The development of antisocial behavior and hypothalamic-pituitary-adrenal axis activity in adolescence

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Chapter five

Combining salivary stress-related parameters in the explanation of adolescent antisocial behavior

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Abstract

Antisocial behavior is increasingly associated with decreased cortisol awakening responses (CAR) and decreased stress reactivity of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary system (SAM). Because these parameters reflect different, yet interrelated components of the stress system, examining them in concert may offer a better explanation of the psychophysiological mechanisms underlying antisocial behavior. Therefore, this study specifically investigated the interplay between the CAR, as well as HPA and SAM reactivity to stress, in association with adolescent antisocial behavior.

Participants were 298 general population boys and girls (mean age 17.27, SD 0.42). Antisocial behavior was assessed through self-report. The level of the CAR was assessed, as well as reactivity of the HPA axis and SAM through cortisol and alpha-amylase responses to a public speaking task.

Neither parameter was independently related to antisocial behavior. The best explanation was provided by a CAR and HPA reactivity interaction; low HPA reactivity was associated with antisocial behavior in those with a high CAR, whereas high HPA reactivity was associated with antisocial behavior in those with a low CAR. Although non-significant, a trend towards an interaction between HPA and SAM reactivity appeared, indicating that low HPA reactivity was associated with antisocial behavior in those with low SAM reactivity.

These findings suggest that the additional value of SAM reactivity to HPA reactivity may be limited in explaining antisocial behavior in general population adolescents. In contrast, the CAR and HPA reactivity may reflect different psychophysiological mechanisms underlying adolescent antisocial behavior, therefore this novel finding requires further research.

Introduction

Increasing evidence points to associations between antisocial behavior and decreased stress reactivity of the sympathetic-adrenal-medullary system (SAM) and the hypothalamic-pituitary-adrenal (HPA) axis, as well as low cortisol awakening responses (CAR) (e.g. Alink et al., 2008; Susman et al., 2010). As these reflect different aspects of the stress system, yet are also interrelated, individual parameters provide an incomplete account of the relation between the stress system and antisocial behavior. In order to better understand associations with antisocial behavior, the interplay between multiple parameters should be taken into account (Bauer et al., 2002). Therefore, in this study it was investigated which combination of stress-related parameters best explains antisocial behavior.

The HPA axis and the SAM are anatomically and physiologically connected, and jointly regulate our response to stress. In reaction to a stressor, both systems show increased activity in normative samples. HPA axis reactivity can be assessed by measuring the increase in its end-product, cortisol, and alpha-amylase has been described as a surrogate marker for SAM reactivity. Because the correlation between these peripheral markers for HPA and SAM reactivity is small (Gordis et al., 2006; El-Sheikh et al., 2008; Susman et al., 2010), they can be considered to reflect coordinated, yet distinguishable components of the stress system. Therefore, assessing reactivity of the two systems 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 and hyperarousal with internalizing behavior problems (i.e. inverted U curve). 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 may be differentially activated depending on (the individuals' perception of) the situation. For example, SAM reactivity has been described as a "defense reaction" to controllable stressors, and HPA axis reactivity as a "defeat reaction" in uncontrollable situations (Henry, 1992; Dickerson and Kemeny, 2004). In this case the responses of the two systems could become dissociated, and such asymmetrical HPA and SAM activation may increase the risk for behavior 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 (Spinrad et al., 2009; Allwood et al., 2011; Huijbregts et al., 2011; Rudolph et al., 2011). As to date only few studies have examined concurrent effects of HPA and SAM reactivity, and samples sizes were generally small (i.e. only Rudolph et al.'s sample exceeded N=100), replication in a large general population sample is required to clarify this relation.

