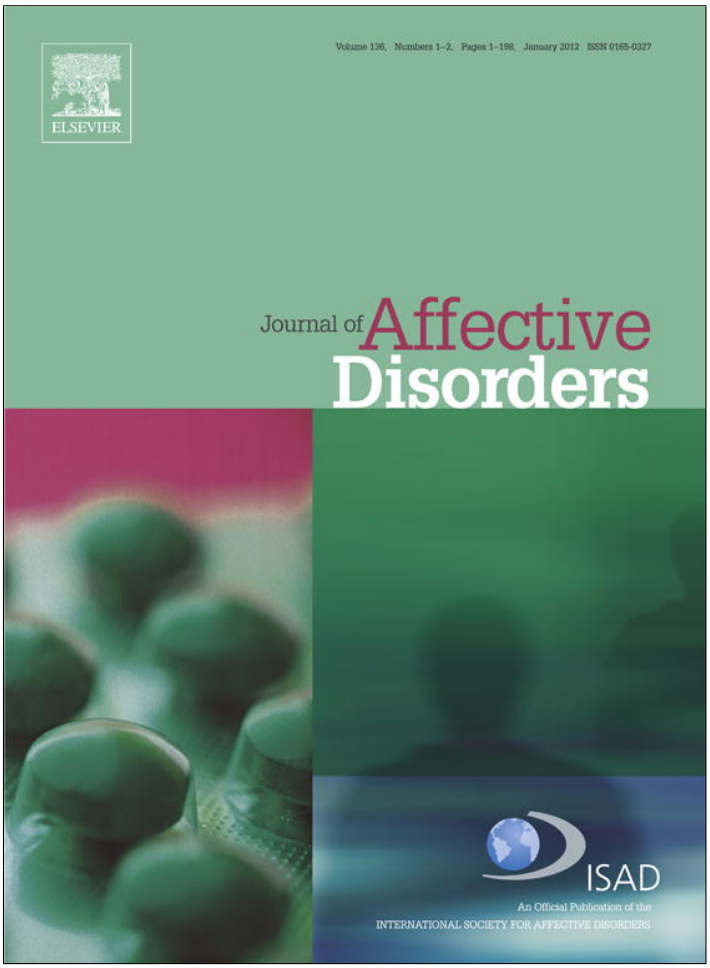
Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/authorsrights

Author's personal copy

Journal of Affective Disorders 196 (2016) 190–199

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research paper

Stress reactivity predicts symptom improvement in children with anxiety disorders

Gwendolyn C. Dieleman [a,*], Anja C. Huizink [c], Joke H.M. Tulen [b], Elisabeth M.W.J. Utens [a], Henning Tiemeier [a,b]

[a] Department of Child and Adolescent Psychiatry/Psychology, Erasmus Medical Center Rotterdam/Sophia Children's Hospital, Rotterdam, The Netherlands
[b] Department of Psychiatry, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands
[c] Department of Developmental Psychology, Faculty of Psychology and Education, VU University, Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 7 July 2015
Received in revised form
30 November 2015
Accepted 6 February 2016
Available online 9 February 2016

Keywords:
Anxiety disorders
Treatment outcome
Autonomic nervous system
Cortisol
Cognitive behavioral therapy
Depressive symptoms

ABSTRACT

Background: We examined the longitudinal associations of autonomic nervous system (ANS) and hypothalamic-pituitary-adrenal (HPA) axis rest and reactivity measures with anxiety and depressive symptoms at one-year follow-up in children with anxiety disorders.
Methods: In a clinical sample of 152 children with a primary DSM-IV anxiety disorder, aged 8 to 12 years, anxiety and depressive symptoms were assessed with the Multidimensional Anxiety Scale for Children and the Children's Depression Inventory at pre-treatment baseline and one year later, after treatment with cognitive behavioral therapy. At baseline, children participated in a 70 min stress task. Salivary cortisol was measured directly prior to and 20 min post stress task. Skin conductance level (SCL), heart rate and high frequency heart rate variability (HRV) were continuously measured during rest and the stress task. To investigate if rest or reactivity measures predicted anxiety and depressive symptoms at one year follow-up, linear regression analyses were conducted for rest and reactivity measures of SCL, heart rate, HRV and cortisol separately.
Results: Higher SCL reactivity predicted less decrease of anxiety symptoms at one-year follow-up. Cortisol reactivity showed a weak association with depressive symptoms at one-year follow-up: lower cortisol reactivity predicted less decrease in depressive symptoms.
Limitations: Only self-reported anxiety and depressive symptoms were used. However, all predictors were objective biological measures, hence there is no risk of shared method variance bias.
Conclusions: These findings suggest that pre-treatment HPA and ANS responsiveness to stress are predictive biomarkers for a lack of symptom improvement in children with a clinical anxiety disorder.
© 2016 Elsevier B.V. All rights reserved.

1. Introduction

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

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

Several studies tried to gain insight into clinical predictors of treatment outcome in children with anxiety disorders. Some studies reported that higher anxiety severity predicts a less favorable outcome (Compton et al., 2014; Hudson et al., 2013; Last et al., 1998; Liber et al., 2010). A few studies showed that children with comorbid mood disorders are more likely to retain their primary anxiety disorder following treatment (Hudson et al., 2013; Liber et al., 2010). Various studies examined the role of parental characteristics as predictors of treatment outcome in children, but an inconsistent pattern of findings resulted (Compton et al., 2014;

* Correspondence to: Gwendolyn Dieleman, Erasmus Medical Center Rotterdam/Sophia Children's Hospital, Department of Child & Adolescent Psychiatry/Psychology, Wytemaweg 12, 3015 CN Rotterdam, The Netherlands.
E-mail address: g.dieleman@erasmusmc.nl (G.C. Dieleman).

http://dx.doi.org/10.1016/j.jad.2016.02.022
0165-0327/© 2016 Elsevier B.V. All rights reserved.

