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Disruptive behaviors and HPA-axis activity in young adolescent boys and girls from the general population

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Abstract

It is important to investigate associations between biological factors and disruptive behaviors in children and adolescents. Antisocial, aggressive, and criminal behaviors in adults often begin early in life. Disruptive behaviors are often thought to be associated with low activity of the hypothalamic–pituitary–adrenal (HPA) axis. Cortisol, the end-product of this axis, can be measured to investigate HPA-axis activity. Previous studies on this topic concerned clinical or high risk samples. The aim of the present study was to investigate to which extent HPA-axis functioning plays a role in disruptive behaviors in pre-adolescents from the general population. One thousand seven hundred and sixty eight 10- to 12-year-olds from the Dutch general population were investigated. Disruptive behaviors were assessed with the Child Behavior Checklist, the Youth Self-Report, and the Antisocial Behavior Questionnaire. Baseline morning and evening salivary cortisol levels were assessed. Unexpectedly, small associations were found between disruptive behaviors, including attention problems, and *higher* cortisol levels. However, all effect sizes of significant effects were very small. Our study indicated that HPA-axis functioning may be more relevant in clinical or high risk samples than at the general population level. The association between HPA-axis functioning and attention problems, that has gotten less attention than that with aggressive or delinquent behaviors, requires further research. Furthermore, because effect sizes were relatively small, it can be concluded that, in pre-adolescence, the measures of baseline HPA-axis functioning that were used for the present study can not be used as biological markers for disruptive behaviors.

Keywords: HPA-axis; Salivary cortisol; Disruptive behaviors; Pre-adolescence; General population

1. Introduction

It is important to investigate associations between biological factors and disruptive behaviors in children and adolescents, because antisocial, aggressive, and criminal behaviors often have their onset early in life (Moffitt, 1993). Disruptive behaviors in children and adolescents are often thought to be associated with low activity of the hypothalamic–pituitary–adrenal (HPA) axis (Van Goozen et al., 2000; McBurnett et al., 2000; Raine, 1993, 1996). Cortisol, the end-product of this axis, is often measured to investigate HPA-axis activity. It is obvious why HPA-axis functioning and antisocial behaviors are often mentioned in the same breath. Two influential theories have postulated an association between disruptive behaviors and low arousal (Raine, 1996). According to the first, the fearlessness theory, a low tendency to become aroused in reaction to fearful stimuli would result in a higher likelihood to become disruptive (Raine, 1993). The immediate fear reaction

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(increased heart rate, blood pressure, sweat production, etc., within seconds) is mediated by sympathetic nervous system activity. The somewhat postponed fear reaction, meant to enable an individual to resist long-term environmental stresses, is mediated by the HPA-axis. Hence, based on the fearlessness theory, an association between high disruptive behavior levels and low HPA-axis activity could be expected (Van Goozen et al., 2000).

A second important theory is the sensation-seeking theory (Eysenck, 1964; Quay, 1965; Raine, 1993; Zuckerman and Neeb, 1979). This theory hypothesizes that low arousal is an unpleasant physiological state. To get rid of this state, individuals with low arousal levels would seek stimulation, for instance by initiating antisocial behaviors that increase physical tension. It could be argued that sensation seeking activities would mainly help to temporarily obtain a higher sympathetic arousal level, and would not induce higher HPA-axis activity. However, mutual functional connections exist between the sympathetic nervous system and the HPA-axis (Chrousos and Gold, 1998). For instance, sympathetic activation results in higher production of corticotropin-releasing factor (CRF) in the hypothalamus (Calogero et al., 1988), which ultimately induces cortisol production. Vice versa, CRF may stimulate noradrenergic neurons as well (Sapolsky et al., 1986). Hence, individuals with low sympathetic arousal levels, who may tend to seek sensation, may display low HPA-axis activity as well.

