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Hypothalamus pituitary adrenal axis and autonomic activity during stress in delinquent male adolescents and controls

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Summary
Objective: Patterns of low autonomic arousal have consistently been related to delinquency and disruptive behavior disorders (DBD) in children and adolescents. Findings on another stress regulating mechanism, the hypothalamic pituitary adrenal (HPA) axis, have been inconsistent, which may partly be due to not considering specific stress reactivity measures. Therefore, the aim of the present study was to investigate the relationship between disruptive behavior in male adolescents and their HPA and autonomic reactivity to a standard public speaking task (PST).
Method: Responsivity to the PST of cortisol, heart rate (HR), skin conductance level (SCL) and self-reported negative feelings was measured, and compared between 12and14-year-old boys who attended a delinquency diversion program (DP), with and without DBD (DP^+, $n = 22$ and DP^-, $n = 49$, resectively), and matched normal controls (NC, $n = 30$). DBD diagnoses were based on a structured psychiatric interview.
Results: The DP+ group, but not the DP− group, showed a significantly decreased cortisol and HR response during the PST as compared with the NC group. No significant effects were found for SCL. All subjects connoted the task negatively.

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Conclusions: The results indicate that low cortisol and HR responsivity to stress may be a neurobiological marker for delinquent boys with DBD, but not for those without DBD. Directions for future research and clinical implications are discussed.

1. Introduction

Antisocial behavior by children is a major public health problem, with disruptive behavior disordered (DBD) children costing society at least ten times as much as normally developing children (Scott et al., 2001). DBD children are at risk for a series of negative outcomes in adulthood, such as criminal behavior, social isolation, unemployment, and psychiatric disorders, including depression, anxiety disorders and substance abuse (Maughan and Rutter, 2001). Research over the past decades, has provided evidence that neurobiological factors are important in explaining individual variation in disruptive behavior and in predicting the outcome of children with DBD (Raine, 2002). Stress regulating mechanisms, e.g. the hypothalamic pituitary adrenal axis and the autonomic nervous system (ANS), have drawn much attention in this respect, representing biological parameters of arousal. Low arousal has been hypothesized to reflect fearlessness and sensation seeking dimensions, which in turn may predispose to antisocial behavior (Raine, 1993; Zuckerman, 1979).

Studies on the relationship between arousal parameters, e.g. HPA-axis and ANS, and antisocial behavior have investigated these parameters under resting conditions and during stress. With respect to HPA activity, though low resting levels of it's final product cortisol have consistently been associated with antisocial behavior in adults (Virkkunen, 1985; Woodman et al., 1978), findings in children and adolescents have been less clear, with some studies demonstrating low resting cortisol levels in children with disruptive behavior problems (Shoal et al., 2003; Pajer et al., 2001; McBurnett et al., 2000), and others not (Azar et al., 2004; Scerbo and Kolko, 1994; Klimes-Dougan et al., 2001). Definitions of disruptive behavior and time of cortisol sampling varied in these studies. Moreover, although these studies focused on basal cortisol levels, factors causing stress (e.g. strange lab environment, performing tasks) were not controlled for. Taken together, these methodological difficulties might partly explain some of the controversial findings.