Author's personal copy

Hudson et al., 2013; Legerstee et al., 2008). Because clinical characteristics are weak or inconsistent indicators of response to CBT, there is an increasing interest in identifying biomarkers to predict differential treatment response (Lester and Eley, 2013).

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

Despite the evidence of altered pre-treatment HPA axis and autonomic functioning in anxiety-disordered children, studies that have assessed stress physiology as a predictor of therapy outcome in childhood anxiety disorders are lacking. This study aims to investigate the longitudinal association of stress physiology at pre-treatment baseline with anxiety and depressive symptoms at one year follow-up in a clinical sample of anxiety disordered children treated with CBT. We hypothesize that anxiety-disordered children with a stronger autonomic stress response, i.e. heightened activity of the sympathetic and diminished activity of the parasympathetic nervous system, show persistence of anxiety symptoms one year later. Also, we explore the longitudinal association between cortisol levels and anxiety symptoms at one-year follow-up, but formulate no specific hypothesis, given the inconsistent results of previous studies. Furthermore, since comorbid depressive symptoms have been associated with a less favorable treatment outcome in children with anxiety disorders (Hudson et al., 2013; Liber et al., 2010) and in previous work we observed that cortisol reactivity was specifically associated with depressive symptoms (Dieleman et al., 2010), we will also explore the longitudinal association of stress physiology with depressive symptoms.

2. Methods

2.1. Participants

This study included 152 children, aged 8–12 years, referred to the outpatient clinic of the Department of Child and Adolescent Psychiatry of Erasmus Medical Center in Rotterdam or the University Medical Center in Leiden, The Netherlands. These hospitals serve as secondary or tertiary referral centers of South-West Netherlands. Children had a primary diagnosis of separation anxiety disorder (n=57), generalized anxiety disorder (n=47), social phobia (n=29) or specific phobia (n=19). All children were diagnosed with the Anxiety Disorders Interview Schedule for DSM-IV (Silverman, 1996).

Exclusion criteria were: IQ < 85, poor command of the Dutch language, serious somatic disease, autism spectrum disorder, selective mutism, psychotic disorders, pharmacotherapy that could interfere with HPA axis or ANS functioning.

Methylphenidate treatment in children with comorbid attention deficit hyperactivity disorder was discontinued the day before physiological measurements (n=7), because methylphenidate treatment can increase heart rate and blood pressure (Ballard et al., 1976). Children on medication for an anxiety disorder were withdrawn from medication, if possible, or otherwise excluded. For children with comorbid attention deficit hyperactivity disorder, the dosage of medication was kept constant during the study as a constant dosage of medication for attention deficit hyperactivity disorder was considered unlikely to confound treatment effects. The Committees for Medical Ethics of Erasmus Medical Center and Leiden University Medical Center approved the study.

2.2. Procedure

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

Physiological assessments took place in the hospital between 12.00 h and 18.30 h, because variation of cortisol levels is least in the afternoon (Wust et al., 2000). After an acclimatization period of 45 min, the session began with a resting period of 10 min. Subsequently, a mental arithmetic task and, after another resting period of 10 min, a social competence interview were administered. Saliva collection took place after resting period 1 (cortisol during rest) and 20 min after the social competence interview (cortisol following stress), because cortisol levels typically peak 20–30 min after stress. Fig. 1 presents the temporal sequence of measures. Parents were asked to report general physical condition, dietary pattern and medication use of their child. For more information on medication use, see Appendix 1.

2.3. Measures

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

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

The *Children's Depression Inventory* (*CDI*) is an age-appropriate 27-item self-report questionnaire (Kovacs, 1992), administered at

Author's personal copy

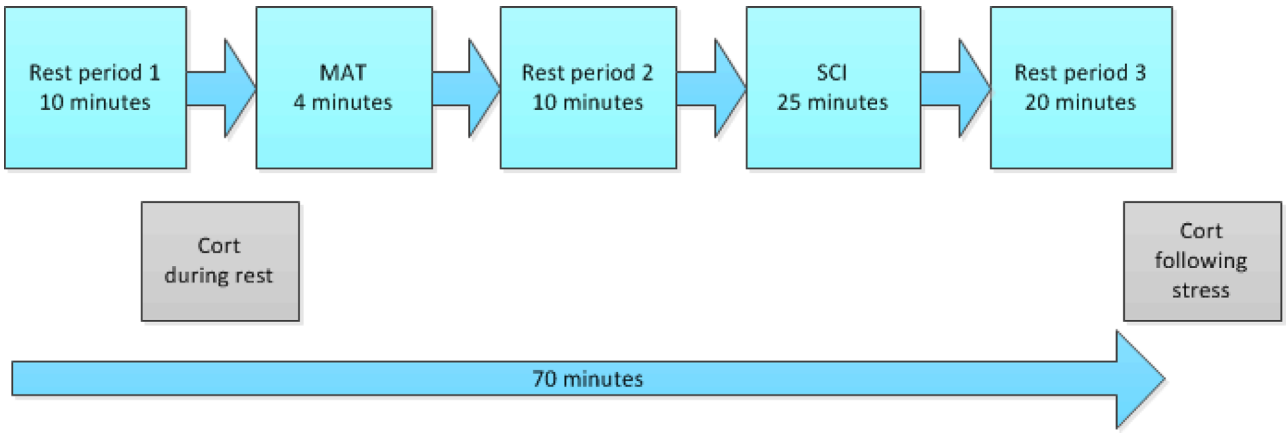


Fig. 1. Procedure. Note. MAT = mental arithmetic task, SCI = social competence interview, Cort = cortisol. Cort following stress: cortisol levels in response to stress typically peak about 20–30 min following a stressor.

