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Schoorl, J.

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Author: Schoorl, Jantiene
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CHAPTER 3

The role of anxiety in cortisol stress response and cortisol recovery in boys with oppositional defiant disorder/conduct disorder

Jantiene Schoorl, Sophie van Rijn, Minet de Wied,
Stephanie H. M. van Goozen, and Hanna Swaab

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ABSTRACT

Children with antisocial and aggressive behaviours have been found to show abnormal neurobiological responses to stress, specifically impaired cortisol stress reactivity. The role of individual characteristics, such as comorbid anxiety, in the stress response is far less studied. Furthermore, this study extended previous studies in that not only baseline and reactivity to a psychosocial stressor were examined, but also recovery from a stressor. These three phases of cortisol could be impacted differentially in boys with oppositional defiant disorder/conduct disorder (ODD/CD) with (+ANX) and without anxiety (-ANX). The results revealed that cortisol patterns in response to psychosocial stress were different for boys with ODD/CD+ANX (n=32), ODD/CD-ANX (n=22) and non-clinical controls (NC) (n=34), with age range of 7.8 to 12.9 years. The ODD/CD-ANX group showed lower overall cortisol levels than the NC group. When considering the three phases of cortisol separately, the ODD/CD-ANX group had lower baseline cortisol levels relative to the other groups, whereas the ODD/CD+ANX showed an impaired cortisol recovery response. Within those with ODD/CD, callous-unemotional traits were predictive of high baseline cortisol levels. Also, anxiety predicted high baseline and recovery cortisol levels, whereas a high number of CD symptoms predicted reduced cortisol stress reactivity. These results clearly indicate that comorbid anxiety is an important factor in explaining differences in stress response profiles in boys with ODD/CD; although boys with CD/ODD are generally characterized by an impaired cortisol stress response, we found that those with comorbid anxiety showed impaired cortisol recovery, whereas those without anxiety showed reduced baseline cortisol levels.

INTRODUCTION

There is increasing evidence that neurobiological deficits play a key role in aggressive and antisocial behaviour in children (Van Goozen et al., 2007). It is argued that stress regulating mechanisms, such as the hypothalamic-pituitary-adrenal (HPA) axis, are important in explaining individual differences in aggressive and antisocial behaviour. The end product, cortisol, has received much attention because of its vital role in enabling adaptive responses to stress, in order to survive and cope with danger (Sapolsky, 1998). Studies have reported mixed findings concerning the relationship between aggressive and antisocial behaviour and cortisol (Alink et al., 2008; Van Goozen et al., 2007). Inconsistencies in findings might be explained by methodological differences, such as different populations (community versus clinical, age, male/female), sampling of cortisol (plasma, urine, saliva) and time of the day, informant (self-, parent- or teacher-report) and type of stressor. However, another explanation might be found in the notion that children with aggressive and antisocial behaviour form a heterogeneous group (Stadler, 2010), and that individual differences in levels of emotional problems vary greatly (Schoorl et al., 2016a).

Nevertheless, studies on primary school-aged children with aggression problems have generally found normal cortisol baselines but reduced cortisol stress reactivity to stress, compared to controls (Snoek et al., 2004; Van Goozen et al., 1998; Van Goozen et al., 2000). This blunted cortisol stress reactivity has been associated with fearlessness and deficient emotion regulation (Van Goozen, 2015), which may be an important mechanism driving behavioural problems in children with oppositional defiant disorder (ODD) or conduct disorder (CD) (Burke, 2012; Cavanagh et al., 2014). Interestingly, studies on ODD/CD and anxiety show different results; cortisol levels were higher in anxious children with CD (McBurnett et al., 1991) and higher cortisol stress reactivity was found in boys with ODD (Van Goozen et al., 1998) and ODD/CD (Van Goozen et al., 2000) with relatively high levels of anxiety compared to low anxious boys. Thus not all children with ODD/CD have low cortisol levels and comorbid anxiety might be an important factor contributing to variability in cortisol responses within ODD/CD samples.

Because in previous studies variation in callous-unemotional (CU) traits has been considered a relevant factor contributing to variability in cortisol responses (Hawes et al., 2009), this was also included in the present manuscript. CU traits have been related to lower baseline cortisol as well as blunted cortisol response to stress (Loney et al., 2006; Stadler et al., 2011). However, two other studies did not find a relation between baseline cortisol and CU traits (Feilhauer et al., 2013; Poustka et al., 2010).