Only a few studies in behavior-disordered children have actually measured HPA responsivity to stress. Moss et al. (1995) studied a sample of sons of fathers with a psychoactive substance use disorder (PSUD) ($n = 81$), and a normal control (NC) group ($n = 103$). Two saliva samples were obtained, just before and 35 min after an event-related potential (ERP) task. The sons of PSUD fathers, who scored high on aggression measures, had lower cortisol levels before the ERP task. The authors assume that this is related to reduced anticipatory anxiety. Another explanation may be that the participants were tested in the early morning. As such, the decline in cortisol over the two samples, observed in both groups, may have been caused by natural diurnal variation, and the low cortisol levels in the sons of PSUD fathers may reflect a decrease in diurnal variation in this group. In another study, comparing a sample of clinical referred DBD children ($n = 26$) with NCs ($n = 26$), Van Goozen et al. (2000) studied cortisol levels in reaction to a task consisting of provocation and frustration by a virtual opponent. Nine cortisol samples were obtained (four before the task and five during and after the task) and all participants were tested between 1 and 4 pm. Although no differences in baseline cortisol levels were reported, DBD children consistently had lower cortisol levels during the stressor than NC children. Applying the same protocol, Snoek et al. (2004) explored the specificity of these findings for DBD in comparison to attention deficit hyperactivity disorder (ADHD). Comparing clinical referred children (age 7–12) with a diagnosis of DBD ($n = 15$), DBD/ADHD (32), ADHD only (23), and NCs (26), an attenuated cortisol response was reported for both DBD groups, but not for the ADHD only group, in comparison with NCs. Thus, decreased cortisol response to stress may be a specific characteristic of clinical referred DBD children.

With respect to ANS, of which two peripheral parameters have been studied predominantly, i.e. heart rate (HR) and skin conductance level (SCL), the literature is more extensive and consistent. A vast number of studies, both in children and adults, has demonstrated a relationship between low resting and stress induced HR/SCL and antisocial behavior both in resting and stressful situations (for a review, see Ortiz and Raine, 2004). Still, Lorber (2004) points out some inconsistencies in the literature due to diversity of behavioral constructs of antisocial behavior studied in relation to autonomic activity, i.e. aggression, psychopathy,

and DBD. In his meta-analysis low HR is most consistently related to DBD. The relatively few studies in DBD children specifically are in line with this, since they reported low levels of ANS activity, both in resting (Lahey et al., 1995) and in stressful situations (Van Goozen et al., 2000; Snoek et al., 2004). Moreover, two studies to date reported that low HR reactivity was specific for DBD, irrespective of comorbid ADHD (Snoek et al., 2004; Herpertz et al., 2005).

As such, there are three main issues that remain to be elucidated. First, it remains unclear whether low HPA and ANS activity is specific for clinical-referred children with a DBD diagnosis or whether it is also a marker non-clinical referred DBD children. Second, the picture may vary depending on whether data are obtained during resting or stressful conditions. Third, findings regarding responsivity might depend on the kind of stressor that is applied. To date, although theories postulate fearlessness in adolescents with disruptive behavior problems, there has been no study in this population measuring both HPA and ANS responsivity to a validated stressor designed specifically to be fearful.

To address these lacunae in the literature, the aim of the current study was to investigate salivary cortisol, heart rate, skin conductance and self-reported negative feelings (NFs) under resting conditions and during a public speaking task (PST), in 12–14 year old males referred to a delinquency diversion project (DP) and in matched NCs (NC). To address the issue whether altered HPA and/or ANS (re)activity is specific for disruptive behavior in general or for DBD in particular, the purpose was to subdivide the DP group into two subgroups based on the presence or absence of DBD (DP+ and DP−, respectivey). Decreased HPA and ANS reactivity during stressful conditions were predicted for the DP group as compared with the NC group, with these effects being be more apparent in the subgroup of DP boys with a DBD.

2. Methods

2.1. Subjects

The sample included adolescents attending a delinquency diversion program (DP group, $n = 71$), and a matched group of NCs (NC group, $n = 30$). In the Netherlands, children from 12 to 17 years old who have committed a minor offence can be sent to a DP to prohibit court intervention. This option is only available for specific types of petty offenses: wanton destructiveness, vandalism, simple theft, hooliganism, infractions on the firework regulations and minor forms of aggression. DP children in the lowest age group (12–14 years) were included for this study, in order to investigate the relationship between cortisol and disruptive behavior in peri-pubertal boys with an increased risk of developing a criminal career and allowing follow-up studies during adolescence.

Contact with subjects and their parents was established by the research team at the offices of the participating DPs within weeks after arrest by the police. Exclusion criteria for participation were a history of neurological or endocrinological disorders, steroid medication, and IQ below 70. The NC boys were recruited from local schools and football clubs. Delinquent boys and NCs were matched group-wise for age, IQ, SES and ethnicity (see Table 1). None of the subjects was on medication.

