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Concurrent attenuated reactivity of alpha-amylase and cortisol is related to disruptive behavior in male adolescents

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ABSTRACT

Attenuated reactivity of salivary alpha-amylase has been proposed as a specific sympathetic marker of disruptive behavior in juveniles and may have additional value to studying other autonomic parameters and hypothalamic–pituitary–adrenal axis activity. Investigating the interrelationships between neurobiological parameters in relation to juvenile disruptive behavior may enhance insight into the complex mechanisms at play.

We investigated salivary alpha-amylase, cortisol, heart rate (HR), and heart rate variability (HRV) in response to a standardized public speaking task, and examined interactions between these parameters in relation to disruptive behavior. Participants were 48 delinquent male adolescents (mean age 18.4 years, SD 0.9), with and without a disruptive behavior disorder (resp. DP+, DP−) and 16 matched normal controls (NC). A structured psychiatric interview as well as the Youth Self Report and Child Behavior Checklist were administered to assess disruptive behavior.

Alpha-amylase and cortisol reactivity, but not HR or HRV, showed significant inverse associations with dimensional measures of disruptive behavior. Moreover, both cortisol and alpha-amylase reactivity were significantly lower in the DP+ group as compared to the NC group. The mentioned relationships remained present when nicotine use was entered as a covariate. Combining alpha-amylase and cortisol in one model explained a larger part of the variance of disruptive behavior than either single parameter. There were no interactions between alpha-amylase and cortisol or HRV in relation to disruptive behavior.

Attenuated alpha-amylase responsivity to stress is a correlate of disruptive behavior in late-adolescent males. Although nicotine use explains a considerable part of the variance of disruptive behavior, both alpha-amylase and cortisol are related to disruptive behavior, over and above the effect of nicotine use. Combining alpha-amylase and cortisol improved insight into neurobiological mechanisms involved with disruptive behavior; concurrent low reactivity of both parameters was related to higher levels of disruptive behavior.

Introduction

Increasing evidence suggests that disruptive behavior in juveniles is associated with decreased activity of stress-related neurobiological systems, such as the hypothalamic–pituitary–adrenal (HPA) axis and the autonomic nervous system (ANS) (Beauchaine, 2001; Raine, 2002; van Goozen et al., 2007). Regarding the ANS, general autonomic measures (i.e. heart rate) as well as parasympathetic measures (i.e. heart rate variability, HRV) and sympathetic measures (i.e. skin conductance, (nor)epinephrine) have been studied. The study of the sympathetic component, however, has been hampered by difficulties in obtaining biological measures such as (nor)epinephrine. Recently, an easily obtainable marker of sympathetic nervous system activity, namely salivary alpha-amylase has been reported on, and an inverse relationship was found with disruptive behavior (Granger et al., 2007; Nater and Rohleder, 2009). Therefore, in the current study we examined the additional value of alpha-amylase to cortisol, heart rate and HRV as potential correlate of juvenile disruptive behavior. Moreover, we investigated the combined activity as well as interactions between the various parameters in relation to disruptive behavior.

Measures of the ANS like heart rate are regulated by both parasympathetic and sympathetic nervous systems (resp. PNS, SNS), and may

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therefore be less specific than 'pure' PNS or SNS measures (Berntson et al., 1991). While parasympathetic measures have been studied rather extensively in relation to disruptive behavior (Beauchaine, 2001), research on measures of SNS reactivity has been hampered by various methodological difficulties. Although skin conductance is determined as a marker of SNS, it appears to be most valuable for measuring phasic responses to stimuli presented for milliseconds to seconds, rather than psychosocial stress experiments lasting for minutes or hours (Lahey et al., 1993; Popma et al., 2006; van Goozen et al., 2000). Catecholamines as SNS measures are relatively difficult to obtain, which may explain the small number of studies so far. Correlations between plasma catecholamines, particularly norepinephrine, and alpha-amylase have been reported (Chatterton et al., 1996; Rohleder et al., 2004), although findings are not overall consistent (Nater et al., 2006). Because norepinephrine stimulates the output of alpha-amylase by the salivary glands in response to adrenergic sympathetic activation (Bosch et al., 2003), salivary alpha-amylase is likely to act as a specific measure of SNS reactivity (Rohleder et al., 2004; Van Stegeren et al., 2006). Although the specificity of alpha-amylase as a sympathetic measure depends on methodological issues like sampling procedures (Bosch et al., 2011; Nater and Rohleder, 2009; Rohleder and Nater, 2009), it may serve as a correlate of juvenile disruptive behavior.

Regarding the relation between alpha-amylase and disruptive behavior, an overview of the current literature by Granger et al. (2007) indicated inverse relationships in healthy children and adolescents. In a recent study in a general population sample of early-adolescent boys and girls, attenuated alpha-amylase reactivity to the Trier Social Stress Test (TSST) was found in relation to parent-reported disruptive behavior (Susman et al., 2010). To date, studies on the relationship between alpha-amylase reactivity and disruptive behavior in clinic-referred and delinquent samples are lacking.

Other parameters of the stress regulation system have been studied extensively in both clinic-referred or delinquent samples and the general population. Measures include the HPA-axis (represented by cortisol), the ANS (represented by heart rate) and more specific the PNS (represented by HRV). Inverse relationships between these parameters and disruptive behavior have been shown frequently (Beauchaine, 2001; Ortiz and Raine, 2004; van Goozen et al., 2007). These associations have often been explained by theories of low (autonomic) arousal. In these theories, attenuated physiological responsivity to stress is regarded as a marker of low levels of fear and low punishment sensitivity. Fearless juveniles are thought to be more likely to engage in disruptive behaviors because they do not fear the negative consequences of their actions (Raine, 1993; Raine, 2002). Genetic vulnerabilities and/or early life adversities may underlie the attenuated stress responsivity (van Goozen and Fairchild, 2008).