The HPA axis has not only been related to antisocial behavior through its decreased 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 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 et al., 2009). Especially the level of the CAR is thought to partly reflect the *trait* physiological HPA axis activity (Hellhammer et al., 2007; Pruessner et al., 1997; Edwards et al., 2001), whereas reactivity of the HPA axis to social stress, is regarded as a *state* phenomenon, largely dependent on situational specific factors, and the individual's perception and processing of a stressor (Gaab et al., 2005; Dickerson and Kemeny, 2004).

However, it is unclear how these distinct components of HPA axis activity relate in explaining antisocial behavior. From a theoretical perspective, three mechanisms have been proposed. According to the sensation seeking theory (Zuckerman and 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 mainly be associated with low trait HPA axis activity (van Goozen et al., 2007). 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 (Raine, 1993). Here, antisocial behavior would mainly be associated with low HPA stress reactivity (van Goozen et al., 2007). Finally, it has been posed that frequent sensation seeking (associated with low trait HPA activity) 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 decreased trait HPA axis activity and decreased HPA stress reactivity. 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.

Within antisocial behavior, aggression and rule-breaking are two main types of behavior often recognized (Achenbach et al., 1989; Burt, 2012; Loeber and Schmaling, 1985). More importantly, the relative influence of neurobiological risk factors may differ for aggression and rule-breaking. Both types are thought to result from complex interactions between environmental, social, cognitive, and biological risk factors (Moffitt, 1993; Raine et al., 2005). However, neurobiological deficits may be more strongly related to aggression than to rule-breaking (Burt, 2012; McBurnett et al., 2000), which has indeed been shown for the CAR (Platje et al., 2013b).

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 (Gordis et al., 2006; de Vries-Bouw et al., 2012), we aim to replicate this design by examining concurrent reactivity in a large general population sample. 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, were studied concurrently. We examined which combination of the three stress-related parameters provides the best explanation of antisocial behavior. We combined parameters in one explanatory model of antisocial behavior, and examined interactions between these parameters.

Methods

Participants

Participants were 298 adolescents (168 boys and 130 girls), with a mean age of 17.27 years (SD=0.42). They were recruited from the RADAR (Research on Adolescent Development And Relationships) study. RADAR is a Dutch population based cohort study, with over-sampling (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 between January 2010 and January 2011. The CAR was assessed at the participant's home in February and March of 2010, when antisocial behavior was assessed as well.

The total cohort consisted of 497 adolescents, and 418 adolescents participated in 2010. Of the 418, 303 participated in the lab session, of whom 227 also participated in the CAR measurement. The 303 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 303, two participants provided insufficient saliva for the assays, and for three participants there were too many missing values on all stress-related parameters, adding up to a sample of 298 in the final analyses. Characteristics of the participants in the final analyses are described in Table 5.1.

Antisocial behavior

Antisocial behavior was assessed by means of the externalizing scales of the Youth Self Report (YSR, Achenbach, 1991b), administered to the adolescents. Within the externalizing dimension, sub-scales differentiate aggression and rule-breaking behavior. Items are scored on a three-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 et al., 1997).

Table 5.1. Characteristics of participants (N = 298).

	Mean / n	**SD / %**	**Range**
Age	17.27	0.42	15.33 - 18.67
Boys	168	56.4	
Nicotine use[a]	75	25.2	
Alcohol use[a]	48	16.1	
Externalizing	48.14	10.04	29 - 77
Rule-breaking	54.14	4.75	50 - 75
Aggression	54.31	7.07	50 - 85