This study was approved of by the Medical Ethical Committee of the VU University medical center Amsterdam, and parents as well as subjects gave written informed consent.

2.2. Instruments

The National Institute of Mental Health (NIMH) Diagnostic Interview Schedule for Children (DISC), version IV (Shaffer et al., 2000) is an extensive structured psychiatric interview, of which the sections on attention-deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), conduct disorder (CD) and post-traumatic stress disorder (PTSD) of both the child and parent versions were administered by trained interviewers to assess current psychiatric diagnoses. Subjects were scored as having a psychiatric diagnosis based on meeting criteria from either youth or parent report (Pajer et al., 2001). Since ODD and CD are highly interrelated (Lahey et al., 1992), subjects who scored either or both of these diagnoses on the DISC were classified as having a DBD. Within the DP group 22 subjects had a DBD diagnosis (DP+), while 49 had not (DP−). Of de DP+ subjects, 9 had ADHD, compared to 5 of the DP− subjects. A χ^2 analysis showed that ADHD was significantly more prevalent in the DP+ group than in the DP− group ($p < 0.01$). Since PTSD has been reported to influence HPA axis activity, this section of the DISC was administered to be able to control for possible PTSD comorbidity. In the current sample though, none of the subjects scored a PTSD diagnosis. Within the control group, no subjects scored a DISC diagnosis. Two subtests (Vocabulary and Block-design) of the WISC-R intelligence test (Wechsler, 1974) were used to estimate subjects' IQ.

Table 1 Characteristics of subjects

	NC ($n = 30$)	DP− ($n = 49$)	DP+ ($n = 22$)
Age	13.30 ± 0.70	13.93 ± 0.78	13.63 ± 0.69
IQ	98.73 ± 11.67	92.83 ± 12.56	93.32 ± 14.43
SES			
Low	11 (36.7%)	23 (46.9%)	10 (45.5%)
Middle	9 (30.0%)	11 (22.4%)	8 (36.4%)
High	10 (33.3%)	15 (30.6%)	4 (18.2%)
Ethnicity			
Caucasian	11 (36.7%)	17 (34.7%)	7 (31.8%)
Surinam/Antillean	9 (30.0%)	13 (26.5%)	7 (31.8%)
Mediterrenean	6 (20.0%)	15 (30.6%)	4 (18.2%)
Other	4 (13.3%)	4 (8.2%)	4 (18.2%)

Data are presented as means and SD or number and percentage with subgroup.

2.3. Stress test procedures

The psychosocial stress test consisted of a PST, with video recording, which has been proven to be an effective stressor in both children and adults (Dickerson and Kemeny, 2004; Kudielka et al., 2004). This task embodies all the major criteria of an anxiety-arousing situation, for it is perceived as a threat, only partially under participant control and implies uncertainty with regard to the outcome and/or consequences. To minimize anticipatory anxiety during the preceding resting period, the exact content of the entire test-session was not explained beforehand, as subjects were asked to take part in a study on 'difficult tasks'. The PST was imbedded in a 125 min test session, consisting of an initial resting period (50 min), the PST (15 min) and a post-test resting period (60 min). Both resting periods were spent as relaxed as possible with subjects filling in questionnaires. Furthermore, subjects were instructed to remain seated and were not allowed to eat or drink during the test session. After the initial resting period, an unfamiliar test assistant entered the room in order to explain PST. It was suggested that a 'jury' of three psychologists was behind a one-way screen, judging the subjects' performance. Subjects were given 10 minutes to prepare a 5-min talk on a topic of their choice. If the subjects stopped talking before the 5 min were over, the test assistant came into the room and motivated the subjects to continue, by saying 'You have some time left, please continue', or 'Try to tell us something more about your topic'. After the talk, subjects were left to wait for 2 min for the judgment of the psychologists. This judgment was always positive, thereby ending the stressful situation.