Similarly, it has been proposed that disruptive children are characterized by a mismatch in the interplay between different physiological systems involved in the regulation of stress. For example, regarding SNS and HPA-axis reactivity, generally, activity of both systems increases in response to stress. Bauer (2002) postulated two models that specifically describe the interrelationship between both systems in relation to disruptive behavior. The *additive* model proposes that low reactivity in both systems concurrently (balanced low activity) is related to elevated levels of disruptive behavior. As such, this fits in with the low arousal theory. Alternatively, the *interactive* model proposes that low reactivity in one system together with concurrent high reactivity in the other system (asymmetrical or unbalanced activity) is associated with greater risk of disruptive behavior (Bauer et al., 2002). In this model it is thus suggested that the relationship between either of the two systems and disruptive behavior is moderated by the other system. Gordis et al. (2006) tested this hypothesis in a study in which they investigated the interaction between alpha-amylase and cortisol in relation to disruptive behavior. In a sample of maltreated early-adolescents and a control group, they found that interactions between the HPA-axis and the SNS are linked with disruptive behavior. The interaction showed that low activity in both systems was associated with more aggression (Gordis et al., 2006).

Moreover, another mismatch between generally well-coordinated physiological stress systems within the ANS, i.e. the interaction between the SNS and the PNS, has been described in disruptive juveniles. It has generally been assumed that SNS and PNS display coupled, reciprocal actions on organ systems. When SNS activity increases, the PNS activity decreases, and vice versa. However, it has also been argued that the SNS and PNS function as two separate dimensions (Berntson et al., 1991). These non-reciprocal actions may result in concurrent increases or concurrent decreases in both branches, leading to ambiguous effects on physiological arousal (Berntson et al., 1993). Indeed, several studies found concurrent low levels of SNS and PNS to be related to juvenile disruptive behavior (Beauchaine et al., 2007; Boyce et al., 2001; El-Sheikh et al., 2009). Findings warrant replication in other age samples like children or late-adolescents, as well as in specific samples like (clinic referred) disruptive behavior disordered juveniles or delinquents.

There are still inconsistencies in the literature on the relationships between neurobiological parameters and disruptive behavior (Dietrich et al., 2007; Lorber, 2004; Sondeijker et al., 2008), for which several explanations have been given. One explanation may be the different methods of measuring disruptive behavior that were used. Although for clinical purposes it is useful to study disruptive behavior categorized in disorders, dimensional measures are able to distinguish between severe or mild forms of disruptive behavior. It is thus important to relate neurobiological parameters to categorical and dimensional measures of disruptive behavior. Another explanation may be that many studies have focused on only one system at the time, not taking into account cumulative effects and interactions between involved systems (Bauer et al., 2002; Gordis et al., 2006). As explained above, it has been proposed not to focus on physiological systems independently, but to take into account interrelationships as well, to enhance understanding of the associations with juvenile disruptive behavior (Bauer et al., 2002).

Improving knowledge on the neurobiological basis of disruptive behavior may ultimately lead to improved identification of juveniles at risk for a deviant development, such as juvenile delinquents. Therefore, in the present study, we concurrently assessed reactivity of alpha-amylase, cortisol, heart rate and HRV during a public speaking task in delinquent male adolescents and matched normal controls. We related neurobiological reactivity to categorical as well as dimensional measures of disruptive behavior. We investigated whether examining combined reactivity of the parameters alpha-amylase, cortisol, heart rate and HRV improves the explanation of disruptive behavior compared to examining one of the parameters alone. Furthermore, we tested which model of Bauer (additive or interactive) best explains disruptive behavior, by investigating interactions between SNS and HPA-axis reactivity as well as between SNS and PNS reactivity in relation to disruptive behavior.

Material and methods

Participants

Participants were 64 male adolescents, mean age 18.4 years, SD 0.9. From this sample, 48 participants attended a delinquency diversion program (DP group) and 16 were matched normal controls (NC group). Boys of the DP group all had a history of committing one or more offenses while in the NC group none had committed offenses (information administered from a police registration system). Groups were matched on IQ, there were no differences between groups in age, SES or ethnicity (see Table 1). The delinquent group showed a higher proportion of participants using nicotine compared to the NC group ($Chi^2 = 13.675$; $p < .001$). Because it is recommended to control for nicotine use when studying measures of ANS and HPA-axis (Granger et al., 2009; Kudielka et al., 2009; Rohleder and Nater, 2009), we incorporated the influence of nicotine use on the studied relationships (see Statistical

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analyses). Both groups were derived from a larger sample that participates in an ongoing study on neurobiological factors of antisocial behavior (for details, see Popma et al., 2006, 2007). Participants and the participating parents were given a thorough verbal and written outline of the procedures and they all gave written informed consent. Participants and the participating parents received a reimbursement for participation. The study was approved by the Medical Ethics Committee of the VU University Medical Center Amsterdam and was conducted in accordance with the Declaration of Helsinki.

Instruments

To obtain dimensional data on disruptive behavior problems, participants and parents filled out respectively the Youth Self Report (YSR) and the Child Behavior Check List (CBCL), which are widely used questionnaires to assess behavioral problems in children and adolescents (Achenbach, 2001; Verhulst et al., 1997). The questionnaires were obtained either at home, or during a visit at the laboratory, prior to a psychosocial stress task procedure (details provided below). The T-scores of the externalizing behavior scales were used.