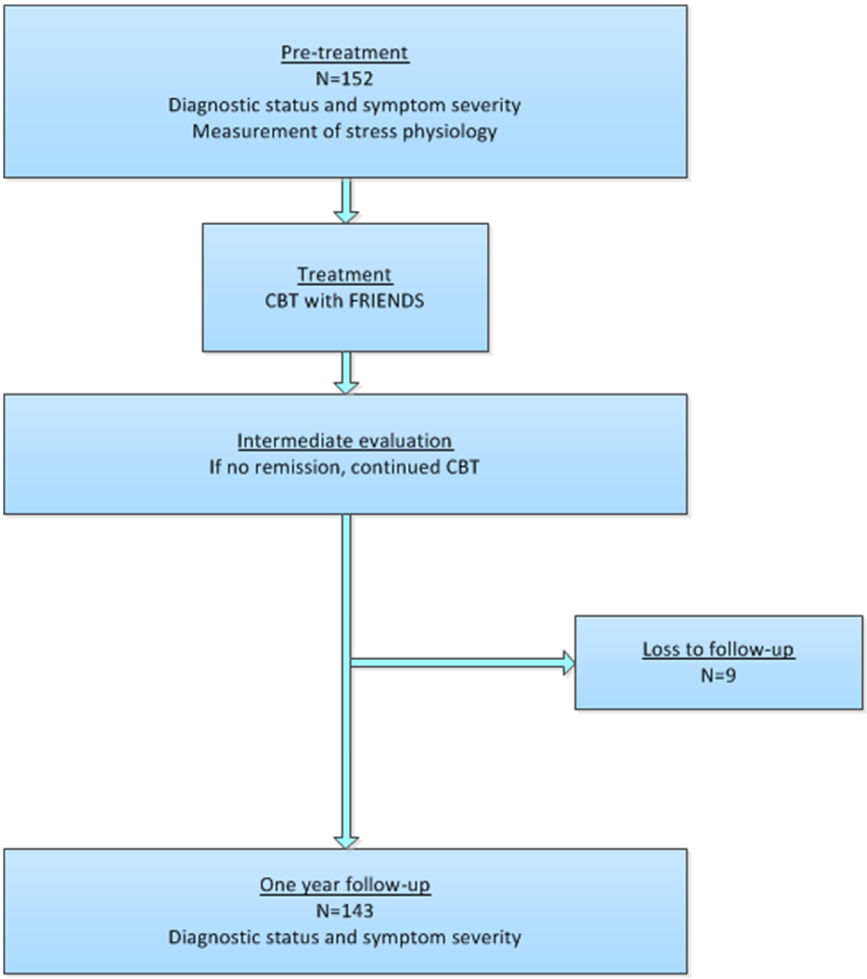


Fig. 2. Flowchart. Note. CBT = cognitive behavioral therapy.

pre-treatment, post-treatment and one-year follow-up, assessing depressive symptoms in the last two weeks in children and adolescents. Items are scored from 0 to 2 (0 = never true, 1 = sometimes true, 2 = always true). The Dutch translation showed good internal consistency for the total score (.82) (Liber et al., 2008), 1-month test-retest reliabilities (.72) are acceptable (Kovacs, 1981).

2.4. Treatment

All children participated in a standardized stepped-care CBT program for childhood anxiety disorders, consisting of two phases (van der Leeden et al., 2011). In the first phase, children were treated with the FRIENDS program, an evidence-based treatment

Author's personal copy

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

The present study is an extension of the data collection described by Liber et al. (2008) with an additional 25 children. Liber et al. studied the effects of individual versus group CBT in children up to 12 years of age, who attended primary school. The additional 25 children comprised 6 children who attended primary school but could not be randomized, because they refused assignment to group treatment (n=2), were absent at the start of the group (n=1), or were treated at an affiliated outpatient clinic near to home (n=3). The other 19 children, aged 12−13 years, attended the first grade of secondary school and received individual therapy because of practical reasons (time schedules differed between the different secondary schools). To summarize, all additional 25 children received individual treatment with the FRIENDS program. The efficacy of group CBT did not differ from the efficacy of individual CBT in children up to 12 years of age (Liber et al., 2008).

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

2.5. Stress tasks

The mental arithmetic task is a standardized laboratory stress task to induce measurable physiological changes (Kirschbaum et al., 1993). The mental arithmetic task lasted 4 min, during which the child was asked to subtract numbers as quickly and accurately as possible. If the child made a mistake or did not respond, he or she was required to start all over again. Dependent on the child's age, the child was asked to subtract 7 from 100 (< 12 years), or 23 from 1021 (≥ 12 years), and to continue this subtraction till no further subtraction was possible.