2.4. Saliva collection and cortisol analysis

Saliva was sampled for cortisol measurements, using the Salivette sampling device (Sarstedt, Nümbrecht, Germany). Each subject participated in the experiment on a week-day between 1 and 4 pm to limit diurnal variation and because afternoon values are more strongly influenced by external stimulation (Kirschbaum and Hellhammer, 1994). Seven saliva samples were collected (see Table 2) at the following time points: (1) 20 min before the start of the PST, (2) before preparing the PST-talk, (3) before the talk, (4) immediately after the talk, and 20, 40 and 60 min after finishing the talk (sample 5, 6, 7, respectively). Cortisol can be measured form saliva in a reliable and stress-free way (Kirschbaum and Hellhammer, 1994), and reflects the biologically active (unbound) fraction of serum cortisol (Aardal and Holm, 1995). After the start of an effective stressor, salivary cortisol increases can be observed 15–20 min later (Kirschbaum and Hellhammer, 1994). Cortisol analyses were performed in duplicate by direct radioimmunoassay, using 125I-cortisol and antiserum made against the 3-CMO-BSA conjugate (Sulon et al., 1978). The lower detection limit of the assay was 7 ng/dl, with mean intra- and inter-assay coefficients of variation of respectively 4.3% ($n = 10$) and 9.4% ($n = 30$).

2.5. HR and SCL registration

HR and SCL were continuously measured as indices of autonomous activity using the VU-AMS (Klaver, 1994). Three electrodes were placed on the chest to measure HR. Ag/AgCl electrodes for SCL measurement were applied to the medial phalanges of

Table 2 Time schedule for the psychosocial stress test session

Initial resting period		Psychosocial stress test		Post-test resting period			
$t = -50$ min	$t = -25$	$t = 0$	$t = 10$	$t = 15$	$t = 35$	$t = 55$	$t = 75$
HR and SCL	Saliva NF[a]	Saliva NF	Saliva NF	Saliva NF	Saliva NF	Saliva	Saliva NF

[a]Von Zerssen self-reported negative feelings.

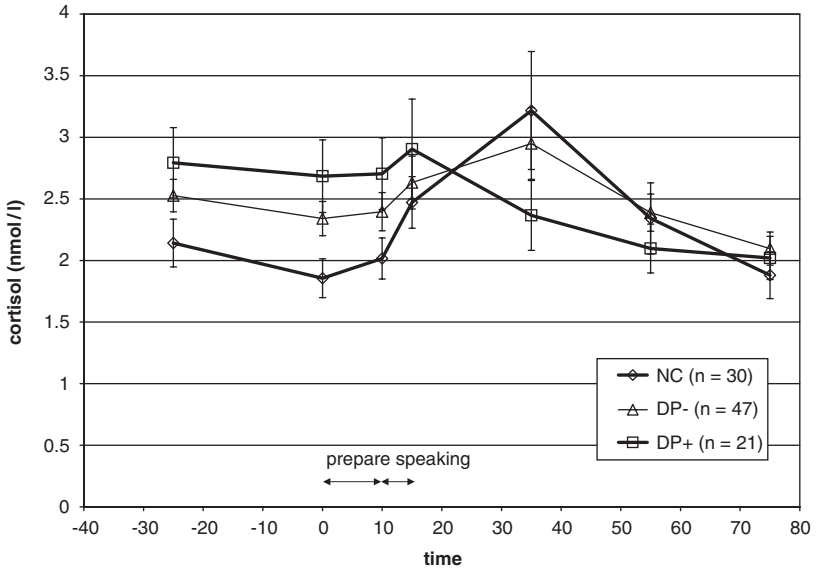

Figure 1 Salivary cortisol during baseline, preparation, speaking and again baseline conditions. Means and standard error scores are indicated.

the middle and ring fingers of the non-dominant hand after applying Redux crème (Fowles et al., 1981).