To assess current psychiatric diagnoses of disruptive behavior disorders, the National Institute of Mental Health (NIMH) Diagnostic Interview Schedule for Children (DISC), version IV (Shaffer et al., 2000) was used, which is an extensive structured psychiatric interview. The sections on oppositional defiant disorder (ODD) and conduct disorder (CD) were administered from both participants and their parents by trained interviewers. Subjects were scored as having a diagnosis, when a diagnosis was scored in either of the separate interviews (Pajer et al., 2001). Subjects with either ODD and/or CD were classified as having disruptive behavior disorder (DBD). Within the delinquent group 15 subjects had a DBD diagnosis (DP+), while 33 had not (DP−). None of the participants in the control group had a DBD diagnosis.

Psychosocial stress test procedure

The participants performed a psychosocial stress test procedure in the laboratory, consisting of a public speaking task (PST) in front of a one-way screen with video recording (Jansen et al., 2000), which is an effective stressor in both children and adults (Dickerson and Kemeny, 2004). In healthy participants, similar psychosocial stress tests elicited increases in alpha-amylase, cortisol and heart rate, and a decrease in HRV (Kudielka et al., 2004a, 2004b; Nater et al., 2005; Strahler et al., 2010). The procedure is described in detail elsewhere (Popma et al., 2006). Briefly, there was a 50 minute resting period prior to the PST and a 60 minute resting period afterwards. After the resting period, an unfamiliar test assistant explained the PST itself, which consisted of a 5 minute speech on a topic of choice preceded by 10 min of preparation. It was suggested that a 'jury' of three psychologists was behind a one-way screen, judging the participants' performance. This judgment was always positive, thereby ending the stressful situation. All participants performed the procedure in the afternoon and all started within 3 SD before or after the mean starting time (13.56 h, SD 0:41). There were no differences in starting time between subgroups ($F=0.490$; $p=0.615$) and there were no correlations between starting time and any of the neurobiological parameters at rest or during stress (all $p>0.11$). Forty participants (62.5%) performed the same procedure in a previous assessment within our ongoing study, with a time lag of five years between the assessments (within subgroups: NC 87.5%; DP− 51.5%, DP+ 73.3%, $Chi^2=6.701$; $p=0.035$). We did not expect a habituation effect, because it has been shown that there was no habituation when repeating after shorter periods (Kirschbaum et al., 1995; Schommer et al., 2003). Indeed, we found no differences in neurobiological stress responsivity and negative affect between those participants who performed the procedure previously and those who did not (independent sample *t*-tests, all $p>0.58$).

Table 1
Characteristics of participants.

	NC (n=16)	DP− (n=33)	DP+ (n=15)	*F*/ Chi^2	*p*
Age	18.42±0.91	18.42±0.83	18.09±0.93	0.85	0.43
IQ	105.88 ±9.54	99.81±9.58	100.27 ±10.26	2.20	0.12
SES					
Low	4 (25.0%)	14 (42.4%)	7 (46.7%)	2.43	0.66
Middle	4 (25.0%)	7 (21.2%)	4 (26.7%)		
High	8 (50.0%)	12 (36.4%)	4 (26.7%)		
Ethnicity					
Caucasian	10 (62.5%)	18 (54.5%)	9 (60.0%)	0.32	0.85
Non-Caucasian	6 (37.5%)	15 (45.5%)	6 (40.0%)		
Externalizing behavior					
YSR T-score	47.38 ±12.14	51.09 ±8.71	62.93 ±10.85	10.16	0.00[a]
CBCL T-score	43.63±8.38	48.72±9.99	62.67±9.84	16.77	0.00[b]
Smoking status					
Use of nicotine	2 (12.5%)	20 (62.5%)	11 (73.3%)	14.16	0.00[c]

Data are presented as means±SD or number and percentage within subgroup.
NC, normal control subjects; DP−, delinquents without disruptive behavior disorder; DP+, delinquents with disruptive behavior disorder; SES, socioeconomic status; YSR, Youth Self Report; CBCL, Child Behavior Checklist.
[a] DP+ >NC ($p<0.01$), DP+ >DP− ($p<0.01$).
[b] DP+ >NC ($p<0.01$), DP+ >DP− ($p<0.01$).
[c] DP+ >NC ($p=0.01$), DP− >NC ($p=0.01$).

Procedure for saliva collection and ANS recording during the stress test procedure

Saliva was sampled using the cotton salivette sampling device without citric acid preparation (Sarstedt, Nümbrecht, Germany) for cortisol and alpha-amylase assessment at the following seven time points: 1) 25 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 (resp. samples 5, 6 and 7). Participants were instructed not to smoke, eat and drink (besides water) during the entire test session. Participants placed the salivette in their mouth for approximately 1 min, and they were instructed to gently chew it.

Heart rate and HRV were measured continuously during the entire procedure as an index of autonomic/parasympathetic activity, using the VU-AMS (Klaver et al., 1994). Three disposable Ag/AgCl electrodes filled with conducting paste were placed on the chest, they were connected with lead wires to the AMS device. The R-top was recognized with a level detector with automatic level adjustment. At each R-peak, a ms counter is read and reset, yielding the raw inter-beat interval. The R–R time accuracy was 1 ms. From the ECG we obtained the inter-beat interval (IBI) time series. For the analysis of HRV, we performed spectral analyses using Kubios HRV software, developed by the Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Finland. The IBI time series was decomposed into component HRV frequencies by using Fourier transformations. The resulting components are expressed in terms of a spectral density function, or the amount of spectral power within a given frequency band. For the purpose of this study, we used high-frequency HRV (0.15–0.40 Hz). High-frequency power provides a frequency-domain index of parasympathetic activity (Berntson et al., 1997). The mean heart rate/HRV during seven time periods, analogous to the moments of saliva sampling, was used in the analyses.

