared, a probe appeared on the location of one of the preceding pictures. Two different probes were used. A probe consisted of two white dots that could be positioned either (1) next to each other or (2) one above the other. In response to the appearing probe, a corresponding key on the keyboard had to be pressed. All but the two response keys on the keyboard were covered. Probes remained on the screen until a response was given. Intertrial intervals varied randomly between 500, 750, 1,000, and 1,500 msec. Completion of the PDT took approximately 10 minutes for each child.

As in the original PDT, the pictures were selected from the International Affective Picture System (Lang et al., 2001). For many such pictures, ratings on Valence, Arousal, and Dominance that have been provided by 11- and 12-year-olds are available (Lang et al., 2001). Valence scores indicate the type of content of a picture. A high Valence represents a pleasant content, e.g., ice cream, clowns; a low Valence indicates an unpleasant content, e.g., fighting men, biting snake. Arousal can range from calm to excited. Dominance indicates the extent of feeling of control. Based on the ratings on Valence and Arousal by children, 20 pictures were selected. Of these, 10 were labeled severely threatening (low on Valence, high on Arousal) and 10 mildly threatening (low to moderate on Valence and moderate on Arousal). Because 48 threatening pictures were needed to construct the task, 28 extra pictures were selected based on the content of the original 20 pictures. For instance, if data regarding Valence and Arousal were available for one picture with a biting snake, another picture with an attacking snake was used as well. A total of 24 Severely threatening, 24 Mildly threatening, and 122 Neutral pictures was selected. Pictures with notably distracting features (brightness, color combinations, etc.) were not included in the picture set of the present study.

The pictures were used to construct 85 trials. In each trial two pictures were combined: either a Mildly or a Severely threatening picture with a Neutral picture, or two Neutral pictures. This yielded 37 Neutral/Neutral, 24 Mild/Neutral and 24 Severe/Neutral picture combinations. The location of the threatening picture (Severe or Mild) was balanced (left or right of the Neutral picture).

To ensure that every participant would focus on the center of the screen before each trial, the task started with an instruction, asking the participant to focus on the white cross in the middle of the screen. Following this instruction the participant was informed that a picture pair would follow the white cross, and that this pair would be replaced by a probe stimulus. Finally, the participant was instructed to react as quickly and accurately as possible to the probe stimulus by pressing the corresponding key with the pointing finger of the dominant hand. This instruction was followed by 10 neutral/neutral practice trials to make the child familiar with the task procedure. In case of a false response the participant got audio feedback (a 'beep'). After the practice trials a second instruction was given. The participant was told that the actual task was about to start, this time without a 'beep' if the response was false. These instructions were followed by the actual Probe Detection Task; 3 buffer (neutral/neutral) and 72 randomized trials, each followed by a probe. The location and the orientation (two dots horizontally or vertically) of the probe were randomized within each condition.

For each trial, reaction time, probe location, the location of the threatening picture (if present), response accuracy (true/false) and threat condition (Neutral, Mild or Severe) were stored.

Task presentation and data storage was done on a pentium 3,800 MHz computer with a 19-inch color monitor, supported by MEL version 2.1 software (Schneider, 1995). The PDT was completed in a dark and empty classroom.

2.2.3. *Statistical analyses*

Response times of trials with false responses (4.2%) and response times faster than 300 or exceeding 2000 msec. were excluded from further analyses. Attentional

bias towards threat is shown by shorter response latencies for probes appearing in the same location as the threat, versus the neutral pictures, whereas attentional bias away from threat results in longer latencies for probes appearing in the same location as the threat, versus the neutral pictures (e.g., Vasey et al., 1995, 1996). Therefore, mean response times were computed for each condition (Severe/Neutral, Mild/Neutral) in which the probe appeared at the location of the threatening picture. For the Neutral/Neutral condition, overall mean response times were computed. The data of the threat condition as presented in this paper are restricted to these analyses. Additionally, when information is presented previous to a probe, the spatial attention has to be shifted either towards or away from the location of the presented information to detect the probe appearing on either the same location (engage attention towards the presented information) or counterlocation (disengage the attention from the location of the previous presented information). Engage and disengage attentional strategies have been shown to be relevant in visual spatial attention (e.g., Posner, 1988; Yiend & Mathews, 2001). In order to analyze these two components of spatial attention in our data, for both the Mild and the Severe threat conditions, mean response times were calculated for trials in which the threat picture and the probe were presented at the same location (Engage), and also for trials in which the probe was counterlocated to the threat picture (Disengage). Because response times to both mildly and severely threatening pictures were significantly longer as compared to the neutral pictures, per subject one average Engage and one average Disengage response time was calculated across trials containing mildly or severely threatening pictures. This procedure reduced the amount of data presented and increased the power of our analyses.

Whole group overall analyses: In order to evaluate the effect of threat conditions on mean response time, a repeated-measures analysis of variance (ANOVA) was applied with response time in the Neutral, Mild and Severe threat conditions as dependent variable. The potential difference in spatial attention (Engage vs Disengage response times) was tested by means of a Student's t-test for pairwise comparisons. In order to investigate gender-related differences in response times to each threat condition and in overall Engage and Disengage average response time, we used one-way Analyses of Variance (ANOVA).

Associations between anxiety scores, and response times to threat condition and spatial attention: Spearman correlations were computed between the scores on the five MASC scales and response times in the Neutral, Mild, and Severe threat condition and the response times in Engage and Disengage trials to investigate the relationships of different aspects of anxiety with responses to threat stimuli and attentional bias. Differences between correlations of anxiety scales and threat condition (Neutral, Mild, Severe), and between anxiety scales and spatial attention (Engage, Disengage), were tested according to Steiger (1980).

Comparison of Low and High Anxious groups regarding response times to threat condition and spatial attention: we divided the sample in a high and a low anxious group, based on the 50% highest and 50% lowest scores on the MASC scale Total Anxiety (high anxious: M = 47.1, SD = 7.1; low anxious: M = 23.8, SD = 6.6). Multivariate Analyses of Covariance (MANCOVA) were used to compare the two groups with respect to (1) response times for Severe/Neutral, Mild/Neutral, and Neutral/Neutral, and (2) Disengage versus Engage response times, both analyses with gender as a covariate. In addition, we used Wilcoxon tests to further investigate differences between Engage and Disengage response times within the highest (above average plus 1 SD, n = 9; M = 54.2, SD = 4.2) and the lowest (under average minus 1 SD, n = 9, M = 17.4, SD = 3.1) scoring groups on the MASC Total Anxiety scale. All of the 9 high anxious subjects scored above the clinical cutting score of 47.

An alpha of .05 was used to indicate significant effects.

2.3. RESULTS

2.3.1. *Sample characteristics*

Data regarding MASC scale sores are presented in Table 2.1. For the MASC scale scores, Cronbach alphas reflecting internal consistencies were .88 for the Total Anxiety scale, .67 for the Physical Symptoms scale, .73 for the Harm Avoidance scale, .82 for the Social Anxiety scale, and .68 for the Separation Anxiety scale.

	Minimum	Maximum	M	SD
MASC				
Total Anxiety	13	64	36.0	13.6
Physical Symptoms	0	20	8.2	4.4
Harm Avoidance	4	25	14.6	4.6
Social Anxiety	0	19	7.8	5.0
Separation Anxiety	0	16	5.4	3.8

Table 2.1. Descriptives of MASC Scores for The Total Group of Non-referred Children.

2.3.2. *Overall analyses*

2.3.2.1. *Threat condition and spatial attention*

Inter-condition Pearson correlation coefficients were high (Neutral with Severe: r = .92; Neutral with Mild: r = .88; Severe with Mild: r = .91, all ps < .01). Still, significant differences in response times between threat conditions were found when the probes were presented at the same location as the threat stimulus ($F_{2, 86}$ = 9.65, p < .0005): the mean response time in the Severe threat condition was significantly higher than the mean response times in the Neutral and Mild threat conditions (t_{43} = 4.47, t_{43} = 2.93, respectively, both ps < .01). Furthermore, the mean response time in the Mild threat condition was significantly higher than in the Neutral threat condition (t_{43} = 2.52, p < .05) (Table 2.2.). The overall mean Engage and Disengage response times were similar (t_{43} = 0.38, ns; Table 2.2.), indicating no significant differences in mean response times to probes presented at the same or at the opposite location of the threat stimulus.

	Total group (*n*=44)		Girls (*n*=28)		Boys (*n*=16)	
	M	***SD***	***M***	***SD***	***M***	***SD***
Threat Condition						
Neutral	982	155	971	160	1003	150
Mild	1003	158	980	149	1044	171
Severe	1032	181	1014	171	1064	198
Spatial Attention						
Engage	1018	165	997	155	1054	182
Disengage	1021	177	990	170	1075	182

Table 2.2. Mean Response Times (RT, msec.) for All Conditions of the Probe Detection Task, for the Total Group and Girls and Boys Separately.

2.3.2.2. *Effects of gender*

Boys and girls showed no significant differences in response times of the Neutral ($F_{1, 42}$ = .42, ns), Mild ($F_{1, 42}$ = 1.73, ns) or Severe ($F_{1, 42}$ = .78, ns) threat condition. Also, no significant differences in response times between boys and girls were found in the Engage condition ($F_{1,41}$ = 2.10, ns), when probes were presented at the same location as the threat stimulus. Girls responded significantly faster in the Disengage condition than boys ($F_{1, 41}$ = 4.19, p < .05), when probes were presented opposite of the threat stimulus (Table 2.2.).

2.3.2.3. *Associations between anxiety scores and response times*

The MASC scales Total Anxiety, Physical Anxiety and Separation Anxiety showed significant positive correlations with the mean response times of all conditions (Table 2.3.). Harm Avoidance showed significant positive correlations with mean response times of the Severe, Engage, and Disengage conditions. Social Anxiety only showed a significant positive correlation with the mean Engage response times, as mirrored by a significant correlation with response times of the Severe condition as well (Table 2.3.). However, when, for each MASC scale, correlations in the Severe/Neutral or Mild/Neutral versus the Neutral/Neutral condition were compared according to Steiger (1980), no significant differences were found. Similarly, when, for each MASC scale, differences in correlations with Engage and Disengage response times were tested, no significant differences were found. Overall, these results indicate that, for each MASC scale, an increase in anxiety was associated with a longer mean response time, irrespective of the presence of threatening stimuli or probe location.

Scale	**Threat Condition**			**Spatial Attention**	
	Severe	**Mild**	**Neutral**	**Engage**	**Disengage**
MASC					
Total Anxiety	.47**	.45**	.44*	.45*	.44*
Physical Anxiety	.36*	.31*	.30*	.35*	.32*
Harm Avoidance	.33*	.29	.28	.34*	.31*
Social Anxiety	.32*	.30	.29	.32*	.25
Separation Anxiety	.36*	.35*	.37*	.38*	.36*

*: p < .05; **: p < .01

Table 2.3. Spearman Correlation Coefficients between MASC Scales and Severe/Neutral, Mild/Neutral, Neutral/Neutral, Engage, and Disengage Response Times.

2.3.2.4. *Response times in High versus Low Anxious children*

In all threat conditions, the children in the High Anxious group showed longer mean response times as compared to the Low Anxious group (Neutral, $F_{1, 42}$ = 13.36, p < .005; Mild, $F_{1, 42}$ = 9.25, p < .005; Severe, $F_{1, 42}$ = 8.91, p <.005; Table 2.4.). There was no significant gender effect ($F_{1, 40}$ = .79, ns).

The mean response times of the High Anxious children were also significantly longer than the mean response times of the Low Anxious children in both the Engage ($F_{1, 41}$ = 14.81, p < .001) and the Disengage ($F_{1, 41}$ = 10.41, p < .005) conditions (Table 2.4.); there was no significant effect of gender ($F_{1, 40}$ = 3.30, ns). These

results reflect longer mean response times of the High Anxious group to probes located both at the same and at the opposite location of the threatening stimulus, as compared to the Low Anxious group.

Condition	Low Anxious (n=21)		High Anxious (n=23)	
	M	*SD*	*M*	*SD*
Threat Condition				
Neutral	908	162	1050	115
Mild	930	178	1064	111
Severe	950	182	1101	153
Spatial Attention				
Engage	946	175	1083	127
Disengage	943	173	1092	152

Table 2.4. Mean Response Times (RT, msec.) for All Conditions of the Probe Detection Task, for the Low Anxious Group (50% Lowest MASC Total Anxiety Score) and High Anxious Group (50% Highest MASC Total Anxiety Score) Separately.

Additionally, High and Low Anxious groups were tested separately to see if within-group differences in response times between Engage and Disengage trials could be found. However, within both groups Engage and Disengage response times did not differ significantly (t_{22} = 0.71 and t_{20} = -.27, respectively, ns). To verify these last findings, mean response times in the Engage and Disengage conditions of children scoring low (under average minus 1 *SD*, *n* = 9) and high (above average plus 1 *SD*, *n* = 9) on the MASC Total Anxiety scale were compared. Within both groups, no significant difference between the response times in the Engage and the Disengage conditions was found either (*Z* = - .30, ns, for the Low Anxious; *Z* = -1.13, ns, for the High Anxious). Thus, within the High and Low Anxious groups, no differences in mean response time to probe location were observed.

2.4. DISCUSSION

We aimed to clarify attentional bias to threatening stimuli in children by using emotional pictures as stimuli in a sample of non-referred children. Response times to probes appearing on the same location as the threatening picture (Mild, Severe) versus the neutral picture, as well as spatial attention (Engage, Disengage) were studied for the whole group and in relation to anxiety scores as quantified by means of the MASC. Overall, when pictures were more threatening, mean response times increased significantly versus mean response times to the neutral pictures, indicating the presence of an attentional bias away from threatening stimuli. This is consistent with earlier findings in adults and in referred children (Yiend & Mathews, 2001; Taghavi et al., 2003). Theoretically, if more cognitive resources are needed to evaluate a stimulus, response times increase (Williams et al. 1988; Crick & Dodge, 1994). Apparently, the more threatening the pictures were, the more cognitive resources were required to evaluate the information presented. No significant differences were observed between mean response times to probes presented at the same location as the threatening picture (Engage) and mean response times to probes presented at the opposite location of the threatening picture (Disengage). In general, differences between engage and disengage attentional strategies can become evident when on several locations new information is presented at the same time. When this happens attention has to be divided and the available resources to process the relevance of the new information have to be distributed quickly to enable a fast and proper judgment regarding potential consequences for the individual involved. Based on cognitive predispositions particular information (e.g., with an obvious emotional or threatening content) will either be favored (engage) or ignored/avoided (disengage). Overall, we observed no evidence for such an attentional differentiation in the present sample of non-referred children. Yet, girls responded significantly faster than boys in the Disengage condition, suggesting a gender specific ability to disengage attention from the location of the threat, but it was unrelated to anxiety scores as quantified by the MASC. Nevertheless, these finding provide support for a potential role of gender on early attentional processes. Consequently, future researchers should consider a possible gender effect when investigating these kind of processes.

2.4.1. *Anxiety symptoms and mean response times*

Mean response times to all threat conditions, including Neutral, correlated significantly positively with MASC Total Anxiety scores. With increasing anxiety scores, response times became longer. Apparently, the more anxious children were, the more time they needed to respond. This finding is consistent with that of Taghavi et al. (2003), who compared response times in anxious children versus normal controls. It is possible that anxious children need more time to evaluate information, especially when the content of the presented information becomes increasingly threatening. According to Williams et al. (1988), anxiety is associated with a cognitive bias, by which ambiguous new information is automatically labeled as potentially threatening during the initial stages of information processing.

Hence, more cognitive resources are allocated to evaluate this information during the following stages, resulting in increasing response times.
No specific differences were found between the subscales of the MASC. Within the clinical context, it is known that high comorbidity rates exist between the different diagnoses of anxiety disorder, such as between generalized anxiety disorder, separation anxiety disorder, social phobia, or specific phobia (Essau et al., 2003) and even, that it can be doubted whether childhood anxiety disorders can be regarded as distinct diagnostic constructs (Ferdinand et al., 2006). This suggestion may be strengthened by the present finding of a general effect of anxiety on cognitive processes at the early stage of information processing during childhood.

2.4.2. *Response times in high versus low anxious children*
We expected high anxiety according to MASC scores to be associated with attentional bias towards threat pictures and low anxiety according to MASC scores to be associated with attentional bias away from threat pictures. Our sample was divided into High and Low Anxious groups based on the MASC Total Anxiety scale scores. About half (n = 11) of the subjects in the High Anxious group scored above a clinical cutting score of 47. The High Anxious group showed significantly longer mean response times than the Low anxious group to probes presented at the same location as the Mild and Severe pictures, but also to probes presented after the Neutral pictures. Both Engage and Disengage mean response times were also significantly longer for the High Anxious group, in comparison to the Low Anxious group. Both results indicate a general lengthening of mean response time with increased test-anxiety, and do not confirm our expectations of attentional bias towards or away in relation to high or low test-anxiety scores. Also when both groups were analyzed separately, no signs of attentional bias were found. Our separate analyses of the highest and lowest scoring subjects within the two groups verified these findings.
Gender did not affect these results. This contrasts, for instance, with Vasey et al. (1996), who reported attentional bias away from threat in low anxious boys. However, our findings are confirmed by Taghavi et al. (1999) and Vasey et al. (1995), who did not find any bias in low anxious children.

2.4.3. *Limitations*
It might be argued that the lack of associations between anxiety levels and attentional bias is due to the sample characteristics, for instance, the use of a school sample instead of a clinical sample, yet half of the subjects of the High Anxious group scored within the clinical range. Nevertheless, studies in clinical samples of referred children with anxiety disorders are needed.
Furthermore, the long mean response times in our sample, compared to other samples, are remarkable (Kindt et al, 1997; Yiend & Mathews, 2001; Taghavi et al., 2003). This may be due to methodological differences: responding to a word versus responding to a combination of pictures, or word-naming latency versus pressing a button on a keyboard. As stated by Schippell et al. (2003) results of studies using different versions of the Probe Detection Task are hard to compare. Nevertheless, the longer response times may indicate the use of more cognitive domains, which are not necessarily associated with attention. The influence of these domains on a child's responses may have reduced attentional bias effects that occur at the very first stages of information processing.

2.4.4. *Conclusions and clinical implications*
Our overall analyses indicated the presence of an attentional bias away from threatening pictures in a sample of non-referred children. However, in relation to anxiety, our study did not confirm that high anxious children show an attentional bias towards threatening pictures or that low anxious children show an attentional bias away from threatening pictures. The children did not appear to have a specific attentional preference when confronted with threatening information. Higher anxiety did seem to be associated with longer mean response times. However, the attention of anxious children in the present sample is not specifically drawn, at least at the early stage of information processing, by potential dangers. It may be true, as stated above, that these longer response times in children reporting more anxiety symptoms originate from the interpretation of a new stimulus as potentially threatening, which may be exaggerated with regard to its actual content but causes the mobilization of extra cognitive resources in the later stages of information processing.
The present findings were observed in a sample of non-referred children. If similar results are found in clinically anxious children, it is useful to develop treatments that aim at these quick and automatic responses. The most efficacious treatment for anxiety disorders in children and adolescents, cognitive behavior therapy, focuses on cognitive restructuring of late conscious cognitive strategies. The results of the present study indicate that development of new treatment strategies that aim at restructuring the very early cognitive strategies might be considered.

3.

Attentional bias in children with an anxiety disorder

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Summary. - Cognitive strategies associated with anxiety seem to originate in childhood. Early attentional processes are assumed to play an important role in the development of such strategies. Information regarding these processes and their association with anxiety symptoms during childhood may help us to better understand the factors involved in the development of anxiety disorders. The performance of thirty-seven children with an anxiety disorder on a Probe Detection Task with fear-related pictures was compared with that of forty-four control children. The trials comprising threat-neutral picture combinations induced a significant increase in mean response time versus the neutral-neutral picture combinations, in both patients and controls. However, the two groups differed significantly in attentional strategy related to threatening pictures: the anxious group, and in particular the anxious girls, showed a significantly larger negative bias score as compared to the controls, indicating an initial avoiding strategy in anxious children when confronted with fear-related pictures. Our findings are in accordance with the 'vigilance-avoidance' theory, suggesting an avoiding tendency for fear-related stimuli in anxious individuals, which may be strongest for anxious girls. Because anxiety related differences in attentional processes during childhood may constitute a risk factor for the persistence of anxiety symptoms into adulthood, emphasis on these processes during treatment should be considered.

3.1. INTRODUCTION

Numerous studies have been performed to identify the cognitive mechanisms involved in the etiology and persistence of anxiety disorders (e.g., Amir et al., 2003; Beck & Clark, 1997; Bradley et al., 1997; Keogh et al., 2001). Original efforts focused mainly on the cognitive processing of threatening information in adults (Mansell et al., 2003; Mogg et al., 1995; Spector et al., 2003; Williams et al., 1996; Yiend & Mathews, 2001). However, because development of high trait anxiety, potentially cumulating towards an anxiety disorder, seems to originate in childhood (Daleiden & Vasey, 1997; Ehrenreich & Gross, 2002) knowledge of the development of cognitive mechanisms during childhood may be essential to better understand the basis and nature of anxiety disorders.

A useful concept for developing theory and conducting research in the field of anxiety is 'attentional bias'. This concept is defined as the attentional preference for (bias toward), or to the contrary, the avoidance of (bias away), emotional visual information (Mogg & Bradley, 1998). This implies that information is prioritized. Subsequently, based on the extent of threat or importance for the individual, cognitive resources are allocated to process the information and to prepare a response (Crick & Dodge, 1994).

Biases in the early stages of this sequence (like specific attentional preferences) have consequences for all following stages. When these biases are present during childhood and adolescence, they may over time lead to deviant and automated cognitive strategies when confronted with ambiguous or fear related information (Daleiden & Vasey, 1997; Ehrenreich & Gross, 2002). Not surprisingly, such automated cognitive schemata are associated with the development of anxiety disorders and may play a prominent role in their etiology and persistence (Mogg & Bradley, 1998; Mathews & MacLeod, 1994; Vasey et al., 1996; Williams et al., 1988). Consequently, attentional biases during childhood and adolescence may indicate a cognitive vulnerability for the development of anxiety disorders (Mogg & Bradley, 1998; Waters et al., 2004; Williams et al., 1988).

Attentional bias can be demonstrated by performance on specific cognitive tasks. During such tasks, individuals are instructed to respond to a probe, e.g. a dot, by pressing a button. The probe appears after threatening and/or neutral information (pictures or words) and is presented either in the same or in the opposite location of the threatening information. If a person is biased towards (i.e. is more vigilant to) the presented threatening information, the response time on a probe emerging on the same position will be reduced and vice versa. Using such Probe Detection Tasks evidence has been found for an attentional bias towards threat stimuli in

adults with high anxiety levels or an anxiety disorder (Bradley et al., 1997; Keogh et al., 2001; Mogg et al., 1995; Mogg & Bradley 1999; Spector et al., 2003).

Until now, empirical data regarding associations between attentional bias and anxiety in children are hardly available and inconclusive. Vasey et al. (1995) compared response times of children with a primary diagnosis of anxiety disorder (n = 12, age 9-14 years) who had been referred to a treatment program, with an age and gender matched group of children without an overt anxiety disorder. A Probe Detection Task with words (threatening – neutral or neutral – neutral combinations) was applied. Compared to the non-referred group, children with an anxiety disorder responded faster to probes emerging on the location of the threatening words, indicating an attentional bias towards threat. Taghavi et al. (1999) used the same probe detection taks to investigate the response times of 24 children and adolescents (mean age 13.6 years, *s.d.* = 3.2) with a diagnosis of general anxiety disorder (GAD) without depressive symptoms and a group of age and gender matched controls. The anxious children and adolescents showed faster response times to probes following threat words compared to probes presented on the neutral location. The results of this study also indicated an attentional bias of the anxious group towards threat when compared to the non-referred sample. However, in the same study the results in the non-referred sample were compared with a sample of adolescents with a combined anxious-depressed disorder as well (n = 19, 12 girls, 7 boys, mean age 14.8, *s.d.* = 2.5). No significant differences between these samples in attentional bias were found.

Other studies did not find differences in attentional processes between high or clinically anxious children and control children. Kindt et al., (1997a) reported a significant attentional bias towards threat words in both high, but sub-clinical, and low anxious girls (age: 8-9 years). Anxiety was assessed using the Trait form of the Spielberger State-Trait Anxiety Inventory for Children (STAIC: Spielberger, 1973) and the Fear Survey Schedule for Children Revised (FSSC-R: Ollendick, 1983). No attentional bias was found in either high or low anxious boys of the same age. Kindt et al., (1997b) neither found a significant difference in attentional bias between 8 to 12 year old children scoring high and children scoring low on the Spider Phobia Questionnaire (SPQ, Klorman et al., 1974) when confronted with spider related words. However, Kindt and Brosschot (1999) reported an attentional bias in children with spider phobia compared to controls (age range: 8-12 years), but only towards spider related words and not towards spider related pictures. Waters et al. (2004) did find an attentional bias towards fear-related pictures using the Probe Detection Task, but this was found in both clinically anxious (n = 23; age range: 9-12 years) and non-referred (n = 105; age range: 9-12 years) children and it did not differ significantly between these two groups.

Based on these studies, we find that the available information on associations between attentional bias and anxiety symptoms in children is inconsistent. Small sample sizes, different methods to allocate children to specific anxiety groups, and/or the different versions of reaction time tasks may explain most of the differences in results. Another issue is the difference in age range between the studies. Cognitive processes like attentional bias may be sensitive to developmental factors and bias effects may increase or change with age (Daleiden & Vasey, 1997; Mogg & Bradley, 1998; Waters et al, 2004). When studying attentional bias in children and/or adolescents limiting the age range of the samples studied seems advisable. Additionally, it has been suggested that the use of words as threat stimuli in children might be inappropriate because words appeal to cognitive skills, like reading ability and verbal processes, which are not associated with early attentional processes (Ehrenreich & Gross, 2002; Kindt & Brosschot, 1999; Waters et al., 2004). Furthermore, anxiety disordered children compared to low anxious children may be more familiar with fear related words because they use them more often. This may accelerate identification when such words are presented. These two effects may influence response times in tasks using words (Bradley et al., 1997; Ehrenreich & Gross, 2002; Yiend & Mathews, 2001). Consequently, when applying a Probe Detection Task to children, preferably other stimuli should be used with simplified response demands, like pressing a button, contrary to reading words or name the color in which a specific word is presented.

In research with adults, Probe Detection Tasks have been developed using pictures or faces as threat stimuli (e.g. Bradley et al., 1997; Yiend & Mathews, 2001). Up to now only a few studies can be found that used a Probe Detection Task with pictures to investigate attentional bias in children (Kindt & Brosschot, 1999; Waters et al., 2004). Considering the contradictory results of these previous studies, we decided to perform the present study by applying a recently developed non verbal Probe Detection task in a sample of children with an anxiety disorder.