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

3. Physiological and hormonal measures

3.1. Cortisol assessment

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

3.2. Autonomic measures

During the experiment, continuous measurements were made of heart rate, respiration rate and skin conductance level (SCL) in order to assess ANS activity. For technical specifics see Ewart and Kolodner (1991).

3.3. Analysis of physiological data

A customised software program calculated the interbeat intervals of the ECG using R-top detection, resulting in interbeat interval time series during rest (minutes 7–10 of the first resting period; a 3 min stationary period) and stress (minutes 17–20 of the re-experiencing part of the social competence interview; a period without speaking and regular breathing). Interbeat interval time series were transformed to heart rate series. We conducted spectral analysis of these intervals using discrete Fourier transformation (van Steenis et al., 1994). For each time segment, we calculated mean heart rate and high frequency heart rate variability (HRV; .14–.50 Hz), the last parameter as a proxy for the parasympathetic component of autonomic cardiac control. Because HRV is strongly correlated with respiratory sinus arrhythmia (Kamath and Fallen, 1993), respiratory frequency was monitored and controlled for. Time segments with more spectral power for respiration in the mid frequency band than in the high frequency band were discarded from HRV analyses. This resulted in the removal of 1 subject for the respective analyses. Mean SCL levels were computed during rest and stress periods as parameters reflecting sympathetic ANS activity.

4. Data analysis

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

The focus of our analyses was on stress reactivity measures. To investigate if reactivity measures of autonomic and HPA axis functioning predicted anxiety symptoms at one-year follow-up, linear regression analyses were conducted for reactivity measures of SCL, HRV, heart rate and cortisol levels, separately. Anxiety

Author's personal copy

symptoms at one-year follow-up were entered as dependent variable. To control for possible effects of age, gender, pre-treatment anxiety symptoms and depressive symptoms at one-year follow-up, all were entered to the first model as independent variables. Subsequently, reactivity measures of SCL, HRV, heart rate and cortisol in response to the social competence interview were additionally entered in the second block as independent variables, all controlled for their respective rest measures. Second, these analyses were repeated separately for rest measures of SCL, HRV, heart rate and cortisol.

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

Effect sizes are reported as R squared, with.01 defined as a small effect size, .09 as a medium effect size and .25 as a large effect size (Cohen, 1988). A post hoc power calculation was performed to compute the achieved power given the final sample size and effect size. The achieved power was .71, based on an α-error probability of .05. All statistical analyses were performed with SPSS 21.0.

5. Results

5.1. Sample characteristics

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

Table 1
Sample characteristics.

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

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

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

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

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

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

The association of reactivity measures with depressive symptoms at one-year follow-up is presented in Table 4. As shown, there was a weak negative association of cortisol reactivity to stress with change of depressive symptoms at one-year follow-up. The effect size was small with a $R^{2\ change}$ of.03. In other words, lower cortisol reactivity was associated with less decrease in depressive symptoms at one-year follow-up. To check that our significant results were not due to medication effects, we did a secondary analysis controlled for medication use. The effect size and p-value were comparable ($R^{2\ change}$=.03, p=.063, N=96), which indicated that our findings were not influenced by the use of medication.

6. Discussion

6.1. Principal findings

This clinical study of pediatric anxiety disorders showed that children with higher pre-treatment SCL reactivity to stress, as a proxy of sympathetic reactivity, responded less to treatment. Their

Author's personal copy

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

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

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

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

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

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

anxiety symptoms decreased less over a one-year period as compared to children with a lower pre-treatment SCL reactivity. Furthermore, children with lower pre-treatment cortisol reactivity showed less decrease of depressive symptoms.

6.2. Autonomic nervous system

To our knowledge, this is the first longitudinal study that uses ANS functioning as a predictor of treatment outcome in children with an anxiety disorder. Heightened activity of the sympathetic nervous system is associated with anxiety disorders in children (Dieleman et al., 2015; Kossowsky et al., 2012; Schmitz et al., 2011). Our study showed that higher sympathetic reactivity in response to a stressor predicted less improvement in anxiety symptoms one year later. The autonomic nervous system regulates critical life functions on a moment-to-moment basis through its sympathetic and parasympathetic branches. To be able to respond to a threatening situation, the body prepares itself for fight or

Author's personal copy

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

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

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

flight. This autonomic activation leads to an increase in heart rate, blood pressure, sweat gland activity, and respiration. Subjectively, the individual feels tense and flushed, has palpitations, shortness of breath and increased perspiration. We carefully speculate that anxiety disordered children with a higher reactivity of the sympathetic nervous system, also experience more physiological and subjective arousal in response to daily fear or stress. As a consequence, higher sympathetic reactivity may influence the natural course of anxiety disorders or the efficacy of standardized CBT.

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

6.3. HPA-axis

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

Glucocorticoids can act both to augment and suppress sympathetically mediated changes in cardiovascular function, metabolism, and immune function. Normally, activation of these stress systems leads to behavioral and physical adaptive changes that improve an organism's ability to survive (Sapolsky, 2002). Possibly, anxiety disordered children with a less responsive HPA-axis to stress might be more susceptible to persistence of depressive symptoms, because a less adaptive HPA-axis leads to more difficulties in mobilizing energy resources to sufficiently cope with stress (Hankin et al., 2010). This might underlie the impaired effects of treatment strategies and, as a result, persistence of depressive symptoms.