Note. [a] nicotine and alcohol use within 24 hours before lab session

Stress reactivity of the HPA axis and SAM

The assessment of stress reactivity was assessed at the end of a lab session (see Fig. 5.1) in which the participants watched empathy-inducing film clips (de Wied et al., 2006) and performed both the Stop Signal task and the Iowa Gambling task. The Leiden Public Speaking Task (Leiden PST; Westenberg et al., 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 and Kemeny, 2004) in normative samples. First, the participants watched a nature documentary (baseline), after which detailed instructions were given. Subsequently, they were given five minutes to prepare their speech. They were asked to deliver their speech in front of a pre-recorded audience consisting of age-matched peers and a teacher. The participants were told that their video-taped 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 and colleagues (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). The moments of collection were just before baseline (sample 1), after baseline (sample 2), immediately after the speech (sample 3), 10 minutes after the speech (sample 4), followed by four samples with intervals of 5 minutes (sample 5 - 8). Reactivity of cortisol in saliva is delayed with approximately 20 minutes, whereas alpha-amylase reactivity is delayed with approximately 10 minutes. The average time of collection of the first sample was 3:58 p.m. (SD 26 min). A graphical representation of the lab session and the sampling moments is provided in Figure 5.1.

The CAR

The CAR was assessed in saliva sampled immediately after awakening (Cort0), and 30 minutes (Cort30) and 60 minutes (Cort60) later. Saliva samples were collected by passive drooling. 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 8:00 a.m., 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 Cort60. They were requested to report the exact sampling times on the instruction form on the day of sampling, and also 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, e.g. when Cort0 was sampled after 8:00 a.m. or the sampling time of Cort30 or Cort60 was over 15 minutes 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 (Cort0) was 7:02 a.m. (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 for example coffee), alcohol, nicotine and drug use, as well as physical exercise, all taken place within the last 24 hours, 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 contra-conceptive (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-assay and inter-assay coefficients of variation of 3.4% and 12.2%. Alpha-amylase samples were diluted 50 times with 9% sodium chlorine, 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 3SD 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 three participants there were too many missing values on all three stress-related parameters, and a total of 298 adolescents participated in the final analyses.

As measures of reactivity of the HPA axis and SAM, area under the curves with respect to increase (AUCi) were computed for cortisol and alpha-amylase over samples 2 to 5 (from just before start, until 15 minutes 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 Cort0, Cort30, and Cort60 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. Next, hierarchical regression analyses were performed with either externalizing, rule-breaking, or aggressive behavior as dependent variable. Of the control variables assessed, only gender and nicotine use were significantly associated with both antisocial behavior and stress-related parameters, these were entered as confounders in step 1. In step 2, main effects of either cortisol reactivity, alpha-amylase reactivity, or the CAR were added to the model. To investigate effects of combining the stress-related parameters in one model, in step 3, concurrent effects of either alpha-amylase reactivity and cortisol reactivity (a), cortisol reactivity and the CAR (b), or all three parameters together (c) were examined. In step 4, 2-way interactions between either cortisol reactivity and alpha-amylase reactivity (a)

or cortisol reactivity and the CAR (b) were added. In step 4c, the two 2-way interactions between cortisol reactivity and alpha-amylase reactivity, as well as cortisol reactivity and the CAR were examined together. In step 5, a 3-way interaction between cortisol reactivity, alpha-amylase reactivity and the CAR was added. Significant interaction effects were probed using simple slope analysis (Aiken and West, 1991).

Results

Reactivity to stress during the public speaking task was significant for both cortisol and alpha-amylase (see Fig. 5.1), as repeated measures ANOVAs revealed a curvilinear main effect of time for cortisol ($F(7) = 17.969$, $p < .001$) and alpha-amylase ($F(7) = 79.122$, $p < .001$)).

Pearson correlations between antisocial behavior and stress-related parameters are shown in Table 5.2. Externalizing, rule-breaking, and aggression showed moderate positive correlations. Reactivity of cortisol and alpha-amylase was also positively correlated, although weak. Correlations between measures of antisocial behavior and stress-related parameters were all non-significant.

