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HPA-axis activity as a predictor of future disruptive behaviors in young adolescents

FROUKE E.P.L. SONDEIJKER, ROBERT F. FERDINAND, ALBERTINE J. OLDEHINKEL, HENNING TIEMEIER, JOHAN ORMEL, and FRANK C. VERHULST

Abstract

Low HPA-axis activity has been proposed as a risk factor for disruptive behaviors. However longitudinal data on this topic are practically lacking. In the present study we investigated if low HPA-axis activity predicted future disruptive behaviors. We included 1,399 boys and girls from the Dutch general population, initially aged 10–12 years. At the first assessment, basal cortisol levels were assessed. At the first assessment and at follow-up 2 years later disruptive behaviors were assessed with parent and self-report questionnaires. The results suggest that the association between low cortisol levels at 8.00 p.m. and future disruptive behaviors according to the parents was only present for boys. More importantly however, the results suggest that low HPA-axis activity is not a good predictor for disruptive behaviors, but could be valuable to identify those with a poor prognosis, once disruptive behaviors are present in preadolescence.

Descriptors: HPA-axis, Disruptive behaviors, Cortisol, Adolescents, General population

Disruptive behaviors in children and adolescents are common, have a negative impact on families, schools, and communities, and predict delinquency and substance abuse in adulthood (e.g., Fergusson, Lyskkey, & Horwood, 1997; Moffitt, Caspi, Dick-son, Silva, & Stanton, 1996; Moffitt, Caspi, Harrington, & Mil-ne, 2002; Nagin & Tremblay, 1999; Robbins, 1966). This warrants research aimed at early risk factors and at factors that determine change in symptoms across time (Côté, Tremblay, Nagin, Zoccolillo, & Vitaro, 2002; Hinshaw, 2002; Loeb, Green, Keenan, & Lahey, 1995; Nagin & Tremblay, 1999).

In the past 10 years, data have become available suggesting that low activity of the HPA-axis is a risk indicator for disruptive behaviors in children and adolescents (Lahey, Hart, Pliszka, Applegate, & McBurnett, 1993). Theoretically, low HPA-axis activity is associated with low levels of arousal of the central nervous system (Chrousos & Gold, 1998; Van Goozen, Matthys, Cohen-Kettenis, Buitelaar, & van Engeland, 2000), which would predispose to disruptive behaviors. More specifically, low cortisol levels have been linked to disruptive behaviors via lowered sympathetic nervous system activity and reduced fear conditioning (Gordis, Granger, Susman, & Trickett, 2006; Van Goozen et al., 1998). The fearlessness theory (Raine, 1993; Zuckerman & Neeb, 1979), which is in line with this theoretical background, implies that low arousal makes individuals fearless. These relatively fearless individuals are, according to this theory, vulnerable to disruptive behaviors. Another theory, the stimulation-seeking theory (Eysenck, 1964; Quay, 1965; Raine, 1993; Zuckerman & Neeb, 1979), suggests that low arousal represents an aversive physiological condition. To attain a higher, more optimal level of arousal, individuals would seek stimulation. Aggressive or delinquent behaviors could be stimulating for some children and adolescents.

Several studies examined cross-sectional associations between activity of the HPA-axis and disruptive behaviors in children and adolescents. Some studies found an association between low basal cortisol levels and disruptive behaviors (McBurnett, Lahey, Rathouz, & Loeb, 2000; Pajer, Gardner, Rubin, Perel, & Neal, 2001; Vanyukov et al., 1993), whereas others did not (Scerbo & Kolko, 1994; Schulz, Halperin, Newcorn, Sharma, & Gabriel,
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Johnson, 2005; Vamvakopoulos & Chrousos, 1993). Disruptive behaviors only concerned boys, whereas gender differences in associations between HPA-axis activity and disruptive behaviors have been found (McBurnett et al., 2000; Shoal et al., 2003; Van Bokhoven et al., 2004). These unequivocal findings may be due to methodological differences between the studies. The time of the day cortisol samples are gathered is important, because cortisol levels follow a diurnal rhythm. In all three studies, cortisol samples were collected at only one time point during the day. Van Bokhoven and colleagues chose to measure cortisol levels immediately upon arrival at the laboratory, between 8:45 and 9:55 a.m., Shoal et al. measured cortisol levels at about 9:00 a.m., and in McBurnett’s study the time of the day of saliva collection could not be controlled and was allowed to vary across the day. Further, it is recommended to sample cortisol levels on a normal day, because special events or stressful circumstances may influence cortisol levels. In all three previous studies participants had to go to a laboratory. Visiting a laboratory may itself be stressful to some participants. Finally, the studies did not provide information regarding illnesses and medications that may affect cortisol levels.