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

6.4. Strengths and limitations

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

Author's personal copy

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

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

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

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

Appendix A

See Figs. A1 and A2.

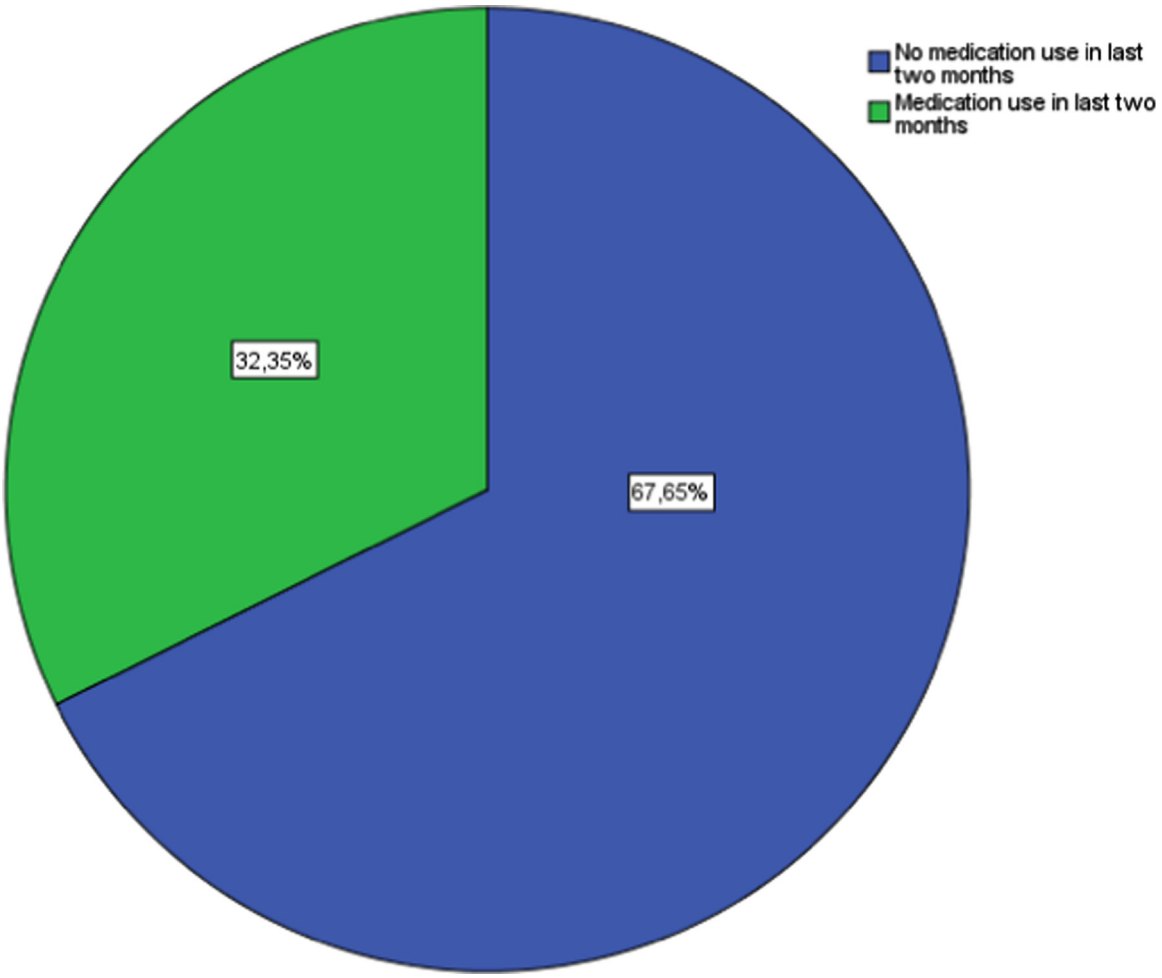


Fig. A1. Medication use during last two months.