Recording of negative affect

At the same time points of saliva sampling except for sample 6, participants filled out the Von Zerssen (1986) scale, modified for children, to measure affect changes. Participants were asked to report their feelings from a list of positive and negative affect labels. Items

could be scored as follows: 0 = positive affect label, e.g. 'good', or 'calm', 2 = negative affect label, e.g. 'bad', or 'nervous', or 1 = 'none of those'. A total negative affect score per time point was calculated by adding scores of the 9 items.

Cortisol and alpha-amylase analyses

Cortisol was analyzed by using electrochemiluminescence immunoassay (ECLIA) in Leiden, the Netherlands. The lower detection limit was 0.5 nmol/l, with mean intra- and inter-assay coefficients of variation of respectively 3.4% and 12.2%. Alpha-amylase was analyzed in the same laboratory. Samples were 50 times diluted with 9% sodium chlorine, using a Hamilton Microlab 500B/C diluter. Diluted samples were analyzed by using enzymatic colorimetric assay. Defined oligosaccharides such as 4,6-ethylidene-(G7) p-nitrophenyl-(G1)-alpha, D-maltoheptaoside (ethylidene-G7PNP) are cleaved under the catalytic action of alpha-amylases. The G2PNP, G3PNP, and G4PNP fragments formed are completely hydrolyzed to p-nitrophenol and glucose by alpha-glucosidase. The color intensity of the p-nitrophenol is directly proportional to the alpha-amylase activity. It is determined by measuring the increase in absorbance at 409 nm. The lower detection limit was 3 U/l, and the mean intra- and inter-assay coefficients of variation were both lower than 2.0%.

Statistical analyses

Analyses were performed using SPSS 17.0. Alpha-amylase, cortisol and HRV values were positively skewed, therefore a square-root transformation was applied, after which all values were normally distributed. Heart rate values were normally distributed. For reasons of physiological meaningfulness, Figs. 1 and 3 show absolute values of the parameters. Participants with more than 2 missing saliva samples/heart rate/HRV periods, or with 2 consecutive missing samples/periods were excluded from the particular analyses. Outliers were defined as values more than 3 SD below or above the group average, participants with more than 2 outlying values were excluded. Single or two non-consecutive missing samples or outliers were replaced by the group average at that time point.

Chi-square or one-way analyses of variance (ANOVAs) were performed to compare demographic characteristics between subgroups (NC vs. DP− vs. DP+). To assess changes in neurobiological parameters during the stress task, repeated measures ANOVAs were conducted with 'time' (consecutive samples) as within-subjects factor. Greenhouse–Geisser corrections were applied when the assumption of sphericity was violated. Difference contrasts were performed to further assess effects of time, i.e. comparing the values of a sample at a certain time point to all previous ones.

As a measure of basal activity of the three neurobiological parameters, sample/period 2 during the psychosocial stress task was used. As a specific measure of responsivity of the biological variables to the stressor, areas under the curve with respect to the increase (AUCi) were computed across samples 2–6, with reference to sample 2 (right before the start of the stressor) (Pruessner et al., 2003).

To assess the relationship between the biological parameters and the dimensional measures of disruptive behavior, single linear regression analyses were conducted on the total group of participants with cortisol, amylase, heart rate or HRV (basal measures or AUCis) as independent variable and T-scores of the externalizing scales of YSR/CBCL as dependent variables. Additional analyses were performed with nicotine use entered as dichotomous covariate in the significant linear regression models, to control for the possible confounding effect.

One-way ANOVAs were conducted for group comparisons (NC vs. DP− vs. DP+) on neurobiological reactivity (AUCis). Simple contrasts were conducted to further explore differences between groups. Based on previous research (Fairchild et al., 2008; Popma et al., 2006), it is expected that the main differences are between NC and DP+ groups. Therefore in case of a non-significant ANOVA, we did perform simple contrasts for the specific comparison of the DP+ and NC groups.