Another gap left behind by previous work is the importance of gender differences. The three previous longitudinal studies that investigated if low HPA-axis activity predicts future disruptive behaviors only concerned boys, whereas gender differences in associations between HPA-axis activity and disruptive behaviors may be present (Kessl et al., 1995; Shirtcliffe, Granger, Booth, & Johnson, 2005; Vamvakopoulos & Chrousos, 1993). Disruptive behaviors are more frequent in boys than in girls (Côté et al., 2002). Additionally, in the present study’s sample, boys had significantly lower morning levels than girls (Rosmalen et al., 2005). Therefore, one could hypothesize that a negative association between basal morning cortisol and disruptive behaviors could be stronger in boys than in girls.

The aim of the present study was threefold. First, it was investigated whether low salivary cortisol levels predicted future disruptive behaviors in young adolescents from the general population. Second, it was investigated if low HPA-axis activity predicted persistence of disruptive behavior problems. Third, it was investigated whether the association between cortisol levels and future disruptive behaviors was different for boys and girls.

Methods

Sample and Procedure

The present study was part of the TRacking Adolescents’ Individual Lives Survey (TRAILS). TRAILS is a prospective cohort study of Dutch early adolescents aged 10-12 years, who were followed across a period of two years. The first assessment took place in 2001–2002, the second in 2003–2004. The main objective of TRAILS is to contribute to the understanding of the development of psychopathology, as well as the underlying etiological mechanisms. TRAILS is a multidisciplinary study with data on various domains of determinants and outcomes, collected in multiple sources, with multiple methods (De Winter et al., 2005). Because the incidence of emotional and behavioral problems increases substantially in adolescence, TRAILS follows preadolescents (ages 10–12) until the age of 24 with biennial assessments.

The TRAILS target sample consisted of young adolescents from five municipalities in the north of the Netherlands, including both urban and rural areas. Of all eligible individuals (N = 2,935), 76.0% participated in the study (N = 2,230, mean age 11.09 years, SD 0.55, 50.8% girls). Participants did not differ from those who refused with respect to the proportion of single parent families, the prevalence of teacher-rated problem behavior, several sociodemographic variables, and mental health outcomes provided by the teacher (De Winter et al., 2005).

At the second assessment wave, information was obtained from 2,149 (96.4%) of those who participated at Wave 1 (mean age 13.56 years, SD 0.53, with 51.0% girls). To assess disruptive behaviors, two questionnaires were used at Wave 1 and Wave 2, the Child Behavior Checklist (CBCL; Achenbach, 1991a; Verhulst, Van der Ende, & Koot, 1996) and the Youth-Self Report (YSR; Achenbach, 1991b, Verhulst, Van der Ende, & Koot, 1997). The number of individuals for whom questionnaires were available at both assessment waves was 1,765 for the CBCL and 1,941 for the YSR. Furthermore, at Wave 1, cortisol levels were determined for 1,768 individuals, of whom 22 were excluded because of use of antibiotics or corticosteroids. In addition, cortisol values that were above 3 SD of the mean were excluded, to reduce the impact of outliers (cortisol directly after waking up [Cort 1]: 21 excluded, 1,666 valid measurements; cortisol half an hour after awakening [Cort 2]: 11 excluded, 1,683 valid measurements; cortisol at 8:00 p.m. [Cort 3]: 18 excluded, 1,683 valid measurements in the final data set). This resulted in 1,399 individuals for whom Wave 1 and Wave 2 CBCLs and YSRs, plus at least one Wave 1 cortisol measure were available.

To examine possible selective attrition using a simple t test is the best and most common way, but not very informative in the present study. The problem was that all of the t tests were significant, probably due to the large sample size, but the means were almost equal for most variables. The two variables that had a really different mean for completers versus noncompleters were the CBCL disruptive scale and SES. As a more informative alternative, a stepwise logistic regression analysis was performed. Some other advantages of this approach are that only one statistical test is needed, which reduces findings based on chance, and we corrected for dependency between different predictors. The dependent variable was “all information available,” and age, gender, socioeconomic status (SES), the different cortisol measures, and the scores on the CBCL and YSR disruptive scale were predictors. Low SES scores (completers mean = 0.09, noncompleters mean = −0.30) and high disruptive behavior...
scores according to the CBCL (completers mean = 4.54, non-completers mean = 4.99) predicted attrition. However, the effect size of the entire model was very small (Cox and Snell $R^2 = 2.4\%$).

