Article

Adolescent Antisocial Behavior Explained by Combining Stress-Related Parameters

Evelien Platje,[1,2,3] Lucres M. C. Jansen,[1] Robert R. J. M. Vermeiren,[1,4] Theo A. H. Doreleijers,[1,5] Pol A. C. van Lier,[6] Hans M. Koot,[6] Wim H. J. Meeus,[7,8] Suzan J. T. Branje,[7] and Arne Popma[1]

[1]VU University Medical Center, Department of Child and Adolescent Psychiatry, Duivendrecht, The Netherlands
[2]Leiden University, Clinical Child and Adolescent Studies, Leiden, The Netherlands
[3]Leiden Institute for Brain and Cognition, Leiden, The Netherlands
[4]Curium – Leiden University Medical Center, Leiden, The Netherlands
[5]Leiden University, Department of Criminal Law and Criminology, Leiden, The Netherlands
[6]VU University, Department of Developmental Psychology, Amsterdam, The Netherlands
[7]Utrecht University, Research Center Adolescent Development, Utrecht, The Netherlands
[8]Tilburg University, Tilburg School of Behavioral and Social Sciences, Tilburg, The Netherlands

Abstract: Many stress-related parameters have been associated with antisocial behavior, including low cortisol awakening responses (CAR), as well as low cortisol and alpha-amylase reactivity to stress. These parameters reflect different, yet interrelated components of the stress system, yet it remains to be determined whether they exert joint or independent effects. Therefore, this study examined them in concert, as this may offer a better explanation of the psychophysiological mechanism's underlying antisocial behavior. Antisocial behavior was assessed through self-report by 197 general population boys and girls (M_{age} = 17.31, *SD* = 0.44). The CAR was assessed, as well as cortisol and alpha-amylase reactivity to a public speaking task. Neither stress-related parameter was independently related to antisocial behavior. The best explanation was provided by a CAR × Cortisol reactivity interaction, indicating that in youth with a low CAR, antisocial behavior was positively associated with cortisol reactivity. In youth with a high CAR, no association between antisocial behavior and cortisol reactivity was found. Between cortisol and alpha-amylase reactivity a trend toward an interaction appeared, indicating a negative association between cortisol reactivity and antisocial behavior in those with low alpha-amylase reactivity, and a positive association in those with high alpha-amylase reactivity. These findings indicate that in order to understand the mechanisms underlying antisocial behavior, the stress system should be studied comprehensively rather than focus on single parameters. Particularly cortisol parameters appear to be jointly related to antisocial behavior, the additional value of alpha-amylase reactivity to cortisol reactivity may however be limited.

Keywords: antisocial behavior, cortisol, alpha-amylase, awakening response, stress reactivity, adolescence

Antisocial behavior peaks in adolescence, to such extent that the vast majority of adolescents report to have committed some form of antisocial behavior (e.g., Junger-Tas, 1994). However, there are large individual differences in the degree to which these adolescents show antisocial behavior. To enhance our understanding of the mechanisms underlying these individual differences in antisocial behavior, neurobiological factors have increasingly been studied. As a neurobiological risk factor, decreased stress-related parameters have often been associated with antisocial behavior (e.g., review by Van Goozen, Fairchild, Snoek, & Harold, 2007). However, there exist a variety of stress-related parameters which have been related to antisocial behavior, such as decreased alpha-amylase reactivity and cortisol reactivity to stress, as well as low cortisol awakening responses (CAR) (e.g., Alink et al., 2008; Platje et al., 2013; Susman et al., 2010). As these reflect different, yet interrelated aspects of the stress system, individual parameters provide an incomplete account of the relation between these parameters and antisocial behavior. In order to further our understanding of associations with antisocial behavior, the interplay between multiple parameters should be taken into account (Bauer, Quas, & Boyce, 2002). Therefore, in this study it was investigated as to which combination of stress-related parameters best explains antisocial behavior.

In response to stress, cortisol and alpha-amylase levels increase as the stress systems, the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary

DOI: 10.1027/0269-8803/a000173

system (SAM), are activated. The HPA axis and the SAM are anatomically and physiologically connected, and jointly regulate our response to stress. Because the correlation between cortisol and alpha-amylase is small (El-Sheikh, Erath, Buckhalt, Granger, & Mize, 2008; Gordis, Granger, Susman, & Trickett, 2006; Susman et al., 2010), the HPA axis and the SAM can be considered to be coordinated, yet distinguishable stress systems. Therefore, assessing reactivity of the HPA axis and the SAM concurrently has been proposed to provide a better explanation of antisocial behavior than either alone (Bauer et al., 2002).

According to Bauer et al. (2002), such concurrent reactivity in relation to antisocial behavior could follow two patterns, additive or interactive. In the additive model, antisocial behavior is associated with concordant low HPA and low SAM reactivity. The additive model connects to the classic theories on arousal, stating that there is an optimal medium level of arousal, whereas hypoarousal is associated with antisocial behavioral problems. In the interactive model, antisocial behavior is hypothesized to arise from low reactivity of one system and high reactivity of the other. This model is based on the suggestion that the HPA and SAM are differentially activated depending on (the individuals' perception of) the situation. For example, SAM reactivity has been described as a "defense reaction" to controllable stressors, that is, fight or flight, while HPA axis reactivity is considered to be a "defeat reaction" in uncontrollable situations, that is, distress (Dickerson & Kemeny, 2004; Henry, 1992). Moreover, it has been posed that the HPA axis suppresses SAM activity (Sapolsky, Romero, & Munck, 2000). Where the initial SAM reactivity promotes adaptation (i.e., allostasis), chronic or inadequate SAM activity may result in cumulative changes leading to a "wear-and-tear" of the body and brain, termed allostatic overload, which is associated with the onset of psychopathology (McEwen, 2004). Hence, asymmetrical HPA and SAM activation may increase the risk for allostatic overload and could eventually lead to behavioral problems (Bauer et al., 2002).