Several studies found low basal HPA-axis activity in disruptive individuals (Vanyukov et al., 1993; Moss et al., 1995; Van Goozen et al., 1998; McBurnett et al., 2000; Pajer et al., 2001; Kariyawasam et al., 2002; Shoal et al., 2003; Van de Wiel et al., 2004). McBurnett et al. (2000) found evidence for an association between low salivary cortisol levels and high symptom levels in 38 referred 7- to 12-year-old boys with conduct disorder. A single saliva cortisol sample – time of sampling was not standardized – was obtained during two visits to the clinic. Vanyukov et al. (1993) studied a high-risk sample of 78 10- to 12-year-old sons of fathers with addiction problems. Low saliva cortisol concentrations – assessed at 9 a.m. – were associated with high levels of conduct problems. Pajer et al. (2001) found lower morning basal plasma cortisol levels in 47 15- to 17- year-old girls with conduct disorder than in 37 control girls from the community. However, there are also studies reporting a lack of associations (Dabbs et al., 1991; Stoff et al., 1992; Scerbo and Kolko, 1994; Schulz et al., 1997; Jansen et al., 1999; Van Goozen et al., 2000; Snoek et al., 2002; Oosterlaan et al., 2005). All in all, evidence for low basal cortisol in children with disruptive behavior problems is inconsistent.

Previous studies mainly concerned relatively small samples, and some suffered from methodological problems with cortisol measurements, such as the fact that cortisol levels were not assessed at a standardized time point during the day (McBurnett et al., 2000), despite the abundant knowledge we have about diurnal fluctuations (Pruessner et al., 1997; Weitzman et al., 1971; Wüst et al., 2000). However, an even more important methodological obstacle is the fact that previous studies mainly investigated clinical or high risk samples, and did not address the importance of HPA-axis functioning as a possible correlate of disruptive behaviors in the general population. Hence, important evidence that may help us to understand etiological mechanisms that determine the occurrence of disruptive behaviors at the level of the general population is lacking. Of course, it would be valuable to gather empirical data regarding the HPA-axis–disruptive behavior association in the general population. If the association that was found in clinical and high risk samples would be confirmed in the general population, this would help us to formulate further hypotheses regarding the mechanisms that might explain this association. Further, the usefulness of early assessment of HPA-axis functioning, for the purpose of early detection of those who are at risk for future adverse development, should be tested as a next step. However, if an association between disruptive behaviors and low HPA-axis activity would not be confirmed in the general population, this would indicate that efforts to reveal putative etiological mechanisms should be made in other directions.

Another area that received too little attention thus far is HPA-axis functioning in girls with disruptive behaviors. Although lower than in boys, the prevalence of disruptive behavior problems in girls is not negligible (Côté et al., 2001; Tremblay et al., 1992). Cortisol levels are associated with pubertal stage (Keiss et al., 1995), and gonadal steroids interact with HPA-axis functioning (Burgess and Handa, 1992; Handa et al., 1994; Roy et al., 1999; Vamvakopoulos and Chrousos, 1993). Hence, associations between disruptive behaviors and HPA-axis functioning might be different in girls than in boys. Studies aimed at revealing etiological mechanisms, in our opinion, are equally important for both sexes. Given the paucity of empirical data on this topic in girls, studies filling this gap are needed.

The aim of the present study was to investigate if high levels of disruptive behaviors are indeed associated with low baseline HPA-axis activity. More specifically, the present study tested if the association between disruptive behaviors and HPA-axis functioning, as previously found in small high risk or clinical samples that mainly consisted of boys, could be confirmed in a large representative general population sample of 10- to 12-year-olds, that did not only contain males, but females as well.

2. Materials and methods

2.1. Sample and procedure

This study was part of the TRacking Adolescents' Individual Lives Survey (TRAILS) study. The target sample of TRAILS consisted of 10- to 12-year-olds from five municipalities in the North of the Netherlands, that includes urban and rural areas, who were assessed between March 2001 and July 2002. Of all eligible individuals ($N = 2935$), 76.0% participated in the study ($N = 2230$, mean age

11.09 years, SD .55, 50.8% (1132) girls, 15.3% (341) single parent families, 9.0% (201) participants without siblings, 7.9% (176) used mental health services). Participants did not differ from those who refused with respect to the proportion of single parent families (15.3% versus 16.5%), and the prevalence of teacher-rated problem behaviors measured with vignettes of the Teacher Report Form (internalizing 22.0% versus 25.1%; externalizing 13.0% versus 14.4%). This supported the representativeness of the TRAILS sample (De Winter et al., 2005).