Using a modified version of the Probe Detection Task of Yiend and Mathews (2001), our aim was to investigate whether children with an anxiety disorder within an age range of 9.5-13.5 years show an attentional bias towards fear related pictures. This bias should not, or to a significantly lesser extent, be present in a relatively low anxious group of controls, because we believe these children to be less vigilant for fear related stimuli. Because in some earlier studies (Kindt et al, 1997a; Vasey et al., 1996) gender-related differences in attentional bias were found, we also evaluated effects of gender, whereas age was included as covariate in our analyses.

3.2. METHOD

3.2.1. *Subjects*

Patients: Thirty-seven children (mean age = 11.5 years; range: 9.5-13.4 years; 18 boys and 19 girls) participated in the present study after being referred for internalizing psychopathology to the outpatient clinic of the department of Child and Adolescent Psychiatry of either the Erasmus MC in Rotterdam or Curium in Leiden. After registration the children conducted the standardized diagnostic assessments for anxiety related psychopathology in these two institutes. Inclusion was done according to consecutive referrals. The diagnosis Anxiety Disorder was based on the Anxiety Disorders Interview Schedule for DSM-IV-Child Version (ADIS-C; Silverman et al., 2001). The standardized interviews were conducted with the parents and the children themselves. Inclusion criteria were a primary diagnosis of anxiety disorder: Generalized Anxiety Disorder (GAD), Separation Anxiety Disorder (SAD), Social Phobia (SOP), or a Specific Phobia (SP). Exclusion criteria were: the use of anxiety medication (e.g. sertraline, paroxetine), a co-morbid Pervasive Development Disorder, Obsessive Compulsive Disorder, Post Traumatic Stress Disorder or substance abuse, and an IQ score under 85 on the Wechsler Intelligence Scale for Children -Revised (WISC-R; Wechsler, 1974). The children with an anxiety disorder did not use any medication before or during the study, neither did they follow therapy aimed at their anxiety symptoms for at least up to 3 months before this study.

Controls: A control group of non-referred children (44 medication-free children; mean age = 11.6 years; age range: 10.8-13.3 years; 16 boys, 28 girls) was recruited from two classes of two primary schools in the Rotterdam area.

The Medical Ethical Committees of the Erasmus MC in Rotterdam and the Leiden University Medical Center in Leiden approved of the protocol. One child of the control group and none of the patients refrained from participation. All participants were informed beforehand about the procedures and gave written consent for their participation.

3.2.2. *Materials and measurements*

The Anxiety Disorders Interview Schedule for DSM-IV-Child Version (ADIS-C; Silverman et al., 2001): The ADIS-C is a semi-structured interview; the interview is conducted with the child or adolescent and with the parents separately. It is specifically developed for the diagnosis of anxiety and related disorders in 7-17 year olds. During both the parent and the child interview, DSM-IV symptoms are judged by the child and its parent as either absent ('no') or present ('yes'). For both the parent and child interview, the number of reported symptoms is compared to the number of symptoms required for a DSM-IV diagnosis. When the minimal requirements for a DSM-IV diagnosis are met, the parent or the child is asked to indicate on a 9-point scale (0–8) to what extent the symptoms interfere with the child's daily life. The reported impairment rating from the parent and/or the child interview must be equal to or exceeding 4 to represent a clinically significant disorder. If a disorder is thus reported by either the parent(s) or the child, the interviewing clinician has to confirm the diagnosis by indicating his or her impression through an interference score on the same 9-point scale, the Clinician Severity Rating (for this patient group: median = 6; range = 4-8). If either the parent(s) and/or the child and the clinician rate a diagnosis on or above 4, the diagnosis is set.

The Youth Self Report (YSR; Achenbach, 1991a): The YSR is a 120-item self-report questionnaire that covers problem behaviors in the preceding 6 months. All items are scored on a 3-point scale (0 = not true, 1 = somewhat or sometimes true, 2 = very or often true). The YSR was derived from the Child Behavior Checklist (CBCL; Achenbach, 1991b) and has good reliability and validity, both in American (Achenbach, 1991a) and Dutch samples (Verhulst et al., 1989). An YSR Total Problems scale score of 56 for boys and 58 for girls is used as clinical cut-off in the Dutch population (Verhulst et al., 1997). The average YSR Total Problems scale score in the control sample was 38.1 (*sd* = 17.4), which made us conclude that, on average, the participants in the control sample scored well below the clinical cutoff.

Probe Detection Task: The Probe Detection Task (PDT) was based on the PDT of Yiend and Matthews (2001). Two modifications were made: pictures more suitable for children replaced the original pictures (see below) and the responses had to be given on a computer keyboard instead of a response box. As for the original PDT, the pictures were selected from the International Affective Picture System (IAPS; Lang et al., 2001). For many pictures in the IAPS, ratings on valence and arousal that have been provided by 11- and 12-year-olds are available (Lang et al., 2001). Valence scores indicate the type of content of a picture. A high valence represents a pleasant content, a low valence indicates an unpleasant content. Arousal can range from calm to excited (Lang et al., 2001). Based on the ratings on valence and arousal by children, 20 pictures were selected. Of these, 10 were labeled severely threatening (low on valence, high on arousal) and 10 mildly threatening (low to moderate on valence and moderate on arousal). Because 48 threatening pictures were needed to construct the task, 28 extra pictures were selected based on the content of the original 20 pictures. For instance, if data regarding valence and

arousal were available for one biting snake, other IAPS pictures with an attacking snake were used as well. Thus, 48 threatening and 122 neutral pictures were selected[1]. Pictures with notably distracting features (brightness, color combinations, etc.) were not included.

In this version of the PDT, 85 trials were constructed. In each trial two pictures, either a threat picture in combination with a neutral picture, or two neutral pictures were presented. This yielded 37 neutral/neutral, and 48 threat/neutral picture combinations. These picture pairs were presented horizontally during 500 milliseconds on a computer screen. The location of the threatening picture was balanced (left, right) over the trials containing a picture of the same category. Before presenting the picture pair, a white cross was presented in the middle of the screen during 500 milliseconds. Immediately after the picture pair disappeared, a probe appeared on the location of one of the preceding pictures. Two different probes were used: two white dots positioned either (1) next to each other or (2) one above the other. The location and the orientation of the probe were randomized within each condition (neutral/neutral, threat/neutral). Probes remained on the screen until a response was given. Inter trial intervals varied randomly between 500, 750, 1000, and 1500 milliseconds.

The task started with an instruction, asking the participant to focus on the white cross in the middle of the screen. Subsequently, the participant was informed that a picture pair was to follow the white cross, and that this pair would be replaced by a probe stimulus. On this probe a response had to be given based on the probe's appearance but not its location. The instruction was followed by 10 trials each with two pictures, both with neutral content to make the child familiar with the task procedure. If a false response was given the participant got audio feedback (a 'beep'). After the practice trials a second instruction was presented. The participant was told that the actual task was about to start, this time without a 'beep' if the response was false. These instructions were followed by the actual Probe Detection Task; 3 buffer (neutral/neutral) and 72 randomized trials, each followed by a probe.

For each trial, reaction time, probe location, the location of the threatening picture, response accuracy (true/false) and condition (neutral, threat) were stored. For task presentation and data storage a pentium 3,800 MHz computer with a 19-inch color monitor was used, supported by MEL software (Schneider, 1995).

[1]) Pictures from IAPS used for threatening condition: 1120; 1280; 1300; 1321; 1660; 1930; 1931; 2120; 2130; 2683; 2780; 2800; 2900.1; 3230; 3280; 3500; 3530; 5950; 6190; 6213; 6230; 6242; 6244; 6250; 6260; 6300; 6370; 6940; 7380; 7390; 8179; 9000; 9041; 9050; 9160; 9280; 9404; 9411; 9421; 9470; 9471; 9480; 9530; 9584; 9630; 9635; 9911; 9920.

3.2.3. *Procedure*

The parents and child were invited to the outpatient clinic of the Department of Child and Adolescent Psychiatry, where the diagnostic screening and test session took place. The child performed the PDT in a darkened, isolated and quiet room in one of the two institutes, during the week prior to their first therapy session.

The control group of non-referred children completed the YSR in the classroom, supervised by the class teacher, one week prior to performing the Probe Detection Task (PDT). The school children conducted the PDT in a darkened and empty classroom in their own school. For all children, the researcher explained the test procedure before the start of the PDT. In addition, a practice session took place (see above) before the actual start of the test. The children were instructed to react as quickly and accurately as possible to the probe stimulus by pressing the corresponding key on the keyboard with the pointing finger of the dominant hand. Completion of the PDT took approximately 10 minutes for each child.

3.2.4. *Statistical Analyses*

Response times of trials with false responses (4.2% for the controls, 2.3% for the patients) and response times faster than 300 or exceeding 2000 milliseconds were excluded from further analyses. For each individual, overall mean response times were calculated for neutral/neutral and threat/neutral trials, irrespective of probe location. Additionally, when information is presented previous to a probe, the spatial attention has to be shifted either towards or away from the location of the presented information to detect the probe appearing on either the same location (engage attention towards the presented information) or counter location (disengage the attention from the location of the previous presented information). Engage and disengage attentional strategies have been shown to be relevant in visual spatial attention (Posner, 1988; Yiend & Mathews, 2001). Therefore, in relation to probe location, the following parameters were computed: 1) probe right/threat right, PRTR, 2) probe left/threat left, PLTL, 3) probe right/threat left, PRTL, and 4) probe left/threat right, PLTR. Based on these parameters, for each participant a bias score was computed using the formula developed by MacLeod and Mathews (1988):

[(PLTR - PLTL) + (PRTL - PRTR)] / 2

If the result of this formula equals zero, the position of the threat compared to the position of the following probe does not influence response times, or bias attention. If a participant attends selectively to the location of the threat, thus detecting probes emerging in that area disproportionately faster, the formula will result in a large positive value. If a participant tends to avoid the location of a threatening picture an equivalent negative bias score will result from the formula. Addition-

ally, irrespective of left or right position, overall mean response times were calculated for trials in which threat and probe were presented on the same location (engage) and trials in which threat and probe were counter located (disengage).
Similarity of age between the two groups was confirmed by means of an independent sample t-test ($p > .05$).
An analysis of variance (ANOVA) for repeated measures was used to investigate overall differences in mean response times between the two groups (factor Group: patient, control), the two conditions (factor Condition: neutral, threat), and between boys and girls (factor Gender: boys, girls), as well as the interaction effects between these factors, with age included as a covariate. Additionally, an ANOVA was used to evaluate differences in the bias scores between the patients and controls (factor Group), and between boys and girls (factor Gender), as well as the interaction effect between Group and Gender, including age as a covariate.
To evaluate if the bias score, per subgroup, differed significantly from zero (i.e., the position of the threat compared to the position of the following probe does not influence response times), one-sample t-tests were performed.
All analyses were conducted using SPSS (version 10.1). A p-value of .05 was used to indicate a significant effect.

3.3. RESULTS

3.3.1. *Neutral versus Threatening pictures*

Patients and controls did not differ significantly in mean response times to the pictures (Group: $F(1,76) = .04$, n.s.), however, a significant main effect was found for Condition ($F(1,76) = 11.37$, $p = .001$), indicating significantly larger mean response times to trials including a threatening picture as compared to trials including only two neutral pictures, for both patients and controls (table 3.1.). Overall, no significant differences were found between boys and girls (Gender: $F(1,76) = .07$, n.s.). Yet, a significant three-way interaction effect Group x Condition x Gender was observed ($F = 5.4$, $p = .023$), suggesting that response times to threatening pictures were largest for the girls with an anxiety disorder and for the boys of the control group (table 3.1.). Overall, response times to neutral and threatening pictures became faster with increasing age (covariate age: $F(1,76) = 8.12$, $p = .006$).

	Anxiety disorder			Controls		
Condition	**Boys (n=18)**	**Girls (n=19)**	**Total (n=37)**	**Boys (n=16)**	**Girls (n=28)**	**Total (n=44)**
Neutral/neutral	1003 (161)	1012 (168)	1008 (163)	999 (152)	969 (158)	980 (155)
Threat/neutral	1018 (182)	1033 (177)	1026 (177)	1050 (156)	985 (152)	1009 (155)
Engage	1023 (180)	1053 (168)	1039 (172)	1038 (152)	987 (146)	1006 (149)
Disengage	1014 (190)	1012 (190)	1013 (187)	1062 (163)	983 (163)	1012 (165)

Table 3.1. Overall mean response times (*sd*) in milliseconds to the neutral/neutral and threat/neutral picture trials of the Probe Detection Task, for the patient and control group and for boys and girls separately. Mean response times to probes presented at similar location of the threat pictures (engage) and mean response times to trials in which probes and threat pictures were counter located (disengage) are also presented in relation to group and gender.

3.3.2. *Bias score*

Table 3.2. presents the mean response times for every combination of threat and probe location, for patient and control group separately. Overall mean response times for trials in which threat and probe were presented on the same location (engage) and trials in which threat and probe were counter located (disengage) are included in table 3.1.
Regarding the bias scores, the scores of the control group did not differ significantly from zero (mean: 6.33; $t(43) = .76$, n.s.), but the scores of the anxious group did (mean: -25,5; $t(36) = -2.29$, $p = .028$; figure 3.1.). In line with these analyses, we observed a significant main effect for Group ($F(1,76) = 6,59$, $p = .012$) on the bias scores, with the anxiety disordered children showing significantly larger negative bias scores, indicating an avoiding tendency, as compared to the controls. Table 3.1. and 3.2. illustrate that the mean response times to probes presented at similar location of the threat pictures (engage) were larger for the patients, as compared to the controls.

		Anxiety disorder		Controls	
		Location threat picture		**Location threat picture**	
		left	**right**	**left**	**right**
Location probe	**left**	1031 (182	1013 (194)	984 (153)	1001 (172)
	right	1014 (185)	1046 (175)	1023 (174)	1028 (157)

Table 3.2. Mean response times (*sd*) in milliseconds within every combination of threat and probe location, for patient and control group separately.

A significant main effect was also found for Gender ($F(1,76) = 4.69$, $p = .034$), which could be explained by the significantly larger negative bias scores in girls, as compared to boys (figure 3.1.). In particular, the bias score of the anxious girls was significantly reduced versus zero (mean: -41.0; $t(18) = -2.86$, $p = .01$, figure 3.1.); whereas for the boys in the control group, a trend towards a significant increase versus zero was observed (mean: 24.2; $t(15) = 2.05$, $p = .06$). However, the interaction between factors Group and Gender was not significant ($F(1,76) = .014$, n.s.). The bias scores were not significantly influenced by age ($F(1,76) = .014$, n.s.).

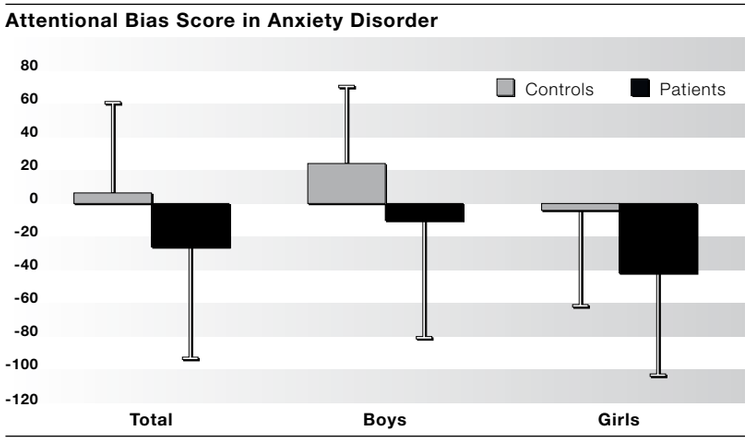

Figure 3.1. Mean and *sd* of the bias scores for the total patient and control group, and for boys and girls separately.

3.4. DISCUSSION

The aim of the current study was to investigate attentional bias to fear-related pictures in children with an anxiety disorder. Overall, the trials comprising threat-neutral picture combinations induced a significant increase in mean response time versus the neutral-neutral picture combinations, in both patients and controls. However, the two groups differed significantly in attentional strategy when responding to threatening pictures, as reflected in the significantly larger negative bias score in the anxious group, particularly anxious girls, as compared to the controls.

The observed increase in mean response time to threatening pictures is consistent with previous findings in adults and in referred children (Yiend & Mathews, 2001; Taghavi et al., 2003), and may reflect an increase in cognitive resources that are needed to evaluate a stimulus (Williams et al. 1988; Crick & Dodge, 1994). For both patients and controls, it appears that the more threatening the pictures were, the more cognitive resources were required to evaluate the information presented, leading to prolonged response times. Yet, the significant interaction effect Group x Condition x Gender revealed that responses to threatening pictures were largest for anxious girls and control boys, indicating the importance of gender in these processes.

The significant bias score in the anxious children is in accordance with earlier results (Taghavi et al., 1999; Vasey et al., 1995). However, the negative direction of the bias score, indicating an avoidance tendency in anxious children when confronted with fear-related pictures, was remarkable. On first sight this seems to be the first study to report a negative bias score in anxious children, which is contrary to earlier studies reporting a bias towards threat stimuli (a positive bias score) in anxious children (Kindt & Brosschot, 1999; Waters at al., 2004). However, Kindt and Brosschot (1999) also reported a negative bias score in children with spider phobia when confronted with spider related pictures. These scores exceeded the also negative bias scores of the control children, although this difference in bias scores between groups was not significant. In this study only children reporting phobic symptoms for spiders on the SPQ were included, who were confronted with stimuli related to their phobia. A direct relationship between specific anxiety disorders and stimulus content may partly explain the contrast between these findings and the currently found significant difference in bias scores. Our sample consisted of a broader range of anxiety disorders, based on parent and child reports, as well as the judgment of a clinician, which were assessed using the ADIS-C. Additionally, the stimuli used in the present study consisted of a broad range of fear-related pictures, not only appealing to one specific anxiety domain. Next to the current results there is strong evidence from earlier studies that attentional bias, either towards or away from threatening stimuli, is generally present in highly anxious children, and does not depend on the match of specific anxiety symptoms with fear-related stimuli (Taghavi et al., 1999; Vasey et al., 1995; Waters et al., 2004).

An additional similarity between the present results and the study of Kindt and Brosschot (1999) is the prolonged mean response time (>800 milliseconds) as compared to studies reporting positive bias scores (range: 500-800 milliseconds; Kindt et al., 1997b; Taghavi et al., 1999; Vasey et al., 1996; Waters et al., 2004). The prolonged response times may be due to methodological differences: responding to a word versus responding to a combination of pictures, or word-

naming latency versus pressing a button on a keyboard. As stated by Schippell et al. (2003) results of studies using different versions of the Probe Detection Task are hard to compare. Nevertheless, it may implicate that cognitive processes other than strictly early attentional processes are influencing the response times. For instance, processes such as Inhibition of Return (IOR: Los, 2004; Prime & Ward, 2004; Spalek & Hammad, 2004) may be responsible for the observed negative bias scores in both our study and the study of Kindt and Brosschot (1999). IOR indicates that attention is inhibited from returning to a previously attended location (Posner, Rafal, Choate, & Vaughan, 1985). With regard to the present results this implies that, although the threat picture may have captured attention initially, an inhibiting effect towards the initially attended location delays the response to probes emerging there (Prime & Ward, 2004). This is in accordance with the suggestion of Mogg and Bradley (1998) that clinically anxious individuals may show a 'vigilance-avoidance' pattern of bias, which indicates that after an initial vigilance for threat, clinically anxious individuals try to avoid detailed processing of this information to minimize potential discomfort.

Processes like IOR or the 'vigilance-avoidance' bias pattern may have masked an initial vigilance for the fear-related pictures. But if we assume these cognitive systems to be operational, we must conclude that they primarily affect response times in the engage condition of the anxious group, resulting in negative bias scores. This does not undermine the fact that a significant difference in cognitive strategy was found between non-referred children and children with an anxiety disorder. Further research focused on the time line of cognitive steps after fear-related stimuli have been presented is desirable.

Another possible explanation for differences between studies regarding both direction and extent of the reported bias scores in anxious children compared to controls, is the role of age in the development of attentional bias. Childhood is known to be a very sensitive period for developing all kinds of automated cognitive schemata to respond appropriately and efficiently to all kinds of different stimuli (Crone et al., 2004; Ehrenreich & Gross, 2002; Mogg & Bradley. 1998). Some authors suggest that these automated processes may not yet be fully developed in children up to 12 years old, which may account for the wide variety of results obtained when investigating attentional bias in children (Ehrenreich & Gross, 2002; Kindt & Brosschot, 1999; Kindt et al., 1997b; Vasey et al., 1996; Waters et al., 2004). Our data underline this assumption: although we restricted the age-range within our sample of children (9.5-13.4 years), an increase in age significantly reduced mean response times. However, as a derived measure the bias score did not show this effect of age.

In conclusion, a significant difference has been found between the early attentional strategies used by children with an anxiety disorder and non-referred control children when confronted with fear-related stimuli. Contrary to earlier findings and our original hypothesis, our result indicates an initial avoiding strategy for fear-related locations in children with an anxiety disorder. This is in accordance with a 'vigilance-avoiding' strategy aimed at minimizing confrontation with threat, that may maintain or cause a higher vigilance for fear-related stimuli in highly anxious individuals (Mogg & Bradley, 1998). The role of early attentional processes in anxiety disorders from childhood into adulthood may be considerable, which supports the need for an emphasis on these processes in the treatment of anxiety disorders in children.

4.

Associations between HPA-axis functioning and level of anxiety in children and adolescents with an anxiety disorder

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In press: Depression and Anxiety, 2007

Summary - The Hypothalamus-Pituitary-Adrenal (HPA) axis becomes active in response to stress. Hence, increased levels of anxiety in children and adolescents may be associated with changes in HPA-axis functioning. The aim of this study was to test if level of anxiety or specific anxiety disorders were associated with basal HPA-axis activity in children and adolescents with an anxiety disorder.
Methods: In 99 8- to 16-year-olds with an anxiety disorder, basal cortisol levels were assessed. It was tested if (1) cortisol levels correlated with the level of self-reported anxiety, and (2) if cortisol levels were different for individuals with different anxiety disorders. Results: In girls, low levels of anxiety were associated with a stronger rise in early morning cortisol concentrations. In both boys and girls, harm avoidance predicted low cortisol concentrations after awakening. Separation anxiety and physical anxiety symptoms predicted cortisol concentrations at noon. Differences between individuals with different anxiety disorders were not found. Conclusions: More research is needed regarding mechanisms that explain the associations that were found, and to investigate if treatment may influence HPA-axis functioning in children and adolescents with an anxiety disorder.

4.1. INTRODUCTION

Several studies have provided evidence for an association between Hypothalamus-Pituitary-Adrenal (HPA)-axis functioning and psychiatric problems (Chrousos, 1997; Kirschbaum & Hellhamer, 1994; Levine, 2000; Preussner et al., 2003a). Research on this topic is based on the role of the HPA-axis in stress regulation. In stressful situations, the hypothalamus secretes corticotrophin-releasing hormone (CRH), which stimulates the pituitary gland to secrete adrenocorticotrophic hormone (ACTH). Subsequently, ACTH is released which causes the adrenal glands to produce cortisol. Changes in cortisol concentrations influence immunity, metabolism, growth, reproduction and other important physiological processes (Chrousos, 1997; De Kloet, 2003; Sapolsky et al., 2000).
Studies in adults have revealed evidence for increased cortisol secretion in stressful situations (Francis, 1979; Kirschbaum et al., 1993; Van Eck et al., 1996). Some authors hypothesized that increases in cortisol concentrations due to regular, or occasional but severe, stress may cause down-regulation of the HPA-axis (De Kloet, 2003; Sapolsky et al., 2000), which is in accordance with findings in animals (McEwen, 2001; Spencer et al., 1996). Down-regulation of basal HPA-axis activity can be reflected in changes in rise of cortisol concentrations after awakening (Cortisol Awakening Rise, CAR: Grossi et al., 1998; Ockenfels et al., 1995; Preussner et al., 1997; Preussner et al., 2003a; Vingerhoets et al., 1996; Wüst et al., 2000a; Zarkovic et al., 2003), the Area Under the Curve (AUC), which is constructed by using multiple cortisol assessments over the day (Preussner et al., 2003b; Rosmalen et al., 2005), or cortisol concentrations on specific moments of the day, for example immediately after awakening or late in the evening (Rosmalen et al., 2005; Yehuda et al., 2005a).
HPA-axis functioning has not only been investigated in the context of immediate stress, but also in association with psychiatric problems that are associated with severe or chronic stress, or regular distress. For example, there is evidence for altered HPA-axis functioning in children and adults with a post traumatic stress disorder (PTSD) (Anisman et al., 2001; Bonne et al., 2003; Bremner et al., 2003; Goenjian et al., 2003; Hageman et al., 2001; Yehuda et al., 1996; Yehuda et al., 2005b; Wessa et al., 2006). Studies in adults showed that other disorders were associated with HPA-axis functioning as well (Francis, 1979; Heim & Nemeroff, 1999; Mason et al., 1986; Ockenfels et al., 1995; Penza et al., 2003), whereas information in children is scarce (Coplan et al., 2002; Feder et al., 2004; Gerra et al., 2000; Granger et al., 1994).
Granger et al. (1994) studied 102 7- to 17-year olds who had been referred to an

outpatient clinic for behavioral and emotional problems. They found that social anxiety was associated with a higher increase in salivary cortisol concentrations after a Parent-Child Conflict Discussion Task. Gerra et al. (2000) assessed changes in saliva cortisol concentrations induced by a public speaking task and did not find differences between 22 male adolescents with an anxiety disorder versus 20 male healthy controls. Feder et al. (2004) assessed 76 6- to 12-year-olds with a depressive disorder, 31 with an anxiety disorder, and 17 controls. Those with an anxiety disorder had lower nighttime cortisol levels and a steeper rise of cortisol concentrations after awakening, compared to the depressed and the healthy control children. Coplan et al. (2002) found significantly higher cortisol concentrations prior to Carbon Dioxide (CO_2) inhalation in children and adolescents with an anxiety disorder who had symptoms of anxiety or panic during inhalation, compared to those with an anxiety disorder who did not display anxiety or panic, and compared to those without an anxiety disorder.

In summary, two studies found that high levels of anxiety in children and adolescents are associated with higher cortisol concentrations during stressful conditions, whereas one did not. Further the study of Feder et al. (2004) showed that high anxiety levels are associated with changes in basal HPA-axis activity as well. To our knowledge, other studies that investigated the relation between high levels of anxiety and basal HPA-axis activity in children and adolescents are not available.

In the present study, HPA-axis functioning was assessed in 8- to 16-year-olds with Social Phobia, Generalized Anxiety Disorder, Specific Phobia or Separation Anxiety Disorder. It was investigated if severity of anxiety symptoms during the previous two weeks was associated with CAR, AUC or cortisol concentrations at awakening, 30 minutes later, at noon or at 8 p.m.

We presume that individuals with high anxiety levels feel stressed regularly. This could result in down-regulation of the HPA-axis. Hence, we hypothesized that high levels of anxiety would be associated with low cortisol concentrations immediately after awakening, a lower CAR, and reduced AUC. With respect to CAR, this may seem to contradict the findings of Feder et al. (2004) because these authors reported a steeper rise in cortisol levels after awakening in anxious children. However, this steeper rise did not indicate that anxious children had higher cortisol levels, but instead, low cortisol concentrations just before and immediately after awakening.