Since Bauer postulated this multisystem approach in 2002, researchers set out to investigate these systems together. The additive model seems to find most support in explaining antisocial behavior, both in delinquent boys (De Vries-Bouw et al., 2012) and in general population boys and girls (Gordis et al., 2006). However, other studies in general population samples did not find additive or interactive effects in relation to antisocial behavior (Allwood, Handwerger, Kivlighan, Granger, & Stroud, 2011; Huijbregts, van Berkel, Swaab-Barneveld, & van Goozen, 2011; Rudolph, Troop-Gordon, & Granger, 2011). As to date, only few studies have examined concurrent effects of HPA and SAM reactivity, and generally with small sample sizes (i.e., only Rudolph et al.'s sample exceeded $N = 100$). Therefore, replication in a larger general population sample is required to clarify this relation.

The HPA axis has not only been related to antisocial behavior through its decreased cortisol response to stressful situations, a low cortisol awakening response has also frequently been associated with antisocial behavior (e.g., Popma et al., 2007; Sondeijker et al., 2008). The CAR is a distinct increase in cortisol levels occurring in the first half hour after awakening. The level of the CAR reflects basal activity of the HPA axis, and additionally, the rise in cortisol levels as a response to awakening reflects the flexibility of the HPA axis (Fries, Dettenborn, & Kirschbaum, 2009). Especially the level of the CAR is thought to partly reflect the *trait* physiological HPA axis activity (Edwards, Clow, Evans, & Hucklebridge, 2001; Hellhammer et al., 2007; Pruessner et al., 1997), whereas cortisol reactivity of the HPA axis to stress is regarded as a *state* phenomenon, largely dependent on situation-specific factors, and the individual's perception and processing of a stressor (Dickerson & Kemeny, 2004; Gaab, Rohleder, Nater, & Ehlert, 2005).

However, it is unclear whether the CAR level and cortisol reactivity to stress, as distinct components of HPA axis activity, are jointly or independently related to antisocial behavior. From a theoretical perspective, three mechanisms have been proposed. According to the sensation seeking theory (Zuckerman & Neeb, 1979), low HPA axis activity is thought to constitute a negative physiological state, which could be increased (i.e., normalized) by seeking sensation through antisocial behavior. Hence, antisocial behavior would be mainly associated with low CAR levels triggering sensation seeking (Van Goozen et al., 2007), in combination with an increase in cortisol reactivity as a result of the sensation. Alternatively, according to the fearlessness theory, low HPA axis activity is thought to reflect an individual's fearlessness, as a result of which youngsters may not fear the negative consequences of antisocial behavior (Blair, 2004; Lykken, 1995; Patrick, 1994; Van Goozen, Matthys, Cohen-Kettenis, Buitelaar, & van Engeland, 2000; Van Goozen, Snoek, Matthys, van Rossum, & Van Engeland, 2004). Here, antisocial behavior would be mainly associated with low cortisol reactivity to stress (Van Goozen et al., 2007). Finally, it has been posed that frequent sensation seeking (associated with low CAR levels) may lead to habituation, and eventually to a blunted stress response (and thereby fearlessness; Van Goozen et al., 2007). In that case, antisocial behavior would be associated with both low CAR levels and low cortisol reactivity to stress. However, both components of HPA activity have not previously been studied in concert in relation to antisocial behavior. Therefore, in the current study both HPA axis components will be

assessed in the same subjects to better explain the stress-related mechanisms involved in antisocial behavior.

It is important to further extend concurrent assessment of stress-related parameters to elucidate the psychophysiological explanation of antisocial behavior. As recent evidence suggests that concurrent low stress reactivity of the SAM and HPA axis may explain antisocial behavior better than either system alone (De Vries-Bouw et al., 2012; Gordis et al., 2006), we aim to replicate this design by examining concurrent reactivity of the two stress systems in a larger general population sample within this cross-sectional study. Moreover, as to date it is unknown how the CAR and stress reactivity of the HPA axis relate in the explanation of antisocial behavior, we aim to fill this gap in the literature. Therefore, in this study reactivity of the SAM and the HPA axis, as well as the CAR, was studied concurrently. It is expected that low reactivity to stress of both the HPA axis and the SAM system is associated with increased antisocial behavior, that is, in line with Bauer's additive model (Bauer et al., 2002; De Vries-Bouw et al., 2012; Gordis et al., 2006). As for reactivity of the HPA axis and the CAR, both parameters have been found to be decreased in relation to antisocial behavior (e.g., Popma et al., 2006, 2007; Sondeijker et al., 2008). It could therefore be expected that assessed together, both parameters are also decreased in relation to antisocial behavior ("habituation"). However, as these parameters have not previously been combined, it may be possible that some individuals show decreased HPA reactivity to stress (fearlessness), whereas others show decreased CAR levels and increased HPA reactivity to stress (sensation seeking) in relation to antisocial behavior.