Author's personal copy

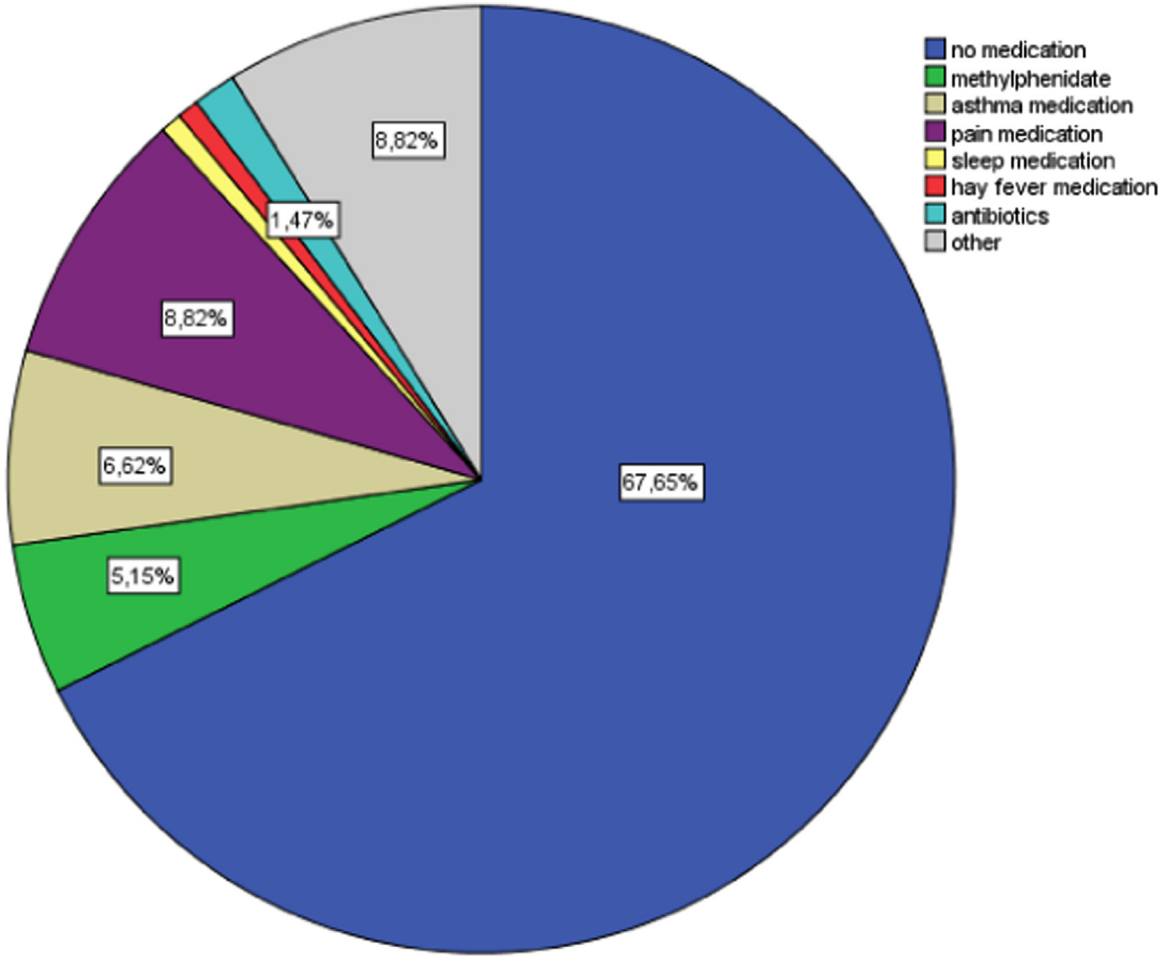


Fig. A2. Type of medication use in last two months.

References

Alpers, G.W., Sell, R., 2008. And yet they correlate: psychophysiological activation predicts self-report outcomes of exposure therapy in claustrophobia. J. Anxiety Disord. 22, 1101–1109.

Ballard, J.E., Boileau, R.A., Sleator, E.K., Massey, B.H., Sprague, R.L., 1976. Cardiovascular responses of Hyperactive children to methylphenidate. J. Am. Med. Assoc. 236, 2870–2874.

Barrett, P.M., Lowry-Webster, H., Turner, C., 2000. FRIENDS for Children. Group Leader Manual and Workbook for Children. Australian Academic Press, Bowen Hills, Queensland, Australia.

Beesdo-Baum, K., Knappe, S., 2012. Developmental epidemiology of anxiety disorders. Child. Adolesc. Psychiatr. Clin. N. Am. 21, 457–478.

Benjamin, C.L., Harrison, J.P., Settipani, C.A., Brodman, D.M., Kendall, P.C., 2013. Anxiety and related outcomes in young adults 7 to 19 years after receiving treatment for child anxiety. J. Consult. Clin. Psychol. 81, 865–876.

Bittner, A., Egger, H.L., Erkanli, A., Jane Costello, E., Foley, D.L., Angold, A., 2007. What do childhood anxiety disorders predict? J. Child. Psychol. Psychiatry 48, 1174–1183.

Bornas, X., Llabrrés, J., Tortella-Feliu, M., Fullana, M.A., Montoya, P., Lopez, A., Noguera, M., Gelabert, J.M., 2007. Vagally mediated heart rate variability and heart rate entropy as predictors of treatment outcome in flight phobia. Biol. Psychol. 76, 188–195.

Burke, H.M., Davis, M.C., Otte, C., Mohr, D.C., 2005. Depression and cortisol responses to psychological stress: a meta-analysis. Psychoneuroendocrinology 30, 846–856.

Cohen, J., 1988. Statistical power Analysis for the Behavioral Sciences, 2nd ed. Erlbaum, Hillsdale, NJ.

Compton, S.N., Peris, T.S., Almirall, D., Birmaher, B., Sherrill, J., Kendall, P.C., March, J.S., Gosch, E.A., Ginsburg, G.S., Rynn, M.A., Piacentini, J.C., McCracken, J.T., Keeton, C.P., Suveg, C.M., Aschenbrand, S.G., Sakolsky, D., Iyengar, S., Walkup, J.T., Albano, A.M., 2014. Predictors and moderators of treatment response in childhood anxiety disorders: results from the CAMS trial. J. Consult. Clin. Psychol. 82, 212–224.

Davies, C.D., Niles, A.N., Pittig, A., Arch, J.J., Craske, M.G., 2015. Physiological and behavioral indices of emotion dysregulation as predictors of outcome from cognitive behavioral therapy and acceptance and commitment therapy for anxiety. J. Behav. Ther. Exp. Psychiatry 46, 35–43.

Dieleman, G.C., van der Ende, J., Verhulst, F.C., Huizink, A.C., 2010. Perceived and physiological arousal during a stress task: can they differentiate between anxiety and depression? Psychoneuroendocrinology 35, 1223–1234.