Because HPA-axis functioning is associated with age and gender, effects of these factors on cortisol levels were investigated as well (Bartels et al., 2003; Feder et al., 2004; Kudielka and Kirschbaum, 2003; Rosmalen et al., 2005).

Finally, because evidence for specific associations between the type of anxiety disorder and HPA-axis activity is scarce, we also investigated if the indices of HPA-axis activity differed among the DSM-IV anxiety disorders.

4.2. METHOD

4.2.1. *Participants*

Ninety-nine 8- to 16-year-old children and adolescents who had been referred to the outpatient clinic of the department of Child and Adolescent Psychiatry of either the Erasmus Medical Center in Rotterdam (n = 67) or Leiden University Medical Center – Curium (n = 32) participated in the study (53 boys, 46 girls, average age: 10.8 years, standard deviation: 2.2 years). All consecutive referrals to these departments were diagnosed with the Anxiety Disorders Interview Schedule for DSM-IV-Child Version (ADIS-C; Silverman et al., 2001; see below). All individuals who fulfilled the criteria for a primary diagnosis of one of the following anxiety disorders constituted the target sample: Generalized Anxiety Disorder (GAD; n = 33), Separation Anxiety Disorder (SAD; n = 24), Social Phobia (SOP; n = 24) or a Specific Phobia (SP; n = 18). Exclusion criteria were: current medication for an anxiety disorder, co-morbid Pervasive Development Disorder, Obsessive Compulsive Disorder, Post Traumatic Stress Disorder, substance abuse, and an IQ score below 85 on the Wechsler Intelligence Scale for Children- Revised (WISC-R; Wechsler, 1974). Three participants were diagnosed with a co-morbid depression, 8 with co-morbid dysthymia, and 55 participants had at least one other anxiety disorder (GAD, SAD, SOP, or SP). Ten participants had co-morbid ADHD. From the initial 103 children and adolescents who met the inclusion criteria, four refrained from participation.

The Medical Ethical Committees of the Erasmus MC in Rotterdam and the Leiden University Medical Center in Leiden approved the protocol. All parents and each adolescent provided written consent.

4.2.2. *Instruments*

The ADIS-C (Silverman et al., 2001), the Multidimensional Anxiety Scale for Children (MASC; March et al., 1997), and the Children's Depression Inventory (CDI; Kovacs, 1992) were administered routinely to all consecutive referrals, independently of inclusion in the study.

ADIS-C

The Anxiety Disorders Interview Schedule for DSM-IV (ADIS-C; Silverman et al., 2001; Siebelink & Treffers, 1998) is a semi-structured interview that can be used to assess DSM-IV anxiety disorders in 7- to 17-year-olds. The interview was conducted by a graduated psychologist with the child or adolescent and with the 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 and child, and according to the interviewer, are taken into account.

MASC

The Multidimensional Anxiety Scale for Children (March et al., 1997) is a 39-item self-report questionnaire for assessment of anxiety symptoms in children and adolescents, and covers the past two weeks. The scoring format is; 0 = never true, 1 = rarely true, 2 = sometimes true, 3 = often true. Scores on five scales can be derived: Total Anxiety, Physical Symptoms, Harm Avoidance, Social Anxiety, and Separation Anxiety (March et al., 1997).

CDI

The Children's Depression Inventory (Kovacs, 1992; Sitarenios & Kovacs, 1999; Koot and Van Widenfelt, 2000) is a 27 item self-report questionnaire that covers depressive symptoms in the past two weeks. The scoring format is 0 = never true, 1 = sometimes true, 2 = always true. A CDI Total score can be derived by adding up the item scores.

4.2.3. *Study procedure*

After having signed informed consent, the parents and children received an information brochure, 4 plastic saliva tubes and sampling instructions.

Participants were instructed to collect saliva samples: (1) immediately after awakening in the morning, when the child was still in bed; (2) 30 minutes later; (3) at 12.00, and (4) at 8 p.m. The time of awakening had to be written on the label of the first saliva tube immediately after sampling. An extra instruction for the children stressed that they should not eat during half an hour before sampling, and refrain from dairy products from one hour before sampling. All samples were collected on a regular school day, stored in the freezer at home, and taken to the clinic one day later, when the next appointment at the clinic was scheduled. Every saliva sample was collected by pushing the tube against the lower lip and having some saliva flow into the tube. Participants were instructed to fill the tubes till a marker (at 500 µL).

4.2.4. *Cortisol assessment*

At the clinic, saliva samples were stored at -20 °C, and transported afterwards to the laboratory for storage (at -20 °C) and analysis. Cortisol concentrations were determined in duplicate 200 µL samples by solid-phase radioimmunoassay with iodinated cortisol that competes with cortisol for antibody sites (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, USA). The lower limit of detection was.1 nmol/l, the intra-assay variation was 4.4% and the inter-assay variation was 5.2%.

On average, 9.2% of the samples were missing due to insufficient saliva or lack of reliability due to significant intra-assay differences. These intra-assay differences may be explained by violation of the protocol, eating or tooth brushing, which may have polluted the samples with low concentrations of blood or other substances (like dairy products). Of the available data, samples with extremely high cortisol concentrations were excluded from further analysis to control for outliers. For this purpose, for each sample values 2 standard deviations above the average were omitted (sample 1, 5 omissions; sample 2, 3; sample 3, 1; sample 4, 3). CAR was calculated for each subject by subtracting sample 1 concentrations from sample 2 values.

Adults with a rise of at least 2.5 nmol/l in cortisol concentrations during the first 30 minutes after awakening are often classified as responders (Wüst et al., 2000b). Sixty-four percent of the individuals in the current sample were classified as 'responder' according to this definition (criterion 1, see table 4.1.). This is lower than in adults, for whom percentages around 75% have been reported (Federenko et al., 2004; Kunz-Ebrecht et al., 2004; Wüst et al., 2000b). It is unclear why certain individuals do not show a rise in cortisol concentrations after awakening. This may be due to neurobiological factors, but also to methodological problems. Lack of compliance to the research protocol, that is collecting saliva samples at the wrong time, is often mentioned as a possible reason for the inability to detect a (nevertheless present) rise in cortisol concentrations (e.g. Kunz-Ebrecht et al., 2004). Additionally, the younger a child, the lower the cortisol awakening rise, which seems to be due to higher night and early morning cortisol concentrations in younger children (Bartels et al., 2003; Feder et al., 2004; Kudielka & Kirschbaum, 2003; Rosmalen et al., 2005). For this reason, it has been suggested to consider children and adolescents with an increase in early morning cortisol concentrations, independently of the extent of the increase, as responders (criterion 2: Rosmalen et al., 2005). We conducted analyses regarding CAR in the complete sample. However, because a lacking CAR could be associated with assessment problems as mentioned above, we also conducted analyses for the participants who could

be regarded as responders according to criterion 2. AUC (area under the curve) is a measure that represents the total amount of cortisol that is produced during a specific period. AUC was calculated according to Preussner et al. (2003b) by computing the area between a cortisol level of zero, and the curve that was constituted by sample 1, 2, 3, and 4 levels. If sample 1, 2, or 3 values were missing, AUC was not calculated. If sample 4 values were missing, the sample 4 value for those with missing values was replaced by the average sample 4 value of the other individuals. We considered this appropriate because sample 4 values were generally low, did not differ much between individuals, and therefore did not influence AUC to a large extent.

4.2.5. *Statistical analyses*

An independent samples t-test was applied to investigate the difference in age between boys and girls. A one-way analysis of variance (ANOVA) and post-hoc t-tests were used to assess age differences between individuals with different primary anxiety diagnoses.

To investigate if the level of anxiety predicted the CAR, a stepwise regression analysis was conducted. CAR was included as dependent variable. MASC Social Anxiety, Separation Anxiety, Harm Avoidance, and Physical Symptoms scores, as well as the CDI Total score we entered as candidate predictors. Because of their putative effects on CAR, gender and age were entered as covariates (Kunz-Ebrecht et al., 2004; Rosmalen et al., 2005). A similar regression analysis was conducted with the MASC Total Anxiety scale instead of the four MASC subscales as candidate predictor. Finally, to assess differences in the association between anxiety and HPA-axis functioning in boys versus girls, interaction terms between MASC scale scores and gender were entered as well.

For CAR all regression analyses were conducted (1) with the complete data set and (2) only using the data from individuals who showed an increase in cortisol concentrations after awakening (criterion 2).

To investigate whether CAR was different for individuals with different anxiety diagnoses (GAD, SOP, SP, SAD) an ANOVA was conducted. Like the other analyses regarding CAR, we repeated this analysis while only including the participants who showed an increase in cortisol concentrations after awakening.

Two independent stepwise regression analyses were conducted with AUC as dependent variable and either the four MASC scales or the MASC Total Anxiety scale as candidate predictors, together with gender, CDI Total score and age. An ANOVA was applied to investigate differences in AUC between the distinct anxiety diagnoses.

Finally, sample 1, 2, 3, and 4 cortisol concentrations were analyzed separately. First, four independent sets of stepwise regression analyses were conducted, with consecutively each of the samples 1, 2, 3, and 4 cortisol levels as dependent variables, and the four MASC subscales as candidate predictors, as well as gender, CDI Total score and age. These analyses were repeated with the MASC Total Anxiety scale as candidate predictor, instead of the four subscale scores. For each cortisol sample an ANOVA was conducted to investigate differences in cortisol concentrations between anxiety diagnoses. All statistical analyses were conducted with SPSS 11.0 (SPSS Inc., Chicago, USA).

4.3. RESULTS

General information regarding the sample is presented in table 4.1. The age difference between boys and girls was not significant: $t(97) = -1.73$. Participants with social phobia were significantly older than those with other diagnoses ($F(3,93) = 10.0$, $p < .0005$). Further, those with a primary diagnosis of generalized anxiety disorder (10.7 years) were significantly older than those with specific phobia (9.3 years) ($t(47) = 2.4$, $p < .05$), see table 4.1.

	Age in years		SAD		GAD		SOP		SP
	(s.d.)	n	Age (s.d)	n	Age (s.d.)	n	Age (s.d.)	n	Age (s.d.)
Boys	10.4	12	9.9	15	10.3	12	12.3	14	9.0
(n = 53)	(2.2)		(1.5)		(1.6)		(2.8)		(1.1)
Girls	11.2	12	10.5	18	11.0	12	12.8	4	9, 10, 11, 13
(n = 46)	(2.1)		(1.5)		(2.5)		(1.3)		
Total	10.8	24	10.2	33	10.7	24	12.5	18	9.3
	(2.2)		(1.5)		(2.2)		(2.3)		(1.4)

Table 4.1. General information.

Note: s.d. = standard deviation. SAD = Separation Anxiety Disorder; GAD = Generalized Anxiety Disorder; SOP = Social Phobia; SP = Specific Phobia

4.3.1. *Cortisol Awakening Rise (CAR)*

The first set of regression analyses, including the entire sample, did not reveal a significant association between scores on MASC scales and CAR, whereas an age effect was found ($F(1,68) = 4.8$, $p < .05$, $R^2 = .07$) (figure 4.1.). Similarly, an association between MASC Total Anxiety scale scores and CAR was not found. However, a non-significant trend was found for the regression model that included the interaction term of the MASC Total Anxiety scale score and gender: $F(2, 67) = 2.7$, $p = .08$.

	Awakening (s.d.)	Awakening +30 minutes (s.d.)	noon (s.d.)	8 p.m. (s.d.)	AUC (s.d.)	CAR (s.d.)	Responders	
							Criterion 1	Criterion 2
Boys	12.29 (6.58)	18.76 (9.43)	4.33 (2.12)	1.60 (2.04)	91.6 (37.1)	7.86 (6.40)	67.3%	79.6%
(n = 53)								
Girls	14.98 (6.94)	20.80 (9.43)	5.33 (3.33)	1.41 (1.44)	100.1 (53.7)	9.13 (7.25)	59.5 %	78.4%
(n = 46)								
Total	14.03 (6.75)	19.65 (8.30)	4.79 (2.77)	1.51 (1.79)	94.7 (45.0)	8.41 (6.75)	64.0 %	79.1%

Table 4.2. Mean (s.d.) day time cortisol concentrations, the Area Under the Curve (AUC), Cortisol Awakening Rise (CAR), all in nmol/l.
Note: Criterion 1 responder: an increase in cortisol concentrations of at least 2.5 nmol/l during the first 30 minutes after awakening. Criterion 2 responder: an increase in cortisol concentrations during the first 30 minutes after awakening.

In the sub-sample of responders according to criterion 2, which consisted of 39 boys and 31 girls (average age: 10.8 years, s.d.: 2.3 years; diagnoses: 16 SAD, 24 GAD, 16 SOP, 14 SP; see table 4.2.), somewhat different associations were found.

No significant effects were found for the MASC scales Social Anxiety, Separation Anxiety, Harm Avoidance, and Physical Symptoms. However, in this sub-sample, the interaction between gender and scores on the MASC Total Anxiety scale ($\beta = -.24$, 95% CI = -.47 - -.01, $p = .04$) predicted the rise in cortisol concentrations after awakening at a borderline level of significance ($F(5, 63) = 2.25$, $p = .06$). This effect accounted for 16% of the variance. To clarify this interaction, we divided both the boys and the girls in two groups, based on the 50th percentile score on the MASC Total Anxiety scale score for each gender. Girls in the low anxiety group showed a higher morning rise than the other groups (see figure 4.2.).

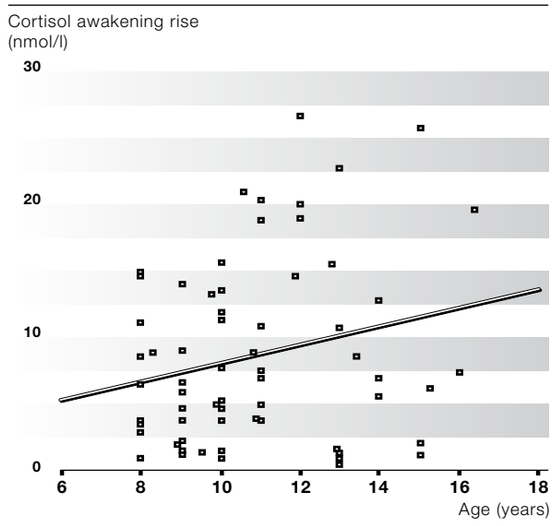

Figure 4.1. Rise in cortisol concentrations after awakening and age, with regression line.

Post hoc analyses using the Kolmogorov-Smirnov test showed that the difference in rise in cortisol concentrations between high anxious girls (n = 15, average age: 10.9 years, s.d.: 1.9 years; diagnoses: 4 SAD; 6 GAD; 3 SOP; 2 SP) and low anxious girls (n = 16, average age: 11.4 years, s.d.: 2.2 years; diagnoses: 4 SAD; 7 GAD; 4 SOP; 1 SP) was significant ($Z = 2.04$, $p < .05$). These two groups of girls did not differ with respect to age ($Z = -.89$, n.s). The difference in rise in cortisol concentrations after awakening was explained by significantly higher cortisol concentrations 30 minutes after awakening in the low anxious girls (average = 27.02 nmol/l), compared to girls who were high on anxiety (average = 17.91 nmol/l; $Z = 1.99$, $p < .05$). No significant differences between these two groups were found in cortisol concentrations immediately after awakening ($Z = 1.46$, n.s.). The difference in rise of cortisol concentrations between boys and girls with low scores on the MASC Total Anxiety scale was not significant ($Z = 1.11$, n.s.).
The four diagnostic groups (SAD, GAD, SOP, SP) did not differ with respect to CAR. ANOVAs yielded non-significant results, independently of the use of the complete data set ($F(3, 73) = 1.9$, n.s), or the use of data only from responders according to criterion 2 ($F(3, 67 = 1.7$, n.s.).

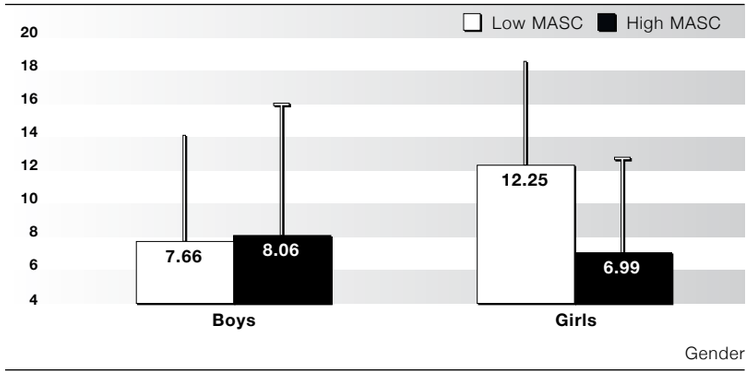

Figure 4.2. Mean and standard deviation of the morning rise in saliva cortisol concentrations for boys and girls with low and high scores on the MASC Total Anxiety scale.

4.3.2. *Area Under the Curve (AUC)*

Regression analyses using the complete data set (responders and non-responders, n = 99), did not reveal significant associations between scores on the MASC Physical Symptoms scale, Social Anxiety scale, Separation Anxiety scale, or the Harm Avoidance scale ($F(7, 79) = 1.44$, n.s.). Similarly MASC Total Anxiety scale and AUC were not associated ($F(4, 80) = 1.66$, n.s.). The four diagnostic groups (SAD, GAD, SOP, SP) did not differ with respect to AUC ($F(3, 83) = .12$, n.s).

4.3.3. *Separate daytime cortisol samples*

Regression analyses based on the full data set (responders and non-responders) revealed an association between scores on the MASC Harm Avoidance scale and cortisol concentrations after awakening ($F(1, 79) = 5.5$, $p < .05$, $\beta = -.26$, $R^2 = .07$). No association was found between the MASC Total Anxiety scale and cortisol concentrations after awakening ($F(1, 78) = .004$, n.s.). No significant associations were found between the scores on the MASC scales Physical Symptoms, Separation Anxiety, Harm Avoidance, and Social Anxiety and cortisol concentrations 30 minutes after awakening ($F(7, 75) = .85$, n.s.), nor between scores on the MASC Total Anxiety scale and cortisol concentrations 30 minutes after awakening ($F(1, 78) = .04$, n.s.). Age ($\beta = .27$, $p < .05$), MASC Separation Anxiety scale scores ($\beta = .22$, $p = .06$), and MASC Physical Symptoms scale scores ($\beta = -.26$, $p = .04$) predicted cortisol concentrations at noon ($F(3, 80) = 2.73$, $p < .05$, $R^2 = .09$). Hence, a positive association was found between Separation Anxiety scores and cortisol concentrations at noon, whereas a negative association was found between Physical Symptoms scores and cortisol concentrations at noon. No association was found between scores on the MASC scale Total Anxiety and cortisol concentrations at noon ($F(1, 78) = .21$, n.s.). Furthermore, age ($\beta = .38$, $p = .001$) predicted cortisol concentrations at 8.00 p.m. ($F(1, 80) = 9.5$, $p < .005$, $R^2 = .11$).

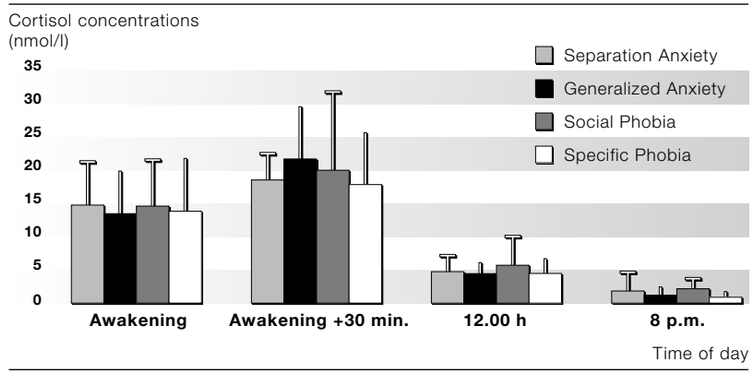

Figure 4.3. Cortisol concentrations across the day for each ADIS-C / DSM-IV anxiety disorder.

No significant differences were found between children with SAD, GAD, SOP, or SP, in sample 1, 2, or 3 cortisol values (sample 1: $F(3, 88) = .5$, n.s.; sample 2: $F(3, 88) = 1.4$, n.s.; sample 3: $F(3, 88) = .2$, n.s). However, differences were found for sample 4 cortisol concentrations ($F(3, 88) = 4.5$, $p < .01$). Post hoc analyses showed that individuals with a primary diagnosis of SOP had a significantly higher sample 4 cortisol concentration (GAD: 1.1 nmol/l; SAD: 1.1 nmol/l; SOP: 1.9 nmol/l; SP: .9 nmol/l). However, because participants with SOP were significantly older while a strong effect of age on sample 4 cortisol concentrations was already found, we added age as a covariate. In this way it was found that the effect of SOP on sample 4 cortisol concentrations was explained entirely by age (statistics full model: $F(1, 85) = 11.34$, $p < .0005$), see figure 4.3.

4.4. DISCUSSION

The present study investigated associations between levels of anxiety symptoms and basal cortisol levels in children and adolescents with an anxiety disorder. Analyses for the group of responders revealed that an interaction between gender and MASC Total Anxiety scale scores predicted the rise in cortisol concentrations during the first 30 minutes after awakening. Additionally, associations were

found between scores on the MASC Harm Avoidance scale and cortisol concentrations immediately after awakening, and between scores on the MASC Physical Symptoms and Separation Anxiety scales and cortisol concentrations at noon. Next to significant associations between age and CAR, which is in accordance with previous findings (Rosmalen et al., 2005), an association between age and cortisol concentrations at 8 p.m. was found, which has not been reported earlier. Significant associations between either anxiety symptoms or diagnoses and AUC were not found, or disappeared after adding age as a covariate. In general, the results indicated that basal HPA-axis activity in children with anxiety disorders is associated with scores on dimensional anxiety scales, but not with the type of categorical DSM-IV anxiety disorder. It should be emphasized that the study sample was fairly homogeneous with respect to levels of anxiety, since it did not contain individuals with low anxiety levels. However, the fact that, despite the homogeneity of the sample, associations between cortisol concentrations and anxiety levels were still found underscores the significance of the findings. The variance in anxiety symptoms in the present sample appeared to be sufficient to find significant associations between HPA-axis activity and level of anxiety. Nevertheless, it is important to compare the results of the present study with findings in low anxious samples in the future.

4.4.1. *MASC scores and cortisol levels*

In the sub-sample of children and adolescents with a rise in cortisol concentrations after awakening, girls with higher anxiety levels showed a lower morning rise in cortisol levels than girls with lower self reported anxiety levels. Effects of chronic stress and anxiety on the rise of cortisol concentrations after awakening have been reported earlier in women. However, in previous studies, the rise in cortisol concentrations after awakening became higher if the level of chronic stress increased (Kunz-Ebrecht et al., 2004; Schulz et al., 1998). In the girls from the present study's sample, higher levels of anxiety, which may be related to regular experiences of stress, were associated with a lower rise of cortisol concentrations after awakening. This was reflected by significant differences in cortisol concentrations 30 minutes after awakening, whereas no significant differences were found between these two groups in cortisol concentrations immediately after awakening. The fact that previous studies investigated women from the general population, instead of children with an anxiety disorder, may partly explain the discrepancy between the present versus previous findings. The findings of the present study seem to be in accordance with the hypothesis that extremely anxious children and adolescents may show hypo-, instead of hypercortisolism (Gunnar & Vazquez, 2001). Future research is needed to find out if CAR is also lower in girls with high anxiety levels, compared to girls from the general population. Nevertheless, the present findings do suggest that girls with an anxiety disorder only show significant chances in CAR if anxiety symptoms are severe, contrary to what was found in boys. The differences in CAR between boys and girls are in accordance with earlier findings indicating significant differences in CAR between men and women on working days (Kunz-Ebrecht et al., 2004) and in children and adolescents (Rosmalen et al., 2005).

In the sub-sample of participants who showed an increase in cortisol concentrations after awakening, the interaction between MASC Total Anxiety scores and gender seemed to predict CAR. This effect was not found if those without a rise in morning cortisol levels were also included in the analyses. Previous studies have shown that CAR increases with age, as does the number of individuals with a positive CAR (Bartels et al., 2003; Kudielka & Kirschbaum, 2003; Rosmalen et al., 2005). It is subject of debate whether a lack of CAR in an individual is due to methodological shortcomings, such as not collecting saliva at the right moment, or that instead, in some individuals, cortisol levels do not increase after awakening. Further investigations, in which morning rise in cortisol levels are assessed in controlled conditions in a lab, would shed more light on this topic. Without such investigations it remains unknown if our finding that CAR was associated with anxiety levels in girls was merely an artifact, or, instead, a significant finding. In any case, this finding can serve for formulating hypotheses for future research.

The negative association between scores on the MASC Harm Avoidance scale and cortisol concentrations immediately after awakening was in accordance with earlier findings of lower early morning cortisol concentrations in children with an anxiety disorder (Feder et al., 2004) and with studies that found a negative association between early morning cortisol concentrations and chronic stress in adults (Preussner et al., 1999; Zarkovic et al., 2003). According to Feder et al. (2004), lower cortisol concentrations during the night and early morning in children with an anxiety disorder may be associated with disturbances in sleep dynamics. Evidence has been found for associations between signs of restlessness during sleep, reflected by stronger EEG delta activity, increased rapid-eye-movements, and nocturnal awakenings and suppressed HPA-axis activity (Bierwolf et al., 1997; Feder et al., 2004; Luboshitzky, 2000; Seifritz et al., 1995). The influence of anxiety or chronic stress on sleep dynamics may have caused lower early morning cortisol concentrations, possible due to changes in CRH regulation (Feder et al., 2004).

Higher MASC Separation Anxiety scale scores and lower MASC Physical Symptoms scale scores predicted higher cortisol concentrations at noon. Previ-

ous studies found an association between other anxiety symptoms and cortisol concentrations during the day (Granger et al., 1994: Wüst et al., 2000a). To our knowledge, this is the first time that high levels of separation anxiety were found to be associated with an increase in daytime cortisol concentrations. This finding may reflect the HPA-axis dynamics normally seen in relation to immediate stress; children reporting high levels of separation anxiety who are separated from their parents may react with increased HPA-axis activity. Future longitudinal studies are needed to investigate if, with persisting separation anxiety, the initial hyper-secretion of cortisol at noon will still be found, or will have been reversed into hypo-secretion caused by the chronic presence of these symptoms. It may seem remarkable that there were no significant differences between participants with a primary diagnosis of SAD versus the other DSM-IV diagnostic groups. The reason for this discrepancy may be that the MASC covers the past two weeks, whereas the diagnosis SAD was based on the past 6 months. So, the association between the MASC Separation Anxiety scale and cortisol levels at noon may reflect responses to recently experienced distress related to symptoms of separation anxiety, being fairly independent of the actual presence of SAD. Comorbidity between symptoms of separation anxiety and other anxiety symptoms may be another explanation. Scores on the MASC scale Physical Symptoms were associated negatively with cortisol levels at noon. This scale is not specifically related to a well defined anxiety (DSM-IV) diagnosis, but it can perhaps be regarded as a construct underlying childhood anxiety.