Materials and Methods

Participants

Participants were 197 adolescents (115 boys and 82 girls), with a mean age of 17.31 years (*SD* = 0.44). They were recruited from the RADAR (Research on Adolescent Development And Relationships) study. RADAR is a Dutch population based cohort study, with oversampling (50%) of boys and girls with a borderline clinical score on the externalizing scale of the Teacher's Report Form (TRF; Achenbach, 1991a) at age 11. All participants and their parents have provided written informed consent and received a reimbursement for their participation. The RADAR study has been approved by the responsible Medical Ethics Committee and was conducted in accordance with the Declaration of Helsinki. Stress reactivity of the HPA axis and SAM was assessed during a lab session, and all adolescents participated within a time frame of 361 days. The CAR was assessed in the same year at the participant's home in February and March, when antisocial behavior was assessed as well.

Table 1. Characteristics of participants (*N* = 197)

	Mean/*n*	*SD* (%)	Range
Age	17.31	0.44	16.08–18.67
Boys	115	58.4	
Nicotine use[a]	46	23.4	
Alcohol use[a]	31	15.7	
Externalizing[b]	48.01	10.25	29–74

Notes. [a]Nicotine and alcohol use within 24 hr before lab session; [b]*T*-scores.

In the lab session 303 adolescents participated, of whom 227 also participated in the CAR measurement. These 227 participants did not differ from those not participating in the lab session on the level of antisocial behavior (Youth Self Report externalizing scale), age, gender, and nicotine or alcohol use on a regular basis (all $p > .1$). Of the 227 adolescents participating in both the lab session and the CAR measurement, two participants provided insufficient saliva for the assays, and for 28 participants there were too many missing values on the stress-related parameters, adding up to a sample of 197 in the final analyses. Characteristics of the participants in the final analyses are described in Table 1.

Antisocial Behavior

Antisocial behavior was assessed by means of the externalizing scales of the Youth Self Report (YSR; Achenbach, 1991b), administered to the adolescents. Externalizing behavior consists of aggression and rule-breaking behavior. Items are scored on a 3-point scale (0 = *not true*, 1 = *somewhat true*, 2 = *very true or often true*). Scores were transformed to T-scores, which are normalized standard scores based on gender and age. Good reliability and validity have been reported for the Dutch YSR version (Verhulst, Van der Ende, & Koot, 1997), in the current sample, Cronbach's α was .90 for the externalizing scale.

Stress Reactivity of the HPA Axis and SAM

The assessment of stress reactivity was assessed at the end of a lab session (see Figure 1) in which the participants watched empathy-inducing film clips (De Wied, van Boxtel, Zaalberg, Goudena, & Matthys, 2006) and performed both the Stop Signal task and the Iowa Gambling task, data from these tasks were not used in the current study. The Leiden Public Speaking Task (Leiden PST; Westenberg et al.,

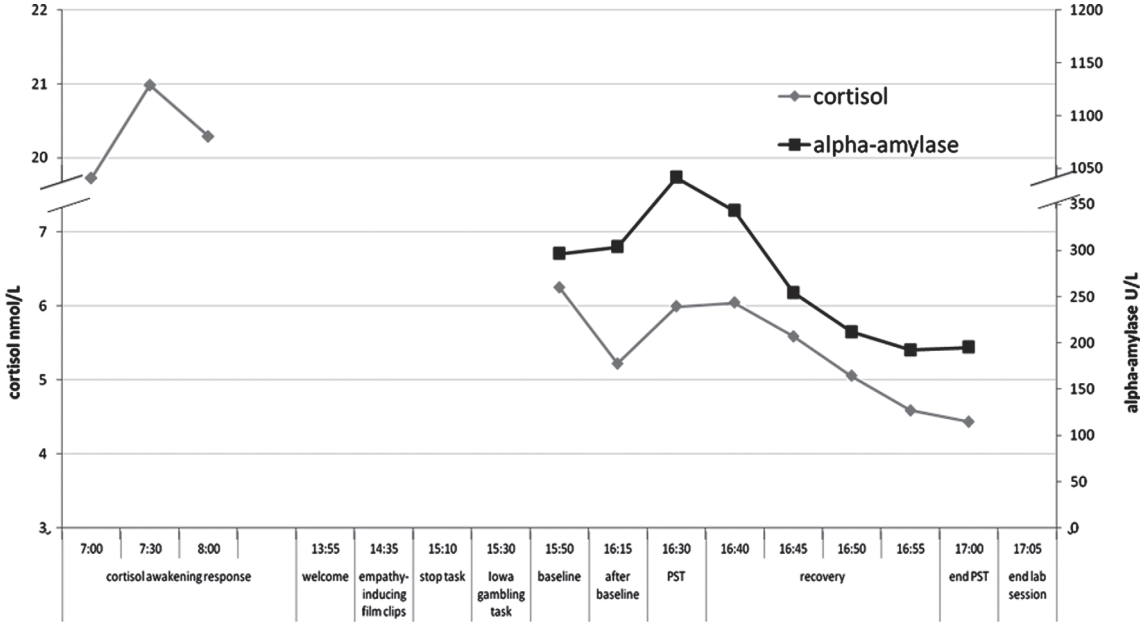

Figure 1. Overview of the lab session including the Public Speaking Task. The CAR and Public Speaking Task were generally not assessed on the same day. For representational purposes, untransformed cortisol and alpha-amylase values are shown in the figure, whereas a square root transformation was performed before analyses. CAR = Cortisol Awakening Response.

2009) was used to measure physiological stress reactivity to social evaluation. Social evaluative threat has been shown to elicit great physiological and psychological stress in a laboratory situation (Dickerson & Kemeny, 2004) in normative samples. First, the participants watched a nature documentary (baseline), after which detailed instructions were given. Subsequently, they were given five min to prepare their speech. They were asked to deliver their speech in front of a prerecorded audience consisting of age-matched peers and a teacher. The participants were told that their videotaped speech would be judged afterwards by a teacher and peers from another school. Finally, during the recovery phase, they watched another nature documentary. For an extended description of the task, readers are referred to Westenberg et al. (2009).