Saliva samples were received from 1768 children (79.3% of all TRAILS participants). Those who did not provide saliva samples did not differ from those who did with respect to gender (48.5% male versus 49.4% male, $\chi^2(df = 1) = 0.13$; $p = .72$), pubertal development (average Tanner stage score = 1.92 versus 1.86, $t = -1.39$; $p = .16$), or levels of disruptive behaviors (CBCL ADH Problems scale $t = 1.40$; $p = .16$, OD Problems scale $t = .14$; $p = .89$, CD Problems scale $t = .21$; $p = .83$, YSR ADH Problems scale $t = -1.20$; $p = .23$, OD Problems scale $t = -.36$; $p = .72$, CD Problems scale $t = 1.10$; $p = .27$). However, those who did not provide saliva samples were slightly older (11.16 years versus 11.08 years, $t = -3.08$; $p = .002$), had a higher BMI (18.50 versus 17.92 kg/m^2, $t = -3.22$; $p = .001$), and had a slightly higher ASBQ total score $t = 3.23$; $p = .001$; explained variance = .7%). However, given the fact that the differences between those who provided saliva samples versus those who did not were very small, the sub-sample that was used for the present manuscript can be regarded as representative of the TRAILS sample at large.

2.2. Measures

The *Child Behavior Checklist* (CBCL; Achenbach, 1991a) is a parent questionnaire for assessing problems in 6- to 18-year-olds. The *Youth Self-Report* (YSR; Achenbach, 1991b) is a self-report questionnaire that was modeled on the CBCL. The questionnaires contain – respectively – 113 and 112 items on behavioral or emotional problems in the past six 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 American version of the CBCL and YSR were confirmed for the Dutch translations (De Groot et al., 1994; Verhulst et al., 1997; Verhulst et al., 1996).

The original empirical syndrome scales for the CBCL and the YSR were based on multivariate statistical analysis on data from large samples. To fit more closely to the clinical-diagnostic approach, represented by the DSM (APA, 1994), the following DSM-IV scales were constructed for the CBCL and its derivatives: Affective Problems, Anxiety Problems, Somatic Problems, Attention Deficit/Hyperactivity (ADH) Problems, Oppositional Defiant (OD) Problems, and Conduct (CD) Problems (Achenbach and Dumenci, 2001; Achenbach et al., 2003). A confirmatory factor analyses proved the good fit of these scales (Sondeijker et al., 2005).

Antisocial behavior pertains to behavior that results in physical or mental harm, property loss, or damage to others. It is behavior that lowers the well being of other persons to a large degree (Loeber and Schmaling, 1985; Rutter et al., 1998; Coie and Dodge, 1998). Although the CBCL and YSR contain the OD and CD Problems scales, it can be argued that these scales do not contain enough items that reflect extreme antisocial behaviors. The more severe the disruptive behaviors, the stronger associations with cortisol levels might be. Therefore, scores on the *Antisocial Behavior Questionnaire* (ASBQ), that contains a large number of items on severe antisocial behaviors, were also used for the present study. The ASBQ is comparable to the Self-Report Delinquency Scale (Moffitt and Silva, 1988), and consists of 31 items on lifetime antisocial behaviors (e.g. 'Have you ever destroyed something on purpose?', 'Have you ever used a weapon?', 'Have you ever used drugs?', 'Have you ever been in contact with the police?'). Questions can be rated as (1) no, never, (2) once, (3) two or three times, (4) four to six times, (5) seven times or more. The internal consistency of total score of the items of the ASBQ in the TRAILS sample was .88.