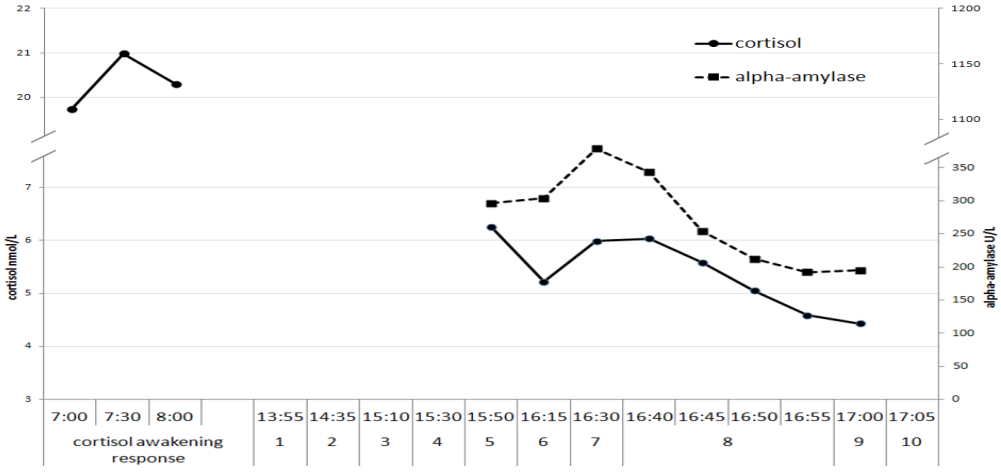

Figure 5.1. Overview of the lab session including the Public Speaking Task. 1: welcome, 2:empathy inducing film clips, 3:STOP task, 4:IOWA gambling task, 5:baseline, 6:after baseline, 7:PST, 8:recovery, 9:end PST, 10:end lab session.

Note. 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.

Table 5.2. Correlations between antisocial behavior and stress-related parameters.

	1	2	3	4	5
1. Externalizing					
2. Rule-breaking	.555**				
3. Aggression	.575**	.593**			
4. Cortisol reactivity	-.020	.005	.009		
5. Alpha-amylase reactivity	-.005	-.012	-.018	.197**	
6. CAR	.059	.026	.011	-.023	-.048

Note. Two-tailed Pearson correlations. ** $p \leq .01$.

As gender and nicotine use were associated with both antisocial behavior and stress-related parameters, these were entered in the regression analyses as covariates. First, main effects of cortisol reactivity, alpha-amylase reactivity, and the CAR level predicting externalizing, rule-breaking, and aggressive behavior were investigated. As shown in Table 5.3, linear regression analyses revealed that neither of these parameters independently showed a significant association with any type of antisocial behavior.

Next, it was examined which combination of parameters provides the best explanation of antisocial behavior. Therefore, we combined parameters in one explanatory model of antisocial behavior, and examined interactions between these parameters. For overall externalizing behavior, interactions between the stress-related parameters were found, these are described below. For rule-breaking and aggression, combining stress-related parameters did not reveal significant associations.

Table 5.3. Results of regression analyses predicting antisocial behavior by cortisol reactivity, alpha-amylase reactivity and the CAR, as well concurrent effects of these three parameters.

			Externalizing		**Rule-breaking**		**Aggression**	
			β	**adj R^2**	**β**	**adj R^2**	**β**	**adj R^2**
Step 1		Covariates		.038		.051		.046
		Main effects						
Step 2		cortisol reactivity	-.016	.034	.007	.048	-.004	.042
		alpha-amylase reactivity	.004	.025	.000	.050	-.009	.042
		CAR	.058	.013	.027	.043	.029	.053
		Combined effects						
Step 3	a.	cortisol reactivity	-.018	.026	.003	.044	.001	.034
		alpha-amylase reactivity	.000		-.013		-.009	
	b.	cortisol reactivity	.031	.009	.037	.040	.061	.052
		CAR	.058		.027		.030	
	c.	cortisol reactivity	.046	.004	.039	.034	.080	.055
		alpha-amylase reactivity	-.058		.025		-.083	
		CAR	.034		-.011		.008	
Step 4	a.	cortisol reactivity * alpha-amylase reactivity	**.125†**	.034	.047	.042	-.014	.031
	b.	cortisol reactivity * CAR	**-.492****	.043	-.224	.043	-.263	.058
	c.	cortisol reactivity * alpha-amylase reactivity	**.148†**	.043	-.102	.038	.051	.055
		cortisol reactivity * CAR	-.411*		.125		-.205	
Step 5		cortisol reactivity * alpha-amylase reactivity * CAR	-.153	.064	-.298	.045	-.271	.051