2.6. Recording of negative affect

At the same time points of cortisol sampling, subjects filled out the Von Zerssen scale (Von Zerssen, 1986), modified for children, to measure affect changes. Participants were asked to report their feelings from a list of positive and negative affect labels: good/bad, happy/sad, cheerful/not cheerful, pleasant/unpleasant, satisfied/dissatisfied, afraid/not afraid, strong/weak, calm/nervous, and angry/not angry'. Items could be scored as follows: 0 = positive affect label, e.g. good, 2 = negative affect label, e.g. bad, or 1 = 'none of those'. A total negative affect score per time point was calculated by adding scores of 9 items. The mean Cronbach α was 0.73.

2.7. Data analyses

Because cortisol values were positively skewed, they were transformed to the natural logarithm scale after which all cortisol values were normally distributed as tested with a Kolmogorov–Smirnov analysis. For reasons of physiological meaningfulness, the graphical representation of the diurnal curve in Fig. 1 shows absolute cortisol values instead of log(cortisol) values. All other variables were normally distributed. Repeated measures analyses of variance (MANOVAs) with 'group' (DP+ vs. DP− vs. NC) as between subjects factor and 'time' (consecutive samples) as within-subjects factor were conducted, applying greenhouse-geisser correction when appropriate, to assess differences between groups with respect to changes over time in log(cortisol), HR, SCL and negative affect. Main effects of time, as well as interactions between 'time' and 'group' were further analyzed by conducting difference contrast tests,

i.e. comparing the values of a sample at a certain time point to all previous ones. To explore main effects of group, simple contrast tests were employed. As a specific measure of responsivity of the biological variables to the stressor, area under the response curves (AURC) were computed, with reference to the pre-test value right before the start of the stressor. As such, for cortisol the AURC was computed between samples 2 and 7, with reference to sample 2. As ANS parameters react without substantial latency-time to the onset and ending of a stressor, for both HR and SCL the AURC was computed between samples 2 and 5, with reference to sample 2. One-factor ANOVAs were used to assess the effect of group (DP+ vs. DP− vs. NC) on the AURC for cortisol, HR and SCL. Cortisol could not be analyzed for three subjects (2 DP− and 1 DP+) due to a lack of saliva in the salivettes. HR and SCL were not recorded for one DP− subject because of technical problems with the VU-AMS. Outliers were defined as values more than 3 SD above or below the group average. As such, one subject (DP−) was found to have two HR outliers and was excluded from the HR analyses. All post hoc analyses were corrected with the Bonferroni method for multiple comparisons.

3. Results

3.1. HPA: cortisol

A graphic representation of salivary cortisol levels by subgroup is given in Fig. 1. Repeated-measures MANOVA revealed a significant main effect of time [$F = 11.21$, $p < 0.001$], a significant group by time interaction [$F = 3.32$, $p < 0.01$], but no main effect of group [$F = 1.02$, $p = 0.366$]. Difference contrast tests, comparing each sample with all previous ones, showed that the significant main effect of time was mainly attributable to significant effects for sample 2, 4, 5, 6, and 7. The significant group by time interaction was mainly attributable to a significant difference between groups for sample 4 versus previous samples [$F = 3.66$, $p = 0.029$], sample 5 versus previous samples [$F = 6.52$, $p < 0.0022$], while the effect for sample 6 versus previous samples approached significance [$F = 2.84$, $p = 0.062$]. An ANOVA on HPA reactivity to the stressor, represented by the AURC for cortisol, by subgroup (DP+ vs. DP− vs. NC), revealed a significant difference between groups [$F = 4.80$, $p = 0.010$]. Post hoc analysis showed a significantly smaller AURC for DP+ compared with NC [$p < 0.008$].

3.2. ANS: heart rate and skin conductance

Heart rate (HR): a graphic representation of heart rate levels by subgroup is given in Fig. 2. Repeated-measures MANOVA revealed a significant main effect of time [$F = 41.70$, $p < 0.001$], no group by time interaction [$F = 1.67$, $p = 0.15$], and no main effect of group [$F = 0.15$, $p = 0.86$]. Difference contrast tests showed significant time-effects at all time points. An ANOVA on AURC for heart rate, by subgroup (DP+ vs. DP− vs. NC), revealed a significant group difference [$F = 3645$, $p = 0.030$]. Post hoc analysis showed a significant smaller AURC for DP+ compared with NC [$p < 0.040$], and a trend toward a smaller AURC for DP+ as compared with DP− approaching significance [$p = 0.064$].