Collection of salivary *cortisol* does not induce stress, which is an advantage compared to collection via venipuncture. Furthermore, total plasma cortisol levels represent all the cortisol that is present in the blood, whereas the effect of plasma cortisol is only caused by the proportion of free cortisol, that is not attached to carrier-proteins. Salivary cortisol levels represent free cortisol only, because free cortisol is able to pass to saliva, and correlate considerably with free plasma cortisol levels (Kirschbaum and Hellhammer, 1994; Van Goozen et al., 1998). TRAILS participants provided two samples of saliva in the morning, shortly after waking up (Cort 1) and half an hour later (Cort 2), and one at 8.00 p.m. (Cort 3), by means of salivettes. All participants were instructed to collect saliva on a normal day, without special events or stressful circumstances, when they were not ill, did not have a cold, and, preferably, did not take any medication. If any of these requirements were not met, this could be noted down on an accompanying form. The saliva samples were stored at −20 °C until analysis. Previous studies suggested that salivary cortisol levels are stable for prolonged periods of time at −20 °C (Aardal and Holm, 1995). After completion of the data collection, all samples were sent in one batch (frozen, by courier) to the laboratory (Department of Clinical and Theoretical Psychobiology, University of Trier, Germany) for analysis.

Cortisol levels were determined with a competitive solid phase time-resolved fluorescene immunoassay with fluorometric end point detection (DELFIA). Ninety-six well Maxisorb microtiterplates (Nunc) were used, that were coated with rabbit-anti-ovine immunoglobulin. After an incubation period of 48 hours at 4 °C, the plates were washed with washbuffer (pH 7.4), coated with an ovine anti-cortisol antibody and incubated again. Synthetic saliva mixed with cortisol in a range from 0 to 100 nmol/l served as standards. Standards, controls (saliva pools) and samples were tested

in duplicate wells. Fifty microliters of biotin-conjugated cortisol was added and after 30 min of incubation the non-binding cortisol/biotin-conjugated cortisol was removed by washing. Two-hundred microliters europium-streptavidin (Wallac, Turku, Finnland) was added to each well and after 30 min and 6 times of washing 200 μl enhancement solution was added (Pharmacia, Freiburg, Germany). Within 15 min on a shaker the enhancement solution induced fluorescene that could be detected with a DELFIA-Fluorometer (Wallac, Turku, Finland). A standard curve was generated and the cortisol concentrations of the samples were calculated with a computer program. The intra-assay coefficient of variation was between 4.0% and 6.7%, and the corresponding inter-assay coefficients of variation between 7.1% and 9.0% (Rosmalen et al., 2005).

Cortisol samples were obtained from 1768 children. Twenty-two of those were excluded because of use of antibiotics or corticosteroids. Furthermore, for each time-point, cortisol values that were above 3 SD of the mean were excluded, to reduce the impact of outliers (Cort 1 21 excluded, 1666 valid measurements; Cort 2 11 excluded, 1683 valid measurements; Cort 3 18 excluded, 1683 valid measurements in the final dataset). The area under the curve (AUC) was computed for the first two cortisol measures by using the following formula (Pruessner et al., 2003):

$$\mathrm{AUC} = \frac{(\mathrm{Cort2} - \mathrm{Cort1}) * 0.5}{2} + (0.5 * \mathrm{Cort1})$$

This AUC yielded a measure of morning cortisol concentration. In other studies, AUC is often computed based on cortisol concentrations that cover an entire day, to obtain a cortisol measure that represents the total cortisol production on a day. Because, in TRAILS, only morning and evening cortisol samples were obtained due to financial constraints, it was not possible to compute such an AUC.

Given possible confounding effects, pubertal stage and BMI were assessed as well. Pubertal stage was assessed using schematic drawings of secondary sex characteristics associated with the five Tanner stages of pubertal development (Tanner, 1962). Within a questionnaire, individuals were provided with gender-appropriate sketches and were asked to select the sketches that looked most like themselves. These ratings have been widely used and have demonstrated good reliability and validity (Brooks-Gunn et al., 1987; Dorn et al., 1990). Height and weight were measured in school on the day that the young adolescents completed the questionnaires. Height was measured in meters and weight in kilograms. The same height meter and weighing scale were used throughout the study. Body mass index (BMI), a standard index of a person's weight in relation to height, was determined for each subject by dividing weight (kg) by the square of height (m^2).