In addition to child factors that may contribute to variability, it may also be relevant to distinguish several phases of stress responses, which could be impacted differentially in these children. The degree to which children are able to regulate

stress is not only evident in a blunted or sharpened cortisol response to stress, but also in their ability to recover from stress. The ability to recover after a stressor is an important indicator of the quality of an individual's emotion regulation (Freeman, 1939; Ji et al., 2015). Infants of mothers whose interactions with their infants were most disrupted, e.g. highly unresponsive, ineffective or inappropriate, did not recover from a stressor; their cortisol levels kept increasing after the stressor was gone (Crockett et al., 2013). Also, faster cortisol recovery after daily stressors was related to maternal sensitivity in infants, indicating that sensitive mothers helped their infants indirectly to regulate their cortisol response (Albers et al., 2008; Blair et al., 2008). Healthy individuals are able to rapidly down regulate emotions after a stressor has ended, as a means of adapting to environmental challenges without the severe biological cost of keeping stress levels high (Hastings et al., 2011). Recovery from stress is thus an important mechanism in behavioural adaptation. The aggressive and antisocial behaviour that children suffering from ODD/CD show might be the result of impaired recovery. However, the literature examining cortisol recovery separate from cortisol response to stress in children with emotional and behavioural problems is sparse, and in relation to ODD/CD, to our knowledge, non-existent. Therefore, the aims of the study were to further investigate the role of anxiety within those with ODD/CD and to examine cortisol under baseline, stress and recovery conditions. To this end we included boys with ODD/CD with and without a comorbid anxiety disorder and also a sample of typically developing boys as controls.

METHOD

The current study was approved by the Medical Ethical Committee of Leiden University Medical Centre (LUMC). Prior to participation signed informed consent according to the declaration of Helsinki was obtained from the parents. Eleven boys with ODD/CD and two controls from the larger study were not able to produce saliva samples, missed one or more saliva sample or had one or two saliva samples that were inadequate for analyses, i.e. 3 SD above mean, and were excluded from the current study.

Participants

The ODD/CD group (*n*=54) was recruited at clinical health centres (*n*=19), special education schools (*n*=26) and regular elementary schools (*n*=9). All boys had an IQ over 70, were aged between 7.8 and 12.9, and a diagnosis of ODD or CD on the Diagnostic Interview Schedule for Children (DISC-IV; Shaffer et al., 2000). All boys met criteria for ODD diagnosis and 17 boys (32%) also met CD criteria. Other comorbid diagnoses were: attention deficit hyperactivity disorder (ADHD) (*n*=38, 70%), depression (*n*=8, 15%), and other disorders, e.g. eating or tic disorders (*n*=15,

28%), as based on the DISC-IV. Twenty-two boys (41%) used psychostimulants and two (4%) were on risperidone.

Using the DISC-IV boys with ODD/CD were divided into the *ODD/CD+ANX group* if they met criteria for a comorbid anxiety disorder (*n*=32). Boys in the ODD/CD+ANX group met criteria of one or more of the following anxiety disorder: separation anxiety disorder, social phobia, generalized anxiety disorder, panic disorder, agoraphobia and specific phobia. If they did not meet criteria for any of these anxiety disorders they were included in the *ODD/CD-ANX group* (*n*=22).

T*he non-clinical control group (NC)* (*n*=34) was recruited at regular elementary schools. All boys had an IQ over 70 and were aged between 8.0 and 12.7. None of them used medication or showed severe aggressive behaviours, expressed as a diagnosis of ODD or CD, a score outside the normal range (T>60) on the externalizing scale of the Child Behavior Checklist (CBCL/6-18) or Teacher Report Form (TRF/6-18) (Achenbach and Rescorla, 2001).

Recruitment
Boys referred through clinical centres were first screened with the CBCL (Achenbach and Rescorla, 2001). Those who scored above the borderline cut off point on the externalizing scale were subsequently administered the DISC-IV interview Module E (section on ODD and CD) (Shaffer et al., 2000). Only those children who met criteria of either ODD or CD were asked to take part in this study.

Special educational needs schools and regular elementary schools were selected based on their location, no further than one hour's drive from Leiden University. Headmasters were contacted by one of the researchers and if the headmaster agreed to take part, information brochures for parents and response-cards were distributed by the teachers to the children in their class.

Participating boys were asked to visit Leiden University for one day with one of their parents. During this day parents signed an informed consent, filled out questionnaires and completed the DISC-IV interview.

Measures
IQ was estimated using the Vocabulary and Block Design subtests of the Dutch version (Kort et al., 2005) of the Wechsler Intelligence Scale for Children (WISC-III) (Wechsler, 2005). These subtests have been found to provide a good estimation of full scale IQ scores (Sattler, 1992).

