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

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ABSTRACT

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

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

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

Conclusions: Some evidence was found in support of the tripartite model. Our findings indicate that perceived arousal to a challenge might be a useful tool to assess the PH component of the tripartite model. Reactive HPA-axis functioning might be able to differentiate between anxiety and depressive problems in children in a general population sample, but effect sizes are small and replication is needed.

INTRODUCTION

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

An essential step forward for research in this field is to better define anxiety and depression in children. Much evidence has shown that anxiety and depressive symptoms in children and adolescents occur frequently and are often comorbid, with comorbidity rates ranging from 21 to 54% in population based studies (e.g. Essau, Conradt, Petermann, 2000; Costello et al., 2003; Ferdinand et al., 2005). Anxiety and depression might be two different valid constructs that often co-occur, or they could be different manifestations of the same underlying vulnerability. There are many models that have tried to disentangle these constructs. An interesting theoretical framework to address this question is the tripartite model, proposed by Clark and Watson (1991) and Watson et al. (1995a,b) in which symptoms of anxiety and depression are viewed along three dimensions. This model groups symptoms of depression and anxiety into 3 subtypes: negative affectivitiy (NA), a measure for general affective distress, positive affectivity (PA), a measure representing pleasurable engagement with the environment, and physiological hyperarousal (PH), a measure representing somatic tension and arousal, e.g. racing heart, sweaty palms and dry mouth. NA is associated with both anxiety and depression. A lack of PA, is hypothesized to be specifically associated with depression, while the third component, PH, is hypothesized to be specific for anxiety (Watson et al., 1995a,b). Research regarding the validity of the tripartite model in child and adolescent, clinical and general population samples also supports a three-factor structure (e.g. Joiner, Catanzaro, Laurent, 1996; Chorpita, Daleiden, 2002; Turner, Barrett, 2003; Cannon, Weems, 2006).

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

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

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

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

Anxiety and depressive symptoms are highly comorbid, but most previous studies did not adjust for the effect of comorbidity on HPA-axis functioning. Only one study addressed

comorbidity in children. Young, Abelson and Cameron (2004) found that only depressed children with a comorbid anxiety disorder showed an exaggerated adrenocorticotropic hormone (ACTH) response and a similar but non-significant effect of a cortisol to a stressor. Subjects with pure mood or anxiety disorders showed normal ACTH and cortisol responses to a stressor.

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

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

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

METHODS

Participants

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

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

This article focuses on children in this age group because beyond the age of 12 years hormonal changes due to the onset of puberty might have an effect on HPA-axis functioning (Matchock, Dorn, Susman, 2007) and this results in a less homogeneous sample. Furthermore, the stress test we used is not applicable below the age of 8 years. To examine possible selective attrition, a stepwise logistic regression analysis was performed with 'participation yes or no' as a dependent variable, and age, sex, socioeconomic status (SES) and the internalizing and externalizing problem scales on the CBCL as possible predictors. Sex, age and the externalizing and internalizing problem scales of the CBCL did not predict attrition. However, lower SES predicted attrition significantly (model $\chi 2 = 8.621$, d.f. = 2, $p = .013$). Cox and Snell R-square of the regression model was .029, which indicated that the effect of SES was small. Furthermore the level of psychopathology at the initial assessment, assessed with the externalizing and internalizing problem scales of the CBCL, was not associated with cooperation.