Written consent was obtained from the children’s parents. The study was approved by the Central Dutch Medical Ethics Committee.

**Measures**

**Questionnaires.** The Child Behavior Checklist (Achenbach, 1991a; Verhulst et al., 1996) is a parent questionnaire for assessing problems in 4- to 18-year-olds. The Youth Self-Report (Achenbach, 1991b; Verhulst, Van der Ende, et al., 1997) 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 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 American version of the CBCL and YSR were confirmed for the Dutch translations (De Groot, Koot, & Verhulst, 1994; 1996; Verhulst, Dekker, & van der Ende, 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 Diagnostic and Statistical Manual of Mental Disorders (DSM; American Psychiatric Association, 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, Dumenci, & Rescorla, 2003). For the present study we used the scales OD Problems, CD Problems, Affective Problems, and Anxiety Problems. A confirmatory factor analyses proved the good fit of these scales (Achenbach & Rescorla, 2001; Sondeijker et al., 2005). The internal consistency of these DSM oriented scales ranged from 0.75 to .84, test–retest reliability ranged from .79 to .89, and cross-informant agreement ranged from .20 to .75 (Achenbach et al., 2003).

**Cortisol.** At Wave 1, participants provided salivary cortisol. 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 & 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.

Concerning the sampling procedure itself, subjects were instructed to keep a glass of water next to their bed and to thoroughly rinse their mouth with tap water before sampling saliva, and not to consume sour products or brush their teeth shortly before that. Saliva samples were stored by the participants in their freezer directly after sampling and mailed to the institute as soon as possible (but not on Fridays and Saturdays in order to prevent unnecessary delay due to the weekend). Participants who did not return the salivettes within a couple of months were sent a reminder letter. In total, we received saliva samples of 1,768 children (79.3% of all TRAILS participants). After exclusion of the outliers, cortisol levels followed a normal distribution (Cort 1 skewness = -.70, kurtosis = .63; Cort 2 skewness = -.43, kurtosis = .24; Cort 3 skewness = 1.22, kurtosis = 2.01). For more characteristics of this study population, see Rosmalen et al. (2005). The other 20.7% did not respond, not even after a reminder letter was sent. Nonresponders did not differ from responders in terms of gender (48.4% male vs. 49.4% male for non-responders vs. responders, respectively, $\chi^2(df = 1) = 0.132; p = .716$) or pubertal development (average tanner score $= 1.92$ vs. 1.86, $t = -1.394; p = .164$); nonresponders were slightly older (11.16 years vs. 11.08 years, $t = -3.084; p = .002$) and had a higher mean BMI (15.80 vs. 17.92 kg/m$^2$, $t = -3.224; p = .001$). The saliva samples were stored at $-20^\circ$C until analysis. Previous studies suggested that salivary cortisol levels are stable for prolonged periods of time at $-20^\circ$C (Aardal & 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 fluorescent immunoassay with fluorometric end point detection (DELFIA). Ninety-six-well Maxisorb microtiterplates (Nunc) were used that were coated with rabbit-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 were added and, after 30 min of incubation, the nonbinding cortisol/biotin-conjugated cortisol was removed by washing. Two hundred microliters of europium-streptavidin (Wallac, Turku, Finnland) were added to each well and, after 30 min and 6 times of washing, 200 µl of enhancement solution were added (Pharmacia, Freiburg, Germany). Within 15 min on a shaker, the enhancement solution induced fluorescence 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 were between 7.1% and 9.0% (Rosmalen et al., 2005).

Besides the three cortisol measures (Cort 1, Cort 2, and Cort 3), we also computed the area under the curve (AUC). The AUC was computed for the first two cortisol measures by using the following formula (Pruessner, Kirschbaum, Meinschmidt, & Hellhammer, 2003):

$$AUC = \left(\frac{(Cort\ 2 - Cort\ 1) \times 0.5}{2} + (0.5 \times Cort\ 1)\right)$$

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.

**Socioeconomic status.** SES (Wave 1) was computed for each family by calculating the mean of the scores on a number of questions about education, salary, and profession of father and mother. Cronbach’s alpha of SES is .84.