Eight saliva samples were collected before, during, and after the task in order to measure cortisol reactivity (HPA axis reactivity) and alpha-amylase (surrogate marker for SAM reactivity). Saliva samples were obtained by passive drool. The moments of collection were just before baseline (sample 1), after baseline (sample 2), immediately after the speech (sample 3), 10 min after the speech (sample 4), followed by four samples with intervals of 5 min (samples 5–8). The average time of collection of the first sample was 15h58 (*SD* 26 min). A graphical representation of the lab session and the sampling moments is provided in Figure 1.

The CAR

The CAR was assessed in saliva sampled immediately after awakening (CAR0), and 30 min (CAR30) and 60 min (CAR60) later. Saliva samples were obtained by passive drool. Participants were first given detailed verbal and written information regarding cortisol measurements. Subsequently, saliva sampling was planned for a suitable morning on a regular weekday. The first sample (at awakening) was planned before 8 am, while taking into consideration the participant's normal schedule. Sampling times were set and written on a detailed instruction form.

Participants were instructed to rinse their mouths with water before sampling, and not to eat, drink milk or juice, smoke, or brush their teeth before completing CAR60. They were requested to report the exact sampling times on the instruction form on the day of sampling, and to report if mistakes were made in any of the above instructions. After collection, participants were asked to store the samples in the refrigerator and send them by mail to the research center the same day.

At the research center, all samples were checked for correctness of sampling. When necessary, for example when CAR0 was sampled after 8 am or the sampling time of CAR30 or CAR60 was over 15 min late, or mistakes were made in any of the other instructions, participants were asked to collect new saliva samples, and a new sampling day was scheduled. If, despite this, participants had still

not sampled correctly, the incorrect samples were excluded. The average time of collection of the first sample (CAR0) was 7h02 (*SD* 40 min).

Control Variables

Control variables were assessed at the lab session. During the lab session temperature and humidity of the test room were recorded. Food, drinks (including, e.g., coffee), alcohol, nicotine, and drug use, as well as physical exercise, all taken place within the last 24 hr, were assessed. Stressful situations, medication use, diseases, physical and dental conditions (including allergies and oral bleeding), body mass index (BMI), as well as menstrual phase and contraconceptive (OC) use for females were assessed as well.

Cortisol and Alpha-Amylase Analyses

Saliva was stored uncentrifuged at −20 °C until analysis. Cortisol and alpha-amylase were analyzed in Leiden, the Netherlands. Cortisol was analyzed using electrochemiluminescence immunoassay (ECLIA). The lower detection limit was 0.5 nmol/L, with mean intra- and inter-assay coefficients of variation of 3.4% and 12.2%. Alpha-amylase samples were diluted 50 times with 9% sodium chloride, using a Hamilton Microlab 500B/C diluter. Diluted samples were analyzed 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 Analysis

Outliers were defined as 3 *SD* above the mean (16 samples for cortisol reactivity, 36 for alpha-amylase reactivity, and 14 for the CAR). As both cortisol and alpha-amylase reactivity were positively skewed, a square root transformation was performed, after which values were normally distributed. Single missing values of the stress-related parameters were replaced by regression analysis using the missing value as dependent variable and the remaining values as predictors, for boys and girls separately. For cortisol reactivity 16 samples were replaced, 22 for alpha-amylase reactivity, and 19 for the CAR. For 28 participants there was more than one missing value on the stress-related parameters, hence these could not be included in the analyses, a total of 197 adolescents had data available on all variables and were included in the final analyses.

Table 2. Correlations between antisocial behavior and stress-related parameters

	1	2	3
1. Externalizing			
2. Cortisol reactivity	.05		
3. Alpha-amylase reactivity	−.05	.17*	
4. Basal cortisol	.04	−.01	−.06

Notes. Two-tailed Pearson correlations. *$p \leq .05$.

As measures of reactivity of the HPA axis and SAM, area under the curves with respect to increase (AUCi) was computed for cortisol and alpha-amylase over samples 2–5 (from just before start, until 15 min after the stressor), with reference to sample 2. As a measure of the CAR level the area under the curve with respect to ground (AUCg) was computed over CAR0, CAR30, and CAR60 of the cortisol awakening response.

First, it was confirmed that stress reactivity to the PST was present for both cortisol and alpha-amylase with repeated-measures analyses of variance (ANOVAs). Pearson correlations between the stress-related parameters and externalizing behavior were analyzed. Next, hierarchical regression analyses were performed with externalizing behavior as dependent variable. Of the control variables assessed, only gender and nicotine use were significantly associated with both externalizing behavior and stress-related parameters, these were entered as covariates in Step 1. In Step 2, main effects of cortisol reactivity, alpha-amylase reactivity, and the CAR were added to the model. In Step 3, the two 2-way interactions between cortisol reactivity and alpha-amylase reactivity, as well as cortisol reactivity and the CAR were examined together and added to the model. In Step 4, a three-way interaction between cortisol reactivity, alpha-amylase reactivity, and the CAR was added. Significant interaction effects were probed using simple slope analysis at mean ± 1 *SD* (Aiken & West, 1991).

Results

Reactivity to stress during the public speaking task was significant for both cortisol and alpha-amylase (see Figure 1), as repeated-measures ANOVAs revealed a curvilinear main effect of time for cortisol, $F(1, 189) = 13.45$, $p < .001$, and alpha-amylase, $F(1, 192) = 74.42$, $p < .001$.