Note. In step 1 gender and nicotine use were entered in the model as covariates. The number of participants varied per analysis; n = 292 for cortisol reactivity, n = 278 for alpha-amylase reactivity, n = 207 for the CAR, n = 272 for step 3a, n = 207 for step 3b, and n = 197 for step 3c. Significant effects and trends are shown in bold face. †p ≤ .10, * p ≤ .05, ** p ≤ .01.

A trend towards an interaction between cortisol and alpha-amylase reactivity appeared in association to externalizing behavior (see Table 5.3). Single slopes were not significant, but these suggested that when alpha-amylase reactivity was low, the association between cortisol reactivity and externalizing behavior was negative (β = -.157, p = .115) and when alpha-amylase reactivity was high, this association was absent (β = .059, p = .430).

A significant interaction was found between cortisol reactivity and the CAR level for externalizing behavior (see Table 5.3). Single slopes for the interaction between the CAR level and cortisol reactivity in relation to externalizing behavior are plotted in Figure 5.2. It can be seen that when the CAR level was low (i.e. 1SD below the mean), a positive relation between cortisol reactivity and externalizing behavior was found (β = .210, p = .025). Conversely, when the CAR level was high (i.e. 1SD above the mean), a trend towards a negative relation between cortisol reactivity and externalizing behavior was found (β = -.163, p = .095). Combining stress reactivity of cortisol and the CAR level in an interaction, explained a larger part of the variance in externalizing behavior (ΔR^2 = .039, p = .017), than the CAR alone.

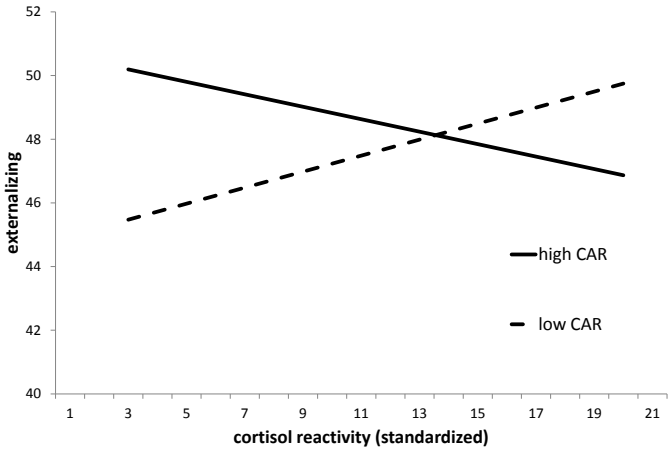

Figure 5.2 Simple slopes for externalizing predicted by cortisol reactivity at 1 SD above and below the mean for the CAR.

Combining the two 2-way interactions between alpha-amylase and cortisol reactivity, as well as the CAR level and cortisol reactivity, did not explain more variance in externalizing behavior than the interaction between the CAR and cortisol reactivity alone.

Discussion

Stress reactivity of the HPA axis and the SAM, as well as the CAR, reflect different aspects of the stress system. Examining them individually thus provides an incomplete account of the relation with antisocial behavior. Therefore, in the current study, these stress-related parameters were examined together 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, i.e. 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.

We did not find a significant additive or interactive effect of low HPA and SAM reactivity with antisocial behavior, as posed by Bauer et al. (2002), although we did find a trend towards an additive effect in the expected direction. 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 (Spinrad et al., 2009; Allwood et al., 2011; Huijbregts et al., 2011; Rudolph et al., 2011, but see Gordis et al., 2006), while in a delinquent sample an additive effect was found (de Vries-Bouw et al., 2012).