**Pubertal stage.** Stage of pubertal development was assessed in the Wave 1 parent interview using schematic drawings of secondary sex characteristics associated with the five standard Tanner stages of pubertal development (Marshall & Tanner, 1969, 1970). Tanner stages are a widely accepted standard for assessment of physical development, and have demonstrated good reliability, validity, and parent-child agreement (Dorn, Sussman, Nettelmann, Inoffgermain, & Chrousos, 1990). A parent (usually the mother) was provided with gender-appropriate sketches and asked to select which of the sketches “looked most like the child.” Based on the parent ratings, children were classified into five stages of puberty, in which stage 1 corresponds to infantile and stage 5 to complete puberty (Tanner & Whitehouse, 1982).

### Statistical Analyses

In preliminary analyses we did not find any differences between OD and CD problems with respect to their association with cortisol levels. Therefore scores on the **DSM-IV** CBCL scales OD Problems and CD Problems were summed. This combined scale was designated as CBCL disruptive scale. The same was done for the **DSM-IV** YSR scales OD and CD Problems, which yielded the YSR disruptive scale.

To investigate whether cortisol levels predicted future disruptive behaviors, stepwise regression analyses were performed with the CBCL disruptive scale scores at Wave 2 as the dependent variable and Wave 1 AUC as an independent variable. To test effects of gender, Wave 1 CBCL Internalizing Problems, Wave 1 CBCL Disruptive Problems (this was a dichotomous variable: above P90 yes or no), pubertal stage, and SES, these variables were added to the model in a second step as independent variables. To examine whether Wave 1 AUC predicted the persistence of disruptive problems, we added the interaction variable AUC × Wave 1 CBCL Disruptive Problems to the model in a third step. To achieve the third aim, examining whether gender differences we added gender as an interaction variable to the regression models. There was one significant interaction. Cortisol levels at 8:00 p.m. (Cort 3) predicted future parent-reported disruptive problems, but only in boys (p = .022). The effect size was 0.3%.

Concerning the third aim, gender differences, we added gender as an interaction variable to the regression model as an interaction variable. The results indicated that internalizing problems did not moderate the association between cortisol levels and future disruptive behavior problems (all ps for CBLC and YSR > .25).

### Discussion

Results of previous studies that examined the association between cortisol levels and disruptive behaviors in children and adolescents are mixed (McBurnett et al., 2000; Shool et al., 2003; Van Bokhoven et al., 2004). Methodological differences between previous studies may constitute one of the reasons for this. More importantly, however, in previous studies only one cortisol sample was taken, whereas to be sure to measure basal cortisol levels it is advisable to take more than one sample a day or at least control for time of sampling. In this way the diurnal rhythm cortisol levels follow during the day can be taken into account. Additionally, it is important that saliva is collected on a day without special events or stressful circumstances. In the present study we therefore gathered three cortisol samples on a normal school day. Furthermore, information from boys and girls was obtained, whereas previous work only concerned boys. Other strengths of this study are that identical instruments were used to assess behavioral problems at the initial and follow-up assessments, which enabled us to detect changes in problems levels across time, that parent and child information about disruptive problems was obtained at both assessments, which reduced the likelihood of informant biases, and that internalizing problems were taken into account.

Low HPA-axis activity in the sample as a whole did not predict future disruptive behaviors. However, as was also found by Shirlcliff et al. (2005), low evening HPA-axis activity in boys predicted parent-reported disruptive problems 2 years later. This finding was opposite to what was found by Van Bokhoven et al. (2004), who found that higher cortisol levels predicted disruptive behaviors. Furthermore, in boys and girls with high levels of disruptive behaviors at the beginning of the study, low morning cortisol levels predicted future behavior problems as indicated by self-reports. Hence, low HPA-axis activity seemed to become a significant predictor of future behavior problems, once preadolescents have begun to display disruptive behaviors, but did not predict the new cases. This corroborated results of previous authors (McBurnett et al., 2000; Shool et al., 2003) but also indicated that low HPA-axis activity is a risk factor in high risk groups, but not at a general population level. This may also
explain why, in referred samples, associations between HPA-axis activity and disruptive behaviors are being found more often.