Dieleman, G.C., Huizink, A.C., Tulen, J.H., Utens, E.M., Creemers, H.E., van der Ende, J., Verhulst, F.C., 2015. Alterations in HPA-axis and autonomic nervous system functioning in childhood anxiety disorders point to a chronic stress hypothesis. Psychoneuroendocrinology 51, 135–150.

Dietrich, A., Ormel, J., Buitelaar, J.K., Verhulst, F.C., Hoekstra, P.J., Hartman, C.A., 2013. Cortisol in the morning and dimensions of anxiety, depression, and aggression in children from a general population and clinic-referred cohort: an integrated analysis. TRAILS Study Psychoneuroendocrinol. 38, 1281–1298.

Ewart, C.K., Kolodner, K.B., 1991. Social competence interview for assessing physiological reactivity in adolescents. Psychosom. Med. 53, 289–304.

Ewart, C.K., Kolodner, K.B., 1993. Predicting ambulatory blood pressure during school: effectiveness of social and nonsocial reactivity tasks in black and white adolescents. Psychophysiology 30, 30–38.

Ewart, C.K., Jorgensen, R.S., Kolodner, K.B., 1998. Sociotropic cognition moderates blood pressure response to interpersonal stress in high-risk adolescent girls. Int. J. Psychophysiol. 28, 131–142.

Feder, A., Coplan, J.D., Goetz, R.R., Mathew, S.J., Pine, D.S., Dahl, R.E., Ryan, N.D., Greenwald, S., Weissman, M.M., 2004. Twenty-four-hour cortisol secretion patterns in prepubertal children with anxiety or depressive disorders. Biol. Psychiatry 56, 198–204.

Forbes, E.E., Williamson, D.E., Ryan, N.D., Birmaher, B., Axelson, D.A., Dahl, R.E., 2006. Peri-sleep-onset cortisol levels in children and adolescents with affective disorders. Biol. Psychiatry 59, 24–30.

Gunnar, M.R., Vazquez, D.M., 2001. Low cortisol and a flattening of expected daytime rhythm: potential indices of risk in Human development. Dev. Psychopathol. 13, 515–538.

Harkness, K.L., Stewart, J.G., Wynne-Edwards, K.E., 2011. Cortisol reactivity to social stress in adolescents: role of depression severity and child maltreatment. Psychoneuroendocrinology 36, 173–181.

James, A.C., James, G., Cowdrey, F.A., Soler, A., Choke, A., 2013. Cognitive behavioural therapy for anxiety disorders in children and adolescents. Cochrane Database Syst. Rev. 6, CD004690.

Hankin, B.L., Badanes, L.S., Abela, J.R., Watamura, S.E., 2010. Hypothalamic-Pituitary-Adrenal axis dysregulation in dysphoric children and adolescents: cortisol