Separation anxiety levels were associated with higher, and scores on the Physical Symptoms scale were associated with lower cortisol levels at noon. Hence different aspects of anxiety were associated with cortisol levels in a different way. This may indicate that separation anxiety and physical symptoms reflect two different dimensions of internalizing problems. This is in accordance with the tripartite model for anxiety and depression (Clark & Watson, 1991; Clark et al., 1994). According to this model, physiological anxiety symptoms represent a dimension that can be separated from a negative affectivity dimension that represents nonphysical symptoms like worrying or tension. The present study indicated that these different dimensions may be associated with different types of HPA-axis activity.

Physical symptoms were associated with lower HPA-axis activity during the day, whereas one might also expect an increased activation of the HPA-axis in those who are aroused physically. This finding may be explained by negative feedback loops that may become more activated in those who initially tend to respond to stressors with physiological hyper-arousal (Sapolsky et al., 2000).

In addition to the two MASC scale scores, age predicted cortisol levels as well, which strengthened the hypothesis that HPA-axis functioning is age dependent (Gunnar & Vazquez, 2001). However, (severe) anxiety could moderate the development of the HPA-axis through adolescence into adulthood. If this would be true, it might be a reason for the discrepancies found between cortisol studies in youth and adults with an anxiety disorder. Longitudinal studies aimed at the development of both anxiety symptoms as well as HPA-axis functioning seem necessary to shed more light on this matter.

4.4.2. *CDI scores and cortisol levels*

Depression has been associated with HPA-axis functioning, generally reflected by hyper-cortisolism during the day (Gold et al., 1988a, 1988b; Parker et al., 2003; Preussner et al., 2003; Tiemeier, 2003; Voderholzer et al., 2004). For this reason, the CDI Total score was included as covariate in all analyses. No significant effects of this depression score on cortisol concentrations were found in the present study. Preussner et al. (2003) reported that the association between depressive symptoms and cortisol levels became stronger when individuals with lower depression scores were studied. The sample of the present study was a clinical one, which may have weakened associations between depression scores and cortisol levels.

4.4.3. *Limitations*

No significant differences in basal HPA-axis functioning were found between the distinct anxiety disorders. However, given high rates of comorbidity between the anxiety disorders that constituted the inclusion criteria (Essau et al., 2000; Verduin & Kendall, 2003), it can be doubted if individuals with these different anxiety disorders really represent different types of individuals. The different diagnostic groups may still have been fairly similar. Empirical evidence even showed that it can be doubted whether the different childhood anxiety disorders can really be regarded as distinct diagnostic constructs (Ferdinand et al., 2005).

Compliance to time of sampling has been reported to be a significant methodological drawback of studies like the present one (Broderick et al., 2004; Gunnar Vazquez, 2001; Kudielka et al., 2003). Especially the timing of sampling during the first 45 minutes after awakening is crucial to be able to draw reliable conclusions with regard to HPA-axis functioning. In every participant without a CAR it is unclear whether this can be attributed to non-compliance or if there are other reasons for the absence of the CAR. This topic is the subject of an ongoing debate in the field of psychoneuroendocrinology. For this reason the analyses regarding CAR in the present study were conducted including the complete sample but also for individuals with a positive CAR.

Referral biases constitute another possible limitation. Especially in adolescence, it may be the case that only the more severe cases tend to visit a university outpatient clinic. This may have limited the representativeness of the study sample and underscores the need for investigations in large general population samples. Comparison of cortisol levels in children and adolescents with an anxiety disorder with levels in a control group of non-anxious individuals would also be important. For instance, such a comparison might shed more light on the interaction between anxiety symptoms and gender with respect to prediction of the cortisol morning rise. Now, the question remains if this is an effect specific for the highly anxious children and adolescents in the present study or a more general phenomenon.

4.4.4. *Clinical implications*

It can be questioned if the association between anxiety levels and HPA-axis functioning is stable, or changes if anxiety symptoms are reduced, for example as a result of treatment. According to Gunnar and Vazquez (2001), cognitive, psychological, and physiological developments go hand in hand during childhood and adolescence. As a consequence, it may be that adverse development in one domain influences the other domains (Charmandari et al., 2003; Gunnar & Vazquez, 1997; Heim & Nemeroff, 1999). If alterations in HPA-axis functioning in children with an anxiety disorder might be reversed, the risk of adverse outcome might decrease. However, further research is needed to investigate this hypothesis.

4.4.5. *Conclusions*

Several associations between levels of anxiety symptoms and cortisol concentrations were found in the present study. However no significant differences based on ADIS-C/DSM-IV anxiety disorders were found. Because no relation between anxiety symptoms and cortisol concentrations at 8 p.m. were found it seems that associations between anxiety levels and HPA-axis functioning are most profound during the morning and afternoon. Although the present study may have provided some interesting new insights in the relation between childhood anxiety and basal HPA-axis functioning, future studies in both clinical and general population samples are needed to put the present study's findings in the right perspective.

5.

Physiological stress reactivity in children and adolescents with an anxiety disorder, and its associations with specific anxiety symptoms

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Submitted for publications

Summary. - Anxiety disorders are among the most common psychiatric diagnoses in childhood and adolescence and may be related to altered physiological reactivity in response to stress. Altered physiological reactivity may be a factor that determines vulnerability for anxiety disorders throughout life. Studies regarding physiological reactivity in children and adolescents with an anxiety disorder are therefore highly relevant.
The physiological response (skin conductance level: SCL, heart rate, blood pressure) to a cognitive stressor (mental arithmetic) and the social competence interview (specific stressor) was assessed in 97 8- to 16-year-olds with an anxiety disorder.
Generalized anxiety was associated with decreased baseline high-frequency-band power of the heart rate and an increased SCL during the interview. Separation anxiety was associated with stronger SCL responses to both stressors. Harm avoidance was significantly associated with a decrease in diastolic blood pressure during mental arithmetic. Finally, social phobia was related to a stronger diastolic blood pressure response to the interview.
The degree of anxiety in children and adolescents with an anxiety disorder appeared to be associated with reduced baseline parasympathetic cardiac control. In response to stressors, the degree of anxiety symptoms was primarily associated with the reactivity of physiological variable that are associated with sympathetic activity.

5.1. INTRODUCTION

Anxiety disorders are among the most common psychiatric diagnoses in childhood and adolescence (Bernstein & Brochardt, 1991) and physiological systems in children and adolescents with anxiety disorders may react differently, compared to non-anxious individuals, in case of adverse experiences and/or stress (Beidel, 1988; Boyce et al., 2001; Garralda et al., 1991; Gerra et al., 2000; Matthews et al., 1986; Monk et al., 2001; Pine et al., 1998; Scheeringa et al., 2004). Persistent distress in youths may reduce the physiological ability to respond adequately to stress (Musante et al., 2000), which may result in vulnerability for anxiety disorders and/or depression (Garralda et al., 1991; Kagan & Snidman, 1999; Penza et al., 2003; Pine et al., 1998) and negative physical health outcomes like cardiovascular events later in life (Allen et al., 1997; Dobkin et al., 2000; Jackson et al., 1999; Jemerin and Boyce, 1990; Kawachi et al., 1994; Monk et al., 2001; Treiber et al., 2001; Yeragani et al., 2001).
Physiological reactivity to stress is determined by activation or inhibition of the sympathetic and the parasympathetic nervous system. The combined activity of both systems in response to stress generally results in heightened skin conductance level (SCL), blood pressure (BP), and heart rate (HR), which are associated with the 'flight or fight' response (Cannon, 1932). Variability in HR and BP is frequently analyzed by means of spectral techniques to obtain noninvasive estimates of sympathetic and parasympathetic regulation of the cardiovascular control system (Akselrod et al., 1981, 1985; Malliani et al., 1991; Task Force, 1996; Tulen et al., 1994, 1996). HR variability, that is related to respiratory variations, usually between 0.20-0.35 Hz (respiratory sinus arrhythmia), results from centrally mediated cardiac vagal (parasympathetic) activity (high frequency band power: HF HR). BP variability in the frequency domain around 0.10 Hz (Mayer waves) reflects alterations in peripheral resistance due to baroreflex-mediated sympathetic control. HR variability in this frequency domain represents changes in the baroreflex response and similarly reflects sympathetic activity, although an influence of vagal activity has also been suggested. Disturbances in vagal activity and baroreflex-mediated sympathetic control have been associated with anxiety and depressive symptoms in adults (Stein et al., 1992; Tulen et al., 1996).
Experimentally induced stress is commonly used to investigate the responses of physiological systems, both in youth as in adults, generally in relation to anxiety, depression or disruptive behavior. Research conducted with children and adolescents generally combined physical stressors (e.g. CO_2 challenge: Monk et al., 2001; Pine et al., 1998), competitive stressors (e.g. computer gaming: Jackson et

al., 1999), cognitive stressors (e.g. Mental Arithmetic: Dorn et al., 2003; Ewart et al., 1991; Gerra et al., 2000, memory tasks: Boyce et al., 1995; Boyce et al., 2001, or mirror tracing: Allen et al., 1997; Chen et al., 2002; Ewart et al., 2004; Salomon et al., 2000), and social stressors, being either public speaking (Beidel, 1988; Dorn et al., 2003; Gerra et al., 2000), or a Social Competence Interview (SCI: Allen et al., 1997; Barbeau et al., 2003; Dobkin et al., 2000; Dorn et al., 2003; Ewart and Kolodner 1991; Ewart et al., 2004; Jackson et al., 1999; Liang et al., 1997; Musante et al., 2000; Salomon et al., 2000). Childhood anxiety seems to be associated with heightened activity of the sympathetic nervous system in response to such tasks (Gerra et al., 2000; Rogeness et al., 1990), but even more with a decrease of parasympathetic control (Boyce et al., 2001; Monk et al., 2001).

Gerra et al. (2000) compared HR, BP and several hormone levels, during a baseline period and in response to various stressors (Mental Arithmetic, Stroop Color-Word Interference Task, and public speaking) of 20 male adolescents with generalized anxiety disorder (with and without co-morbid separation anxiety disorder), and 20 age matched adolescents 'without overt psychiatric disorders'. No significant differences were found during baseline but the adolescents with an anxiety disorder displayed significantly higher HR and BP responses, which, together with the found hormonal responses (mainly noradrenergic) suggested an overactive sympathetic system in response to stress.

Dobkin et al. (2000) did not find significant differences in baseline levels of Diastolic or Systolic Blood Pressure (DBP, SBP) between anxious adolescent boys (n = 19), anxious-disruptive (n = 24), disruptive (n = 30), and control (n = 16) boys. However, in response to the Social Competence Interview (SCI: Ewart et al., 1991) anxious and anxious-disruptive boys displayed a significantly higher SBP responsiveness compared to the other two groups. The lack of significant differences in baseline conditions contrasted with other studies (Monk et al., 2001; Rogeness et al., 1990). According to Dobkin and colleagues this may be due to the method and the informant (teacher) used to classify anxiety in their study.

Monk et al. (2001) exposed 22 children and adolescents (9- to 18-year olds) with an anxiety disorder (Separation Anxiety Disorder; Overanxious Disorder; Panic Disorder; Social Phobia) and 12 healthy 9- to 18-year olds to 15 minute 5% CO_2 inhalation. In the preceding baseline period the participants with an anxiety disorder displayed a significantly higher HR and reduced HF HR. Both groups showed a similar HR response during inhalation. Additionally, the participants without an anxiety disorder displayed a significant reduction of HF HR during inhalation. The initial significant differences between the two groups in HF HR during baseline and in response to CO_2 inhalation disappeared when respiration rate was included as covariate. Nevertheless, the authors conclude that cardiac parasympathetic (vagal) control may be reduced in children and adolescents with an anxiety disorder.

Yeragani et al. (2001) did not find significant differences between children with an anxiety disorder (n = 7) and children without a psychiatric disorder (n =15) in HF HR during 10 minutes in supine posture position. However, other Electrocardiographic (ECG) derivatives, primarily the variability in QT-interval, did show significant differences. Consequently, Yeragani and colleagues concluded that these results could be explained by increased sympathetic activity in children with an anxiety disorder.

Boyce et al. (2001) concluded that children (n = 122) classified as 'internalizing' (depression, overanxious and separation anxiety), based on the MacArthur Health and Behaviour Questionnaire (HBQ: Ablow et al., 1999), showed significantly higher parasympathetic activity in response to various stressors compared to the other groups ('externalizing', 'both', and 'low symptoms'). Their conclusion was based on a significant reduction in Respiratory Sinus Arrhythmia (RSA), a variable highly correlated with HF HR (Akselrod et al., 1981; Akselrod et al., 1985; Houtveen et al., 2002).

These studies provided valuable information regarding associations between anxiety and physiological reactivity in children and adolescents. However, they can not be compared easily with each other, primarily for two reasons.

1. Differences in classification of 'anxious children and adolescents'. Gerra et al. (2000) included adolescents with a generalized anxiety disorder, some with co-morbid separation anxiety disorder. Yeragani et al. (2001) included only 7 children with an anxiety disorder. No information regarding diagnostics was provided. The Social Behavior Questionnaire (SBQ; Tremblay et al., 1992) used in the study of Dobkin et al. (2000) to assess anxiety may not be suitable to identify clinical anxiety or distinguish different forms of anxiety, as acknowledged by the authors themselves. Boyce et al (2001) divided their sample in children scoring high on 'internalizing', 'externalizing', or 'both', and a group scoring low on both scales of the HBQ. Further differentiation within the 'internalizing' group to discriminate between depression and (different forms of) anxiety was not applied, probably because the used instrument is not suitable to do so. Finally, the patient sample of Monk et al. (2001) consisted of children and adolescents with several anxiety disorders. The primary reason to include a broad range of anxiety disorders (contrary to Gerra et al., 2000) is the frequent co-morbidity among children with anxiety disorders. This in line with the suggestion of Ferdinand et al. (2005), who stated that it can be doubted whether the different childhood anxiety disorders

can be regarded as distinct diagnostic constructs. For this reason the discrimination between different kinds of symptoms related to childhood anxiety (like somatic symptoms, symptoms of withdrawal or harm avoidance) may be a more fruitful approach when looking for associations of childhood anxiety and physiological reactivity.

2. The parameters used to investigate physiological reactivity, and even more particular the parasympathetic responses to stressors. RSA or HF HR are generally regarded as valid and reliable parameters of parasympathetic cardiac activity (Akselrod et al, 1981; Akselrod et al., 1985). Until now, only a few studies used these variables to investigate parasympathetic reactivity in samples of anxious children. Yeragani et al (2001) only assessed these parameters in baseline conditions. Monk et al. (2001) initially reported anxiety related differences in reactivity, which disappeared when respiration rate was included as covariate. Boyce et al. (2001) reported significant changes in RSA related to internalizing symptoms on a combination of various tasks.

Both heightened activation of the sympathetic nervous system (Gerra et al., 2000) as well as a decreased cardiac parasympathetic control (Monk et al., 2001) have been reported in children and adolescents with an anxiety disorder in response to stress. These results may, however, be influenced by some methodological weaknesses, being small sample sizes, inaccurate assessment procedures, and the absence of (valid) parasympathetic parameters.

In this study, we used two commonly used stress evoking tasks in children and adolescents: the Mental Arithmetic Task (MAT, standardized cognitive stressor) and the SCI (specific social stressor) to assess physiological responsiveness in 97 children and adolescents with an anxiety disorder. Our aim was to investigate the associations between specific anxiety symptoms and baseline values of physiological parameters, as well as the response of these parameters to both stressors. Special attention was paid to indices of sympathetic and parasympathetic cardiovascular control as assessed by means of spectral analysis of cardiovascular variability.

5.2. METHOD

5.2.1. *Participants*

Ninety-seven 8- to 16-year-old children and adolescents, referred to the outpatient clinic of the department of Child and Adolescent Psychiatry of either the Erasmus Medical Center in Rotterdam (n = 66) or Leiden University Medical Center – Curium (n = 31) participated in the study (51 boys, 46 girls; mean age: 10.8 years, standard deviation: 2.2 years). All consecutive referrals for internalizing psychopathology to these departments were diagnosed with the Anxiety Disorders Interview Schedule for DSM-IV-Child Version (ADIS-C; Silverman et al., 2001). The interviews were conducted by trained graduated psychologists. All individuals who fulfilled the criteria for a primary diagnosis of one of the following anxiety disorders constituted the target sample: Generalized Anxiety Disorder (GAD; n = 32), Separation Anxiety Disorder (SAD; n = 24), Social Phobia (SOP; n = 24) or a Specific Phobia (SP; n = 17). Exclusion criteria were: current medication for an anxiety disorder, (co-morbid) Pervasive Developmental Disorder, Obsessive Compulsive Disorder, Post Traumatic Stress Disorder, substance abuse, an IQ score below 85 on the Wechsler Intelligence Scale for Children-Revised (WISC-III; Wechsler, 1991), and insufficient comprehension of the Dutch language. If a patient received any form of treatment for their anxiety during the previous 3 months (e.g. Cognitive Behavioral Therapy, CBT) he or she was not invited to participate either.

Three participants were diagnosed with a co-morbid depression, 8 with co-morbid dysthymia, and 55 participants had at least one other anxiety disorder (GAD, SAD, SOP, or SP). Ten participants had co-morbid ADHD. From the initial 103 children and adolescents who met the inclusion criteria, six refrained from participation.

The Medical Ethical Committees of the Erasmus MC in Rotterdam and the Leiden University Medical Center in Leiden approved the protocol. All parents and each adolescent provided written consent.

5.2.2. *Instruments*

The ADIS-C (Silverman et al., 2001) and the Multidimensional Anxiety Scale for Children (MASC; March et al., 1997) were administered routinely to all consecutive referrals, independently of inclusion in the study.

ADIS-C

The Anxiety Disorders Interview Schedule for DSM-IV (ADIS-C; Silverman et al., 2001; Siebelink and Treffers, 2001) is a semi-structured interview. The interview was conducted by a graduated psychologist with the child or adolescent and with the parents separately. It was specifically developed to assess anxiety and related disorders in 7- to 17-year olds. During the interviews with both the parent and the child, DSM-IV symptoms were judged by child and parent as either absent ('no') or present ('yes'). For both interviews, the number of reported symptoms was compared to the number of symptoms required for a DSM-IV diagnosis.

If the minimal requirements for a DSM-IV diagnosis were met, the parent or the child was asked to indicate on a 9-point scale (0–8) to what extent the symptoms interfered with the child's daily life. The reported impairment rating from interviews with the parent and/or the child had to be 4 or higher to allow diagnosis of a disorder. Finally, the interviewer had to confirm the diagnosis by indicating an interference score on the same 9-point scale, the Clinician Severity Rating (CSR). If the CSR was 4 or higher a diagnosis was assigned.

MASC

The Multidimensional Anxiety Scale for Children (March et al., 1997) is a 39-item self-report questionnaire for children and adolescents that assesses anxiety symptoms occurring during the past two weeks. All items are scored on a 4-point scale (0 = never true, 1 = rarely true, 2 = sometimes true, 3 = often true). A Total Anxiety score and scores on four scales can be obtained; Physical Symptoms, Harm Avoidance, Social Anxiety, and Separation Anxiety (March et al., 1997).

5.2.3. *Stress Tasks*

The Mental Arithmetic Task (MAT) is generally applied as a standardized laboratory stress task to induce measurable physiological changes (Jorgensen et al., 1990; Kirschbaum et al., 1993). The task lasts 4 minutes, during which a child is asked to subtract numbers as quickly and accurately as possible. If the child makes a mistake, the researcher says 'wrong, we start all over again', which is often experienced as very stressful. Dependent on the child's age, the child is asked to subtract 7 from 100 (< 12 years), or 23 from 1021 (≥ 12 years). If a child managed to reach zero within 4 minutes in the 100-minus-7 version, the researchers continued with the 1021-minus-23 version until the 4 minutes were completed.

The Social Competence Interview (SCI) was derived from the standardized interview developed by Ewart and Kolodner (1991). During this interview a topic which regularly causes stress for the participant is discussed in detail, based on the description of a recent experience during which the subject felt distressed or uncomfortable.

In the original interview the child or adolescent has to chose a subject from six areas (work, family, school, neighborhood, money, and friends) which frequently cause stress in urban adolescents (Ewart & Kolodner, 1991). Based on a pilot study (n = 8) in which all participants chose a topic which appeared to be independent from their anxiety disorder, which suggested avoidance, we decided to use the ADIS-C / DSM-IV anxiety diagnosis with the highest CSR as basis for the interview. In the first stage of the interview (4 minutes), the interviewer inquired to what extent the present anxiety disorder causes recurring distress in the participant. Based on the participants report, a specific and recent stressful experience is discussed in detail. Initially the focus is on general information like: where did it happen?, what happened?, who was present?, what did they say?, how did you respond? Based on this information the chronology of the event is reconstructed by the interviewer. Following this stage more emotional details of the event are explored using questions like: what did you think when your mother said that?, and how did you feel on that moment?, how did you respond when that happened? (8 minutes). In the next stage of the interview (4 minutes) the participant is asked to imagine that he or she is in the situation again and to re-experience step by step the reported emotions again. The instruction is given not to speak and tell about their experiences immediately, just to remember and re-experience the reported event and accompanying emotions again, so that the participant can tell more details afterwards. The participant is guided by the interviewer who recalls the stages of the earlier discussed event followed by the question 'How did you feel on that moment and what did you think then?'. During the final stage of the interview (4 minutes), the interviewer focuses on the competence of the child or adolescent to formulate alternative strategies to the strategy actually followed in the given situation. This stage was used to reduce the induced stress during the interview. The interview lasted approximately 20 minutes and all interviews were conducted by the same graduated psychologist. This psychologist practiced the interview repeatedly during an extensive pilot study. If parents and the participant consented, the complete procedure was taped. The tape was used to obtain regular feedback from the research team.

5.2.4. *Experimental test procedure*

When a child or adolescent met the inclusion criteria, he/she was invited for the study by the diagnostician. An appointment for the physiological assessment was made by phone and an information brochure regarding the research project was sent by mail.

All autonomic measurements were conducted between 2 p.m. and 6 p.m. Before the experimental test procedure started a short introduction was given and the Informed Consent was signed by the participants and his or her parents. Then the participant was brought to the test room where the physiological equipment was connected and the test procedure was explained to the child by a test assistant (being a medical student). After installation the experimental procedure started with a baseline period in which the participant was instructed to sit relaxed, not to speak, to move as little as possible and breathe regularly (10 minutes). Then the MAT was applied (4 minutes) which was followed by a second baseline period (10

minutes). After the second baseline period the SCI was conducted (20 minutes). The procedure ended with a third baseline period (10 minutes). The total procedure took 1.5 hours for each participant.

5.2.5. *Measurement of the physiological variables*
Skin Conductance Level (SCL), ECG, respiration, and BP were recorded continuously during the experiment. SCL was measured using two Ag/AgCl electrodes, one positioned on the middle phalanx of the ring finger and one on the middle phalanx of the index finger of the non-dominant hand. The analogue SCL signal was digitized at a sampling frequency of 8 Hz. The ECG was derived using a precordial lead and was sampled at 512 Hz. Blood pressure was assessed by means of a pulsing cuff around the middle phalanx of the middle finger of the non-dominant hand (Portapres, TNO, Amsterdam, The Netherlands). Blood pressure data was sampled at 512 Hz. The non-dominant hand was kept at heart level by a supportive arm-rest to optimize the correspondence with intra-brachial pressure changes (Parati et al., 1989). An elastic thoracic belt was put around the chest of the subject to monitor respiration (Respiratory effort Velcro® straps; Protech International Inc., San Antonio, USA). During inhalation the elastic belt stretches, and it shortens during exhalation to a proportional degree. The respiratory data was stored with a frequency of 8 Hz. All physiological data were recorded and stored using an ambulatory digital data recorder (Vitaport 2®, TEMEC, Kerkrade, The Netherlands). A test assistant was continuously present in the room during the protocol to monitor the physiological data acquisition on a laptop and to help and support the participant.

5.2.6. *Processing of the physiological variables*
After the experimental testing, the physiological data file was downloaded to a personal computer (2,4 GHz Intel pentium 4). Particular periods were selected for further analyses: minutes 7-9 (baseline period 1); the complete MAT of 4 minutes; minutes 7-9 after the MAT (baseline period 2); minutes 10-12 of the SCI for the following physiological variables: mean levels of HR, DBP, SBP, and SCL. Additionally, baseline period 1 and 2 (minutes 7-9) and minutes 13-15 of the SCI were analyzed for specific parameters of cardiovascular variability: Low Frequency band power of the SBP (LF SBP) and High Frequency band power of the HR (HF HR).
After visually checking the original signals in these periods for artifacts, R-R top detection was conducted on the ECG using custom made software (Van Steenis, Erasmus Medical Center Rotterdam, The Netherlands) to calculate interbeat intervals (IBI's) and mean HR levels with standard deviation in each period. The same software was used to define for each consecutive Inter-Beat-Interval (IBI) both DBP and SBP with an accuracy of 0.1 mmHg. For each time-series an average DBP and SBP with standard deviation could then be calculated. Mean SCL and standard deviation in each period were calculated using Vitagraph® software (TEMEC, Kerkrade, The Netherlands).

5.2.7. *Spectral analyses of the cardiovascular time-series*
Time-series of HR, respiration, and SBP of baseline 1, of baseline 2, and the 3 minutes of the re-experiencing period of the SCI were used for spectral analysis. These periods were selected because the participants did not speak and the breathing rhythm could be regarded as reasonable stationary. The HR and SBP time-series were processed using discrete Fourier transformations, based on non-equidistant sampling of the R-wave incidences (CARSPAN, Mulder & Schweizer, 1993; Van Steenis et al., 1994). For each selected period, the power was calculated for the low frequency band (0.07-0.14 Hz) of SBP and the high frequency band (0.15-0.50 Hz) of HR. Data of the LF SBP and the HF HR appeared to be not normally distributed. Consequently, the natural logarithms of these variables were computed for statistical analyses: the natural logarithm of LF SBP (LF SBP (ln)) was regarded as parameter of sympathetic activity, and the natural logarithm of HF HR (HF HR (ln)) as parameter of parasympathetic activity. Because breathing irregularities can seriously influence the frequency distribution of the power spectra (e.g. Monk et al, 2001), a respiration power spectrum was calculated as well. Only spectral data of subjects showing a predominant breathing frequency in the high frequency band (0.15-0.50 Hz) in the analyzed periods were used.

5.2.8. *Statistical analysis*
All physiological variables were tested for normality using the Kolmogorov-Smirnov test and log transformed if the distribution within the present sample was not normal (LF SBP and HF HR).
Differences were calculated between baseline 1 and the MAT for mean levels of SCL, HR, DBP and SBP (ΔMAT). Then, differences in mean levels of SCL, HR, SBP, and SBP were calculated between baseline 2 and minutes 10-12 of the SCI (immediately preceding the re-experiencing period) (ΔSCI). Additionally, difference scores were calculated for LF SBP (ln) and HF HR (ln) between baseline 2 levels and the levels during minutes 13-15 of the SCI (re-experiencing period) (ΔSCI2).
Paired sample t-tests were applied to investigate the difference between ΔMAT

and ΔSCI for HR, DBP, and SBP responses. However, because the assumption of normality was violated in ΔSCI for the SCL response, a Wilcoxon's signed Ranks test was applied to investigate differences in SCL response on the MAT and the SCI.