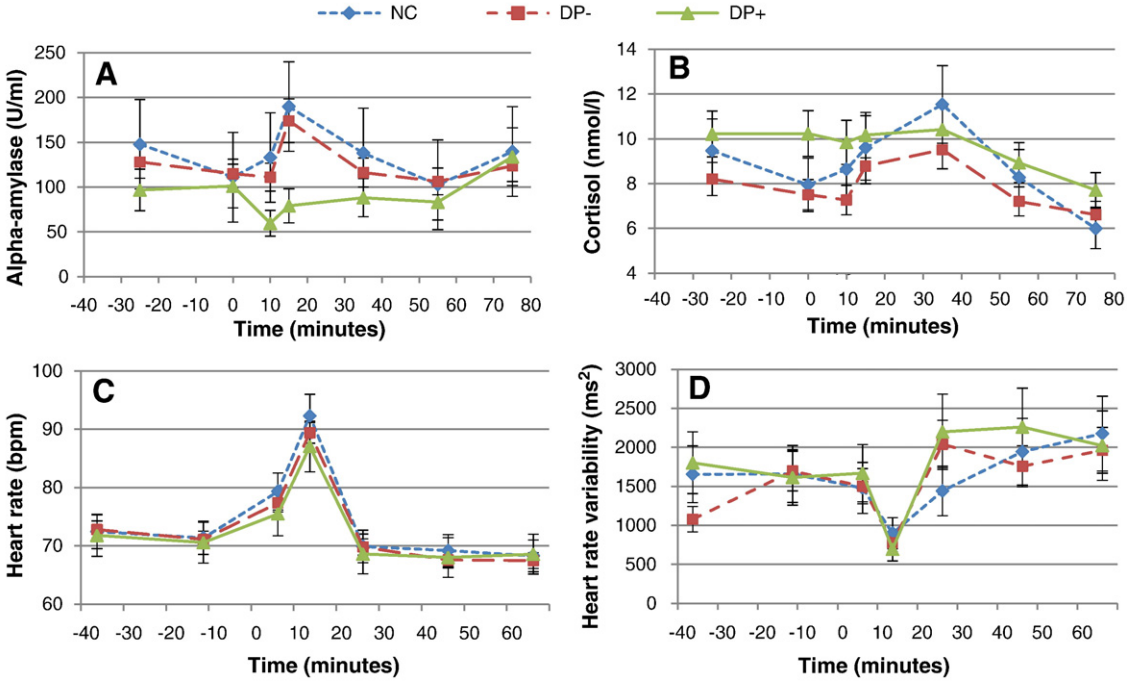

Fig. 1. Number of participants varied per analyses, N for respectively NC, DP−, DP+: alpha-amylase 15, 33, 12; cortisol 16, 32, 12; heart rate 15, 27, 15; and heart rate variability 15, 29, 15. The mean (±SEM) levels are displayed for A. alpha-amylase, B. cortisol, C. heart rate, D. heart rate variability. Levels are by subgroup during the stress test procedure, showing seven time points during baseline, preparation (0–10), speaking (10–15) and again baseline.NC, normal control subjects; DP−, delinquents without disruptive behavior disorder; DP+, delinquents with disruptive behavior disorder.

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Group sizes were too small to enter nicotine use as a covariate. As an alternative, we compared nicotine users in the DP− and DP+ groups on neurobiological parameters.

Stepwise forward multiple linear regression procedures were performed to test which (combination) neurobiological factors (AUCis) best predicted self- or parent-reported disruptive behavior. We only put the parameters $p<0.1$ in the model, based on the single linear regression analyses. In both single and multiple regressions, we used standardized values for all measures. Pearson correlations were conducted to assess bivariate correlations between the neurobiological parameters. As a first test of the Bauer models, we examined the interaction between alpha-amylase and cortisol in relation to disruptive behavior. For that purpose, we used multiple linear regression with disruptive behavior as dependent variable and alpha-amylase, cortisol and their interaction as independent variables. As a second test of the Bauer models, the interaction between alpha-amylase and HRV in relation to disruptive behavior was examined in a multiple linear regression with alpha-amylase, HRV and their interaction as independent variables.

Results

Repeated measures ANOVAs showed significant main effects of time for all four neurobiological parameters, attributable to the time points during the stress test procedure, revealing significant changes in parameters during the stress test (alpha-amylase: $F=4.88$; $p<0.01$; cortisol: $F=12.93$; $p<0.01$; heart rate: $F=147.64$; $p<0.01$; HRV: $F=18.31$; $p<0.01$).

Neurobiological parameters related to dimensional measures of disruptive behavior

We used single linear regression analyses to test the bivariate relationships between cortisol, alpha-amylase, heart rate and HRV with self- and parent-reported disruptive behavior in all participants. There were no relationships between basal levels of any of the four neurobiological parameters and self- or parent-reported disruptive behavior. As a specific measure of reactivity to stress, we related AUCis of the four parameters to the dimensional measures of disruptive behavior (see Table 2). Alpha-amylase reactivity showed a significant inverse association with self- and parent-reported disruptive behavior. Furthermore, we found a significant inverse relationship between cortisol reactivity and self-reported disruptive behavior. There were no relationships between heart rate or HRV reactivity with self- and parent reported disruptive behavior.

When nicotine use was entered as covariate, the relationship between alpha-amylase and self-reported disruptive behavior remained significant (Beta = −0.26; $p=0.03$; Adj. $R^2=0.05$) whereas the relationship with parent-reported disruptive behavior did not remain significant (Beta = −0.24; $p=0.08$; Adj. $R^2=0.04$). The relationship between cortisol and self-reported disruptive behavior remained significant (Beta = −.23; $p=0.05$; Adj. $R^2=.04$). In all three models, nicotine use was significantly related to higher levels of disruptive behavior (Beta ranging from 0.30 to 0.47, all $p<.03$).

Differences between subgroups in neurobiological parameters and negative affect

Besides dimensional measures of disruptive behavior, we investigated differences between subgroups (NC/DP−/DP+) in reactivity of the four neurobiological parameters. Graphic representations of neurobiological levels before, during and after the stress task by subgroup are given in Figs. 1A–D. A one-way ANOVA revealed significant differences between subgroups in alpha-amylase reactivity (AUCi, $F=4.10$; $p=0.02$). Simple contrast tests showed a significant attenuated AUCi for the DP+ group relative to both the NC and DP− groups (resp. $p=0.01$; $p=0.02$). Although visually the DP+ group showed an attenuated cortisol response to stress compared to the NC and DP− groups (see Fig. 1B), the one-way ANOVA was not significant ($F=2.18$; $p=0.12$). Simple contrast tests however revealed a significantly smaller AUCi for DP+ relative to NC ($p=0.04$). We found no differences between subgroups in heart rate or HRV reactivity.

Table 2
Bivariate and multivariate relationships between neurobiological parameters during stress (AUCis) and dimensional measures of disruptive behavior.

	Self-reported disruptive behavior			Parent-reported disruptive behavior		
	Beta	p	Adj. R^2	Beta	p	Adj. R^2
Bivariate analysis						
Cortisol	−0.39	0.01	0.14	−0.24	0.09	0.04
Alpha-amylase	−0.38	0.01	0.13	−0.34	0.01	0.10
Heart rate	−0.06	0.67	−0.01	−0.15	0.28	0.01
Heart rate variability	0.15	0.27	0.01	0.01	0.95	−0.02
Multivariate analysis						
Cortisol	−0.27	0.05	0.17[a]	−0.11	0.48	0.09[b]
Alpha-amylase	−0.26	0.06		−0.29	0.06	

Adj. R^2: Adjusted R^2.
[a] ΔR^2 relative to bivariate cortisol analysis = 0.036.
[b] ΔR^2 relative to bivariate cortisol analysis = 0.049.