Our study may have important implications for theory building. Well-known theories are the sensation-seeking theory, which was mentioned in the introduction, or the fearlessness theory, which implies that low arousal levels—associated with low HPA-axis activity—would make people fearless and therefore vulnerable to disruptive behaviors (Raine, 1993; Zeckman & Neeb, 1979). These theories imply that low arousal levels put individuals at risk for future behavior problems. The findings of the present study argue against fearlessness as being a sufficient cause of future disruptive behaviors, because individuals with low cortisol levels were not at risk for future behavior problems. Moreover, only in those who displayed high levels of behavior problems already was low HPA-axis activity a risk factor for future problems. This may even imply that low HPA-axis activity may be a consequence of persistent behavior problems, instead of a cause (Gunnar & Vazquez, 2001). Further, it is in line with previous studies in boys from clinical or high-risk groups (McBurnett et al., 2000; Shoal et al., 2003) that probably contained a high proportion of individuals with persistent disruptive behavior problems (Sayal, 2004). Moreover, low HPA-axis activity could be valuable to identify those with a poor prognosis, once disruptive behaviors are present in preadolescence.

Some authors have explained the association between low HPA-axis activity and persistent disruptive behaviors by postulating that a high threshold for activation of the HPA-axis may be a useful protection against high levels of environmental stress resulting from angry teachers or parents who yell at them that children with disruptive behaviors often have to cope with (Van de Wiel, van Goozen, Matthys, Snoek, & van Engeland, 2004). In our opinion, on the one hand, having a high threshold for activation of the HPA-axis is indeed useful to protect the body against high levels of stress. On the other hand, these children need more and more arousal or environmental stress to activate the HPA-axis. This, according to the sensation seeking theory, might promote disruptive behaviors. Viewed from that angle, having a high threshold for activation of the HPA-axis can also be involved in the persistence of disruptive behaviors. These mechanisms may well play a role in individuals diagnosed with disruptive behavior disorders, given their long-lasting high levels of environmental stress, but these kinds of alterations in HPA-axis sensitivity and activity may play a less prominent role in a general population sample.

The present study shows that the association between HPA-axis functioning and disruptive problems is very weak and not as straightforward as we may have thought. Apparently, it is possible that biological alterations are not the cause but the consequence of disruptive problems, and the well-known existing theories do not necessarily pertain to the population as a whole. However, our findings still may also indicate that low HPA-axis activity causes persistence of high problem levels but does not cause the appearance of new externalizing problems. Use of interaction models as a framework helps to explain why this could be the case. It could be, for instance, that high levels of externalizing problems at Wave 1 are present due to factors such as peer influences, interaction with parents, or living in a poor neighbourhood. Once externalizing problems are there, those with low tendency toward arousal (as could be reflected by low HPA-axis activity) could be less likely to be hampered by a high arousal level and, as a consequence, might continue behaving badly, whereas those with a higher tendency toward arousal might be protected against this prognosis. In individuals with low externalizing problem levels at Wave 1, HPA-axis activity might not be relevant as a risk factor, because externalizing problems are not present, so their course cannot be influenced by HPA-axis functioning anyway. However, we only assessed one aspect of fear and one physiological variable, which suggests caution in interpreting the present study. Furthermore, cortisol samples were collected at home, which is a drawback of this study, despite the fact that other studies that succeeded in gathering information regarding HPA-axis activity and future behavior problems in such a large representative sample of preadolescent, do not exist. Despite thorough instructions, it can be expected that especially the morning levels of cortisol may have been more unreliable than we would wish, given problems with sampling immediately after awakening, level of physical activity in the morning (ideally, individuals should lay down for 30 min between Cort 1 and Cort 2), poorer compliance with the instructions, or problems with timing between Cort 1 and Cort 2. Although it is valuable to collect cortisol levels on a normal day, collection of cortisol levels in a laboratory would probably have resulted in higher reliability of cortisol data. This may indicate that the findings of the present study represent an underestimation of the associations between HPA-axis activity and disruptive behaviors.

Second, it could be speculated that noncompliance in participants with disruptive behavior problems might actually have created the effects observed here. Notwithstanding the slightly selective attrition, there still are > 100 children with high levels of disruptive problems in this sample. The group of adolescents with severe disruptive problems is large enough to detect even very small effects.

Third, it could be that HPA-axis activity itself does not constitute a strong risk factor for disruptive behaviors, but that, in combination with other factors, such as parental rearing practices, early adversities, or adverse peer relationships, low HPA-axis activity may become more important. This is not entirely unlikely, given the growing knowledge about the importance of interactions between biological/individual factors and environmental influences (Moffitt, Caspi, & Rutter, 2005; Rutter & Silberg, 2002). However, the results of this study could also indicate that lower or higher HPA-axis activity does not really put individuals at risk for future behavior problems. Of course, replication studies are needed in other countries, using other measures, to put the findings of the present study to the test.

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