Skin conductance level (SCL): a graphic representation of SCL by subgroup is given in Fig. 3. Repeated-measures MANOVA revealed a significant main effect of time [$F = 62.93$, $p < 0.001$], no group by time interaction [$F = 0.74$, $p = 0.60$], and no main effect of group [$F = 2.32$, $p = 1.03$]. Difference contrast tests showed significant time-effects at all time points.

3.3. Self-reported negative feelings

A graphic representation of NF scores by subgroup is given in Fig. 4. Repeated-measures MANOVA, with greenhouse-geisser correction, revealed a significant main effect of time [$F = 37.60$, $p < 0.001$], no group by time interaction [$F = 0.50$, $p = 0.82$], and no main effect of group [$F = 0.46$, $p = 0.63$]. Difference contrast tests showed significant time-effects at all timepoints.

In a series of post hoc analyses, Kruskall–Wallis tests were used to examine the individual items of the NF scale. At the time point before speaking, significant group differences were found, with both DP groups reporting more 'unpleasant' feelings ($\chi = 8.37$, $p < 0.015$) and the NC group reporting more 'nervous' feelings ($\chi = 6.99$, $p < 0.03$).

3.4. Covariate analyses

As mentioned before, ADHD was more prevalent in the DP+ group than in the DP− group. Rerunning analyses with ADHD as a co-variate did not affect significancy of any of the results, and ADHD was not significant as a co-variate in any of the analyses.

4. Discussion

The aim of the present study was to investigate the relationship between HPA and ANS (HR and SCL)

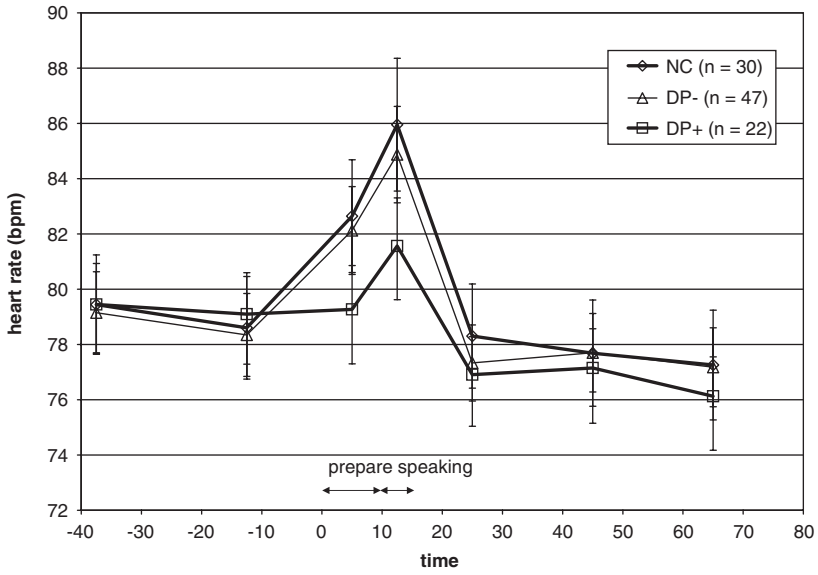

Figure 2 Heart rate during baseline, preparation, speaking and again baseline conditions. Means and standard error scores are indicated.