To control for the effect of nicotine use, we compared the nicotine using participants in the DP− and DP+ groups (respectively n = 20 and n = 12). Independent sample t-tests showed a significantly lower alpha-amylase reactivity (AUCi) in the nicotine using part of the DP+ group compared to the nicotine using part of the DP− group ($t=2.21$; $p=.04$, Cohen's $d=0.92$). No other differences between the groups were found.

Repeated-measures ANOVA on negative affect revealed a significant main effect of time ($F=7.93$; $p<0.01$), yet no effect of group ($F=2.75$; $p=0.072$) or group by time interaction ($F=0.71$; $p=0.68$) (see Fig. 2). Simple contrast tests showed significant higher levels of negative affect for the DP+ group relative to the NC group ($p=0.03$).

Combining neurobiological parameters in relation to dimensional measures of disruptive behavior

Using multiple linear regression, we tested the advantage of combining alpha-amylase and cortisol reactivity (AUCis) in one model over and above the use of a single parameter as predictor of disruptive behavior. Although in the multivariate regression model the regression coefficients of alpha-amylase and cortisol slightly decreased, the R^2 of the total model improved, showing that the addition of alpha-amylase to cortisol in one model explained a larger part of the variance than either single parameters (see Table 2).

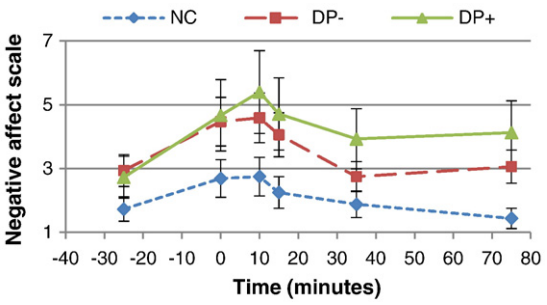

Fig. 2. Mean (±SEM) levels of negative affect by subgroup during the stress test procedure, showing six time points during baseline, preparation (0–10), speaking (10–15) and again baseline. The range of possible values on the negative affect scale was 0 to 18. NC, normal control subjects (n = 16); DP−, delinquents without disruptive behavior disorder (n = 32); DP+, delinquents with disruptive behavior disorder (n = 15).

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Interactions between neurobiological parameters and disruptive behavior: testing the Bauer models

As a first test of the Bauer models, we tested the interaction between alpha-amylase and cortisol reactivity (AUCis) in relation to disruptive behavior. We found a significant positive correlation between alpha-amylase and cortisol reactivity ($R = 0.44$; $p = 0.01$). There was no interaction between alpha-amylase and cortisol reactivity in relation to self-reported disruptive behavior (Beta = 0.01; $p = 0.92$) or parent-reported disruptive behavior (Beta = −0.09; $p = 0.54$). This means that the relationship between either of the two neurobiological parameters and disruptive behavior is not moderated by the other parameter. A plot of these relations appears in Fig. 3A. This figure shows that concurrent low alpha-amylase and cortisol reactivity is related to highest levels of disruptive behavior.

As a second test of the Bauer models, we tested the interaction between alpha-amylase and HRV reactivity (AUCis) in relation to disruptive behavior. We found a significant inverse correlation between alpha-amylase and HRV ($R = -0.32$; $p = 0.02$). There was no interaction between alpha-amylase and HRV in relation to self-reported disruptive behavior (Beta = 0.05; $p = 0.69$) or parent-reported disruptive behavior (Beta = −0.16; $p = 0.26$). A plot of these relations appears in Fig. 3B, showing that the relation between alpha-amylase reactivity and disruptive behavior is similar for high and low values of HRV.

Discussion

In the present study, we investigated whether examining concurrent reactivity of the parameters alpha-amylase, cortisol, heart rate and HRV improves the explanation of disruptive behavior compared to taking into account only one of these parameters. Furthermore, we investigated whether the interrelationship between different neurobiological parameters in relation to disruptive behavior is either additive or interactive. We studied delinquents with and without a disruptive behavior disorder as well as normal controls. Furthermore, we related neurobiological reactivity to dimensional measures of disruptive behavior.

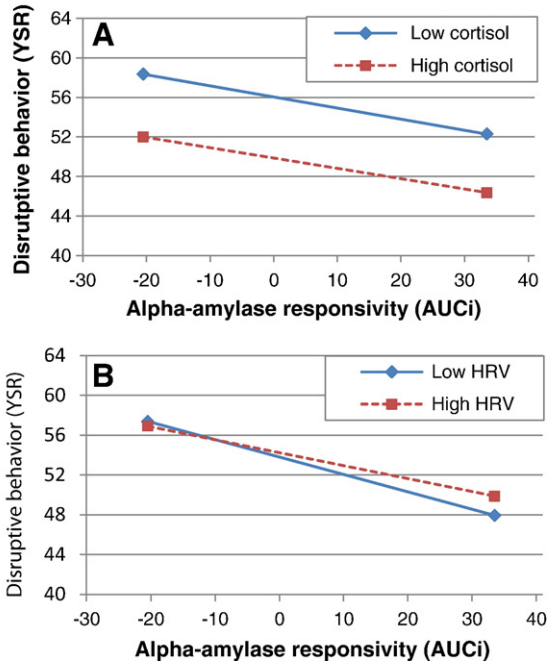

Fig. 3. Relation between alpha-amylase AUCi and self-reported disruptive behavior at high (+1 SD) and low (−1 SD) values of A. cortisol AUCi and B. heart rate variability AUCi. Marking points relate to alpha-amylase values 1 SD below and above the mean.YSR = Youth Self Report. HRV = heart rate variability.