2.3. Statistical analyses

Descriptives for the CBCL/YSR scales ADH Problems, OD Problems, and CD Problems scores, for the total score of the ASBQ, and for Cort 1, Cort 2, Cort 3, and AUC were computed.

Then, a set of 11×4 linear regression analyses was conducted (11 candidate predictors, 4 dependent variables), with scores on the CBCL/YSR ADH, OD, and CD Problems scales, the ASBQ total score, and gender (coded '0' for girls and '1' for boys), age, BMI, and Tanner stage as predictors, and Cort 1, Cort 2, Cort 3, and AUC as dependent variables.

Subsequently, linear regression analyses were conducted to test the predictive power of the interactions between gender and disruptive behaviors in one statistical model (but still separately for Cort 1, Cort 2, Cort 3, and AUC). First, a linear regression analysis was performed with Cort 1 as dependent variable and age, Tanner stage, and BMI as candidate predictors. Subsequently, gender was added to the model as predictor, and after that, scores on the CBCL scale ADH Problems were added. Finally, the interaction between the CBCL ADH Problems scores and gender was added. Exactly the same analyses were performed with the CBCL ADH Problems scale as predictor and the other cortisol measures (Cort 2, Cort 3, and AUC) as dependent variables.

Then, similar as for the ADH Problems scale of the CBCL, regression analyses were conducted for the CBCL scales OD Problems and CD Problems, the YSR scales ADH Problems, OD Problems, and CD Problems, and ASBQ total score.

3. Results

Descriptive information, including raw data separately for boys and girls, regarding the CBCL/YSR ADH, OD, and CD Problems scales, the ASBQ total score, and Cort 1, Cort 2, Cort 3, and AUC is presented in Table 1.

3.1. Separate regression analyses

Results of the set of 11×4 linear regression analyses are presented in Table 2. Effect sizes (explained variance $= R^2$) are presented for those associations that were significant.

Table 2 showed that the association between scores on the YSR scale ADH Problems and Cort 3 was significant. This means that self-reported ADH Problems are positively associated to cortisol levels at 8.00 p.m. Hence, the more ADH problems are present, the higher cortisol levels are at 8.00 p.m. Individuals with ADH problems seemed to get overaroused instead of underaroused, but only in the evening. The effect size of this association was very small.

No significant associations were found between any of the cortisol measures and CBCL scale scores or ASBQ total scores, or with BMI or Tanner stage. Thus, parent reported disruptive behaviors and self reported disruptive behaviors as measured with the CBCL or ASBQ were not related to cortisol levels at all. Age explained 1.0% of the variance in Cort 3; hence, older individuals had higher

Table 1
Means, standard deviations, and ranges of predictor and dependent variables

	Instrument	Mean (SD)		Range
		Boys	Girls	
ADH Problems scale	CBCL	4.53 (3.34)	3.37 (2.99)	0–14
OD Problems scale	CBCL	3.14 (2.15)	2.66 (1.97)	0–10
CD Problems scale	CBCL	2.34 (2.71)	1.26 (1.87)	0–22
ADH Problems scale	YSR	4.09 (2.57)	4.11 (2.42)	0–14
OD Problems scale	YSR	2.36 (1.82)	2.08 (1.64)	0–9
CD Problems scale	YSR	4.25 (3.29)	2.81 (2.34)	0–20
ASBQ total score	ASBQ	13.35 (12.63)	6.56 (7.26)	0–88
Cort 1	–	11.20 (4.72)	11.83 (4.71)	.71 – 29.42 nmol/l
Cort 2	–	14.72 (6.27)	16.02 (6.77)	.22 – 38.42 nmol/l
Cort 3	–	1.90 (1.33)	1.99 (1.34)	.01 – 8.17 nmol/l
AUC	–	6.48 (2.21)	6.95 (2.24)	.52 – 14.36 nmol/l

Note. CBCL, Child Behavior Checklist; YSR, Youth Self Report; ASBQ, Antisocial Behavior Questionnaire; ADH, Attention Deficit Hyperactivity; OD, Oppositional Defiant; CD, Conduct Disorder; Cort 1, cortisol directly after awakening; Cort 2, cortisol half an hour after awakening; Cort 3, cortisol at 8.00 p.m.; AUC, area under the curve.