To investigate associations between psychophysiological measures and anxiety symptoms linear regression analyses were applied. First, MASC scale scores, gender and age were entered separately in a set of analyses, in which physiological functioning during baseline, ΔMAT, ΔSCI and ΔSCI2 were entered as dependent variables. Based on the results of these analyses, potential predictors were identified: each independent variable or covariate with a p-value < .10 was regarded as a possible predictor of physiological functioning and included in a forward stepwise regression model together with all the other candidate predictors for a specific physiological variable. Only predictors in significant models (p < .05) will be reported.

All statistical analyses were conducted using SPSS 11.0 (SPSS Inc., Chicago, USA).

5.3. RESULTS

Due to technical problems or unreliable data, the data regarding 94 children and adolescents were available for the SCL analyses, 87 for the HR analyses, and 76 for DBP and SBP. Due to the restriction of a predominant breathing frequency in the high power band (.15 - .50 Hz) the data of 64 children and adolescents were used for spectral analyses. MASC data were available for all 97 participants, see table 5.1. Physiological data are presented in table 5.2.

Age in years	10.8	(2.2)
MASC:		
Total Axiety	41.2	(16.9)
Physcial symptoms	8.1	(5.9)
Harm Avoidance	16.0	(5.2)
Social Phobia	8.8	(7.1)
Separation Anxiety	8.2	(4.7)

Table 5.1. Mean age and mean scores (standard deviations) on the MASC scales.

5.3.1. *Responses to the MAT and the SCI*

Difference scores between both baseline periods and the following tasks (MAT and SCI) are presented in table 5.3. For all the physiological parameters, the responses to the SCI were significantly lower than the responses to the MAT (see table 5.3.). Post hoc pair wise analyses (baseline 2 versus SCI, minutes 13–15) proved that LF SBP (ln) significantly decreased during the interview (t(60) = -3.65, p < .005). HF HR (ln) did not change significantly during the SCI (t(60) = - .60, n.s.).

	Baseline 1	MAT	Baseline 2	SCI, min. 10-12	SCI, min. 13-15
SCL (µS)	4.16 (3.57)	5.89 (4.30)	5.00 (3.90)	6.02 (4.43)	
HR (bpm)	81.00 (8.41)	84.93 (10.45)	80.77 (8.77)	82.50 (9.54)	
DBP (mmHg)	64.17 (18.51)	78.06 (20.57)	70.97 (19.97)	80.42 (22.79)	
SBP (mmHg)	106.89 (20.25)	125.32 (23.92)	115.82 (22.16)	128.69 (26.09)	
LF SBP (ln)	6.13 (.92)		6.27 (.97)		5.80 (1.03)
HF HR (ln)	7.79 (.82)		7.6 (.74)		7.65 (.76)

Table 5.2. Mean (standard deviations) for the physiological variables in each period for the children and adolescents with an anxiety disorder.

SCL, Skin Conductance Level; HR, Heart Rate; DBP, Diastolic Blood Pressure; SBP, Systolic Blood Pressure; LF SBP (ln), natural logarithm of the low frequency band power of SBP; HF HR (ln), natural logarithm of the high frequency band power of HR; MAT, Mental Arithmetic Task; SCI, Social Competence Interview.

5.3.2. *Associations between anxiety symptoms and physiological variables*
The beta's of possible predictors with a p-value smaller or equal to .10 resulting from the linear regression analyses are presented in table 5.4.

	ΔMAT	ΔSCI	test statistic	p-value
SCL (μS)	1.73 (1.81)	.99 (1.54)	Z = -5.07	< .0005
HR (bpm)	3.90 (7.46)	1.91 (5.41)	t = 2.78	= .007
DBP (mmHg)	13.90 (7.11)	9.42 (5.89)	t = 5.60	< .0005
SBP (mmHg)	18.67 (12.34)	13.17 (9.98)	t = 3.67	< .0005

Table 5.3. Differences in responses of SCL, HR, DBP and SBP to the MAT and the SCI.
SCL, Skin conductance Level; HR, Heart Rate; DBP, Diastolic Blood Pressure; SBP, Systolic Blood Pressure.
ΔMAT: Difference score between baseline 1 levels (minutes 7-9) and the Mental Arithmetic Task.
ΔSCI: Difference score between baseline 2 levels (minutes 7-9) and minutes 10-12 of the Social Competence Interview

Baseline: Combining the candidate predictors in one stepwise forward regression analysis revealed two significant models using anxiety scale scores to predict HF HR during baseline: 1. An increase in MASC Total Anxiety scores was associated with a decrease in HF HR: (standardized beta (β) = - .30), $F(1, 63) = 6.5$, $p < .05$, $R^2 = .09$.2. Higher MASC Social Phobia scores were associated with decreased HF HR: (β = - .40), $F(1, 63) = 12.7$, $p < .005$, $R^2 = .17$.
Additionally, age was associated with an increase in DBP (β = .26, $F(1, 76) = 5.5$, $p < .05$ $R^2 = .07$) and SBP (β = .24, $F(1, 76) = 4.8$, $p < .05$, $R^2 = .06$), though with a decrease in SCL (β = - .25, $F(1, 93) = 6.00$, $p < .05$, $R^2 = .06$) and HR (β = - .24, $F(1, 89) = 5.5$, $p < .05$ $R^2 = .06$). These age effects were independent of the presence of specific anxiety symptoms.
ΔMAT: Two significant models could be formulated with regard to ΔMAT:
1. Gender (β = - .22, girls responding less than boys) combined with the MASC Separation Anxiety scale score (β = .19) predicted the SCL response on the MAT: $F (2, 91) = 4.4$, $p < .05$, $R^2 = .09$.
The contribution of gender in the explained variance (R^2) was 4.9%, leaving 4.1% explained variance for the MASC Separation Anxiety scale score.
2. In the second model, the difference in DBP between baseline 1 and the MAT could be predicted by the combination of age (β = .30) and the MASC Harm Avoidance scale score (β = - .27), $F (2, 73) = 6.1$, $p < .005$, $R^2 = .15$. In this model the MASC Harm Avoidance scale score accounted for 7.4% of the explained variance and age for 7.2%.
ΔSCI: Combining the candidate predictors delivered two significant predictor models with SCL on ΔSCI:
1. Gender (β = - .35, indicating girls showing a lower response than boys) together with the MASC Total Anxiety scale score (β = .35), $F(2, 87) = 10.9$, $p < .0005$, $R^2 = .21$. Gender accounted for 8.8% of the explained variance, with the MASC Total Anxiety accounting for 11.7%.
2. Gender (β = - .31) and the MASC Separation Anxiety scale score (β = .36) predicted the SCL response to the SCI: $F(2, 87) = 11.8$, $p < .0005$, $R^2 = .22$. Gender accounted for 9.6% of the explained variance and the MASC Separation Anxiety scale score for 12.1%. Additionally, the MASC Social Phobia scale score (β = .24) predicted the DBP response to the SCI: $F(1, 71) = 4.2$, $p < .05$, $R^2 = .06$.
ΔSCI2: No significant predictors were found for ΔSCI2.

	Baseline							MAT				ΔSCI			ΔSCI2		
	SCL	HR	DBP	SBP	LF SBP (ln)	HF HR (ln)	SCL	HR	DBP	SBP	SCL	HR	DBP	SBP	LF SBP (ln)	HF HR (ln)
Gender	–	–	–	–	–	-.26 (.03)	-.22 (.03)	–	–	–	-.30 (.00)	–	–	–	–	–
Age	-.25 (.02)	-.24 (.02)	.26 (.02)	.24 (.03)	–	-.37 (.00)	–	–	.28 (.02)	.23 (.05)	–	–	–	.27 (.02)	–	–
MASC scales:																
Total Anxiety	–	-.19 (.08)	–	–	–	-.34 (.01)	–	–	–	–	.29 (.01)	–	–	–	–	–
Harm Avoidance	–	–	–	–	–	–	–	–	-.24 (.04)	–	.24 (.02)	–	–	–	–	–
Physical Symptoms	–	-.22 (.04)	.20 (.09)	.20 (.10)	-.22 (.09)	-.31 (.01)	–	–	–	–	.19 (.08)	–	–	–	–	–
Separation Anxiety	–	–	–	–	–	–	.19 (.07)	–	–	–	.35 (.00)	–	–	–	–	–
Social Phobia	–	-.21 (.05)	–	–	-.21 (.09)	-.46 (.00)	–	–	–	–	–	–	.24 (.05)	.23 (.06)	–	–

Table 5.4. Results of the separate regression analyses to predict physiological outcomes using MASC scale scores. Only the standardized Beta's (p-values) of each candidate predictor with p < .1 are presented.

SCL, Skin Conductance Level; HR, Heart Rate; DBP, Diastolic Blood Pressure; SBP, Systolic Blood Pressure; LF SBP (ln), natural logarithm of the low frequency band power of SBP; HF HR (ln), natural logarithm of the high frequency band power of HR.

ΔMAT: Difference score between baseline 1 levels (minutes 7-9) and the Mental Arithmetic Task.

ΔSCI: Difference score between baseline 2 levels (minutes 7-9) and minutes 10-12 of the Social Competence Interview.

ΔSCI2: Difference score between baseline 2 levels (minutes 7-9) and minutes 13-15 of the Social Competence Interview.

5.4. DISCUSSION

The aim of the present study was to investigate the associations between specific symptoms of anxiety in children and adolescents with an anxiety disorder, and physiological functioning, either during baseline and in response to stress. For this purpose, the reactivity to a cognitive (MAT) and a social stressor (SCI) was assessed in 97 children and adolescents with an anxiety disorder.

With regard to symptoms of anxiety, significant negative associations between generalized anxiety and social phobia with baseline HF HR were found. Additionally, significant associations with specific anxiety symptoms were found for the SCL and DBP responses to the MAT and the SCI: higher separation anxiety scale scores, especially in boys, resulted in an increased SCL response to the MAT. Harm avoidance was negatively associated with the DBP response to the MAT. Generalized anxiety and separation anxiety were associated with an increased SCL response to the SCI. Finally, a stronger DBP response to the SCI was associated with more, or more severe symptoms of social phobia.

Significant effects of age were found in the current sample. Baseline SCL, HR, and HF HR decreased with age, while BP increased. The BP responses to both stressors increased with age as well.

5.4.1. *Anxiety symptoms and physiological activity during baseline*

During baseline, two regression models including symptoms of anxiety could be formulated for HF HR. The models indicated a decrease in parasympathetic cardiac control with either increasing MASC Total Anxiety or MASC Social Phobia scale scores.

An association of anxiety with baseline HF HR in children and adolescents was reported earlier by Monk and colleagues (2001). Our finding corroborates this finding; it may be that symptoms of social phobia are primarily responsible for this association. If we compare the explained variances of both models, the predictive power of the MASC Social Phobia scale exceeds that of the MASC Total Anxiety scale. Apparently the other scales did not increase the predictive power already obtained with the subscale reflecting social phobia.

5.4.2. *Physiological reactivity*

It appeared that the responses to the MAT on all physiological variables significantly exceeded the responses to the SCI. Earlier results suggested that HR responses to the SCI were comparable to MAT responses, whereas BP responses exceeded the responses to the MAT in children and adolescents (Ewart & Kolodner,

1991; Ewart et al., 1998). According to Chen et al. (2002) a strong cardiovascular response to a nonsocial task like the MAT may be associated with strivings aimed at stopping (hostile) criticism and abuse. On the other hand, cardiovascular responses to the SCI have been associated with heightened mental vigilance (instead of active coping) (Ewart et al., 2004), the striving to gain someone's sympathetic support, affection or understanding (Chen et al., 2002), and a more agonistic (interpersonal) coping style, associated with decreased social competence when confronted with stress (Ewart & Jorgensen, 2004; Ewart et al., 2004). However, all these findings have been reported in non-clinical samples. The present findings, showing a significantly stronger cardiovascular response to the MAT, may indicate that a coping style aimed at avoiding or ending (presumed) hostility or criticism may be predominant in children and adolescents with an anxiety disorder. However, because we did not include instruments to assess coping styles in the present research, more research on the relation between coping styles and cardiovascular responses to stress in children and adolescents with an anxiety disorder seems relevant.

5.4.3. *Associations with symptoms of anxiety*

MAT: In response to the MAT, two regression models could be formulated: one for the SCL response, combining gender and the MASC Separation Anxiety scale score, the other combining age and the MASC Harm Avoidance scale score in predicting the DBP response. With an explained variance of 7.4%, especially the negative attribution of symptoms associated with harm avoidance in the DBP response may be considerable.

Negative relationships between the mean BP responses and an avoiding coping style have been reported earlier in response to the SCI (Ewart & Jorgensen, 2004). However, for DBP this association was not significant (Ewart & Jorgensen, 2004: p.82, fig. 3). In this study, we report for the first time significant negative associations of harm avoiding symptoms and the DBP response to the MAT. However, it is not easy to explain the nature of this relationship, and more research on this topic is appropriate.

Gerra et al. (2000) reported a heightened sympathetic reactivity in response to a stress inducing protocol including the MAT in adolescent males with a generalized anxiety disorder. Some adolescents in this study were diagnosed with a co-morbid separation anxiety disorder as well. In the present study a significant sympathetic response, mirrored by an increased SCL, was found which could be related to symptoms of separation anxiety. This effect was stronger for boys than for girls. The present finding suggests that the earlier found associations of anxiety with sympathetic reactivity in adolescent boys may be explained by the presence of symptoms related to separation anxiety.

SCI: In response to the SCI, anxiety symptoms mainly influenced the changes in SCL. Combined with gender two prediction models could be formulated, one with the MASC Total Anxiety scale score and the other with the MASC Separation Anxiety scale score. When comparing the explained variances of both models, we can conclude that symptoms of separation anxiety (like: 'I try to stay near my mom or dad') in the current sample more accurately predicted SCL responses to the social stressor.

The MASC Social Phobia scale score predicted changes in DBP response. Ewart and Kolodner (1991) and Ewart et al. (1998, 2004) reported that the SCI elicits BP changes that exceeded responses to nonsocial stressors (like the MAT). Although in the present sample the MAT induced significantly stronger physiological responses, the association of social phobic symptoms in particular with the DBP response to the SCI may be complementary to this finding: the influence of anxiety symptoms (primarily symptoms of social phobia) is most pronounced during the SCI, indicating that in the present sample social phobic symptoms may be associated with the striving to gain sympathetic support or affection from the interviewer and/or a heightened vigilance (Ewart & Jorgensen, 2004; Ewart et al., 2004)..

Additionally, only a marginally significant effect of symptoms of social phobia was found for SBP responsiveness to the SCI in the present sample (see table 5.4.). Thus, we can neither confirm nor contradict the findings of Dobkin et al. (2000). Nevertheless, the association of symptoms of social phobia with BP responses to the SCI was remarkable and strengthens the suggestion of a relationship between anxiety symptoms and/or coping style with blood pressure responses on a social stressor (Dobkin et al., 2000; Ewart et al., 1998; Ewart & Jorgensen, 2004).

Finally, no models could be formulated using anxiety symptoms to predict the response of LF SBP and HF HR to the SCI in children and adolescents with an anxiety disorder. Although no significant differences between baseline 2 HF HR and SCI HF HR could be found, significant differences were found for LF SBP between these two periods. These combined results suggested the absence of parasympathetic cardiac responsiveness combined with sympathetic reactivity in response to the SCI.

5.4.4. *Limitations*

In the present study, no control group was included without anxiety disorders. Because it was our aim to investigate associations with specific anxiety symptoms, and a wide variance in anxiety symptoms was thus desirable, the presence of a

control group was not necessary. Nevertheless, the homogeneity of the present sample should be taken into account when drawing conclusions regarding populations with less severe forms of anxiety.

Depression may be a factor influencing physiological responsiveness to stressors in children and adolescents as well (Boyce et al., 2001). However, childhood depression is rare and generally, if present, high co-morbidity rates exist with anxiety disorders (Axelson & Birmaher, 2001; Inderbitzen et al., 1995). In the present study all participants had an anxiety disorder as primary diagnosis. Only 3 participants were diagnosed with a co-morbid depression and 8 with co-morbid dysthymia. In addition, there is as yet no evidence that depressive symptoms may influence the association of anxiety and autonomic control in children and adolescents with an anxiety disorder. For these reasons depression scores were not included in the present study.

5.4.5. *Conclusions*

The present study is the first to assess physiological reactivity to a social and a nonsocial stressor in a large sample of children and adolescents with an anxiety disorder. Because state of the art diagnostic instruments have been used to classify this sample, we were able to investigate the relationships between specific anxiety symptoms and physiological responsiveness to stress, contrary to the more categorical approach used in other studies. Such an approach may be desirable because the discrimination between pathological domains like 'internalizing' and 'externalizing' behavior does not yield insight in anxiety specific processes and secondly, discriminating between anxiety disorders in childhood and adolescence may be disputable (Ferdinand et al., 2005; Monk et al., 2001).

The present findings confirm earlier studies suggesting that (symptoms of) anxiety may be associated with decreased parasympathetic cardiac control in children and adolescents during baselines preceding a stress invoking protocol. In children and adolescents with an anxiety disorder the physiological responses to the cognitive stressor (MAT) may exceed the responses to a social stressor, which may be related to a specific coping style aimed at ending critism. Additionally, specific symptoms of anxiety appeared to primarily influence the sympathetic responses to the social stressor. The association of symptoms of anxiety with primarily sympathetic activity to the social stressor was strengthened by the lack of significant associations with HF HR. Consequently, our finding contradicted the suggestion of anxiety related parasympathetic reactivity in response to stress (Boyce et al., 2001).

Based on the present findings, we conclude that children and adolescents with an anxiety disorder display diminished parasympathetic cardiac control during baseline conditions with increasing frequency or severity of anxiety symptoms. Additionally, in these children and adolescents specific symptoms of anxiety may primarily affect sympathetic reactivity to social stressors.

6.

Autonomic and HPA-axis functioning in relation to severity of anxiety symptoms in children with an anxiety disorder

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Summary. - Anxiety disorders in children and adolescents have been associated with alterations in Hypothalamus – Pituitary gland – Adrenal gland (HPA) axis functioning and disturbed functioning of the autonomic nervous system (ANS). Although these stress regulatory systems may be independently related to anxiety symptoms, given the nature of both systems involved, the aim of the present study was to investigate their combined effects in relation to childhood anxiety symptomatology.
Ninety-nine 7- to 16-year-olds diagnosed with an anxiety disorder, collected saliva cortisol samples during a regular school day. Additionally, ANS reactivity (heart rate and skin conductance level (SCL)) to a mental arithmetic stress task was assessed during a visit to the clinic. Anxiety symptoms during the previous two weeks were assessed using the Multidimensional Anxiety Scale for Children (MASC).
A significant interaction-effect of SCL reactivity to the stressor and saliva cortisol concentrations on MASC Total Anxiety scores was found. Specifically, the combination of high sympathetic reactivity and high cortisol concentrations over the day appeared to be related to anxiety symptoms during the previous two weeks. This confirmed our hypothesis that although independent relations for ANS and HPA-axis functioning with symptoms of severe anxiety in youth have been reported earlier, severe childhood anxiety may be specifically related to the combination of HPA-axis function and sympathetic stress responsiveness.

6.1. INTRODUCTION

Anxiety disorders in children and adolescents are common (Verhulst et al., 1997), are associated with impaired functioning and decreased quality of life (Bastiaansen et al., 2006), and tend to persist into adulthood (Keller et al., 1992; Ollendick & King, 1994). Research aimed at detecting physical correlates of anxiety disorders could help to identify the biological mechanisms that may be associated with their occurrence and persistence. Dysfunctions in the systems involved in regulating an individual's response to stressful events may make an individual vulnerable for high levels of anxiety (Gunnar & Vazquez, 2001; Garralda et al., 1991; Kagan & Snidman, 1999; Musante et al., 2000).
The two primary systems involved in stress regulation are the Autonomic Nervous System (ANS) and the Hypothalamus – Pituitary gland – Adrenal gland (HPA) axis. ANS reactivity to stress is the result of the activation or inhibition of the sympathetic and the parasympathetic nervous system, respectively. In response to stress the combined activity of these two ANS systems generally results in immediate heightening of Skin Conductance Level (SCL), and Heart Rate (HR), as part of the 'fight or flight' response (Cannon, 1932). After the need for a physiological state of emergency has passed, these ANS parameters generally decrease back to baseline levels within minutes. High ANS tonus and regular or severe activation of the ANS, e.g. by chronic stress, may have serious implications for future cardiovascular health (Allen et al., 1997; Dobkin et al., 2000; Jackson et al., 1999; Jemerin & Boyce, 1990; Kawachi et al., 1994; Monk et al., 2001; Treiber et al., 2001; Yeragani et al., 2001).
In response to stress the hypothalamus secretes Corticotrophin-Releasing-Factor (CRH), which stimulates the pituitary gland to produce Adreno-Corticotrophic Hormone (ACTH). ACTH is released into the bloodstream and stimulates cortisol production and release by the adrenal gland (Chrousos, 1997; Sapolsky et al., 2000). The involvement of multiple biochemical mechanisms in these endocrine organs causes a significant delay in HPA-axis responsiveness. It takes approximately 15 to 20 minutes to find significant increases in cortisol concentrations after a stressor has been presented. Short-lasting stressors can cause the activation of the ANS, but will not stimulate the HPA-axis sufficiently to induce a significant rise in cortisol concentrations, whereas more prolonged and social stressful events have been proven to increase cortisol concentrations significantly (Granger et al., 1994; Kirschbaum et al., 1993; Schommer et al., 2003). If such events happen regularly, it may cause down regulation of corticosteroid-sensitive receptors in the organs of the HPA-axis, eventually resulting in chronically decreased cortisol

concentrations (Chrousos, 1997; Sapolsky et al., 2000).
Consequently, remarkable differences exist between the functionality of these two systems involved in stress regulation. Whereas ANS reactivity can be recorded immediately, responsiveness of the HPA-axis can only be monitored with some delay and under more specific circumstances, like public speaking (e.g. Schommer et al., 2003). On the other hand, ANS reactivity is restricted to specific organs, whereas changes in cortisol concentrations can affect all kinds of bodily processes, like immunity, metabolism, growth, reproduction and other important physiological functions (Chrousos, 1997; De Kloet, 2003; Sapolsky et al., 2000).
In relation to ANS functioning, anxiety disorders in children have been associated with diminished baseline parasympathetic activity (Kallen et al., 2006; Monk et al., 2001), with increased overall sympathetic activity (Yeragani et al., 2001), and with increased sympathetic reactivity to a stressor (Gerra et al., 2000; Kallen et al., 2006; Rogeness et al., 1990). Both Kallen et al. (2006) and Monk et al. (2001) reported an anxiety related significant decrease in high frequency band power of the heart rate (HF HR), a parameter of parasympathetic cardiac control (Akselrod et al., 1981; Akselrod et al., 1985; Houtveen et al., 2002), during the baseline period before a standardized stressor. Anxiety disorders (Gerra et al., 2000) or specific anxiety symptoms (Kallen et al., 2006) have been associated with increased sympathetic reactivity to diverse tasks (e.g. mental arithmetic and public speaking) which is generally mirrored in significant increases in Skin Conductance Levels (SCL: Kallen et al., 2006; Van Lang et al., 2006; Wallin, 1981) or plasma noradrenaline levels (Gerra et al., 2000).
With respect to HPA-axis functioning, Granger et al (1994) reported enhanced increases in cortisol concentrations after a parent-child conflict task related to social anxiety. Feder et al. (2004) found lowered nighttime and early morning cortisol levels in children with an anxiety disorder compared to children with a depressive disorder and healthy controls. Kallen et al. (2007) found that symptoms of generalized anxiety were associated with elevated saliva cortisol levels at noon in children and adolescents with an anxiety disorder. Together these studies suggest that high anxiety levels in children and adolecents may be associated with low nighttime and higher daytime HPA-axis activity.
There is some evidence that the activity of the HPA-axis and that of the ANS interact (Morilak et al., 2005; Nater et al., 2005; Nyklocek et al., 2005). Both systems may be triggered by the central release of the neurotransmitter noradrenaline from the nucleus coeruleus which rapidly spreads through the spinal cord and to diverse brain regions, or by CRH, being released from the hypothalamus in response to stress and stimulating both the pituitary gland to release ACTH and the locus coeruleus to produce noradrenaline (Heim & Nemerhoff, 1999, 2001). Additionally, glucocorticoids have been suggested to peripherally restore homeostasis and provide feedback to the central nervous system to control both the intensity and the duration of the stress responses, not only in the HPA-axis itself, but also in other systems, like the forebrain, limbic system and the ANS (Boyle et al., 2006). Somewhat contrary to this finding, Schommer et al. (2003) reported habituation of the HPA-axis response to repeated psychosocial stressors. For the parameters of ANS reactivity habituation over time was not found, indicating a dissociation between the habitual abilities of the two systems in response to repetitive psychosocial stress. Additional to the argument of peripheral homeostasis (Boyle et al., 2006) continuing high ANS activity, especially when it is sympathetic in nature, demands extra energetic resources which may be provided by increased glucose metabolism stimulated by cortisol (De Kloet, 2003; Sapolsky et al., 2000). This may be mirrored by the remarkable difference in time course after either system is stimulated (typically by a stressor).
Interactions between changes in HPA-axis functioning over time and ANS responses to stressors seem to be particularly relevant in relation to anxiety disorders. Sanchez et al. (2005) reported flattened circadian concentrations of cortisol over time and elevated acoustic startle reactivity in relation to behaviours associated to mood and /or anxiety disorders (like screaming and vocalizations) in macaques. According to Sher (2005) cortisol may even be a mediator in the association between anxious personality (type D personality) and cardiovascular disease.
Gerra et al. (2000) investigated the baseline levels and responsiveness of both systems to diverse stressors in 20 boys (average age 12 years) with an anxiety disorder compared to 20 age matched healthy male volunteers. Baseline ACTH levels were significantly higher in the anxious boys, but in response to diverse stressors only the sympathetic responses appeared to be significantly stronger in the boys with an anxiety disorder, whereas no differences were found in HPA-axis responsivity.
If dysfunctions in the circadian rhythm of the HPA-axis combined with the tendency towards hyper-arousal of the sympathetic branch of the ANS in response to stressors is indeed associated with high anxiety in children and adolescents, this should mean that children and adolescents with simultaneous dysfunctions or hypersensitivity in both systems would be particularly at risk for the negative consequences of chronic stress, and the development of severe anxiety. However, despite the possibility of an interaction between the functioning of both systems in relation to the development of high anxiety in childhood and/or adolescence, studies that addressed this interaction in clinical

samples of high anxious youth have not been conducted thus far.
The aim of this study was to investigate if an interaction between the functioning of the HPA-axis and the ANS was associated with the high anxiety levels of children and adolescents with an anxiety disorder. More specifically, we hypothesized that particularly hyperarousal of the sympathetic branch of the ANS in response to a standardized stress task, in combination with structurally heightened cortisol concentrations, was a predictor of anxiety levels in these children and adolescents.