Author's personal copy

reactivity to psychosocial stress from preschool through middle adolescence. Biol. Psychiatry 68, 484–490.
Herman, J.P., Ostrander, M.M., Mueller, N.K., Figueiredo, H., 2005. Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. Prog. Neuropsychopharmacol. Biol. Psychiatry 29, 1201–1213.
Hudson, J.L., Lester, K.J., Lewis, C.M., Tropeano, M., Creswell, C., Collier, D.A., Cooper, P., Lyneham, H.J., Morris, T., Rapee, R.M., Roberts, S., Donald, J.A., Eley, T.C., 2013. Predicting outcomes following cognitive behaviour therapy in child anxiety disorders: the influence of genetic, demographic and clinical information. J. Child. Psychol. Psychiatry 54, 1086–1094.
Jongerden, L., Simon, E., Bodden, D.H., Dirksen, C.D., Bögels, S.M., 2015. Factors associated with the referral of anxious children to mental health care: the influence of family functioning, parenting, parental anxiety and child impairment. Int. J. Methods Psychiatr. Res. 24, 46–57.
Juster, R.P., McEwen, B.S., Lupien, S.J., 2010. Allostatic load biomarkers of chronic stress and impact on health and cognition. Neurosci. Biobehav. Rev. 35, 2–16.
Kamath, M.V., Fallen, E.L., 1993. Power spectral analysis of heart rate variability: a noninvasive signature of cardiac autonomic function. Crit. Rev. Biomed. Eng. 21, 245–311.
Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1993. The 'Trier social stress test'-a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28, 76–81.
Klasen, F., Otto, C., Kriston, L., Patalay, P., Schlack, R., Ravens-Sieberer, U., BELLA study group, 2015. Risk and protective factors for the development of depressive symptoms in children and adolescents: results of the longitudinal BELLA study. Eur. Child. Adolesc. Psychiatry 24, 695–703.
Kossowsky, J., Wilhelm, F.H., Roth, W.T., Schneider, S., 2012. Separation anxiety disorder in children: disorder-specific responses to experimental separation from the mother. J. Child. Psychol. Psychiatry 53, 178–187.
Kovacs, M., 1981. Rating scales to assess depression in school-aged children. Acta Paedopsychiatr. 46, 305–315.
Kovacs, M., 1992. Children's Depression Inventory (CDI); Manual. Multi-Health Systems, North Tonawanda, NY.
Krämer, M., Seefeldt, W.L., Heinrichs, N., Tuschen-Caffier, B., Schmitz, J., Wolf, O.T., Blechert, J., 2012. Subjective, autonomic, and endocrine reactivity during social stress in children with social phobia. J. Abnorm. Child. Psychol. 40, 95–104.
Kristensen, H., Oerbeck, B., Torgersen, H.S., Hansen, B.H., Wyller, V.B., 2014. Somatic symptoms in children with anxiety disorders: an exploratory cross-sectional study of the relationship between subjective and objective measures. Eur. Child. Adolesc. Psychiatry 23, 795–803.
Last, C.G., Hansen, C., Franco, N., 1998. Cognitive-behavioral treatment of school phobia. J. Am. Acad. Child. Adolesc. Psychiatry 37, 404–411.
Legerstee, J.S., Huizink, A.C., Gastel, W., van, Liber, J.M., Treffers, P.D., Verhulst, F.C., Utens, E.M., 2008. Maternal anxiety predicts favourable treatment outcomes in anxiety-disordered adolescents. Acta Psychiatr. Scand. 117, 289–298.
Lester, K.J., Eley, T.C., 2013. Therapygenetics: using genetic markers to predict response to psychological treatment for mood and anxiety disorders. Biol. Mood Anxiety Disord. 3, 4.
Liber, J.M., Van Widenfelt, B.M., Utens, E.M.W.J., Ferdinand, R.F., Van Der Leeden, A.J.M., Van Gastel, W., Treffers, P.D.A., 2008. No differences between group versus individual treatment of childhood anxiety disorders in a randomised clinical trial. J. Child. Psychol. Psychiatry 49, 886–993.
Liber, J.M., Widenfelt, B.M., van, Leeden, A.J., van der, Goedhart, A.W., Utens, E.M., Treffers, P.D., 2010. The relation of severity and comorbidity to treatment outcome with cognitive behavioral therapy for childhood anxiety disorders. J. Abnorm. Child. Psychol. 38, 683–694.
Lopez-Duran, N.L., Kovacs, M., George, C.J., 2009. Hypothalamic-pituitary-adrenal axis dysregulation in depressed children and adolescents: a meta-analysis. Psychoneuroendocrinology 34, 1272–1283.
Luby, J.L., Heffelfinger, A., Mrakotsky, C., Brown, K., Hessler, M., Spitznagel, E., 2003. Alterations in stress cortisol reactivity in depressed preschoolers relative to psychiatric and no-disorder comparison groups. Arch. Gen. Psychiatry 60, 1248–1255.
March, J.S., Parker, J.D., Sullivan, K., Stallings, P., Conners, C.K., 1997. The multidimensional anxiety scale for children (MASC): factor structure, reliability, and validity. J. Am. Acad. Child. Adolesc. Psychiatry 36, 554–565.
Pervanidou, P., 2008. Biology of post-traumatic stress disorder in childhood and adolescence. J. Neuroendocrinol. 20, 632–638.
Sapolsky, R.M., 2002. Endocrinology of the Stress-Response. In: Becker, J.B., Breedlove, S.M., Crews, D., McCarthy, M.M. (Eds.), Behavioral Endocrinology.. The MIT Press, Cambridge, pp. 409–451.
Schmitz, J., Krämer, M., Tuschen-Caffier, B., Heinrichs, N., Blechert, J., 2011. Restricted autonomic flexibility in children with social phobia. J. Child. Psychol. Psychiatry 52, 1203–1211.
Shortt, A.L., Barrett, P.M., Fox, T.L., 2001. Evaluating the FRIENDS program: a cognitive-behavioral group treatment for anxious children and their parents. J. Clin. Child. Psychol. 30, 525–535.
Silverman, W.K., 1996. The Clinician Manual for the Anxiety Disorders Interview Schedule for DSM-IV: Child Version. In: Albano, A.M. (Ed.), 1996. The Psychological Corporation, San Antonio.
Silverman, W.K., Saavedra, L.M., Pina, A.A., 2001. Test-retest reliability of anxiety symptoms and diagnoses with the anxiety disorders interview schedule for DSM-IV: child and parent versions. J. Am. Acad. Child. Adolesc. Psychiatry 40, 937–944.
Suzuki, H., Belden, A.C., Spitznagel, E., Dietrich, R., Luby, J.L., 2013. Blunted stress cortisol reactivity and failure to acclimate to familiar stress in depressed and sub-syndromal children. Psychiatry Res. 210, 575–583.
van der Leeden, A.J., van Widenfelt, B.M., van der Leeden, R., Liber, J.M., Utens, E.M., Treffers, P.D., 2011. Stepped care cognitive behavioural therapy for children with anxiety disorders: a new treatment approach. Behav. Cogn. Psychother. 39, 55–75.
van Steenis, H.G., Tulen, J.H., Mulder, L.J., 1994. Heart rate variability spectra based on non-equidistant sampling: the spectrum of counts and the instantaneous heart rate spectrum. Med. Eng. Phys. 16, 355–362.
Verhulst, F.C., Ende, J. van der, Ferdinand, R.F., Kasius, M.C., 1997. The prevalence of DSM-III-R diagnoses in a national sample of Dutch adolescents. Arch. Gen. Psychiatry 54, 329–336.
Woodward, L.J., Fergusson, D.M., 2001. Life course outcomes of young people with anxiety disorders in adolescence. J. Am. Acad. Child. Adolesc. Psychiatry 40, 1086–1093.
Wust, S., Wolf, J., Hellhammer, D.H., Federenko, I., Schommer, N., Kirschbaum, C., 2000. The cortisol awakening response-normal values and confounds. Noise Health 2, 79–88.