Table 2
Independent associations between CBCL, YSR, and ASBQ scores, and age, gender, BMI, and Tanner stage as predictors, and cortisol measures as dependent variables

	Cort 1		Cort 2		Cort 3		AUC	
	β	R^2	β	R^2	β	R^2	β	R^2
CBCL								
ADH Problems scale	−.024	–	−.041	–	.021	–	−.038	–
OD Problems scale	−.012	–	.001	–	.005	–	−.003	–
CD Problems scale	−.008	–	−.017	–	−.004	–	−.018	–
YSR								
ADH Problems scale	−.005	–	.042	–	.057*	0.3%	.024	–
OD Problems scale	.007	–	.045	–	.035	–	.029	–
CD Problems scale	−.021	–	−.029	–	.006	–	−.038	–
ASBQ total score	−.027	–	−.035	–	.031	–	−.040	–
Age	.046	–	.018	–	.098*	1.0%	.043	–
Gender	−.067*	0.4%	−.099*	1.0%	−.036	–	−.106*	1.1%
BMI	−.013	–	.026	–	−.016	–	.011	–
Tanner stage	−.015	–	.026	–	.008	–	.012	–

Note. Betas are standardized betas: Effect sizes (R^2) are reported for significant effects only. CBCL, Child Behavior Checklist; YSR, Youth Self Report; ASBQ, Antisocial Behavior Questionnaire; ADH, Attention Deficit Hyperactivity; OD, Oppositional Defiant; CD, Conduct Disorder; Cort 1, cortisol directly after awakening; Cort 2, cortisol half an hour after awakening; Cort 3, cortisol at 8.00 p.m.; AUC, area under the curve.
* Indicates that β is significant ($p < .05$).

cortisol levels in the evening. Gender was negatively associated with cortisol and explained .4% of the variance in Cort 1, 1.0% of the variance in Cort 2, and 1.1% of the variance in AUC. In other words, girls had higher cortisol levels than boys in the morning, but in the evening there was no difference in cortisol levels between boys and girls.

3.2. Interaction models

Results of the subsequent linear regression analyses are presented in Table 3. Results regarding age, gender, BMI, and Tanner stage are not presented in Table 3, because these possible confounders did not change the regression models in these analyses, and because separate effects of these predictors were already given in Table 2.

It is shown that the interaction between scores on the ADH Problems scale of the CBCL and gender contributed significantly to the prediction of Cort 1. This indicated that the association between ADH problems and cortisol directly after waking up was different for boys and girls. When levels of ADH Problems were low, girls showed higher cortisol levels than boys. When levels of ADH Problems were high, boys had higher cortisol levels than girls. Moreover, it seemed that girls with attention problems were underaroused, but boys were not. This effect accounted for .3% of the variance in Cort 1. No associations were found between scores on the parent-reported OD or CD Problems scales or the interaction terms and any of the cortisol measures.

A significant association was found between scores on the YSR scale ADH Problems and Cort 3. Another

Table 3
Standardized betas and effect sizes for associations between CBCL, YSR, and ASBQ scores, and interactions with gender, as predictors, and cortisol measures as outcome

	Cort 1		Cort 2		Cort 3		AUC	
	β	R^2	β	R^2	β	R^2	β	R^2
CBCL								
ADH Problems scale	−.027	–	−.035	–	.024	–	−.037	–
Gender * ADHD	.108*	0.3%	.011	–	−.006	–	.050	–
OD Problems scale	−.016	–	.007	–	.015	–	−.003	–
Gender * ODD	.017	–	−.008	–	−.017	–	−.015	–
CD Problems scale	−.018	–	−.015	–	−.001	–	−.023	–
Gender * CD	.005	–	−.019	–	−.109	–	−.056	–
YSR								
ADH Problems scale	−.006	–	.048	–	.052*	0.3%	.029	–
Gender * ADHD	−.074	–	−.084	–	.010	–	−.090	–
OD Problems scale	.011	–	.051*	0.3%	.033	–	.036	–
Gender * ODD	−.091	–	−.071	–	−.003	–	−.118*	0.3%
CD Problems scale	−.025	–	−.030	–	−.002	–	−.040	–
Gender * CD	−.027	–	−.083	–	.006	–	−.066	–
ASBQ								
ASBQ total score	−.038	–	−.028	–	.027	–	−.040	–
Gender * ASBQ	−.101	–	−.103	–	−.086	–	−.140*	0.3%