Bivariate analyses

We studied a relatively new, easily obtainable marker of SNS reactivity in relation to disruptive behavior, namely alpha-amylase. To our knowledge, our study is the first in which this relationship is studied in delinquent late-adolescents. We found a significant inverse relationship between alpha-amylase reactivity and dimensional measures of disruptive behavior in delinquent adolescents and controls. Furthermore, attenuated alpha-amylase reactivity was related to a categorical diagnosis of disruptive behavior disorder. Our findings extend the existing literature on other measures of SNS reactivity (Lahey et al., 1993; McBurnett et al., 2005), as well as findings on alpha-amylase in relation to disruptive behavior in an early-adolescent general population sample (Susman et al., 2010).

Next to alpha-amylase, we also studied established neurobiological markers of juvenile disruptive behavior. In our sample, cortisol reactivity was inversely related to dimensional and categorical measures of disruptive behavior. These findings are consistent with results in delinquent and conduct disordered samples (Fairchild et al., 2008; Popma et al., 2006) and provide further evidence of cortisol as correlate of disruptive behavior in non-population samples.

When nicotine use was entered as a covariate, the relationships between alpha-amylase respectively cortisol with disruptive behavior remained present, although the strength of the relationships decreased. This indicates that nicotine use explains a considerable part of the variance of disruptive behavior. This is not surprising, since it has been found previously that nicotine use is associated with disruptive behavior (Elkins et al., 2007; Riala et al., 2011). Notwithstanding this, our results show that alpha-amylase and cortisol are related to disruptive behavior, over and above the effect of nicotine use.

In our study we did not find a relationship between HRV or heart rate reactivity and disruptive behavior. Our results on HRV reactivity are not surprising, since most previous studies found decreases in HRV in response to psychosocial stress, but no differences between antisocial and control groups (Beauchaine, 2001; Beauchaine et al., 2008; Dietrich et al., 2007; Mezzacappa et al., 1997). However, the absent relationship between heart rate reactivity and disruptive behavior is not in line with previous studies (Fairchild et al., 2008; Popma et al., 2006). An explanation may be found in our relatively small sample, which may well be too small to detect differences in a general ANS marker such as heart rate. Since SNS reactivity but not PNS reactivity was related to disruptive behavior in our study, the previously found relationship between heart rate reactivity and disruptive behavior may mainly be driven by disturbed SNS reactivity.

Despite the relationships we found between some of the neurobiological parameters in response to stress, we did not find relationships between any of the four parameters in resting conditions with disruptive behavior. Most remarkable is the absence of a relationship with resting heart rate, since low resting heart rate is considered a robust neurobiological correlate of juvenile disruptive behavior (Ortiz and Raine, 2004; van Goozen et al., 2007). Our measure of resting heart rate as well as the other resting parameters was taken prior to the public speaking task. Although participants were instructed to spend this time as relaxed as possible and they did not know the content of the task beforehand, neurobiological levels may have been influenced by anticipatory stress. It is recommended for future studies to optimize resting conditions, for example by measuring parameters in a familiar environment, apart from the context of an upcoming task.

The attenuated responsivity we found in both HPA-axis and SNS measures can be explained by the theories of low arousal. The attenuated physiological response may be associated with lower levels of fearfulness and with low responsivity to social cues in general, e.g. punishment (Raine, 1993). Hence, fearless juveniles are more likely

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to engage in disruptive or delinquent behaviors because they are not concerned about the negative consequences of their actions. Notably, the results of our study show that the subjective wellbeing, as represented by negative affect scale, is more negative for the DP+ group than the NC group. This is in line with previous studies, where attenuated cortisol responses in juveniles who displayed disruptive behavior were also accompanied by higher, or at least similar, emotional reactions compared to control subjects (Fairchild et al., 2008; Popma et al., 2006; Snoek et al., 2004; van Goozen et al., 2000). The attenuated physiological response is thus not explained by a diminished emotional response.

Multivariate analyses

Our study provided new insights on the associations between neurobiological parameters and disruptive behavior, by measuring the reactivity of several neurobiological parameters in concert. In our study, the addition of alpha-amylase to cortisol explained a larger part of the variance of disruptive behavior than either single parameter alone. Alpha-amylase and cortisol showed a moderate correlation, and their regression coefficients slightly decreased in the multivariate analyses compared to the bivariate analyses. Although both parameters share a part of the explained variance of disruptive behavior, adding alpha-amylase to cortisol improved the explanation of variance in disruptive behavior. Therefore, it is recommended for studies on juvenile disruptive behavior where saliva is being collected for cortisol analyses, to analyze alpha-amylase as well, although one has to be aware of methodological pitfalls (Bosch et al., 2011; Nater and Rohleder, 2009; Rohleder and Nater, 2009).