Pearson correlations between antisocial behavior and stress-related parameters are shown in Table 2. Reactivity of cortisol and alpha-amylase was positively correlated,

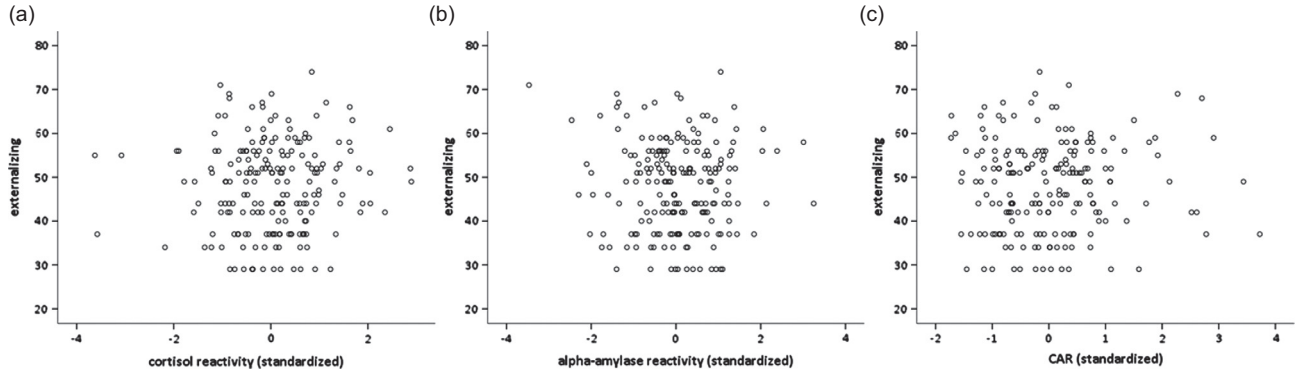

Figure 2. Scatterplots for (a) cortisol reactivity, (b) alpha-amylase reactivity, and (c) the CAR in relation to externalizing behavior. CAR = Cortisol Awakening Response.

although weak. Correlations between externalizing behavior and individual stress-related parameters were nonsignificant, see also Figure 2.

As gender and nicotine use were associated with both antisocial behavior and stress-related parameters, these were entered in the regression analysis as covariates. First, main effects of cortisol reactivity, alpha-amylase reactivity, and the CAR level predicting externalizing behavior were investigated. As shown in Table 3, linear regression analyses revealed that neither of these parameters showed a significant association with antisocial behavior.

Next, the two 2-way interactions between cortisol reactivity and alpha-amylase reactivity, as well as cortisol reactivity and the CAR, were added to the model. A trend toward an interaction between cortisol and alpha-amylase reactivity appeared in association with externalizing behavior (see Table 3). Single slopes were assessed to probe the direction of the interaction at high (i.e., 1 *SD* above the mean) and low (i.e., 1 *SD* below the mean) levels. These were not significant, but suggested that when alpha-amylase reactivity was low, the association between cortisol reactivity and externalizing behavior was negative ($\beta = -.16$, $p = .180$), and vice versa, when alpha-amylase reactivity was high, this association was positive ($\beta = .16$, $p = .077$).

A significant interaction was found between cortisol reactivity and the CAR level for externalizing behavior (see Table 3). Single slopes for the interaction between the CAR level and cortisol reactivity in relation to externalizing behavior are plotted in Figure 3. It can be seen that when the CAR level was low, a positive relation between cortisol reactivity and externalizing behavior was found ($\beta = .21$, $p = .032$). Conversely, when the CAR level was high, no association between cortisol reactivity and externalizing behavior was found ($\beta = -.16$, $p = .126$).

The three-way interaction between cortisol reactivity, alpha-amylase reactivity, and the CAR level was not related to externalizing behavior.

Discussion

Stress reactivity of the HPA axis and the SAM system, as well as the CAR, reflect different, yet interrelated aspects of the stress system. Examining each parameter individually thus provides only a partial account of the relation with antisocial behavior. Therefore, in the current cross-sectional study, these stress-related parameters were examined together in relation to antisocial behavior in general population adolescents. Specifically, it was investigated which combination of stress-related parameters provides the best explanation of antisocial behavior. Our results showed that relationships were found only when interactions between parameters were taken into account, that is, neither parameter was independently related to antisocial behavior. The best explanation of antisocial behavior was provided by the interaction between the level of the CAR and reactivity of the HPA axis, with those with a low CAR showing a positive association between high HPA reactivity and antisocial behavior.

We did not find a significant additive or interactive effect of HPA and SAM reactivity with antisocial behavior, as posed by Bauer et al. (2002), although we did find a trend toward an interaction between HPA and SAM activity, which suggests that when SAM reactivity is low, the association between HPA reactivity and antisocial behavior is negative, that is, in line with an additive effect of concurrent low HPA and low SAM reactivity (Bauer et al., 2002). However, as there were no main effects for either system, the results do not support the additive model. The relation between additive low HPA and SAM reactivity and antisocial behavior may only be weak in general population samples, as most studies in general population samples could not find such an effect (Allwood et al., 2011; Huijbregts et al., 2011; Rudolph et al., 2011; Spinrad et al., 2009, but see Gordis et al., 2006), while in a delinquent sample an additive effect was found (De Vries-Bouw et al., 2012).