Note. Betas are standardized betas: Effect sizes (R^2) are reported for significant effects only. CBCL, Child Behavior Checklist; YSR, Youth Self Report; ASBQ, Antisocial Behavior Questionnaire; ADH, Attention Deficit Hyperactivity; OD, Oppositional Defiant; CD, Conduct Disorder; Cort 1, cortisol directly after awakening; Cort 2, cortisol half an hour after awakening; Cort 3, cortisol at 8.00 p.m.; AUC, area under the curve.
* Indicates that β is significant ($p < .05$).

significant association was found between YSR OD Problems scores and Cort 2. Both associations were positive, which means that disruptive individuals were overaroused instead of, as is described in the arousal theories, underaroused. The effect size was .3% for both associations. Furthermore, for the AUC, a significant association was found with the interaction-term between YSR OD Problems scores and gender (effect size = .3%). This indicated that the association between the area under the curve and self-reported OD Problems was different for boys versus girls. The more OD Problems were present, the higher cortisol levels in girls were, whereas cortisol levels in boys decreased when levels of OD Problems became higher.

Results of the linear regression analyses with the ASBQ as predictor and the different cortisol measures as dependent variables were also presented in Table 3. The interaction between ASBQ total score and gender was associated with AUC (effect size = .3%). This indicated that, if ASBQ scores became higher, cortisol levels in girls increased, whereas those in boys decreased.

4. Discussion

The present study indicated that, in a large representative general population sample of pre-adolescent boys and girls, the association between disruptive behaviors and indices of basal HPA-axis functioning were weak, and not always in the direction we expected a priori (McBurnett et al., 2000; Pajer et al., 2001; Vanyukov et al., 1993). Hence, the findings from previous studies, that were conducted with clinical or high risk samples, could not be generalized to this general population sample. Furthermore, given the finding that effect sizes were relatively small, it can be concluded that, in pre-adolescence, the measures of baseline HPA-axis functioning that were used for the present study cannot be used as biological markers for disruptive behaviors.

To explain discrepancies with previous studies, the severity of problems in high risk groups versus general population samples could be of importance. In clinical samples, problems are more severe and likely to have persisted for several years before referral to mental health services takes place (Sayal, 2004). Hence, the HPA-axis may have become less sensitive to stress (Van de Wiel et al., 2004). This could be a useful form of protection against long-term high cortisol levels, due to the stress that accompanies disruptive behaviors. As a result, in these individuals, much more stress may be needed to activate the HPA-axis, which would result in decreased basal levels of cortisol (under-arousal) in individuals with severe disruptive behaviors. Such a phenomenon might play a less important role in the general population.

Although associations between disruptive behaviors and cortisol levels apparently were weaker in general population samples than in clinical or high-risk samples, we did find an association between ADH Problems and cortisol levels at 8.00 p.m. In contrast to what we expected based on previous studies (McBurnett et al., 2000; Pajer et al.,

2001; Vanyukov et al., 1993; King et al., 1998), it was a positive association. The more ADH Problems were present, the higher the cortisol levels we found. The finding that cortisol levels were associated with ADH Problems scores and not with OD or CD scores may indicate that, despite high comorbidity rates between attention problems and aggressive or delinquent behaviors (Angold et al., 1999), the biological antecedents or consequences of these different areas of behavior problems may not be similar. Although the size of the effect that was found was small, results of the present study indicate that the association between HPA-axis functioning and attention problems, that has gotten less attention than that with aggressive or delinquent behaviors, requires further research.