6.2. METHOD

6.2.1. *Participants*

The sample consisted of 99 7- to 16-year-old children and adolescents who 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 (53 boys, 46 girls; mean age: 10.8 years, standard deviation: 2.2 years). All consecutive referrals for internalizing psychopathology to these departments were diagnosed with the Anxiety Disorders Interview Schedule for DSM-IV-Child Version (ADIS-C; Silverman et al., 2001). The interviews were conducted by trained psychologists. Invited to participate in the study were all individuals assigned with a primary diagnosis of Generalized Anxiety Disorder (GAD; n = 33), Separation Anxiety Disorder (SAD; n = 24), Social Phobia (SOP; n = 24) or a Specific Phobia (SP; n = 18), who were not currently, or during the past 6 months, under treatment for their disorder. Exclusion criteria were: current medication for an anxiety disorder, co-morbid Pervasive Developmental Disorder, Obsessive Compulsive Disorder, Post Traumatic Stress Disorder, substance abuse, an IQ score below 85, and insufficient comprehension of the Dutch language.
The Medical Ethical Committees of the Erasmus MC in Rotterdam and the Leiden University Medical Center in Leiden approved the protocol. All parents and each adolescent provided written consent.

6.2.2. *Instruments*

The ADIS-C (Silverman et al., 2001) and the Multidimensional Anxiety Scale for Children (MASC; March et al., 1997) were administered routinely to all consecutive referred patients independently of inclusion in the study.
ADIS-C: The Dutch version of the Anxiety Disorders Interview Schedule for DSM-IV (ADIS-C; Silverman et al., 2001; Siebelink and Treffers, 2001), a semi-structured interview, was used to establish the anxiety diagnosis. The interview was conducted with the child or adolescent and with the parents separately. It was specifically developed to assess anxiety and related disorders in 7- to 17-year olds. During the interviews with both the parent and the child, DSM-IV symptoms were judged by child and parent as either absent ('no') or present ('yes'). For both interviews, the number of reported symptoms was compared to the number of symptoms required for a DSM-IV diagnosis. If the minimal requirements for a DSM-IV diagnosis were met, the parent or the child was asked to indicate on a 9-point scale (0–8) to what extent the symptoms interfered with the child's daily life. The reported impairment rating from interviews with the parent and/or the child had to be 4 or higher to allow diagnosis of a disorder. Finally, the interviewer had to confirm the diagnosis by indicating an interference score on the same 9-point scale, the Clinician Severity Rating (CSR). If the CSR was 4 or higher, a diagnosis was assigned.
MASC: The Dutch version of the Multidimensional Anxiety Scale for Children (March et al., 1997; Utens & Ferdinand, 2000) is a 39-item self-report questionnaire that assesses anxiety symptoms in children and adolescents that occurred during the past two weeks. All items are scored on a 4-point scale (0 = never true, 1 = rarely true, 2 = sometimes true, 3 = often true). An overall score can be calculated representing Generalized Anxiety (March et al., 1997). In this study, we used the MASC Total score as indicator of anxiety severity.

6.2.3. *Procedure*

If a child or adolescent fulfilled the inclusion criteria he/she was asked to participate in the study. After informed consent from parents and patients, the participants were sent four plastic tubes and instructions for saliva collection via mail. The physiological assessment was scheduled by phone by a member of the research team, generally one week after the instructions were mailed. The actual physiological assessment was always conducted between 2 p.m. and 4 p.m., and combined with a regular visit to the clinic.

6.2.4. *Cortisol data collection*

Participants were instructed to collect four saliva samples on a regular school day by putting the tube against their underlip and have some saliva flow into it: (1) immediately after awakening in the morning, when the child was still in bed; (2) 30 minutes later; (3) at 12.00 a.m.; and (4) at 8 p.m. Time of awakening had to be reported in writing on the label of the first saliva tube immediately after sampling.

An extra instruction for the children stressed that they should refrain from dairy products one hour and all other food thirty minutes before sampling. All samples were stored in the refrigerator at home, and taken to the clinic one day later. Participants were instructed to fill the tubes up to a marker (at 500 µL).

6.2.5. *Stress task and autonomic measurements*

One day after cortisol sampling, the participants were assessed at the clinic. In the laboratory, a test-assistant connected the physiological equipment and explained the test procedure to the child. During the complete assessment the test-assistant stayed in the room to supervise the physiological data acquisition and to help and support the participant. During a baseline period of 10 minutes the participant was instructed to sit relaxed, not to speak, to move as little as possible and breathe regularly. Then the test-leader entered the room and the Mental Arithmetic Task (MAT) was conducted. The MAT is generally applied as a standardized laboratory stress task to induce physiological changes (Jorgensen et al., 1990; Kirschbaum et al., 1993). The task lasts 4 minutes, during which a child is asked to verbally subtract numbers as quickly and accurately as possible. If the child makes a mistake, the researcher says 'wrong, we start all over again', and the participant has to start from the beginning. Dependent on the child's age, the child is asked to subtract 7 from 100 (< 12 years), or 23 from 1021 (≥ 12 years). If a child managed to reach zero within 4 minutes in the 100-minus-7 version, the test-leader continued with the 1021-minus-23 version. After the test, the test-leader indicated it was the end of the task and left the room The MAT was followed by a recovery period of 10 minutes, during which the participant was instructed to sit as relaxed as possible and breathe regularly.

During the baseline period, the MAT, ad the recovery period, Skin Conductance Level (SCL), electrocardiogram (ECG), and respiration were recorded continuously. SCL was measured using two Ag/AgCl electrodes, one positioned on the middle phalanx of the ring finger and one on the middle phalanx of the index finger of the non-dominant hand. The analogue SCL signal was digitized at a sampling frequency of 8 Hz. The ECG was derived using a precordial lead and was sampled at 512 Hz. An elastic thoracic belt was put around the chest of the subject to monitor respiration (Respiratory effort Velcro® straps; Protech International Inc., San Antonio, USA). During inhalation the elastic belt stretches, and it shortens during exhalation to a proportional degree. The respiratory data were stored with a frequency of 8 Hz. All physiological data were recorded and stored using an ambulatory digital data recorder (Vitaport 2®, TEMEC, Kerkrade, The Netherlands).

6.2.6. *Processing of the physiological variables*

After the test procedure, the physiological data-file was downloaded to a personal computer. After visually checking the original signals for artifacts, R-R top detection was conducted on the ECG using custom made software (Van Steenis, Erasmus Medical Center Rotterdam, The Netherlands) to calculate mean HR levels for minutes 7-9 of the baseline period, the entire MAT period and minutes 3-5 of the relaxation period after the task, as period for immediate recovery after the stressor. For each of these three periods mean SCL was calculated as well, using Vitagraph® software (TEMEC, Kerkrade, The Netherlands).

Following these procedures, spectral analyses were used to calculate the High Frequency power of the HR (HF HR) during minutes 7-9 of the baseline period and minutes 3-5 of the recovery period after the MAT. The HR time-series were processed using discrete Fourier transformations, based on non-equidistant sampling of the R-wave incidences (CARSPAN, Mulder and Schweizer, 1993; Van Steenis et al., 1994). For the baseline period (minutes 7-9) and the recovery period (mutes 3-5), the power was calculated for the high frequency (.15 - .50 Hz) band of HR. Because breathing irregularities can seriously influence the frequency distribution of the power spectra (e.g. Monk et al, 2001), a respiration power spectrum was calculated as well. Spectral data were used only of those individuals whose respiration rate was predominant in the high frequency band (.15 - .50 Hz). Given the fact that the children and adolescents had to speak during the MAT, causing irregular respiration patterns, HF HR was not calculated for this period.

SCL was used as an index of sympathetic activity (Jorgensen & Zachariae, 2006; Wallin, 1981) whereas HF HR can be considered as an index of parasympathetic functioning (Finley et al., 1987; Jorgensen and Zachariae, 2006; Akselrod et al., 1981; Akselrod et al., 1985).

6.2.7. *Cortisol assessment*

At the clinic, saliva samples were stored at -20 °C, and transported afterwards to the laboratory for storage (at -20 °C) and analysis. Cortisol concentrations were determined in duplicate 200 µL samples by solid-phase radioimmunoassay with iodinated cortisol that competes with cortisol for antibody sites (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, USA). The lower limit of detection was .1 nmol/l, the intra-assay variation was 4.4% and the inter-assay variation was 5.2%.

On average, 9.2% of the samples was missing due to insufficient saliva or lack of reliability due to significant intra-assay differences. These intra-assay differences may be explained by violation of the protocol, for instance eating or tooth

brushing, which may have polluted the samples with low concentrations of blood or food.
Cortisol concentrations in saliva show a strong circadian rhythm, with a significant rise of concentrations immediately after awakening and a gradual decrease over the day. A frequently used parameter of HPA-axis functioning is the Area Under the Curve calculated using multiple standardized sampling points over the day (AUC; Preusner et al., 2003; Rosmalen et al., 2005). The AUC was calculated, which in our study was an index of total cortisol secretion from waking up (first sample collected) until 8 p.m. (last sample collected).

6.2.8. *Statistical analyses*
For baseline and recovery periods, mean HF HR was not distributed normally. Thus, the natural logarithm of HF HR (HF HR(ln)) was used in statistical analyses.
To investigate how physiological parameters responded to the MAT, difference scores were calculated for SCL and HR during baseline (minutes 7-9) versus MAT (ΔSCL1 and ΔHR1). To assess recovery of the physiological parameters after the MAT, difference scores were calculated by subtracting SCL and HR levels during the recovery period (minutes 3-5) from the SCL and HR levels during the MAT (ΔSCL2 and ΔHR2). Strong recovery can thus be associated with a high positive value.
To assess associations between basal HPA-axis activity and physiological parameters irrespective of anxiety scores, Pearson correlation coefficients were calculated between the AUC and the physiological parameters (SCL, HR, and HF HR(ln)) during baseline and recovery. Further correlations were computed between AUC and the responses of SCL and HR to the MAT: ΔSCL1; ΔHR1; ΔSCL2; and ΔHR2.
To investigate the predictive power of HPA-axis functioning on anxiety levels, a regression analysis was conducted with AUC, gender, and age as independent variables and the MASC Total score as dependent variable. The physiological variables were entered simultaneously in three separate backward regression analyses. One including baseline SCL, HR, and HF HR(ln), the second including SCL and HR during the mental arithmetic task, and the third for SCL, HR, and HF HR(ln) during the recovery period. Gender and age were included as independent variables in these three analyses as well. The physiological responses (ΔSCL1 and ΔHR1) to the mental arithmetic and the physiological recovery after the task (ΔSCL2 and ΔHR2) were included in two separate backward regression analyses, gender and age were included as covariates in both analyses.

Because the aim of the present study was to investigate whether interactions between autonomic stress reactivity and HPA-axis functioning are related to severe anxiety in children and adolescents, firstly interaction terms were calculated between each physiological variable (HR, SCL, and HF HR (ln)) during baseline and the AUC. These interaction terms were used as independent variables for multiple backward regression analyses, with the main effects implemented as well, with gender as factor and age as covariate, and MASC Total score as dependent variable.
Secondly, interaction terms were calculated between the stress-reactivity and stress-recovery of HR, SCL and the AUC. These terms were implemented as independent variables in backward regression analyses, including the main effects as well, with gender as factor and age as covariates, and MASC Total score as dependent variable.
Finally, we calculated the interaction term between the AUC and HF HR (ln) during the recovery period after the MAT. To shed more light on possible parasympathetic correlations this term was used in a backward regression analysis, together with AUC and HF HR (ln) in this period (as main effects), gender as factor and age as covariates, and MASC Total score as dependent variable.

6.3. RESULTS

Of all participating children (n = 99), the MASC data were present (mean = 41.9, SD = 16.9) and of 84 participants an AUC could be calculated (mean = 83.9, SD = 33.9). Due to technical problems or unreliable data, the data regarding 94 children were available for the SCL analyses, 90 for the HR analyses. Due to the restriction of a predominant breathing frequency in the high power band (.15 - .50 Hz) the data of 60 participants were used for spectral analyses of the baseline and the recovery period (table 6.1.).
No significant correlations were found between any of the physiological variables (HR, SCL, and HF HR (ln)) during baseline, recovery, or in response to the MAT and the AUC of the preceding day.

	Mean	Standard deviation
Baseline:		
Skin Conductance Level (µS)	4.2	3.6
Heart Rate (bpm)	81.0	8.4
HF HR (ln)	7.8	.8
Mental Arithmetic Task:		
Skin Conductance Level (µS)	5.9	4.3
Heart Rate (bpm)	85.0	10.5
Recovery:		
Skin Conductance Level (µS)	5.2	3.9
Heart Rate (bpm)	79.7	10.0
HF HR (ln)	7.6	.7

Table 6.1. Mean and SD of the physiological variables during baseline, MAT and recovery in children with an anxiety disorder.
Note: µS = micro-siemens; bpm = beats per minute; mmHg = millimeter mercury; HF HR (ln), natural logarithm of the high frequency band power of HR.

6.3.1. *Effects of HPA-axis functioning and Physiological variables on Anxiety scores*

The regression analysis including AUC, gender and age, resulted only in a significant effect for age on MASC Total scores: $F(1, 81) = 10.60$, $p < .005$, indicating higher MASC scores with increasing age ($r = .31$, $p < .005$).
The regression analyses with the physiological parameters (SCL, HR, and HF HR(ln)) during baseline, gender and age, provided a significant model ($F(1, 55) = 3.95$, $p = .05$, $R^2 = .07$) including HF HR(ln) (standardized beta (ß) = - . 26, $p = .05$), indicating a negative correlation between baseline high frequency power of the heart rate and MASC Total scores. No significant effects were found for any physiological parameter during the mental arithmetic task (SCL, HR) or the recovery period (SCL, HR, HF HR(ln)). The response in skin conductance level (ΔSCL1) or heart rate (ΔHR1) to the mental arithmetic did not significantly predict the MASC Total scores. However, the regression analysis with recovery after the mental arithmetic task (ΔSCL2 and ΔHR2) delivered a significant model ($F(2, 84) = 7.69$, $p < .005$, $R^2 = .16$) including age (ß = .36, $p < .005$) and ΔSCL2 (ß = .20, $p < .05$), indicating higher MASC Total scores to be associated with a stronger SCL recovery, being mirrored by a high positive difference score between SCL during the MAT and during the recovery period.

6.3.2. *Combined effects of ANS reactivity and HPA-axis functioning on Anxiety scores*

No significant prediction model could be found including either the interaction effect of AUC with heart rate response (ΔHR1) or recovery to the MAT (ΔHR2). However, a significant prediction model was found for MASC Total scores, based on the interaction effect of the AUC and skin conductance response to the mental arithmetic task: $F(4, 78) = 7.37$, $p < .0005$, $R^2 = .29$. The interaction of AUC and ΔSCL1 (β = 1.08, $p = .002$), ΔSCL1 (β = - .82, $p = .01$), AUC (β = - .25, $p = .06$) and age (β = .38, $p < .0005$) were incorporated in the model, see figure 6.1.
Especially, the combination of higher basal cortisol concentrations and stronger sympathetic reactivity to the mental arithmetic task, seemed to be related to higher anxiety scores (figure 6.1.).

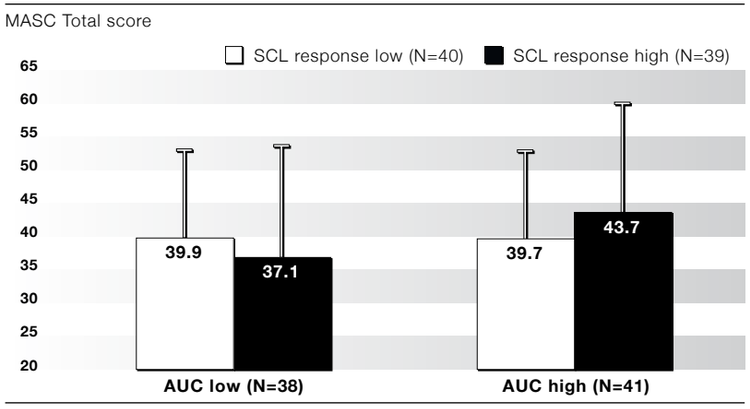


Figure 6.1. Mean MASC Total scores with standard deviations for high versus low Skin Conductance Level (SCL) responses on the Mental Arithmetic Task and high versus low cortisol concentrations over the day (AUC).
Note: SCL = Skin Conductance Level. Groups (high / low) are based on the 50th percentile score.
AUC = Area Under the Curve: measure of cortisol concentrations over the day. Groups (high / low) are based on the 50th percentile score.

The interaction of ΔSCL2 (recovery after the MAT) and AUC delivered a significant model as well: $F(4, 78) = 10.89$, $p < .0005$, $R^2 = .37$. Next to the interaction term (ß = 1.44, $p < .0005$), ΔSCL2 (ß = -1.14, $p = .001$), AUC (ß = - .19, $p = .08$) and age (ß = .42, $p < .0005$) were included in the model. Although again not significant ($F(1, 79) = .01$ for ΔSCL2 high versus low; $F(1, 79) = .54$ for AUC high versus low), a higher AUC combined with a stronger SCL recovery appeared to be related to higher anxiety scores (figure 6.2.). However, additional to this finding and although again not significant, low AUC with only a moderate SCL recovery seemed to be related to low anxiety scores (figure 6.2.).
No significant prediction model for MASC Total scores could be formulated using the interaction of AUC and HF HR(ln) in the recovery period ($F(5, 47) = 1.79$, n.s.) other than including age ($F(1, 47) = 9.33$, $p < .005$), again indicating increasing MASC Total scores with age.

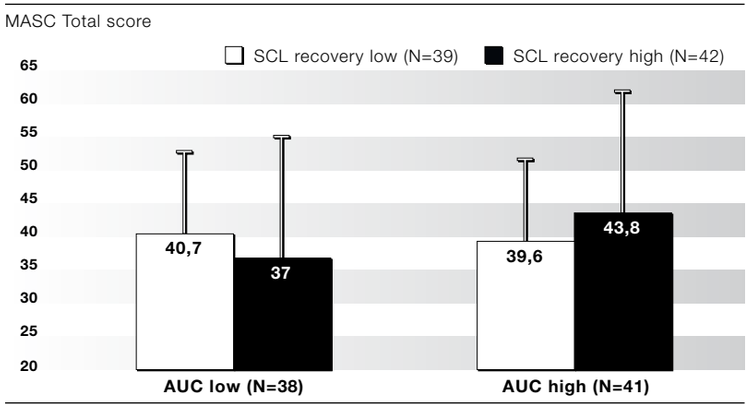


Figure 6.2. Mean MASC Total scores with standard deviations for high versus low Skin Conductance Level (SCL) recovery after the Mental Arithmetic Task and high versus low cortisol concentrations over the day (AUC).
Note: SCL = Skin Conductance Level. Groups (high / low) are based on the 50th percentile score.
AUC = Area Under the Curve: measure of cortisol concentrations over the day. Groups (high / low) are based on the 50th percentile score.

6.4. GENERAL DISCUSSION

It was our intention to find out whether the combination of reactivity of the ANS to a stressor and basal HPA-axis functioning was associated with severe anxiety scores in children and adolescents. For this purpose, we investigated ANS reactivity to a standardized stressor, cortisol concentrations over the day and their interaction in relation to self-reported symptoms of anxiety in children and adolescents with an anxiety disorder.
Within the present sample of children and adolescents with an anxiety disorder no significant correlations were found between cortisol concentrations over the day and either sympathetic or parasympathetic variables, neither during baseline conditions, nor during experimentally induced stress. This suggests that there is no direct relationship in our sample between basal HPA-axis functioning, which can be considered a trait condition, and ANS activity in relation to a stressor, a state condition.
Of the physical variables (HPA-axis functioning and physiological parameters) only reduced parasympathetic tonus was related to anxiety levels in the present sample. This was mirrored by a negative relationship between HF HR(ln) levels during baseline and MASC Total scores. Although this finding does confirm earlier findings (Boyce et al., 2001; Monk et al., 2001) it should be considered with some precautions. Due to methodological reasons (breathing frequency disrupting the reliability of the HF HR) the data of only 60 out of 99 participants (approximately 61%) could be used for analyses. Additionally, the found explained variance was only moderate ($R^2 = .07$). Finally, age was an important factor for all findings of the present sample. Childhood and adolescence are periods of extensive development, both physically and psychologically (Gunnar and Vazquez, 2001). This may not be surprising, but should be taken into account when interpreting the current findings. Both physiological systems associated with stress regulation as well as the anxiety related cognitions may be developing rapidly through childhood and adolescence. The way disturbances in the development of one domain may influence the development in the other is yet unknown. However, based on the present results we must conclude that with age the severity of the anxiety symptomatology in this sample of already high scoring individuals, increased further. This seems to be related to the parallel physiological development in both the HPA-axis and the autonomic nervous system. It underlines that, when interpreting results of studies like the present one, developmental factors should always be taken into account. Consequently, other studies (like longitudinal ones) are important to find out what the developmental risks of the presently described

factors in relation to childhood anxiety and general physical health may be.
This is expressed by the highly significant interaction effect of sympathetic reactivity to the stressor and basal HPA-axis functioning on self reported anxiety scores. This finding of a combined effect seems to be in accordance with the notion that sympathetic hyperactivity can generally be associated with increased HPA-axis functioning (Lovallo et al., 1990; Nater at al., 2005; Nyklicek et al., 2005). Additionally, the combination of higher baseline HPA-axis functioning and autonomic responsiveness to a stressor has been reported earlier in relation to childhood anxiety disorders (Gerra et al., 2000). In the present sample, an increased sympathetic response to stress may be experienced regularly, due to the presence of an anxiety disorder. This may have caused the HPA-axis to adjust itself to the repeatedly occurring demands of the sympathetic stress response, especially when frequent or severe anxiety symptoms are experienced over a period of several weeks, as assessed with the MASC.
A significant interaction effect of the skin conductance decrease after the mental arithmetic task and the area under the curve of cortisol concentrations on MASC Total scores was found as well. Like the initial sympathetic response to the mental arithmetic task, strong sympathetic recovery, combined with high cortisol concentrations over the day may be predictive of high anxiety scores. However, our results are not conclusive and this may be something to investigate in future studies.
Given the explained variances of the significant models relating strong sympathetic recovery to high anxiety scores: $R^2 = .16$ for the model with only the skin conductance recovery versus $R^2 = .37$ for the model including the interaction term, this last model may best account for differences in anxiety scores within the current sample. We conclude that this effect underlines and strengthens the effects discussed above in relation to the stress response.
In conclusion: the interaction of sympathetic response and recovery to a stressor (together constituting sympathetic reactivity) and basal HPA-axis functioning appears to be a stronger predictor of recent experienced symptoms of severe childhood anxiety than anxiety related phenomena in each system independently. Consequently, it appears that indeed, as hypothesized, specifically sympathetic-HPA-axis interactions are related to self-reported anxiety symptoms of children and adolescents in the severe anxiety spectrum.

6.4.1. *Limitations*

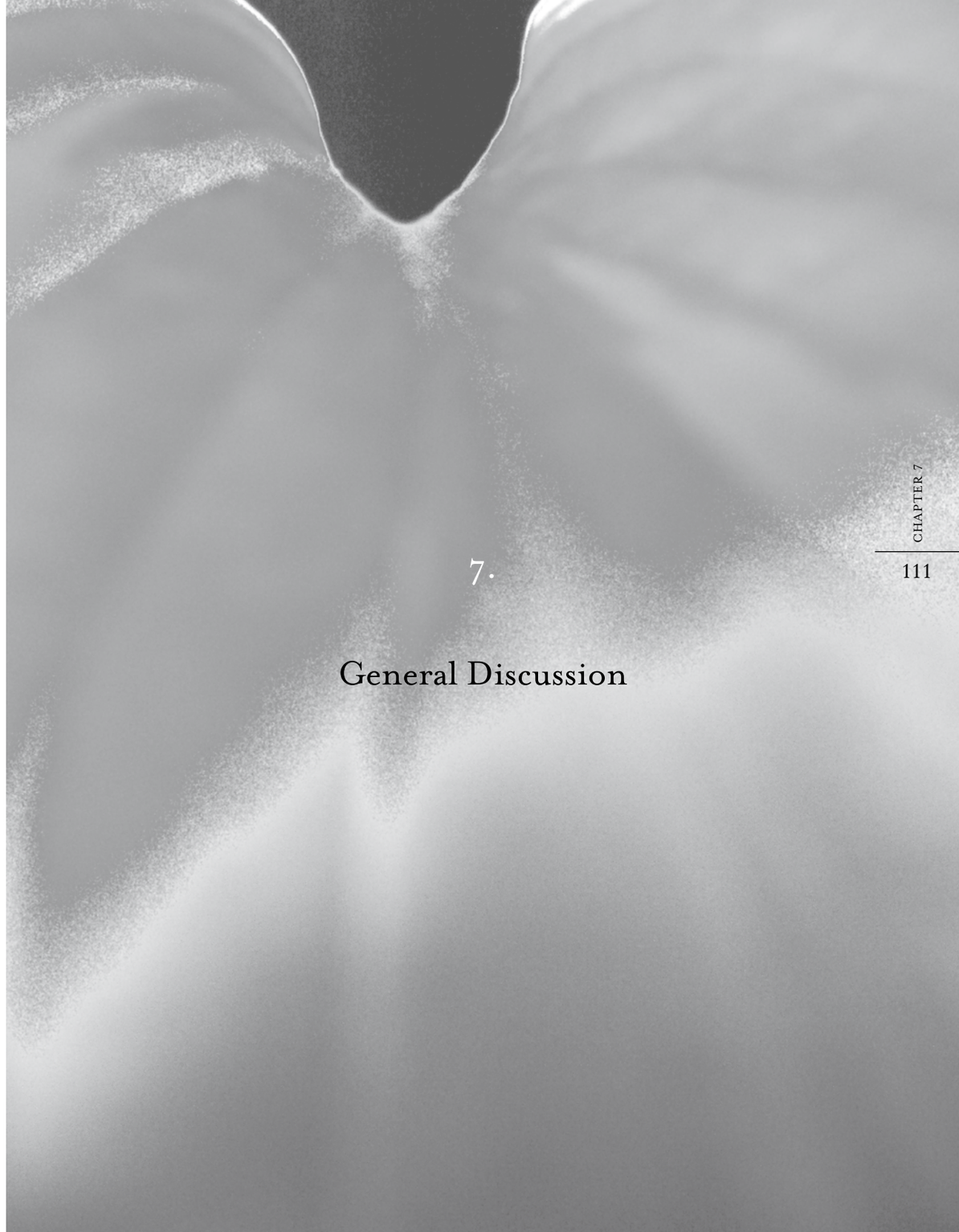
Some restrictions have to be taken into account when evaluating our results.
Firstly, we did not include parasympathetic reactivity to the mental arithmetic task. For methodological reasons it is not easy to assess parasympathetic responses, for example to stressors. Irregularities in breathing caused by talking during the MAT, undermine the reliability of conclusions based on spectral analyses of the HR in this period (e.g. Monk et al., 2001). For this reason no HF HR during the task was calculated in the present study. Additionally, in relation to the analyses of the baseline period we tried to increase the reliability of the parasympathetic parameter by excluding any observation potentially disturbed by breathing irregularities.
Secondly, this study only included children and adolescents with an anxiety disorder. Consequently, the question whether the present findings are specific for children and adolescents with an anxiety disorder or may be generalized to youths in the general population still remains. This may be relevant to investigate in the future.
Finally, significant results have been found in relation to sympathetic responsiveness using the MAT, both in the present research and in earlier studies (e.g. Gerra et al., 2000). However, given the moderate responses found in the present study the use of a MAT as a stressor to induce autonomic responses might be reconsidered when assessing children and adolescents with an anxiety disorder in the future. Maybe other stress tasks are more suitable to induce autonomic responses in high anxious samples.