Table 3. Results of the regression analysis predicting antisocial behavior by cortisol reactivity, alpha-amylase reactivity, and the CAR, as well as concurrent effects of these three parameters

		Externalizing		
		β	*p*	adj R^2
Step 1	Covariates			.01
Step 2	Cortisol reactivity	.05	.531	<.01
	Alpha-amylase reactivity	−.06	.429	
	CAR	.03	.635	
Step 3	Cortisol Reactivity × Alpha-Amylase Reactivity	.13	.076	.04
	Cortisol Reactivity × CAR	−.16	.026	
Step 4	Cortisol Reactivity × Alpha-Amylase Reactivity × CAR	−.05	.533	.06

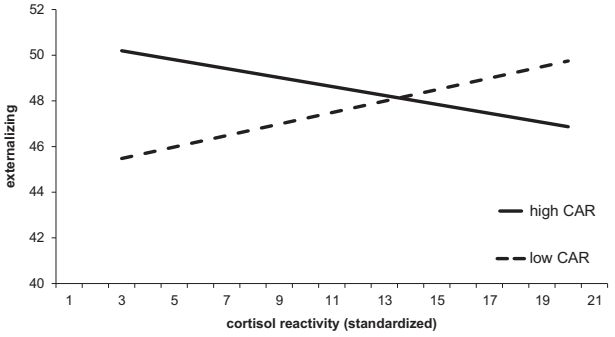

Figure 3. Simple slopes for externalizing behavior predicted by cortisol reactivity at 1 *SD* above and below the mean for the CAR. CAR = Cortisol Awakening Response.

This is, to our knowledge, the first study to examine the CAR and stress reactivity of the HPA axis together in relation to antisocial behavior. It appeared that in youth with a low CAR antisocial behavior was positively associated with HPA reactivity. This could be explained in the light of the sensation seeking theory; those with a low CAR, reflecting low trait arousal, may experience this as an aversive physiological state, which can be normalized (i.e., increased) by seeking sensation through antisocial behavior (Zuckerman & Neeb, 1979). As their HPA reactivity indeed increased during stress, the intended normalization appears to be successful, which may work stimulating to engage in antisocial behavior more often. It would physiologically be plausible that within one individual, the CAR and stress reactivity are regulated differently. Because while part of the same system, these components are considerably different controlled in the brain. Whereas the level of the CAR reflects basal trait functioning of the HPA axis, which is mainly controlled by mineralocorticoid receptors (MRs), stress-induced corticoids activate glucocorticoid receptors (GRs; Heuser et al., 2000; Oitzl, van Haarst, & de Kloet, 1997). Replication is however warranted, especially since the current study has a cross-sectional design, longitudinal studies are necessary. Future studies should also incorporate direct measures of sensation seeking and fearlessness to substantiate the theories underlying antisocial behavior with psychological characteristics.

The current results do not support the fearlessness theory. Possibly, fearlessness, as reflected by low HPA reactivity and/or low SAM reactivity, may be specific for more severely antisocial, clinic-referred, or delinquent populations (De Vries-Bouw et al., 2012; Popma et al., 2006; Van Goozen et al., 1998) compared to general population samples, as fearlessness appears to be characteristic of severe forms of antisocial behavior (e.g., Blair, 2004; Frick & White, 2008; Lykken, 1995; Patrick, 1994).

Some methodological limitations should be mentioned. First, the current study has a cross-sectional design, therefore only correlational associations were assessed. Second, the CAR was assessed in saliva sampled at home on one day only. Correcting for day-to-day variation was therefore not possible. However, previous studies have shown that day-to-day variation of the CAR is relatively low (Edwards et al., 2001; Wust et al., 2000). Although we took all possible precautions in the sampling procedure, among which self-report of exact sampling times, directly monitoring participant's compliance was not possible. However, self-reported sampling times have been found to be preferable to automatic time recording (Kraemer et al., 2006) and sampling of the CAR at home was previously found not to differ from sampling in a controlled laboratory environment (Wilhelm, Born, Kudielka, Schlotz, & Wust, 2007). Third, antisocial behavior was only determined from self-report. Parent-reported antisocial behavior may have resulted in different outcomes. However, in adolescence, youths spend less and less time at home with their parents, and especially antisocial behavior all too often remains unnoticed to others. In adolescence therefore, self-report is expected to give an accurate view of antisocial behavior. Fourth, stress reactivity was assessed at the end of a lab session in which the participants watched empathy-inducing film clips and performed the Stop Signal task

and the Iowa Gambling task. These assessments may have influenced stress reactivity, for instance, highly empathic youth or those performing poor at the tasks may show increased reactivity, moreover, these assessments may have less influenced the reactivity of antisocial youth. Unfortunately, performance on these assessments was not available for the current analyses. Fifth, this study was performed in a general population sample, displaying (mainly) normative levels of antisocial behavior. As such, the results cannot be generalized to for example, clinic-referred youths with disruptive behavior disorders or severe delinquent populations. There are indications that the additive effect of low SAM and HPA reactivity is stronger in such populations (De Vries-Bouw et al., 2012), while CAR and stress reactivity HPA interactions have not been studied previously. This would however be highly relevant to investigate in clinic-referred or severe delinquent populations. Should those with a low CAR show positive associations between stress reactivity and antisocial behavior in these populations as well, they could profit from offering alternative ways of seeking sensation to increase arousal.

Acknowledgments

Data from the RADAR study were used. RADAR has been financially supported by main grants from the Netherlands Organisation for Scientific Research (GB-MAGW 480-03-005, GB-MAGW 480-08-006, GB-MAGW 400-05-212), and Stichting Achmea Slachtoffer en Samenleving (SASS), and the VU University Amsterdam and Utrecht University. This project has been supported by the Foundation "De Drie Lichten" in the Netherlands. We sincerely thank all participating families and schools for taking part in this study.