Child psychopathology was assessed using the Dutch version of the DISC-IV interview (Ferdinand and van der Ende, 2002) with one of the parents. The DISC-IV is a highly structured diagnostic instrument (Shaffer et al., 2000) and was conducted by a clinical trained psychologist with experience. Diagnosis occurred after completion of the

3

interview, at time of measuring symptoms this interviewer was 'blind' to diagnosis. Symptom scores and diagnoses are according to the DSM-IV criteria (DSM-5 had not been published at the start of this study).

CU traits were measured with the CU subscale of the Dutch version (De Wied et al., 2014) of the Antisocial Process Screening Device (APSD; Frick and Hare, 2001). Parent and teacher ratings were combined by taking the highest rated score on each item (see Frick and Hare, 2001). The Cronbach's alpha was .66 for the whole sample.

Psychosocial stress induction procedure The stress paradigm took place in the afternoon. Stress was induced for 90 minutes using an established and ecologically valid psychosocial stressor that involves provocation, frustration and competition to increase emotional arousal. Participating boys were led to believe that they were competing against a videotaped opponent of similar age and sex for best performance and a highly favoured award (for example Lego, a monster truck, a giant toy water pistol or magician tricks box), whilst they were led to believe they were losing out on winning the computer task competition (for details, see Fairchild et al., 2008; Schoorl et al., 2016a; Van Goozen et al., 2000).

Stress manipulation was checked with an adapted version of the Von Zerssen's (1986) clinical self-rating scale, containing eleven moods (happy, well, cheerful, good, liked, satisfied, afraid, worried, embarrassed, ashamed, angry, in control) and feeling of control. Boys rated themselves on a five-point scale ranging from positive towards negative feelings (e.g. 1=happy, 5=gloomy) each time a cortisol sample was taken. All moods were combined into one negative mood score. Mean Cronbach's alpha was .86.

Procedure for cortisol collection Participating boys completed a battery of questionnaires and neuropsychological tests in the morning. At the end of the morning they were asked to provide a baseline cortisol sample (see Fig. 1). In the stress phase four cortisol samples were taken, approximately one every 20 minutes. After the stress phase ended and disclosure was done, the boys remained seated in the same room for one more hour in which they completed questionnaires and watched relaxing cartoons. In this recovery phase three cortisol recovery samples were collected, one every 20 minutes. See Fig. 1 for a schematic representation of the test procedure.

Saliva was collected using a tube in which subjects could spit (passive drool) (0,5ml). Children were instructed to accumulate saliva in the floor of their mouth and collect them directly into sterilized glass tubes. Contamination with food debris was avoided by rinsing the mouth with water before the stress experiment started. After all samples were collected they were stored at -20 °C until analysis.
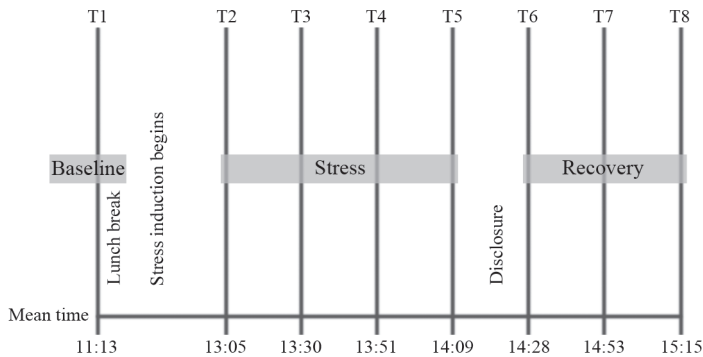
Fig. 1. Schematic representation of the test procedure and mean cortisol and mood rating sampling times.

Assay procedure for cortisol Cortisol concentrations in the saliva samples were determined by using a time-resolved fluorescence immunoassay (DELFIA), for details see (Dressendörfer et al., 1992). The intra- and interassay variability was below 7% and 6%, respectively. Results are reported in nmol/l.

Statistical analysis
The three groups (ODD/CD+ANX, ODD/CD-ANX and NC) were compared on their self-reported mood and all eight cortisol samples with a repeated measures ANOVA. To explore the cortisol pattern in more detail we also examined cortisol during the three phases of the paradigm: baseline, stress and recovery. For the stress phase we calculated a cortisol stress reactivity level by calculating the area under the curve with respect to increase (AUCi) (Pruessner et al., 2003). Cortisol recovery was measured using delta scores of the first and last cortisol measure during the recovery phase (Linden et al., 1997). We applied a Greenhouse Geisser correction if assumptions of sphericity were violated. Finally, a backwards linear regression analysis was performed to investigate the predictive value of clinical characteristics for cortisol baseline, reactivity and recovery. Effect sizes are reported as eta squared (η^2) with .01 being a small, .06 being a medium and .14 being a large effect (Cohen, 1988).