6.4.2. *Clinical implications*

The present study's findings may be of clinical relevance. As such, it is the first study in which evidence is reported for a combined effect of basal levels of HPA-axis functioning and (sympathetic) physiological responsiveness in relation to a psychopathological phenomenon, being experienced anxiety symptoms. Considering the aim of the MASC to assess anxiety symptoms during the previous weeks and given the predictive power of sympathetic reactivity combined with high cortisol concentrations on MASC Total scores we may conclude that, at least in children and adolescents with an anxiety disorder, disturbances in one system may influence the functioning of the other, mediated by the presence of anxiety symptoms or anxious feelings. If recent experiences of anxiety are rare, the coordinated effects of both systems seem diminished, if more anxiety symptoms are reported both systems seem to get more mutually supportive.
The present findings may constitute a rationale for relaxation exercises in interventions aimed at childhood anxiety, like cognitive behavioral therapy. Tackling the initial sympathetic response to anxiety disorder related stressors, may eventually prove to be effective in the reduction of other stress related phenomena as well.

6.4.3. *Conclusions*

Specific information regarding a single biological stress regulatory system may at best only cover a modest percentage of explained variance in relation to severe childhood anxiety. The combination of information from multiple systems related to stress regulation appears to be a fairly good indicator of recently experienced symptoms of anxiety in children and adolescents with an anxiety disorder. Consequently, the hypothesis, that the combination of basal HPA-axis functioning with the sympathetic response to stress might be a better predictor of recently experienced symptoms of anxiety in high anxious children and adolescents than the functioning of both systems independently, was confirmed.



7.

General Discussion

7. GENERAL DISCUSSION

The aim of the present thesis was to further develop our insight in the role of early attentional processes and stress sensitivity in child and adolescent anxiety disorders. The few published studies addressing these issues produced interesting, but inconclusive results (e.g. Feder et al., 2004; Ganger et al., 1994; Gerra et al, 2000; Kindt, & Brosschot, 1999; Monk et al., 2001; Rogeness et al., 1992; Vasey et al., 1996). However, these studies did provide us with sufficient material to stimulate the formulation of specific hypotheses. In this chapter, the most important results of our study will be presented and discussed in relation to the previous findings. The results of this evaluation will be used to formulate the main conclusions of this thesis. Finally, some suggestions about the clinical and scientific implications of the present findings will be put forward.

7.1. *Early attentional processes in children and adolescents with an anxiety disorder*

Based on findings in adults and some smaller studies in children, several authors suggested that high trait anxiety or anxiety disorders in children and adolescents may be related to an attentional bias towards fear related words or pictures when they are presented in a context with neutral information (Ehrenreich & Gross, 2002; Taghavi et al., 1999; Vasey et al., 1995; Vasey et al., 1996). Additionally, it was suggested that low anxiety in children and adolescents should subsequently be related to an attentional bias away from threatening or emotional new information (Vasey et al., 1996). However, results of these studies with children and adolescents were inconclusive, and the scientific evidence overall did not unequivocally support either of these claims. The hypothesis that low anxiety in children may be related to an attentional bias away from threatening information was solely based on the findings of Vasey et al. (1996), who reported such an effect, but only for boys and if the threatening word was presented below the neutral word at the bottom of the screen. Yiend and Mathews (2001) found a bias away from threatening pictures in low anxious students. However, other studies failed to find the suggested bias away in low anxious children and/or adolescents (Ehrenreich & Gross, 2002; Vasey et al., 1995).

Based on these findings, we investigated attentional processes in primary school children (n = 44; age 10-13 year old), and their relationships with anxiety scores (assessed using the Multidimensional Anxiety Scale for Children: MASC). Like the study of Yiend and Mathews (2001), but contrary to earlier studies with children, we assessed attentional bias using a response-time-task with pictures. The primary advantage of using pictures and not words is that response times should not be influenced by reading ability or familiarity with particular words, and that as a result pictures are processed in a more self-referent way than words (Schippell et al., 2003).

Our results did not show an attentional preference for, nor an avoidance of threatening pictures in these children (chapter 2). However, with increasing anxiety scores, or increasing emotional valence of the presented stimuli, response times increased. These longer response times suggest that more cognitive resources are used when either new information is more threatening and consequently likely to be more relevant for the tested individual, or when a child reports heightened (trait) anxiety. Because no differentiation based on MASC scales was found, we concluded that this latter finding was a general effect of childhood anxiety on attentional processes. Children reporting more anxiety symptoms of any kind seem to spend more cognitive resources on the evaluation of novel and potentially threatening information.

Our additional aim was to investigate the hypothesis that clinically anxious children show a significant attentional bias towards new and emotional stimuli compared to their peers without an anxiety disorder (e.g. Taghavi et al., 2003; Vasey et al., 1995; Waters et al., 2004). Again previous results were contradicting and consequently inconclusive. Some authors found an attentional bias towards new emotional stimuli in children with an anxiety disorder, but not in children without an anxiety disorder (Taghavi et al., 1999; Vasey et al., 1995). However, in the Taghavi et al. study (1999), the attentional bias was not found in a group of mixed anxious-depressed children. Other studies did not find differences between children with and without high anxiety scores or even an anxiety disorder (Kindt et al., 1997a; Kindt et al., 1997b; Waters et al., 2004). Kindt and Brosschot (1999) did report an attentional bias towards spider related words in spider phobic children, compared to non-anxious children, however, this attentional bias was not found towards spider related pictures.

To find out whether children with an anxiety disorder show an attentional bias towards new emotional information, we compared their attententional preferences when confronted with emotional pictures with the preferences found in children without an anxiety disorder. In chapter 3, the data presented in chapter 2 were compared with the data of 37 children (age range: 9.5-13.4 years) with an anxiety disorder. The results indicated significant differences between children with and children without anxiety disorders. However, contrary to the original hypothesis we found a significant attentional bias *away* from threatening pictures in the sample with an anxiety disorder, whereas no significant attentional preferences

were found in the children without an anxiety disorder. Furthermore, there may be gender differences underlying this result. Boys without an anxiety disorder showed a tendency of an attentional bias towards emotional pictures, whereas girls with an anxiety disorder showed an attentional bias away from emotional pictures. However, although this seems to be in correspondence with earlier findings (Vasey et al., 1996), no significant interaction effect was found for gender and bias scores on anxiety scores. More research needs to be done in the future to investigate possible gender differences in attentional preferences when confronted with emotional stimuli.

Based on these results, we found no evidence for any attentional bias related to new threatening information in children from the general population, whereas there is significant evidence that children with an anxiety disorder show a bias away from new threatening information. This finding is in accordance with the 'vigilance-avoiding' theory (Mogg & Bradley, 1998), suggesting a cognitive strategy aimed at minimizing confrontation with threat in individuals prone for anxiety. A potential drawback may be that such strategies stimulate avoiding behaviors and may thus maintain or cause an even higher vigilance for fear-related stimuli in highly anxious individuals This may negatively contribute to emotional development, thus completing the vicious developmental circle towards an anxiety disorder.

7.2. *Stress sensitivity, HPA-axis functioning in child and adolescent anxiety disorders*

Previous results suggested that in child and adolescent anxiety (disorders) an overall decrease of cortisol concentrations may be found (Feder et al., 2004; Gunnar & Vazquez, 2001; Rosmalen et al., 2005). This anxiety related hypo-cortisolism in children and adolescents should be most obvious early in the morning in the period just before awaking and during the 30 minutes after (Gunnar & Vazquez, 2001; Feder et al., 2004). We have studied this phenomenon as well in a sample of children and adolescents with an anxiety disorder (n = 99, age range: 8-16 years) (chapter 4). Contrary to earlier findings we tried to find associations with specific anxiety symptoms.. Cortisol levels were assessed using saliva samples, collected on 4 times over a regular school day and recently experienced symptoms of anxiety were assessed using the Multidimensional Anxiety Scale for Children (MASC). Indeed a significant negative correlation was found between the cortisol concentrations in the saliva sample immediately after awakening and the scores on the MASC Harm Avoidance scale. This finding strengthens earlier results both of children with an anxiety disorder (Feder et al., 2004) and of adults in relation to chronic stress (Preussner et al., 1999; Zarkovic et al., 2003).

The rise in cortisol concentrations 30 minutes after awakening did not correlate significantly with MASC scale scores. In our sample, only 79% of the participants showed a rise in cortisol concentrations within this time window. This is a known phenomenon: a rise in cortisol concentrations is reported only in approximately 70% of the published study sample's participants (Kudielka & Kirschbaum, 2003; Preussner et al., 1997; Rosmalen et al., 2005). Presently, it is not clear whether this may be due to methodological shortcomings (strict compliance to the right sampling times), or that approximately 30% of the tested individuals have no morning rise in cortisol concentrations at all (Kudielka et al., 2003). For this reason, we repeated our analyses only including the participants showing a rise in cortisol concentrations between awakening (the saliva sample was collected while still in bed) and 30 minutes later (when the second saliva sample was collected). In this sample, the girls scoring low on the MASC Total Anxiety scale showed a significantly higher rise in cortisol concentrations than the girls with high MASC Total Scores. These finding suggests that girls diagnosed with an anxiety disorder only show a suppressed rise in cortisol concentrations during the 30 minutes after awakening if the recently experienced symptoms of anxiety (mirrored in MASC scores) are severe.

Additionally, a significant positive relationship was found between the saliva cortisol concentrations at noon and the scores on the MASC Separation Anxiety scale, whereas a negative relationship was found with the scores on the MASC Physical Symptoms scale. It is a remarkable finding that two anxiety scales seem to be associated differently with the same endocrine outcome. This finding possibly reflects that the associations with endocrine processes are related to distinct dimensions of anxiety, like the physical dimensions of anxiety and the affective/cognitive dimension of worries and tension, as described in the tripartite model of Clark and Watson (1991).

Based on the present findings, we may conclude that increasing anxiety scores, even within a population of already high scoring children and adolescents, seem to be related to low saliva cortisol concentrations immediately after awakening. This was significant for anxiety symptoms related to harm avoidance (like, 'I usually ask permission'). In girls, the rise in cortisol concentrations 30 minutes after awakening may be suppressed when anxiety scores increase. Because only children and adolescents with an anxiety disorder were included in the present study, it is not possible to conclude if the observed rise in cortisol concentrations after awakening is lower than in children and adolescents from the general population. Future research including low-anxious children and adolescents is necessary to further clarify this matter.

Finally, we still found significant associations between anxiety symptoms and saliva cortisol concentrations at noon. Consequently, we conclude that anxiety related disturbances of the HPA-axis can still be found at noon in children and adolescents selected on their significantly high anxiety levels, and that such disturbances are not restricted to early morning concentrations (Feder et al., 2004).
These findings are in accordance with earlier findings and suggestions that child and adolescent anxiety may be related to lowered morning cortisol concentrations as indication of permanent changes in the functioning of the HPA-axis (Feder et al., 2004; Gunnar & Vazquez, 2001, Rosmalen et al., 2005). Our findings related to the cortisol concentrations at noon are less unequivocal. Nevertheless, this finding indicates that anxiety related disturbances in HPA-axis functioning may still be found at noon. Permanent changes in HPA-axis functioning may undermine the accuracy of the system when the individual is again confronted with adverse experiences and may thus contribute to the subjective feelings of distress during or after the event. As a result, cognitive strategies may be stimulated that are aimed at avoidance of these environments or circumstances. Because HPA-axis functioning is strongly related to public performance, when an individual is socially evaluated, this may become most obvious in situations demanding social interaction (Gruenewald et al., 2004, 2006; Kirschbaum et al., 1993). There are even indications that such disturbances in HPA-axis functioning are related to personality types characterized by the avoidance of social contacts (Sher, 2005).

7.3. *Stress sensitivity, Autonomic functioning in child and adolescent anxiety disorders*

There is evidence for a dysfunctioning of the autonomic nervous system (ANS) in chronic anxiety in children and adolescents (Boyce et al., 2001; Dobkin et al., 2000; Gerra et al., 2000; Monk et al., 2001; Rogeness et al., 1990; Yeragani et al., 2001). In chapter 5, it was investigated whether in children and adolescents with an anxiety disorder (n = 97; age 8-16 year old) anxiety symptoms are associated with ANS functioning, either during baseline conditions or in response to stress. For this purpose, the physiological reactivity to a Mental Arithmetic Task and the Social Competence Interview was assessed, as well as recently experienced anxiety symptoms.
In response to both stressors, self-reported anxiety symptoms were positively related to sympathetic responses. No significant anxiety related parasympathetic responses to either the cognitive, or the social stressor were found. However, a significant negative relationship was found between recently experienced symptoms of anxiety, especially symptoms of social phobia (like 'I worry about other people laughing at me'), and parasympathetic cardiac control during baseline conditions. Because our study was restricted to children and adolescents with an anxiety disorder, we can only conclude that in the high anxiety spectrum differences in severity of recently experienced anxiety symptoms are significantly associated with specific alterations in ANS functioning. However, as these findings are in line with earlier findings of studies including both children and adolescents with an anxiety disorder and non-clinical peers, they strengthen earlier results indicating lowered parasympathetic cardiac control under baseline conditions and increased sympathetic responsiveness to different types of stressors in children with an anxiety disorder as compared to children without overt psychiatric symptoms (Boyce et al., 2001; Gerra et al., 2000; Monk et al., 2001; Rogeness et al., 1990).

7.4. *Combined endocrine and physiological functioning in child and adolescent anxiety disorders*

Given the functioning of the HPA-axis and the ANS, and evidence from studies in adults and animals (Morilak et al., 2005; Nater et al., 2005; Nyklocek et al., 2005), we hypothesized that these two systems may operate reciprocally in relation to stress regulation. If feelings of discomfort and/or stress become chronic, e.g. related to an anxiety disorder, alterations within these systems may influence each other's functioning over time. Because both systems are still developing throughout childhood and adolescence, this may be a serious threat, both for psychological and for physical health. Hypothetically, the reciprocal stimulation may make the combined systems extra vulnerable for anxiety related developmental deviations in either of these two systems. Because decreased basal cortisol concentrations and increased sympathetic responsiveness have been associated with child and adolescent anxiety disorders, and given the nature of both systems involved, it seemed logical that especially the combination of sympathetic reactivity to a stressor and overall cortisol concentrations would present itself as strongly associated with self-reported levels of anxiety. We investigated this in chapter 6 (n = 99,; age range: 7-16 years old) and our hypothesis was confirmed. The interaction of basal cortisol concentrations and sympathetic stress responsiveness, being the immediate reaction to and recovery of skin conductance level after a stressor, proved to be a strong predictor of anxiety levels. Comparing the explained variances of the different models, it appeared that the contribution of the interaction effect of both systems significantly exceeded the contribution of each system independently to the prediction of anxiety levels (as described in the chapters 4 & 5). Based on this result, we conclude that in children and adolescents with an anxiety disorder, deficits of or alterations in the way these systems are functioning in cooperation with

each other, seems to be most indicative for more severe anxiety symptomatology.

7.5. *Strengths and limitations of the present research*

The present study included a large and well-diagnosed sample to investigate attentional processes and stress sensitivity in children and adolescents with an anxiety disorder. The present study's sample outranged all earlier studies, both in sample size and in the diagnostic instruments used to classify the children and adolescents with an anxiety disorder. For example, the studies investigating attentional bias in children with an anxiety disorder included 12 (Vasey et al., 1995) to 24 (Taghavi et al., 1999) patients and the assessment of anxiety was generally based on the Spielberger State-Trait Anxiety Inventory for Children (STAIC: Spielberger, 1973) and/or the Fear Survey Schedule for Children Revised (FSSC-R: Ollendick, 1983). In some studies, information about the diagnostics to classify the participants was not provided (e.g. Vasey et al., 1995). In the present study the diagnosis of all participants was based on a full diagnostic trajectory in one of the two participating institutes (Erasmus MC Rotterdam and Curium Leiden). This trajectory included the Anxiety Disorder Interview Schedule for Children ADIS-C; Silverman et al., 2001), the Multidimensional Anxiety Scale for Children (MASC; March et al., 1997), and the Children's Depression Inventory (CDI; Kovacs, 1992) and eventually, the diagnosis based on these instruments had to be confirmed by a psychiatrist. Consequently, the present research program conducted with these children included state of the art instruments and procedures for assessing anxiety and anxiety disorders. Additionally, the methods used for the assessment of attentional processes, HPA-axis functioning and and ANS functioning were among the most reliable found in literature.

An important limitation of the present study may be that we restricted ourselves to assess stress sensitivity in children and adolescents with an anxiety disorder. As a consequence, it was not possible to compare their data with data obtained in children and adolescents without overt high anxiety. This does imply that particular questions currently can not be answered. On the other hand, it is remarkable to notice that even in this highly anxious sample, differences in self-reported anxiety symptoms are still significantly related to physical phenomena that are suggested to discriminate between children and adolescents with and without an anxiety disorder as well.

Another limitation may be the lack of full control on compliance to the prescribed cortisol sampling times. This is a methodological drawback that is widely recognized. Fortunately, anxious children (contrary to some other types of childhood psychopathology) are not known for their lack of compliance, thus decreasing the risk that this may have disproportionally contributed to our findings.

Finally, because the used parameter of parasympathetic activity is relatively sensitive for changes in breathing frequency, only a restricted percentage of the participants could be included in the analyses investigating anxiety related parasympathetic effects. We are well aware of this, and where applicable we noticed that this may have restricted our findings.

7.6. *Clinical implications*

The present findings may provide us with some important new insights which may have clinical implications as well.

Attentional biases may be associated with anxiety disorders in children. Such biases may influence the allocation of resources to process new information and may consequently contribute to the development of inadequate but automated cognitive strategies for processing new information. Treatment modalities like Cognitive Behavioral Therapy try to make patients aware of such strategies, and aim to change these strategies in more accurate ones. The presently found differences between children with and without an anxiety disorder in the fundamental processes underlying attentional strategies suggest that the deviant development of these strategies starts at an early age. This finding underlines the need for early diagnosis and timely intervention to control for cognitive developments that may eventually lead to a heightened vigilance for anxiety and even anxiety disorders.

This thesis provides more evidence that child and adolescent anxiety disorders may be related with chronically lowered cortisol concentrations, especially in the morning. This hypo-cortisolism may influence the capability of the HPA-axis to cope with (social) stressors, and thus be a factor in the maintenance of high trait anxiety. However, maybe more reason for concern is the potential risk of chronically low cortisol concentrations for long term physical health. Cortisol deficiencies may have a severe impact on immune system functioning, glucose metabolism and cell growth.

The same may be the case with disturbances in autonomic functioning associated with internalizing psychopathology in children and adolescents. The observed alterations in physiological functioning that seem to be related to anxiety disorders may undermine the ability to properly cope with stressful experiences and consequently increase feelings of distress under certain circumstances. This may stimulate cognitions associated with anxiety and thus contribute to develop or sustain an anxiety disorder. Additionally, the found deficiencies in physical stress regulation may constitute a risk for future cardiovascular health, as especially loss of parasympathetic control is associated with long term adverse cardiovascular outcomes (Allen et al., 1997; Jackson et al., 1999; Jemerin and Boyce, 1990).

The observed interaction effect of alterations in both HPA-axis functioning and ANS stress regulation on anxiety levels is interesting because this may suggest that anxiety related dysfunction in one physiological system may stimulate disturbances in the other as well. This may not only strengthen the physiological factors contributing to the maintenance of heightened anxiety, but may significantly increase the risk for the mentioned negative physical outcomes.
Overall, the results described underline the importance of addressing physical phenomena related to stress regulation in treatment programs aimed at reducing anxiety. Relaxation exercises are part of most Cognitive Behavioral Therapy programs, and may add significantly to the treatment success by countering the distress caused by physical signals in stressful situations.

7.7. *Suggestions for future research*

Although the present study provides new insights in two important factors underlying child and adolescent anxiety disorders, it also puts forward some pressing issues for the research agenda in the (near) future.
Although the evidence for anxiety related lowered cortisol concentrations in children and adolescents is getting stronger, decisive data still are lacking. Future studies may compare a sample like the one described in the present thesis with a large sample from the general population.
The present findings describing ANS disturbances in relation with specific anxiety symptoms in children and adolescents with an anxiety disorder strengthen earlier findings. However, up to now all studies used different kinds of methodologies to assess especially parasympathetic functioning. In time it must become clear which method may be the most appropriate, and if it is possible to reliably assess parasympathetic responsiveness to stressors during which respiratory irregularities due to speaking occur (e.g. public speaking). When such techniques become available, more studies should be conducted to explore and investigate these processes underlying feelings of anxiety and distress when confronted with a stressor in more detail.
The observed interaction effect, described in chapter 6, definitely calls for more research to investigate the possible interactions between the functioning of the two systems involved, especially in relation to anxiety in children and adolescents. Interesting topics to address may be the role of the stress responsiveness of the HPA-axis combined with sympathetic and / or parasympathetic phenomena, and the implications of the presently found interaction in the long term in relation to the development of anxiety symptoms.
This brings us to the major issues to be investigated. Age was an important factor in all constructs described in this thesis, either as dependent or as independent variable. It is obvious that there is a strong developmental trajectory underlying the processes investigated. However, clear insight in the developmental trajectory of ANS and HPA-axis functioning is still lacking. Consequently, it is hard to comment on how particular developments in one domain may influence or disturb the development in any other, especially when we would like to get more insight into the association of such developments with psychological phenomena like anxiety. This issue was put forward by Gunnar and Vazquez in 2001 as well. More studies in children and adolescents in the general population should be performed to clarify this matter.
The present study presented some interesting findings regarding attentional bias and physiological functioning in relation to anxiety disorders in children and adolescent. The finding of a suppressed cortisol rise after awakening was the only finding in the present study which showed a significant gender effect. Earlier studies did suggest that gender effects are present in relation to attentional bias (e.g. Vasey et al., 1996), and in our results some indications for such an effect can be found as well. This might also be something to address when conducting studies like the present one in the future.
Although the present study can compete with earlier studies regarding the diagnostics used to classify the participants with an anxiety disorder, still a wide range of anxiety disorders was included. Other scientists may try to specify and differentiate between the different anxiety disorders, i.e., Spider Phobia may have quite different implications than Generalized Anxiety disorder. Practical suggestions may be to specifically include only social phobic adolescents in studies aiming at public speaking, or patients suffering from a Generalized Anxiety Disorder to investigate the role of brooding on basal HPA-axis functioning.
The interaction effect of both the HPA-axis and the autonomic nervous system in relation to childhood anxiety suggests that psychological distress and even psychopathology may have consequences for physical health. However, this development towards significant risks for physical health may start much earlier than expected based on our current knowledge. More research should definitely be done into this matter.
Finally, it may be relevant to know whether the observed attentional and physiological correlates of child and adolescent anxiety disorders recover over time when the anxiety symptoms are treated with success, e.g. by means of Cognitive Behavioral Therapy. On the other hand, the observed attentional and physiological phenomena associated with such disorders may restrain the effect of Cognitive Behavioral interventions, which is an interesting issue to investigate.

7.8. *General conclusions*

The aim of the present thesis was to further clarify the role of early attentional processes and stress sensitivity in child and adolescent anxiety disorders. A significant difference was found between the early attentional strategies used by children with an anxiety disorder and non-referred control children when confronted with fear-related stimuli. Contrary to earlier findings and our original hypothesis, our result indicates an initial avoiding strategy for fear-related stimuli in children with an anxiety disorder. This finding is in accordance with a 'vigilance-avoiding' strategy (Mogg & Bradley, 1998), which aims at minimizing confrontation with threat. A potential drawback may be that such strategies stimulate avoiding behaviors that may restrict the opportunities of the child learn to cope with uncomfortable situations or his or her anxiety.

In relation to stress sensitivity, increased levels of anxiety, even in children and adolescents with an anxiety disorder, were associated with lowered cortisol concentrations immediate after awakening. Additionally, our findings suggested that many recently experienced symptoms of anxiety may be associated with a suppressed rise in cortisol concentrations during the first 30 minutes after awakening. This was the case for girls and may be true for boys.

Earlier findings indicating that child and adolescent anxiety disorders are associated with enhanced sympathetic responsiveness to stressors was strengthened by our data that in children and adolescents with an anxiety disorder, recently experienced symptoms of anxiety are associated with increased sympathetic responsiveness. The previous findings in small samples suggesting that child and adolescent anxiety disorders may be associated with the loss of parasympathetic control were confirmed in our studies as well. Our findings indicated a negative association of parasympathetic cardiac control with recently experienced symptoms of anxiety in children and adolescents with an anxiety disorder.

Finally, the finding of a significant interaction effect of sympathetic stress responsiveness and basal cortisol concentrations on recently experienced anxiety symptoms in children and adolescents with an anxiety disorder was remarkable. The explained variance of this effect indicated that this interaction may be considerably stronger related to child and adolescent anxiety disorders than any other construct investigated in our studies. It implies that recently experienced symptoms of anxiety in children and adolescents, scoring already high on trait anxiety because they are diagnosed with an anxiety disorder, may primarily disrupt the physical capability to accurately cope with stress. However, because the two physiological systems involved have to support each others functioning to some extent, over time anxiety related deficits in one physiological domain are likely to cause mal-functions in the other, especially if the state of anxiety becomes chronic. This is reason for concern. It seems that the more severe cases in an already highly anxious population suffer from deficiencies in two important physiological systems which may have severe consequences, not only in preserving a continuing state of high anxiety and thus the presence of an anxiety disorder, but for future physical health as well.

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Summary
Samenvatting

SUMMARY

The objective of this thesis was to investigate to role of early attentional processes and physiological stress sensitivity in child and adolescent anxiety disorders. In **chapter 1** the current state of knowledge in relation to child and adolescent anxiety, early attentional preferences and physiological stress sensitivity was described. Based on this information, diverse gaps in our knowledge regarding the role of attentional bias and stress sensitivity in the development and maintenance of severe anxiety in youths have been identified. As a result the present study set out to investigate whether:

- Deviant attentional processes are related to childhood anxiety, as suggested by diverse theorists;
- The severity of the symptoms in children and adolescents with an anxiety disorder is related to disturbed Hypothalamus-Pituitary-Adrenal (HPA) axis functioning;
- The severity of the symptoms in children and adolescents with an anxiety disorder is related to either sympathetic hyperarousal to stressors and/or the loss of parasympathetic control;
- Severity of anxiety symptoms is more strongly related to interactive disruptions in hormonal and physiological processes, thus reinforcing each other's deviant development, than to just disruptions in these two stress regulatory systems independently.