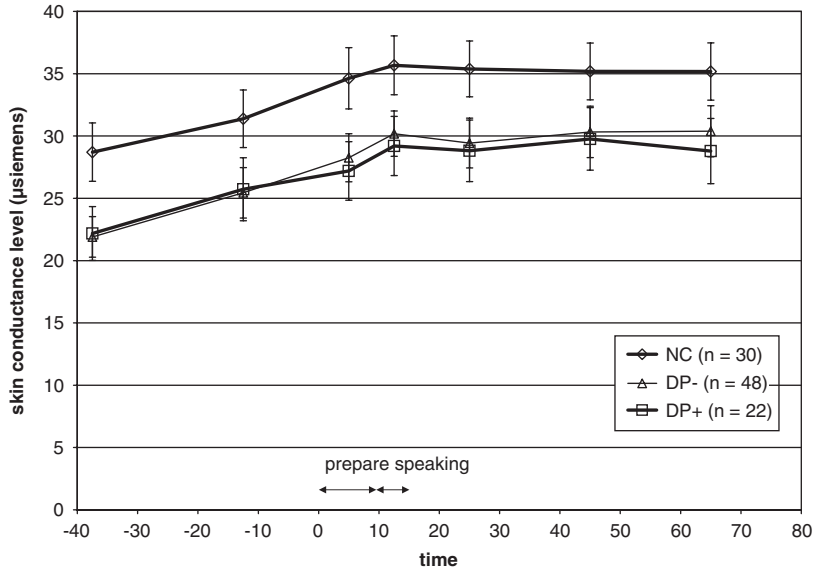

Figure 3 Skin conductance level during baseline, preparation, speaking and again baseline conditions. Means and standard error scores are indicated.

reactivity to a (PST) and disruptive behavior in 12–14 year old males referred to a delinquency (DP), with or without a diagnosis of DBD (DP+ vs. DP−), and matched NC. The DP+ group showed a significantly decreased response of both cortisol and HR to a Public Speaking Task (PST), as compared with the NC group. The DP− group did not differ from the NC group with respect to cortisol and HR response to stress. The differences in cortisol and HR responsivity between the DP+ and NC group were found in spite of similar total scores of reported negative affect in all groups.

The finding of a decreased cortisol and HR response to stress in the DP+ group but not in the DP− group, as compared with the NC group, without a difference in pre-test levels, is in line with findings from earlier studies in clinical referred DBD children, in which a frustrating stressor was used (Van Goozen et al., 1998; Van Goozen et al., 2000; Snoek et al., 2004). As such, this hyporeactivity could be a marker for distinguishing delinquent boys with a DBD diagnosis from both NCs and delinquent boys with less serious disruptive behavior problems, both in clinical- and non-clinical referred samples.

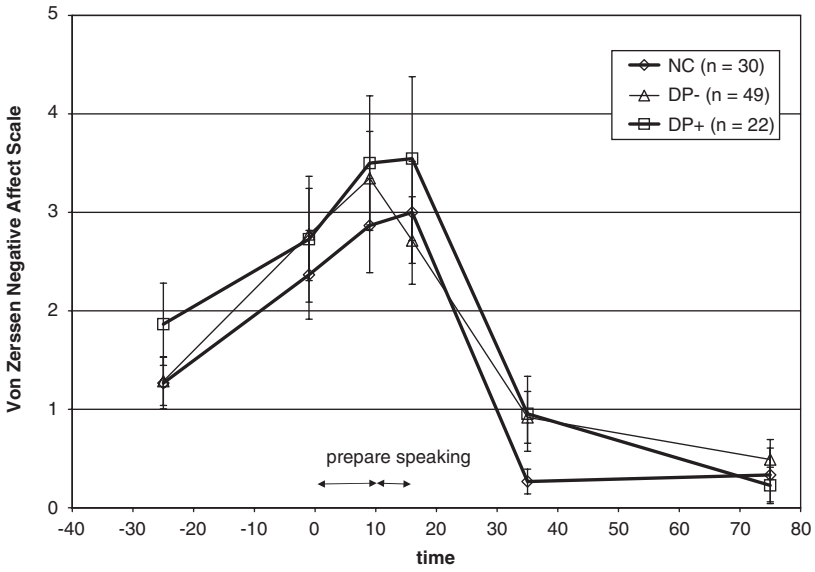

Figure 4 Self-reported negative feelings during baseline, preparation, speaking and again baseline conditions. Means and standard error scores are indicated.