Ethics and Disclosure Statements

All participants of the study provided written informed consent and the study was approved by the Ethics Committees of the responsible universities.

All authors disclose no actual or potential conflicts of interest including any financial, personal, or other relationships with other people or organizations that could inappropriately influence (bias) their work.

References

Achenbach, T. M. (1991a). *Manual for the teachers report form and 1991 profile*. Burlington, VT: University of Vermont, Department of Psychiatry.

Achenbach, T. M. (1991b). *Manual for the youth self-report and 1991 profile*. Burlington, VT: Department of Psychiatry, University of Vermont.

Aiken, L. S., & West, S. G. (1991). *Multiple regression: Testing and interpreting interactions*. London, UK: Sage.

Alink, L. R. A., van Ijzendoorn, M. H., Bakermans-Kranenburg, M. J., Mesman, J., Juffer, F., & Koot, H. M. (2008). Cortisol and externalizing behavior in children and adolescents: Mixed meta-analytic evidence for the inverse relation of basal cortisol and cortisol reactivity with externalizing behavior. *Developmental Psychobiology, 50*, 427–450.

Allwood, M. A., Handwerger, K., Kivlighan, K. T., Granger, D. A., & Stroud, L. R. (2011). Direct and moderating links of salivary alpha-amylase and cortisol stress-reactivity to youth behavioral and emotional adjustment. *Biological Psychology, 88*, 57–64.

Bauer, A. M., Quas, J. A., & Boyce, W. T. (2002). Associations between physiological reactivity and children's behavior: Advantages of a multisystem approach. *Developmental and Behavioral Pediatrics, 23*, 102–113.

Blair, R. J. R. (2004). The roles of orbital frontal cortex in the modulation of antisocial behavior. *Brain and Cognition, 55*, 198–208.

De Vries-Bouw, M., Jansen, L., Vermeiren, R., Doreleijers, T., van de Ven, P., & Popma, A. (2012). Concurrent attenuated reactivity of alpha-amylase and cortisol is related to disruptive behavior in male adolescents. *Hormones and Behavior, 62*, 77–85.

De Wied, W. M., van Boxtel, A., Zaalberg, R., Goudena, P. P., & Matthys, W. (2006). Facial EMG responses to dynamic emotional facial expressions in boys with disruptive behavior disorders. *Journal of Psychiatric Research, 40*, 112–121.

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin, 130*, 355–391.

Edwards, S., Clow, A., Evans, P., & Hucklebridge, F. (2001). Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. *Life Sciences, 68*, 2093–2103.

El-Sheikh, M., Erath, S. A., Buckhalt, J. A., Granger, D. A., & Mize, J. (2008). Cortisol and children's adjustment: The moderating role of sympathetic nervous system activity. *Journal of Abnormal Child Psychology, 36*, 601–611.

Frick, P. J., & White, S. F. (2008). Research review: The importance of callous-unemotional traits for developmental models of aggressive and antisocial behavior. *Journal of Child Psychology and Psychiatry, 49*, 359–375.

Fries, E., Dettenborn, L., & Kirschbaum, C. (2009). The cortisol awakening response (CAR): Facts and future directions. *International Journal of Psychophysiology, 72*, 67–73.

Gaab, J., Rohleder, N., Nater, U. M., & Ehlert, U. (2005). Psychological determinants of the cortisol stress response: The role of anticipatory cognitive appraisal. *Psychoneuroendocrinology, 30*, 599–610.

Gordis, E. B., Granger, D. A., Susman, E. J., & Trickett, P. K. (2006). Asymmetry between salivary cortisol and [alpha]-amylase reactivity to stress: Relation to aggressive behavior in adolescents. *Psychoneuroendocrinology, 31*, 976–987.

Hellhammer, J., Fries, E., Schweisthal, O. W., Schlotz, W., Stone, A. A., & Hagemann, D. (2007). Several daily measurements are necessary to reliably assess the cortisol rise after awakening: State- and trait components. *Psychoneuroendocrinology, 32*, 80–86.

Henry, J. P. (1992). Biological basis of the stress response. *Integrated Physiology and Behavioral Sciences, 27*, 66–83.

Heuser, I., Deuschle, M., Weber, A., Kniest, A., Ziegler, C., Weber, B., & Colla, M. (2000). The role of mineralocorticoid receptors in the circadian activity of the human hypothalamus-pituitary-adrenal system: Effect of age. *Neurobiology of Aging, 21*, 585–589.

Huijbregts, S. C. J., van Berkel, S. R., Swaab-Barneveld, H., & van Goozen, S. H. M. (2011). Neurobiological and behavioral stress reactivity in children prenatally exposed to tobacco. *Psychoneuroendocrinology, 36*, 913–918.

Junger-Tas, J. (1994). *Delinquent behaviour among young people in the western world*. Amsterdam/New York: Kugler Publications.

Kraemer, H. C., Giese-Davis, J., Yutsis, M., O'Hara, R., Neri, E., Gallagher-Thompson, D., … Spiegel, D. (2006). Design decisions to optimize reliability of daytime cortisol slopes in an older population. *American Journal of Geriatric Psychiatry, 14*, 325–333.

Lykken, D. T. (1995). *The antisocial personalities*. Hillsdale, NJ: Erlbaum.

McEwen, B. S. (2004). Protection and damage from acute and chronic stress: Allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Annals of the New York Academy of Sciences, 1032*, 1–7.