Interactions between neurobiological parameters

We found a moderate correlation between alpha-amylase and cortisol. Our findings are in line with studies from Gordis et al. (2006, 2008), while not with other studies in which correlations between the two parameters were absent (El-Sheikh et al., 2009; Susman et al., 2010). Our results did not show an interaction between alpha-amylase and cortisol reactivity in relation to disruptive behavior, meaning that the relationship between either of the two neurobiological parameters and disruptive behavior was not moderated by the other parameter in our study. This and the above mentioned results led us to conclude that the SNS and HPA-axis display balanced reactivity in a population of delinquents and normal controls. In other words, concurrent low alpha-amylase and cortisol reactivity is related to higher levels of disruptive behavior. Consequently, our study provides support for the additive model of Bauer (2002). In contrast, findings from Gordis et al. (2006) did show an interaction between alpha-amylase and cortisol in relation to disruptive behavior, indicating that the relationship between alpha-amylase and disruptive behavior is moderated by cortisol. Although our results did not show an interaction between alpha-amylase and cortisol whereas Gordis' results did, both our and their results showed that concurrent low reactivity of both the SNS and HPA-axis is associated with greater risk of disruptive behavior. Both systems are guided by the same underlying coordination, involving a complex network of brain regions including the amygdala, orbital frontal cortex, and other interconnected regions (Dolan, 2002). A disruption in this coordination may be an important correlate of juvenile disruptive behavior (Bauer et al., 2002). Results from brain imaging studies indeed show evidence for functional and structural abnormalities in the mentioned regions in disruptive juveniles (Stadler et al., 2010). Our results highlight the benefit of having information from both biological stress systems to improve knowledge on the role of neurobiological factors in explaining disruptive behavior.

Regarding the relationship between alpha-amylase and HRV, most healthy children and adolescents exhibit a reciprocal pattern of SNS and PNS activity in response to psychosocial stress (Salomon et al., 2000). We found an inverse correlation between alpha-amylase and HRV, which may suggest reciprocity between SNS and PNS reactivity. Concurrent low reactivity (co-inhibition) of both measures has been found in relation to disruptive behavior (Beauchaine et al., 2007; Boyce et al., 2001; El-Sheikh et al., 2009), a finding we could not replicate. The fact that we did not find an interaction between SNS and PNS in relation to disruptive behavior, may be explained by the absence of a relationship between HRV and disruptive behavior, as discussed above.

Clinical implications

Knowledge on associations between neurobiological parameters and disruptive behavior in juveniles may have potential clinical relevance for intervention purposes. Our results enhance this knowledge by showing that attenuated alpha-amylase reactivity is related to disruptive behavior in juveniles. Moreover, the combination of alpha-amylase and cortisol had advantages over the use of a single parameter. It should be noted that the effect sizes we found were small, indicating that only a small proportion of the disruptive behavior is explained by the (combination of) neurobiological parameters. In fact, many other factors are involved with antisocial behavior, distinguished in individual, family and peer group domains (Loeber et al., 2009). Previous studies showed primary evidence for associations between low cortisol and future disruptive behavior as well as worse treatment outcome (Shoal et al., 2003; Sondeijker et al., 2008; van de Wiel et al., 2004). It is recommended for future studies to expand these findings by studying alpha-amylase longitudinally, and combine multiple neurobiological parameters together with psychosocial measures, preferably from comprehensive biopsychosocial models.

Limitations

There are some methodological limitations of the study that should be considered when interpreting the results. First, we studied a small sample of male adolescents. Especially when dividing the sample in three subgroups, group numbers were small. Results cannot be generalized to other samples like clinic-referred disruptive behavior disordered juveniles, very young offenders or girls. Furthermore, the sample size limited power to incorporate additional parameters to control for confounding effects. This was of particular concern regarding the use of nicotine, because there was a disproportionate use of nicotine in the DP+ group, in particular when compared to the NC group. Although we provided some additional analyses revealing that relationships with alpha-amylase or cortisol and disruptive behavior remained present, we cannot completely rule out an effect of nicotine use on the relationships between the biological parameters and disruptive behavior. Second, we used a cross-sectional design. Although our study adds to the existing literature by examining different neurobiological factors in concert, we cannot provide clarification of the causal relationship between neurobiological reactivity and disruptive behavior. Third, we used a measure of SNS on salivary glands, whereas our measure of PNS was on cardiac activity. It is unclear to what extent the influence of SNS and PNS may differ for different organ systems, being more specific, whether SNS influence on salivary glands differs from SNS influence on cardiac activity. Results may have differed when measures of both SNS and PNS on cardiac activity were used. Fourth, in light of the ongoing discussion on the specificity of alpha-amylase as a measure of sympathetic activity, we may have used a less favorable sampling method for analyzing alpha-amylase. We used salivettes, which may induce measurement errors, since the cotton may retain part of the alpha-amylase (DeCaro, 2008). Furthermore, we instructed the participants to chew on the salivette, which may affect salivary protein composition as well as flow rate (Bosch et al., 2011), which we did not control for. Although our results indicated an additional value of alpha-amylase to

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other ANS measures as a potential biomarker of disruptive behavior in late-adolescents, they need to be interpreted with caution. Future studies that aim to replicate or extend our findings, are advised to take into account the recommendations and considerations provided by Rohleder (2009), Nater (2009) and Bosch (2011).

Concluding remarks

Our study is the first to indicate that attenuated alpha-amylase is a correlate of disruptive behavior in delinquent male adolescents compared to controls. We also extended previous findings by showing attenuated cortisol reactivity in relation to disruptive behavior. Furthermore, when both parameters were combined in one design, a larger part of the variance of disruptive behavior was explained. Specifically, concurrent low reactivity of alpha-amylase and cortisol was related to higher levels of disruptive behavior. Hence, combining neurobiological parameters improves insight into mechanisms involved with disruptive behavior. Still, the results of our study need replication in larger samples. Taking into account methodological issues regarding alpha-amylase, it is recommended for future studies on disruptive behavior to incorporate multiple neurobiological parameters. Improving knowledge on juvenile disruptive behavior may lead to improved intervention strategies, aiming to prevent further development of disruptive behavior at an early stage.

Conflict of interest

All authors declare no conflict of interest.

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