Although the SCL findings in this study were in the predicted direction, no significant effects were observed. In the literature SCL findings in antisocial groups have been less robust than findings on HR (Ortiz and Raine, 2004), which may indicate that parasympathetic regulation, controlling HR but not SCL, is more important than sympathetic influences in explaining disruptive behavior.

Even though no differences were found between groups in total scores of self-reported negative feelings during the stress task, post hoc analyses suggested qualitative differences in affective states. Although these findings should be interpreted with care, given the post hoc nature of the analysis, these results may suggest an alteration in the coordination of emotions and psycho-physiologic mechanisms during stress in boys with disruptive behavior problems.

The observed attenuated endocrinological and physiological responses to a fearful and stressful situation in delinquent boys with a DBD diagnosis relative to NCs, may be associated with lower levels of fearfulness and with low responsivity to social cues in general, e.g. punishment (Raine, 1993). Such mechanisms may be important in explaining the poor prognosis and the limited effectiveness of current treatments of children with a DBD diagnosis. So far, though, only a limited number of studies have demonstrated that HPA and ANS activity may predict the onset and course of disruptive behavior problems. With respect to HR and SCL, low resting levels in children have been found to predict future criminal behavior (Raine et al., 1997; Kruesi et al., 1992). Interestingly, Shoal et al. (Shoal et al., 2003) found that low resting cortisol in 10–12-year-old boys was predictive for correlates of disruptive behavior problems at age 15–17. Furthermore, recent preliminary evidence for the clinical implications of altered HPA responsivity to stress in the treatment of DBD boys was provided by Van de Wiel et al. (2004), who reported low cortisol responsivity during stress to be associated with poor treatment outcome. The authors concluded that, awaiting replication of their preliminary results, in the distant future biological screening could be of value to assign specific treatments to subgroups of DBD children. For example, pharmaceutical interventions aimed at adjusting biological disturbances could be added to psychotherapeutic programmes in those individuals with low HPA and ANS responsivity to stress.

Future research should aim to investigate HPA (re)activity at different levels of the axis (hypothalamus, pituitary and adrenals) and at higher levels (e.g. the amygdala and limbic system) simultaneously to elucidate the neuroendocrinological mechanisms underlying the observed low cortisol levels in DBD populations. More over, to disentangle HPA reactivity per se from HPA reactivity to social stress, studies using non-psychosocial stressors are needed. In this respect, for example, Gispen-de Wied et al. (1998) reported hyporesponsiveness to hydrocortisone, but not to dexamethasone, in a group of 14 clinical referred DBD children as compared with a group of 10 NCs, suggesting feedback disturbances beyond the level of the pituitary. Applying a physical challenge, Jansen

et al. (1999) found no differences in HPA reactivity between 14 clinical referred DBD children and 15 NCs. Future studies seem worthwhile and should include larger sample sizes.

Some methodological limitations of the present study need to be noted. First, no control test was performed to compare measures during the session described here, to measures during a non-stressful session. Still, a body of literature has shown the applied stressor to be highly effective (Dickerson and Kemeny, 2004; Kudielka et al., 2004), and a significant increase of all measures was found during the stressor, with a subsequent decrease after finishing the PST. Moreover, when analyzing responsivity to the applied stressor, we corrected for baseline values measured before the start of the stressor. Second, the current sample of delinquents consisted only of boys. While resting cortisol levels have been related to conduct problems in adolescent girls (Pajer et al., 2001), future studies in girls including measures of HPA responsivity seem warranted. Third, we were not able to control for possible internalizing disorders since the sections of the DISC for these disorders were not administered.

In conclusion, the present findings in a non-clinical referred sample suggest that delinquent boys with DBD, but not delinquent boys without DBD, are characterized by low cortisol and HR responsivity to psychosocial stress. Future research should provide a better understanding of both the biological an psychosocial mechanisms involved in the development, persistence and prognosis of disruptive behavior, to ultimately result in earlier and more effective interventions.

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