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

Procedure

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

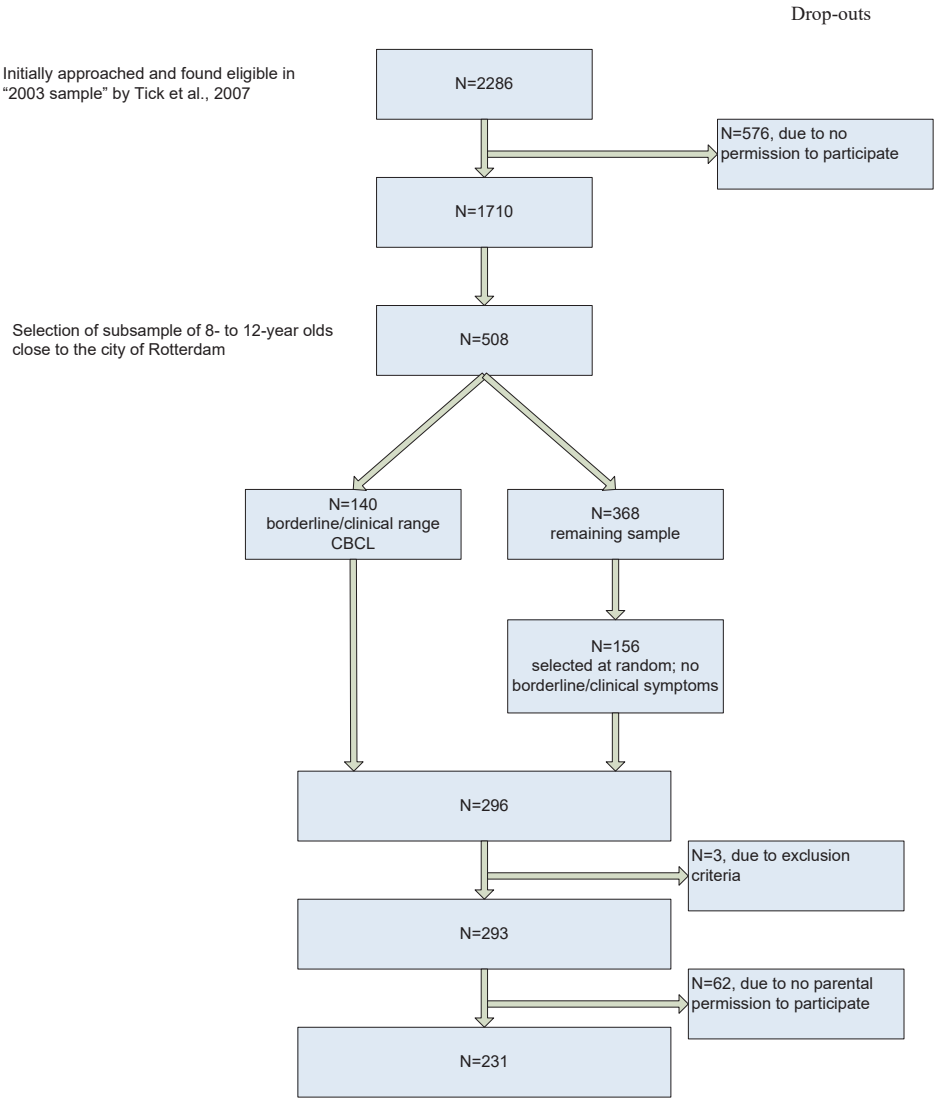

Figure 1. Sample selection
Note. CBCL = Child Behavior Checklist

Participants were instructed to fill the tubes till a marker (at 500 µl). All samples were collected on a regular school day, stored in the freezer at home, and taken to the outpatient department 1 day later, when the physiological assessment took place. Children and parents received a written instruction with drawings and received another instruction by telephone the day before sampling to increase the reliability of cortisol sampling at home. Saliva samples were stored at –20 °C before analysis at the laboratory. Parents were asked

to register general physical condition, activity levels (e.g. sports), consumption pattern (e.g. smoking, alcoholic beverages and caffeine intake), and medication use of the participants.

The physiological assessment took place one day after saliva collection at home. Participants were seated comfortably in a light laboratory room where temperature and humidity levels were kept constant. All assessments took part in the afternoon (between 12 p.m. and 6.30 p.m.). Lunch was eaten before assessment; the gap between lunch and baseline saliva samples was at least 1.5 h. After a rest period of 45 min, the session began with a baseline period of 10 min in which the participant was asked to sit still and relax. Subsequently, a stress test consisting of three subtasks; a mental arithmetic task (MAT) (4 min), a public speaking task (PST) (8 min speech preparation and 6 min public speaking) and a computer task (CT) (5 min) were administered. The public speaking task involved providing the child with a story scenario and then giving him/her 8 min to develop a story (Speech Preparation Period). Then the child had to deliver the speech for 6 min (Speech Period), this was followed by a computer task during 5 min. The story stem used was one in which the child was erroneously accused of stealing and had to describe to a "guard" why they were innocent. During the computer task children had to calculate by heart in how many steps they would be able to order four numbers from lowest through highest. They were told they had to act as quickly as possible and to make as little mistakes as possible. The public speaking task was videotaped and the participant was told that the computer task and the public speaking task were evaluated afterwards. Furthermore, for all tasks there was a time constraint.

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

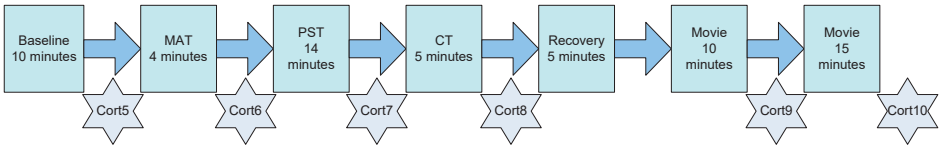

Figure 2. Cortisol sampling
Note. Cort5 = cortisol at the end of the baseline period, Cort6 = cortisol after the mental arithmetic task (MAT), Cort7 = cortisol after the public speaking task (PST), Cort8 = cortisol after the computer task (CT), Cort9 = cortisol 15 minutes after the stress task, Cort10 = cortisol 30 minutes after the stress task

Measures

Child behavior checklist

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

Multidimensional Anxiety Scale for Children

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

Children's Depression Inventory

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

Physiological Arousal Questionnaire (PAQ)

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

Table 1. PAQ items

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

Cortisol assessment

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

In addition to the separate cortisol variables, we chose to use composite cortisol variables to analyze the diurnal and stress cortisol profile. To characterize the diurnal cortisol profile we calculated three composite measures according to Pruessner et al. (2003): cortisol awakening rise with respect to ground (CAR_g) and with respect to increase (CAR_i) and the area under the curve with respect to ground (AUC_g) (Rosmalen et al., 2005). An area under the curve with respect to ground, such as CAR_g or AUC_g, is a measure that represents the total amount of cortisol that is produced during a specific period and is calculated by computing the area between zero and the curve that is constituted by the respective cortisol levels (Pruessner et al., 2003). In the case of CAR_i, the distance from zero is ignored, thereby emphasizing changes over time (Pruessner et al., 2003). To characterize the cortisol profile during stress the area under the curve with respect to ground and increase (respectively AUC_{sg} and AUC_{si}) were calculated using cortisol samples representing baseline (Cort5 and Cort6), representing stress (Cort7, Cort8 and Cort9) and representing recovery (Cort10). For formulas see Appendix A. If one of the cortisol samples was missing, an area under the curve could not be calculated; therefore N=19 AUC_{sg} and N=10 AUC_g were missing.