In accordance with previous work (Klimes-Dougan et al., 2001), girls had higher cortisol levels than boys. There is no clear explanation for the gender differences in basal cortisol levels in 10- to 12-year-olds. Netherton et al. (2004) suggested that gonadal steroids might play an important role. Gonadal steroids, and estrogens in particular, are known to interact with the HPA-axis. Increased HPA-axis activity in girls might be related to the direct effect of estrogens on CRH (Vamvakopoulos and Chrousos, 1993), although, in the present study sample an association between pubertal stage and cortisol levels was not found (Rosmalen et al., 2005). During puberty gender effects might become more clear, at least if gonadal steroids do have an influence on cortisol levels.

More interestingly however, gender interaction-effects were found, indicating that associations between cortisol levels and disruptive behaviors were different in boys versus girls. The first interaction effect concerned cortisol levels just after awakening. High rates of ADH problems were associated with higher early morning cortisol levels in boys, but with lower levels in girls. Several studies indicated that compared with boys, girls with ADH Problems displayed lower levels of hyperactivity and lower rates of other externalizing behaviors; among children with ADH Problems identified from non-referred populations, girls with ADH Problems displayed lower levels of inattention, internalizing behavior, and peer aggression than boys with ADH Problems (e.g. Gaub and Carlson, 1997; Gershon, 2002). Because of the gender differences in ADH Problems, gender differences in arousal levels, and thus gender differences in cortisol levels were expected. However, since ADH Problems seem to be more severe in boys than in girls, we expected lower cortisol levels in boys, but not in girls (Klimes-Dougan et al., 2001).

The other interaction effects, for OD and CD problems remarkably, were opposite to the interaction effect for ADH problems. Higher rates of OD or CD Problems were associated with higher morning cortisol levels (AUC) in girls, and lower cortisol levels in boys. The finding that lower cortisol levels in boys were associated with higher levels of OD or CD Problems was in accordance with previous studies (McBurnett et al., 2000; Vanyukov et al., 1993; King et al., 1998). Hence, in a way, the results provided support for the arousal theories that were mentioned in the introduction (Van Goozen et al., 2000; Raine, 1993; Zuckerman and Neeb, 1979), indicating that low arousal levels put boys at risk for higher rates of disruptive behaviors.

In girls, however, higher levels of disruptive behaviors were associated with higher, and not with lower, morning cortisol levels. Girls did not receive much attention thus far in this field of research. However, the results of our study contrasted with those of Pajer and colleagues (2001), who suggested that conduct disorder in 15- to 17-year-old girls was associated with lower morning cortisol levels. The latter study seemed to support the fearlessness and sensation-seeking theories. However, the results of Pajer et al. (2001) were based on a small and selected sample. Results of the present study did not support the two arousal theories for a large representative sample of girls. Other endocrine mechanisms might be responsible for the positive association that was found between disruptive behaviors and HPA-axis activity in the present study. For instance, estrogens, which are known to influence HPA-axis activity, might play a role (Burgess and Handa, 1992; Handa et al., 1994; Roy et al., 1999; Vamvakopoulos and Chrousos, 1993). Still, this is purely hypothetically. Regardless of the mechanism that is responsible for the gender differences that were found, the present study indicated that different biological factors may be responsible for disruptive behaviors in boys versus girls, and indicated that gender specific research on this topic is needed (Rutter et al., 1998).

The strengths of the present study were the large sample size, use of multiple informants to assess disruptive behaviors, and assessment of three cortisol measures on relevant time points during the day. Yet, the results of the present study should be interpreted against the limitations in our study. First, the cortisol response to stress was not assessed, whereas stress sensitivity may be a key factor in the link between HPA-axis functioning and disruptive behaviors (Bartels et al., 2003). Second, individuals collected cortisol at home, which may be a less standardized procedure than collecting it at a clinic, but also less stressful, which is an important advantage.

It can be concluded that, although studies in high risk groups of mainly boys found evidence for an association between low basal HPA-axis activity and high levels of disruptive behavior problems, this association could hardly be confirmed in a large representative population sample of boys and girls. This casted doubt on the usefulness of cortisol measurements to estimate risk for behavior problems, and on a putative important role for HPA-axis functioning in the etiology of disruptive behavior problems.

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