Oitzl, M.S., van Haarst, A.D., & de Kloet, E.R. (1997). Behavioral and neuroendocrine responses controlled by the concerted action of central mineralocorticoid (MRS) and glucocorticoid receptors (GRS). *Psychoneuroendocrinology, 22*(Suppl. 1), S87–S93.

Patrick, C. J. (1994). Emotion & psychopathy: Startling new insights. *Psychophysiology, 31*, 319–330.

Platje, E., Jansen, L. M. C., Raine, A., Branje, S. J. T., Doreleijers, T. A. H., De Vries-Bouw, M., … Vermeiren, R. R. J. M. (2013). Longitudinal associations in adolescence between cortisol and persistent aggressive or rule-breaking behavior. *Biological Psychology, 93*, 132–137.

Popma, A., Doreleijers, T. A. H., Jansen, L. M. C., van Goozen, S. H. M., van Engeland, H., & Vermeiren, R. (2007). The diurnal cortisol cycle in delinquent male adolescents and normal controls. *Neuropsychopharmacology, 32*, 1622–1628.

Popma, A., Jansen, L. M. C., Vermeiren, R., Steiner, H., Raine, A., van Goozen, S. H. M., … Doreleijers, T. A. H. (2006). Hypothalamus pituitary adrenal axis and autonomic activity during stress in delinquent male adolescents and controls. *Psychoneuroendocrinology, 31*, 948–957.

Pruessner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A., von Auer, K., Jobst, S., … Kirschbaum, C. (1997). Free cortisol levels after awakening: A reliable biological marker for the assessment of adrenocortical activity. *Life Science, 61*, 2539–2549.

Rudolph, K. D., Troop-Gordon, W., & Granger, D. A. (2011). Individual differences in biological stress responses moderate the contribution of early peer victimization to subsequent depressive symptoms. *Psychopharmacology, 214*, 209–219.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions 1. *Endocrine Reviews, 21*, 55–89.

Sondeijker, F. E., Ferdinand, R. F., Oldehinkel, A. J., Tiemeier, H., Ormel, J., & Verhulst, F. C. (2008). HPA-axis activity as a predictor of future disruptive behaviors in young adolescents. *Psychophysiology, 45*, 398–404.

Spinrad, T. L., Eisenberg, N., Granger, D. A., Eggum, N. D., Sallquist, J., Haugen, R. G., … Hofer, C. (2009). Individual differences in preschoolers' salivary cortisol and alpha-amylase reactivity: Relations to temperament and maladjustment. *Hormones and Behavior, 56*, 133–139.

Susman, E. J., Dockray, S., Granger, D. A., Blades, K. T., Randazzo, W., Heaton, J. A., & Dorn, L. D. (2010). Cortisol and alpha amylase reactivity and timing of puberty: Vulnerabilities for antisocial behaviour in young adolescents. *Psychoneuroendocrinology, 35*, 557–569.

Van Goozen, S. H., Fairchild, G., Snoek, H., & Harold, G. T. (2007). The evidence for a neurobiological model of childhood antisocial behavior. *Psychological Bulletin, 133*, 149–182.

Van Goozen, S. H., Matthys, W., Cohen-Kettenis, P. T., Buitelaar, J. K., & van Engeland, H. (2000). Hypothalamic-pituitary-adrenal axis and autonomic nervous system activity in disruptive children and matched controls. *Journal of the American Academy of Child and Adolescent Psychiatry, 39*, 1438–1445.

Van Goozen, S. H., Matthys, W., Cohen-Kettenis, P. T., Gispen-de Wied, C., Wiegant, V. M., & Van Engeland, H. (1998). Salivary cortisol and cardiovascular activity during stress in oppositional-defiant disorder boys and normal controls. *Biological Psychiatry, 43*, 531–539.

Van Goozen, S. H., Snoek, H., Matthys, W., van Rossum, I., & Van Engeland, H. (2004). Evidence of fearlessness in behaviourally disordered children: A study on startle re£ex modulation. *Journal of Child Psychology and Psychiatry, 45*, 884–892.

Verhulst, F. C., Van der Ende, J., & Koot, H. M. (1997). *Handleiding voor de Youth Self- Report* [Dutch manual for the youth self-report (YSR)]. Rotterdam, The Netherlands: Afdeling Kinder-en Jeugdpsychiatrie/Sophia Kinderziekenhuis/ Academisch Ziekenhuis Rotterdam/Erasmus Universiteit Rotterdam.

Westenberg, P. M., Bokhorst, C. L., Miers, A. C., Sumter, S. R., Kallen, V. L., van Pelt, J., & Blote, A. W. (2009). A prepared speech in front of a pre-recorded audience: Subjective, physiological, and neuroendocrine responses to the Leiden Public Speaking Task. *Biological Psychology, 82*, 116–124.

Wilhelm, I., Born, J., Kudielka, B. M., Schlotz, W., & Wust, S. (2007). Is the cortisol awakening rise a response to awakening? *Psychoneuroendocrinology, 32*, 358–366.

Wust, S., Wolf, J., Hellhammer, D. H., Federenko, I., Schommer, N., & Kirschbaum, C. (2000). The cortisol awakening response – normal values and confounds. *Noise Health, 2*, 79–88.

Zuckerman, M., & Neeb, M. (1979). Sensation seeking and psychopathology. *Psychiatry Research, 1*, 255–264.

Received July 7, 2015
Accepted January 22, 2016
Published online September 13, 2016

Evelien Platje
Clinical Child and Adolescent Studies
Leiden University
Wassenaarseweg 52
2333 AK Leiden
The Netherlands
e.platje@fsw.leidenuniv.nl