In **chapter 2** our study to the relation of anxiety and attentional preferences in children without an anxiety disorder was described. Based on the results of a Probe Detection Task conducted in 44 primary school children (aged 10 to 13 years), we concluded that the children showed a tendency away from new and threatening information. However, within this sample the attentional strategy was not related to self-reported anxiety scores, assessed using the Multidimensional Anxiety Scale for Children (MASC). Nevertheless, higher anxiety scores were related to longer overall response times, indicating that more resources were used to process the new information and prepare a response.

In **chapter 3** the results of the non-clinical sample described in chapter 2 were compared to the Probe Detection Task response times of 37 children (aged 9 to 13 years) diagnosed with an anxiety disorder. The two groups differed significantly in attentional strategy. The clinical group showed an initial avoiding strategy when confronted with fear-related pictures. This strategy appears to be most ob-

vious in girls. This finding is in accordance with the 'vigilance-avoidance' theory, but contrary to suggestions made by some theorists that highly anxious children would show a bias towards new and threatening information in the initial stages of information processing.

In **chapter 4** we investigated whether specific anxiety symptoms in children and adolescents with an anxiety disorder could be associated with disturbances in the most prominent generally used parameters of basal HPA-axis functioning, being: the cortisol awakening rise (CAR); the area under the curve (AUC; calculated using multiple samples over a regular day); and the cortisol concentrations on specific times during the day. These were determined by means of repeated saliva samples in 99 children and adolescents (aged 8 to 16 years) diagnosed with an anxiety disorder in one of the participating clinics. The recently experienced symptoms of anxiety were assessed using the MASC.
Higher Harm Avoidance scale scores on the MASC significantly predicted lower cortisol concentrations immediately after awaking. Girls reporting lower levels of recently experienced anxiety showed a stronger CAR than boys and girls reporting considerable levels of anxiety during the previous two weeks. Finally, at noon the scores on the Physical symptoms scale were associated with lower cortisol concentrations, whereas scores on the Separation Anxiety scale were associated with higher cortisol concentrations.

Chapter 5 describes our study to the relation of physiological stress sensitivity and symptoms of anxiety in 97 of children and adolescents from the sample described in chapter 4. Skin Conductance Level (SCL), Blood Pressure (BP), Heart Rate (HR), and respiratory frequency were monitored during baseline, a cognitive, and a social stressor. From the heart rate series, the frequency components were derived as parameters of Heart Rate Variability (HRV). Again, recently experienced symptoms of anxiety were assessed using the MASC.
In these children and adolescents generalized anxiety was associated with a decrease in baseline HRV and increased SCL during the social stressor. Separation anxiety was associated with stronger SCL responses to both stressors. Harm avoidance was significantly associated with a decrease in diastolic BP during the cognitive stressor. Finally, social phobia was related to a stronger diastolic BP response to the stressor. This indicated that in children and adolescents with an anxiety disorder recently experienced symptoms of anxiety may be associated with reduced baseline parasympathetic cardiac control and a heightened sympathetic responsiveness to stressors.

Although significant associations were found between recently experienced anxiety symptoms in children and adolescents with an anxiety disorder, and disturbances in HPA-axis (chapter 4) and autonomic functioning (chapter 5) independently, these dysfunctions may reciprocally strengthen each other. Consequently, we investigated in **chapter 6** whether the recently experienced symptoms of anxiety might be better predicted by the interaction of basal HPA-axis functioning and sympathetic responsiveness to stress.
This appeared to be the case: the interaction of basal cortisol concentrations and sympathetic responsiveness to stress, mirrored by increases in SCL in response to a cognitive stressor, was a stronger predictor of recently experienced anxiety symptoms than anxiety related distortions in both systems independently, as described in chapter 4 and 5. This finding may be explained by heightened cortisol concentrations to support sympathetic hyperarousal by the mobilization of extra energetic resources.

In **chapter 7** the most important findings in of the previous chapters were summarized and discussed in relation to the aims of this thesis. Based on our results we can conclude that:
• Anxiety disorders in children and adolescents are related to deviant early attentional strategies. However, contrary to the regular suggestion of a strategy directed towards new threatening information, we found a strong tendency of avoidance in relation to the presence of an anxiety disorder in children and adolescents.
• Recently experienced symptoms of anxiety can be associated with disturbed basal HPA-axis functioning in children and adolescents suffering from extreme levels of anxiety, and consequently being diagnosed with an anxiety disorder. This is most notable in cortisol concentrations immediately after awakening and in the rise of concentrations during the 30 minutes following awakening.
• In children and adolescents with an anxiety disorder recently experienced symptoms of anxiety are associated with reduced baseline parasympathetic cardiac control and increased sympathetic activity in response to stress.
• The associations between anxiety symptoms in children and adolescents with an anxiety disorder and either disturbed HPA-axis functioning or deviant autonomic functioning become even stronger when the reciprocal functioning of these two physiological systems is taken into account.

For several reasons the present findings may be relevant. The attentional strategy in children and adolescents with an anxiety disorder as described in this study may stimulate avoiding behaviors when confronted with adverse new informa-

tion, what may undermine the ability of a young individual to learn to cope with initially unpleasant situations. However, future research is necessary to investigate the relation between attentional strategies and behaviors.
Additionally, the anxiety related disturbances found in both physiological stress regulatory systems indicate that in children and adolescents with severe levels of anxiety, anxiety symptoms may be related to disturbances in physiological functioning. This may have serious consequences, not only directly related to their anxiety disorder, like increased uncomfortable sensations, but for their present and future physical health as well.

SAMENVATTING

Het doel van het hier beschreven onderzoek was om te onderzoeken welke rol vroege aandachtsstrategieën en fysiologische gevoeligheid voor stress spelen in relatie tot de angstsymptomen van kinderen en adolescenten met een angststoornis.

In **hoofdstuk 1** hebben we de huidige stand van zaken beschreven met betrekking tot angststoornissen bij kinderen en adolescenten, wat er bekend is in relatie tot aandachtsstrategieën binnen deze groep patiënten, en in hoeverre er eerder onderzoek is gedaan naar de fysiologische gevoeligheid voor stress in deze groep. Gebaseerd op deze informatie blijkt er een aantal hiaten in onze kennis over aandachtsstrategieën en fysiologische gevoeligheid voor stress bij deze patiënten te zijn. Daarom was ons doel om te onderzoeken of:
• er een te onderscheiden strategie wordt gevolgd door kinderen met een angststoornis in vergelijking met leeftijdsgenoten zonder een psychiatrische diagnose als ze worden geconfronteerd met nieuwe en bedreigende informatie, zoals door verschillende wetenschappers wordt gesuggereerd;
• de ernst van de angstsymptomen bij kinderen en adolescenten met een angststoornis gerelateerd is aan het verstoord functioneren van de zogenaamde HPA-as, die een belangrijke rol speelt in stressregulatie;
• de ernst van de angstsymptomen bij kinderen en adolescenten met een angststoornis gerelateerd is aan een versterkte sympathische reactie op stress of het verlies aan cardiale parasympathische controle;
• de ernst van de angstsymptomen bij kinderen en adolescenten met een angststoornis mogelijk sterker is verbonden met het gecombineerde (dis)functioneren van de HPA-as en het autonome zenuwstelsel dan met verstoringen in het functioneren van één van beide systemen, onafhankelijk van het functioneren van de ander.

In **hoofdstuk 2** wordt ons onderzoek beschreven naar de relatie tussen aandachtsstrategieën en angstsymptomen bij kinderen zonder een psychiatrische diagnose. Op basis van de resultaten op een ‘Probe Detection Task’, uitgevoerd door 44 kinderen op een basisschool (leeftijd: 8 tot en met 13 jaar) kunnen we concluderen dat de kinderen over het algemeen een vermijdende strategie gebruikten wanneer nieuwe en bedreigende informatie werd gepresenteerd. In deze groep bleek de toegepaste strategie niet gerelateerd te zijn met zelfgerapporteerde angstsymptomen, waarover informatie werd verkregen door middel van de Nederlandse

vertaling van de Multidimensional Anxiety Scale for Children (MASC). Daarentegen waren hogere angstscores op deze lijst wel verbonden met langere reactietijden, wat zou kunnen betekenen dat kinderen met meer angstklachten meer cognitieve middelen inzetten om nieuwe (emotionele) informatie te verwerken en een reactie voor te bereiden.

In **hoofdstuk 3** werden de resultaten bij de niet-klinische populatie vergeleken met een groep van 37 kinderen (leeftijd: 8 tot en met 13 jaar) waarbij een angststoornis was vastgesteld.
De beide groepen bleken significant van elkaar te verschillen in de gebruikte strategie wanneer nieuwe en bedreigende informatie werd aangeboden. In vergelijking met hun leeftijdsgenootjes zonder een psychiatrische diagnose bleken de kinderen met een angststoornis een duidelijk vermijdende strategie te laten zien ten aanzien van de locatie waarop de bedreigende informatie werd aangeboden. Dit bleek het sterkst te zijn voor meisjes. Deze bevinding past bij de 'vigilantie-vermijding' theorie. Maar zij is in strijd met het standpunt van sommige wetenschappers dat als gevolg van het verhoogde angstniveaus de aandacht wordt gericht op de locatie waar bedreigende informatie verschijnt.

Hoofdstuk 4 beschrijft ons onderzoek naar de relatie tussen specifieke angstsymptomen en verstoringen in de meest algemeen gebruikte parameters van het basale functioneren van de HPA-as: de toename van de cortisolconcentratie direct na ontwaken (CAR); de oppervlakte onder de curve, gebaseerd op meerdere cortisolmetingen op één dag; en cortisolconcentraties op specifieke tijden. Deze cortisolconcentraties werden bepaald aan de hand van speekselmonsters die op een reguliere schooldag herhaaldelijk werden verzameld door 99 kinderen en adolescenten met een angststoornis, gediagnosticeerd in één van beide participerende klinieken (Erasmus MC / LUMC). De angstsymptomen werden wederom bepaald met behulp van de Nederlandse vertaling van de MASC.
De Schade Vermijdingsschaal van de MASC bleek verlaagde cortisolconcentraties meteen na ontwaken betrouwbaar te voorspellen. Meisjes die relatief weinig angstsymptomen rapporteerden over de voorgaande 2 weken lieten een sterkere stijging in cortisolconcentraties gedurende de 30 minuten na ontwaken zien dan de jongens en de meisjes die veel angstsymptomen rapporteerden. Tot slot bleken hoge scores op de 'Fysieke symptomen' schaal van de MASC gerelateerd te zijn aan lagere cortisolconcentraties om 12 uur 's middags, terwijl hogere scores op de 'Scheidingsangst' schaal gerelateerd bleken te zijn met hogere cortisolconcentraties op hetzelfde moment.

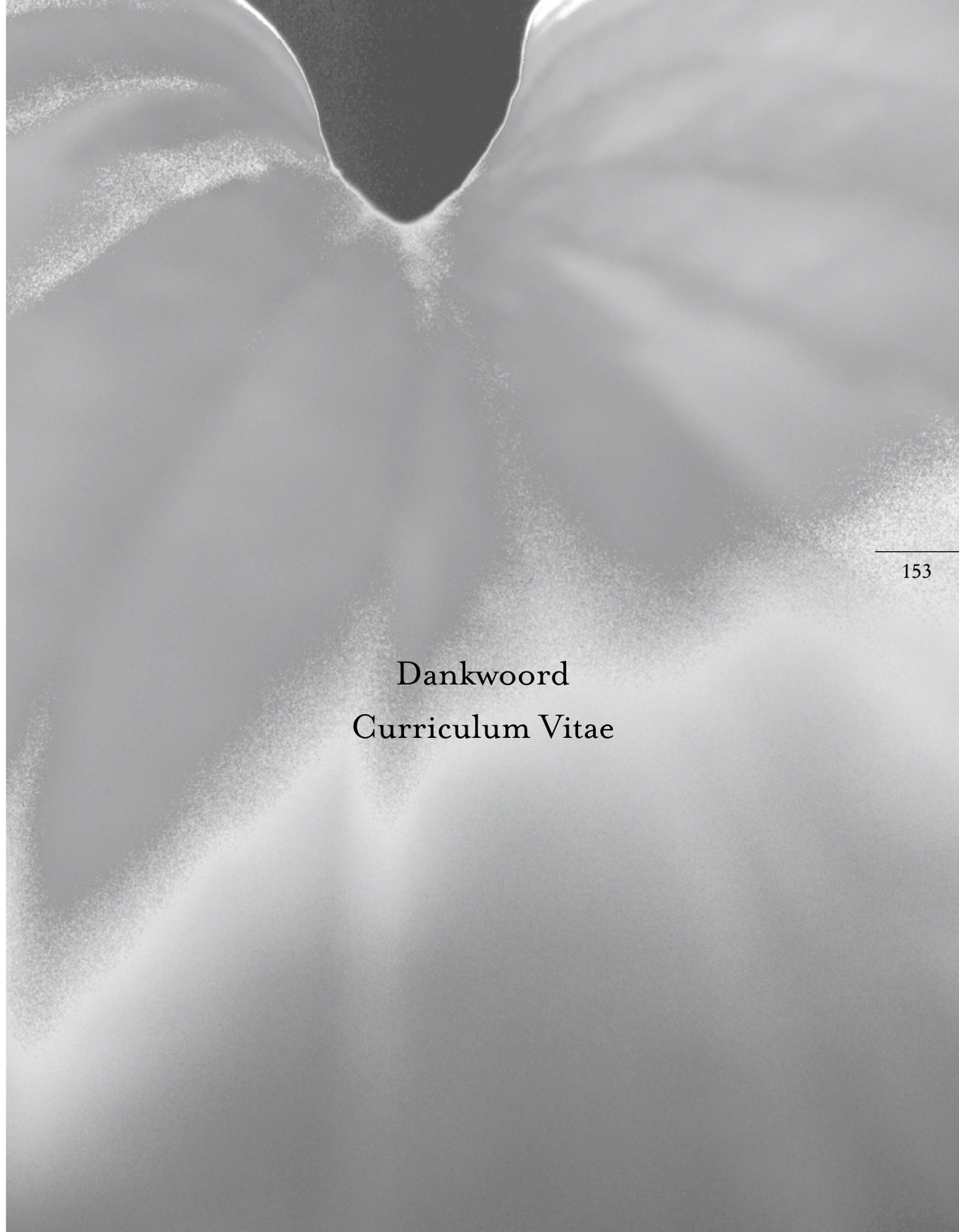
In **hoofdstuk 5** wordt ons onderzoek beschreven naar de relatie tussen fysieke gevoeligheid voor stress en angstsymptomen in 97 kinderen en adolescenten uit de groep beschreven in hoofdstuk 4. Huidgeleiding, bloeddruk, hartslag en ademhalingsfrequentie worden continue gemeten tijdens een ontspanningsperiode, een cognitieve stress taak en een sociale stress taak. Uit de hartslaggegevens werd ook nog de hartritmevariabiliteit bepaald door middel van frequentie bepalingen. Onlangs ervaren angstsymptomen werden wederom onderzocht met Nederlandse vertaling van de MASC.
In deze groep bleek gegeneraliseerde angst (onder ander gekenmerkt door 'piekeren') geassocieerd te zijn met een afname van hartritmevariabiliteit tijdens de ontspanningsperiode. Ook bleek gegeneraliseerde angst verbonden te zijn met een toename van de huidgeleiding gedurende de sociale stressor. Scheidingsangst was gerelateerd aan een toename in huidgeleiding tijdens de cognitieve stress taak en de sociale stress taak. Schadevermijding was gerelateerd aan een verlaagde diastolische bloeddruk tijdens de cognitieve taak. Tot slot was Sociale Angst gerelateerd aan verhoogde diastolische bloeddruk tijdens de sociale stressor. Deze bevindingen suggereren dat bij kinderen en adolescenten met een angststoornis onlangs ervaren angstsymptomen geassocieerd kunnen zijn met een verlaagde parasympathische cardiale controle en een sterkere sympathische reactie op stressoren.

We hebben aanwijzingen gevonden dat angstsymptomen bij kinderen en adolescenten met een angststoornis gerelateerd zouden kunnen zijn aan verstoringen in zowel de HPA-as (hoofdstuk 4) als het functioneren van het autonome zenuwstelsel (hoofdstuk 5). Mogelijk versterken deze effecten elkaar in relatie tot de gerapporteerde angstklachten. Daarom hebben we in **hoofdstuk 6** onderzocht of de relatie tussen angstklachten en fysiologische stressgevoeligheid misschien sterker is als disfuncties in beide systemen tegelijkertijd in beschouwing worden genomen.
Dit bleek het geval te zijn: de interactie tussen basale cortisolconcentraties en de sympathische reactie op stress, die bleek uit de stijging in huidgeleiding in reactie op een cognitieve taak, was sterker verbonden met angstklachten dan met beide parameters afzonderlijk. Een mogelijke verklaring voor deze bevinding is dat verhoogde cortisolconcentraties een versterkte sympathische reactie ondersteunen door het beschikbaar maken van meer energetische middelen.

Uiteindelijk werden in **hoofdstuk 7** de belangrijkste bevindingen van de voorgaande hoofdstukken samengevat en besproken in relatie tot de doelstellingen van dit onderzoek. In relatie tot deze doelstellingen kunnen we concluderen dat:

• angststoornissen in kinderen gerelateerd zijn aan een duidelijk te onderscheiden aandachtsstrategie als zij worden geconfronteerd met bedreigende informatie. Echter, in tegenstelling tot een veel gedane suggestie, bleek dat deze strategie gekenmerkt wordt door de neiging de locatie waarop de betreffende informatie wordt gepresenteerd te vermijden;
• onlangs ervaren angstklachten door kinderen en adolescenten met een angststoornis gerelateerd zijn aan verstoord HPA-as functioneren. Dit bleek het sterkst het geval te zijn voor de cortisolconcentraties meteen na ontwaken en gedurende de 30 minuten hierna;
• onlangs ervaren angstklachten door kinderen en adolescenten met een angststoornis gerelateerd bleken te zijn aan een afname van cardiale parasympathische controle en toename van de sympathische reactie op stress;
• de gevonden verbanden tussen angstklachten bij kinderen en adolescenten met een angststoornis en aan de ene kant verstoringen in HPA-as functioneren en aan de andere kant verstoringen in autonoom functioneren sterker bleken te zijn als ook de wederzijdse beïnvloeding van beide systemen in beschouwing werd genomen.

Om een aantal redenen zijn de resultaten van deze studie relevant. De gevonden alternatieve aandachtsstrategie in kinderen en adolescenten met een angststoornis kunnen vermijdende gedragingen stimuleren dan wel versterken als het individu wordt geconfronteerd met bedreigende nieuwe informatie. Dit kan de mogelijkheid van kinderen en adolescenten om te leren omgaan met moeilijke situaties ernstig belemmeren. Meer onderzoek is echter nodig om te bepalen hoe deviante vroege aandachtstrategieën van invloed kunnen zijn op cognities en gedrag.
Daarnaast duiden de gevonden relaties tussen angstklachten en fysiologisch functioneren op een mogelijk riskante wisselwerking tussen angstige stemmingen en lichamelijk functioneren. Deze aan angst gerelateerde verstoringen in een aantal fysiologische systemen kunnen ernstige gevolgen hebben voor de fysieke gesteldheid van de betrokkene.



Dankwoord

Curriculum Vitae

DANK!

Allereerst en vanzelfsprekend bedank ik mijn ‘coachteam’: dr. Robert Ferdinand, dr. Joke Tulen, prof. dr. Flip Treffers en prof. dr. Frank Verhulst. Dank voor alle wijsheid en vaardigheden die ik dankzij jullie de afgelopen jaren heb kunnen verzamelen en trainen. Dank voor jullie steun, inzet, (schier eindeloze) geduld, humor, inspiratie en bereidheid om op de raarste tijden en plaatsen toch nog even feedback te mailen over artikelen. Dank voor de koffie en het overleg (Frank en Flip, bij meerdere gelegenheden), voor de spreekwoordelijke schop onder mijn kont (Robert), voor de kikkers (Flip), en bovenal Joke voor je kritische blik, je eindeloos goede humeur en je zorgzaamheid, zonder welke ik het proefschrift nooit zou hebben voltooid.

Dr. Lisbeth Utens, beste Lisbeth, je energie en enthousiasme zijn inspirerend. Dank voor de uitstekende samenwerking die we hopelijk in de toekomst kunnen continueren.

Prof. dr. Frank de Jong, dank voor al uw kritische maar ook stimulerende feedback. Het was altijd prettig en zeer leerzaam om met u de endocriene facetten van dit onderzoek te kunnen bespreken.

Prof. dr. Michiel Hengeveld, dank dat u bereid was secretaris te zijn van mijn promotiecommissie, een taak die u uitermate voortvarend ter hand hebt genomen. Daarnaast ook voor de ongekende gastvrijheid van uw afdeling, ik heb me er vanaf de eerste dag helemaal thuis gevoeld.

Prof. dr. Eco de Geus, ik vind het natuurlijk een eer, maar zeker ook heel erg leuk dat u in mijn promotie commissie zitting wilde nemen. Bij deze gelegenheid alleen (helaas) geen bluesband en oesters.

Prof. dr. Jan Passchier, ik heb u de afgelopen jaren regelmatig bewonderd bij presentaties en promoties. Altijd enthousiast en met een scherp oog voor relevantie. Ik waardeer het daarom des te meer dat ook u in mijn promotiecommissie zitting wilde nemen.

Prof. dr. Michiel Westenberg, beste Michiel, dank voor alle morele en praktische steun tijdens de laatste zware loodjes van dit proefschrift. Bijna onvoorstelbaar, maar nu is het dan toch af! De kans die je me hebt gegeven om de kennis en vaardigheden die ik tijdens mijn promotietraject heb opgedaan verder te ontwikkelen is buitengewoon en ook daarvoor heel veel dank. Welkom in mijn promotie commissie!

Juliette, goede collega’s zijn goud waard, zeker op de ‘zware’ momenten. Hoewel we beiden een duidelijk eigen stijl hebben, was het erg goed samenwerken, juist op die momenten. Nu ook jij heel veel succes met het afronden van je proefschrift!

Mijn twee ‘grote’ vrienden, Martijn en Pol, dank dat jullie mij tijdens mijn promotie wederom, en deze keer letterlijk, terzijde willen staan. Martijn voor de vriendschap gedurende mijn hele ‘academische’ ontwikkeling (dat wil zeggen vanaf klas 2 van de basisschool) en Pol voor alle discussies, steun en vriendschap sinds dag één in het Sophia.

Roelie, Hugo, Freddy en Thomas van het ‘J.H.M. Tulen-lab’: we waren samen een klein maar hecht team, wat ik altijd zeer waardeerde. Dank voor jullie collegialiteit en de goede sfeer, vooral gedragen door een gezonde dosis zelfspot en relativeringsvermogen.

Alle collega’s van de afdeling Kinder- en Jeugdpsychiatrie van het Sophia Kinderziekenhuis voor de prettige samenwerking: Ilja, Jolanda, Patricia, Gonneke, Dennis, Karen, Mariëlle, Jan, Susan, Alma, Kirstin, Wendy, Chantal, Natasja, Frouke, Nouchka en Kathleen. Maar zeker ook: Pieter en Jochem (voor de Heeren lunches!), Sonja, Willemijn, Bram, Jeroen, Esther, Gwen, Perlita, Diana, Yashvir, Hélène, Laureen, Donna, Yael, Gerri-Janne, Q en alle anderen.

De collega’s van Curium, met name Adelinde, Birgit, Sophie, Annemarie en Bart, hartelijk dank voor de gastvrijheid en de prima samenwerking.

Arne Popma en Wendy Ooteman, dank voor alle cortisolmomenten en vooral het bezoek aan professor Clemens Kirschbaum.

Dr. Martin Elton, mijn mentor tijdens mijn studie en degene die mijn onderzoeksinteresse heeft laten ontvlammen. Rust in de wetenschap dat je enthousiasme voor onderzoek zeer besmettelijk bleek te zijn.

Familie en vrienden: hier is dan eindelijk het resultaat. Dank voor de jaren geduld, nu zijn er geen excuses meer om niet op al jullie verjaardagen en feestjes te komen!

Dorothée, Charlotte en Herman, dank voor jullie hulp bij de vormgeving van het proefschrift!

Louis en Roos dank voor jullie steun en voor de betrokkenheid op de belangrijke momenten.

Lieve Ans, dit is het dan, meer kan ik er niet van maken. Onvoorstelbaar hoe gedurende al die jaren geen middel te veel was om mij te kunnen laten groeien en ontwikkelen op mijn eigen manier en in het tempo dat ik zelf verkoos. Ongetwijfeld heb je je wel eens achter je oren gekrabd, maar ik hoop dat het de investering (en zorgen) waard was.

Lieve Katinka, dank voor al de uitgesproken en stille steun gedurende de afgelopen jaren. Een belangrijke bijdrage was zeker dat je met enige regelmaat en zonder pardon de wetenschappelijke sleur onderbrak en me mee nam naar een weer een nieuw tropisch land voor een noodzakelijke vakantie, maar ook voor een even zo noodzakelijke verruiming van mijn blik. Dank voor al je inspanningen om ervoor te zorgen dat ik aandacht zou blijven houden voor de andere zaken in het leven, al lukte dat laatste soms maar ternauwernood en vaak ten koste van de nodige frustraties. Hopelijk is nu dan de tijd gekomen om de vruchten te plukken van je geduld. Ik doe mijn best, Kuss

CURRICULUM VITAE

Victor Louis Kallen werd op 4 maart 1975 in Boxtel geboren. In 1993 haalde hij het V.W.O. diploma aan het Eckart College te Eindhoven. Dat zelfde jaar begon hij met de studie Psychologie aan de Katholieke Universtiteit Brabant (KUB) te Tilburg. Na het behalen van de propedeuse en het basis doctoraal vervolgde hij zijn studie per september 1995 bij de vakgroep Psychonomie van de Universiteit van Amsterdam (UvA). In januari 2001 studeerde hij daar af met een studie naar de rol van nicotine in pre-frontale stimulus inhibitie.
Vanaf november 2000 tot en met september 2001 werkte hij als assistent op de Marketing afdeling van Euronext N.V. te Amsterdam waar hij onder andere betrokken was bij de beursgang van dit bedrijf.
In oktober 2001 begon hij het hier beschreven onderzoek als Assistent in Opleiding (AiO) op de afdeling Kinder- en Jeugdpsychiatrie van de Erasmus Universiteit te Rotterdam (afdelingshoofd: Prof.dr. F.C. Verhulst), onder begeleiding van Dr. J.H.M. Tulen, Dr. R.F. Ferdinand en Prof.dr. P.D.A. Treffers (Curium/LUMC).
Sinds 1 oktober 2005 werkt hij bij de afdeling Onderwijs- en Ontwikkelingspsychologie van de Universiteit Leiden (afdelingshoofd: Prof.dr. P.M. Westenberg), tot 1 april 2007 als post-doc en sindsdien als universitair docent.