Data analysis

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

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

First, to investigate if the level of anxiety predicted basal HPA-axis functioning nine separate stepwise regression analyses were conducted. CAR_g, CAR_i, AUC_g, AUC_{sg}, AUC_{si}, Cort4, Cort6, Cort8 and Cort10 were entered in nine separate regression analyses as dependent variables. Age, gender and BMI might confound the cortisol-psychopathology relationship (e.g. see Rosmalen et al., 2005 for an overview). To control for possible effects of age, gender

and BMI, these variables were first entered as a block into the model as independent variables. MASC Total score was entered in a second block as predictor. Nine similar regression analyses were conducted with the log-transformed CDI Total score as independent variable.

Second, to investigate if the level of anxiety predicted perceived arousal, three separate stepwise regression analyses were conducted. PAQ Total score before, during and after the stress test were entered as dependent variables and the MASC Total score was entered as predictor. To control for possible effects of age and gender, these variables were first added to the model as independent variables. Due to overlap between items of the MASC Physical Anxiety Scale and the PAQ, the correlation between the PAQ Total score was calculated. Furthermore the regression analyses were repeated for the MASC Total Score minus the scores on the MASC Phsycical Anxiety Scale. Three similar regression analyses were conducted with the log-transformed CDI Total score as independent variable.

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

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

RESULTS

Descriptives

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

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

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

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

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

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

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

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

Linear regressions

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DISCUSSION

Principal findings

PH as defined by the model of Clark and Watson is a concept based on anxiety and depression questionnaires. This concept is a rather virtual concept that has no quantifiable substrates; therefore it is difficult to assess the usefulness and significance of this model. Furthermore, the model by Clark and Watson is a model that is frequently cited with regard to the disentanglement of anxiety and depression as different constructs. In our opinion it is interesting, although complicated, to view our results with respect to the tripartite model by Clark and Watson; it contributes to the discussion of how the model is constructed and why there are so many versions of it. This study attempted to quantify this concept by using two levels of arousal, a subjective and a physiological, in a specific situation (i.e. in basal conditions and during a stresstask) in order to make it more tangible and applicable. We examined whether, in a general population sample of children, basal and reactive HPA-axis functioning, as a proxy for PH, and perceived arousal before, during and after stress could differentiate anxious from depressive children.

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

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

HPA-axis

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

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

Perceived arousal to a stressor

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

Limitations and strengths

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

The present study tested the validity of the PH component of the tripartite model in a general population sample. Therefore, in comparison to research in clinical samples, results can be generalized due to the lack of selection bias. However, originally, the tripartite model was developed with the use of data from a clinical sample (Clark, Watson, 1991). Clinical and

general population samples might differ in impairment and severity and duration of symptoms. Therefore, the findings found in the present study cannot be generalized to clinical samples. This limitation is confirmed by the post-hoc analyses which show that exceeding the clinical cut-off score of internalizing problems on the CBCL moderates the relationship between perceived arousal and anxiety and depressive symptoms. Furthermore, relations that were not found in this study – for instance, the relation between HPA-axis functioning and anxiety problems- might be established in a clinical sample due to reasons mentioned above. Therefore replication in a clinical sample is needed.

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

In the present study, we used only the child as an informant to assess anxiety and depressive symptoms. One could argue that the use of multiple informants (e.g. parents or teachers) would lead to a more valid assessment of these symptoms (Grills, Ollendick, 2003; Comer, Kendall, 2004). However, multiple informant agreement is higher for externalizing than for internalizing problems; a possible explanation for this phenomenon can be that internalizing problems tend to be inwardly focused upon the self (Grills, Ollendick, 2003). Therefore, self-report questionnaires are valid tools to assess internalizing problems in children and adolescents.

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

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

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

in our study, does not necessarily mean that HPA-axis functioning is not altered in anxious or depressed subjects. These alterations might occur on different levels in the HPA-axis.

CONCLUSION

This study found some evidence in support of the tripartite model of Clark and Watson (1991). Together, our findings indicate that perceived arousal to a challenge might be a useful tool to assess the PH component of the tripartite model in a general population sample of school-aged children. HPA-axis functioning measured by cortisol in stress conditions might differentiate between anxiety and depression, but this result is not very strong and needs replication in a general population sample.

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

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SUPPLEMENTARY MATERIAL

Formulas

The CAR_g and CAR_i were calculated using the following formulas:

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

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

The formula to calculate AUC_g was:

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

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

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

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

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