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. Associations with a low CAR were found only when stress reactivity was high, and associations with low stress reactivity were only found when the CAR was high. In the light of the low arousal theories, two different types of stress-related antisocial behavior could be hypothesized. The first is a type resulting from a high CAR, which could be seen as high trait arousal (Hellhammer et al., 2007), reflecting agitation and irritability, which may make the individual more susceptible to engage in conflict (Dodge and Coie, 1987; Dodge et al., 1997; Scarpa and Raine, 1997). As high arousal is more or less constant, arousal caused by stressful conflict situations may not be recognized, leaving the individual fearless of negative consequences of conflicts (Raine, 1993). The second type has low trait arousal, reflecting an aversive physiological state, which could be increased (i.e. normalized) by seeking sensation through antisocial behavior (Zuckerman and Neeb, 1979). As the individual with this type of stress-related antisocial behavior shows relatively high reactivity, the intended normalization was successful, which may work stimulating to engage in antisocial behavior more often. Although these two types of stress-related antisocial behavior are purely hypothetical until further researched, 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 substantially 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) (Oitzl et al., 1997; Heuser et al., 2000).

As this is the first study to investigate interactions between different components of HPA axis activity in relation to antisocial behavior, replication is warranted. To further substantiate the current findings, future research should incorporate direct psychological measures of sensation seeking and fearlessness. In this respect future studies should also examine whether other known neurobiological deficits corroborate these findings, such as whether amygdala dysfunction, which has often been related to fearlessness (Phelps and LeDoux, 2005; Sterzer and Stadler, 2009), is also associated with stress reactivity as compared to the CAR. Moreover, to examine the mechanisms behind these findings, the relative MR and GR activity should be studied in relation to antisocial behavior, for instance through selective blockade of either receptor type. Should the low CAR/ high reactivity type, and the high CAR/ low stress reactivity type be replicated and corroborated, this may eventually allow for identifying distinct psychophysiological profiles which could be linked to 'sensation seeking antisocial behavior' and 'fearless antisocial behavior' (see e.g. Zuckerman and Neeb, 1979; Raine, 1993; Raine et al., 1998; van Goozen et al., 2007).

For both rule-breaking and aggressive behavior, no effects of combined stress-related parameters were found. This in contrast to suggestions that stress-related parameters may be associated more strongly with aggression than with rule-breaking behavior (Burt, 2012; Platje et al., 2013b). Possibly, this suggestion may specifically pertain to severe and persistent aggression (McBurnett et al., 2000; Shoal et al., 2003; Platje et al., 2013b). In the current design we assessed aggression for the sample as a whole and only at age 17, therefore we could not distinguish severe and persistent aggression from normative aggression. It could also be that the low CAR/ high reactivity type and the high CAR/ low stress reactivity type are linked to other subtypes of antisocial behavior than aggression an rule-breaking, for instance, to "hot" reactive aggression and "cold" proactive aggression (Lopez et al., 2004) or combined with high or low anxiety (Van Goozen et al., 1998).

Some methodological limitations should be mentioned. First, because a cross-sectional design was used, the causal relationship between stress-related parameters and antisocial behavior remains subject to further investigation. 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 (Wust et al., 2000; Edwards et al., 2001). 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 et al., 2007).

Third, this study was performed in a general population sample, displaying (mainly) normative levels of antisocial behavior. As such, the results cannot be generalized to e.g. 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), yet CAR and stress HPA interactions have not been studied previously. This would however be highly relevant to investigate in clinic-referred or severe delinquent populations. Should the different pathways for the CAR and stress reactivity to antisocial behavior also apply to these populations, this could eventually entail that the low CAR/ high reactivity type, and the high CAR/ low stress reactivity type require different interventions.