RESULTS

A MANOVA revealed that medication use was not related to the cortisol measures, *F(3,50)*=.64, *p*=.596. Therefore, medication use was not controlled for in subsequent analyses. The ODD/CD+ANX group had higher levels of comorbid ADHD than the ODD/CD-ANX group, 84% versus 50%, χ^2=7.39, *p*=.007. However, a correlation analysis indicated that ADHD was not related to any of the cortisol measures.

Descriptive statistics for the three groups are presented in Table 1. The three

groups did not differ in age or percentage of Caucasians, respectively $F(2,88)$=1.46, p=.237 and χ^2=2.85, p=.240. The ODD/CD+ANX group had a significantly higher anxiety level than the ODD/CD-ANX group and the NC group, $F(2,88)$=33.75, p<.001, while the other groups did not differ from each other. CU traits were higher in both ODD/CD groups compared to controls, $F(2,83)$=13.84, p<.001, but the ODD/CD groups did not differ from each other. The NC group had a higher IQ score than both ODD/CD groups, $F(2,88)$=6.12, p=.003. Therefore, all subsequent analyses were repeated with IQ included as a covariate. Because results remained the same with or without this covariate, it was chosen to report the analyses without IQ as a covariate.

Table 1. *Descriptive statistics for the ODD/CD+ANX, ODD/CD-ANX and NC group.*

	ODD/CD+ANX	ODD/CD-ANX	NC	*F/ χ^2*
Age	10.1±1.25	10.6±1.35	10.0±1.25	1.46
IQ	94.2±13.46	94.2±13.72	104.0±12.29	6.12**
Caucasian (%)	72%	50%	67%	2.85
Anxiety (CBCL)	9.4±4.32	3.6±3.43	2.4±2.95	33.75***
CU traits	6.8±2.46	7.2±1.57	4.5±2.00	13.84***

ODD, oppositional defiant disorder; CD, conduct disorder; ANX, anxiety; NC, non-clinical control; CBCL, Child Behavior Checklist; CU, callous-unemotional

**: p <.01, *** p <.001

Subjective mood effects
Data of one boy with ODD/CD-ANX was missing, because he refused to fill out the mood questionnaire. There was a significant main effect of time, $F(2.98, 256.52)$=35.28, p<.001, η^2=.29, but not of group, $F(2, 86)$=1.27, p=.287, and no time by group interaction, $F(2.98, 256.52)$=1.62, p=.141, indicating that stress induction was successful and similar in all groups (see Fig. 2).

Cortisol
A repeated measures ANOVA over all 8 cortisol samples showed that there was a significant main effect of time $F(3.93, 334.41)$=11.04, p<.001, η^2=.12, of group $F(2,85)$=3.27, p=.043, η^2=.07, and a time by group interaction $F(3.93, 334.41)$=2.71, p=.007, η^2=.06 (see Fig. 3). The post hoc analyses of the repeated measures ANOVA revealed that both ODD/CD groups did not differ from each other, but the ODD/CD-ANX group did have significantly lower overall cortisol levels than the NC group, p=.013.

Because the significant interaction effect indicates that the three groups had different cortisol patterns over time, which is also evident in Fig. 3, we subsequently did post-hoc ANOVA's to examine group differences for baseline, stress and recovery phase separately in the three groups. The ANOVA for baseline cortisol revealed that the
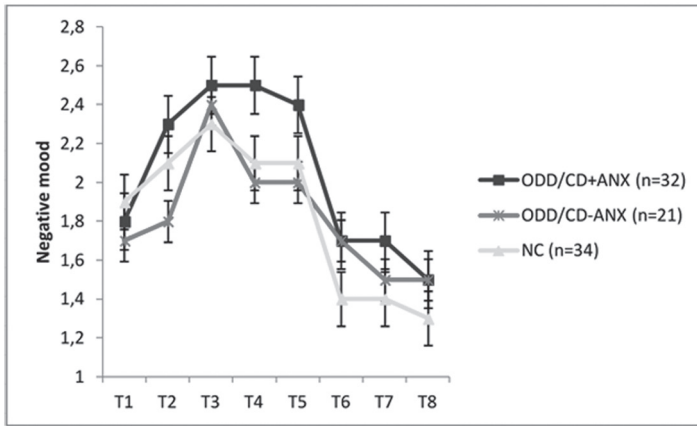
Fig. 2. Mean and standard error of negative mood scores during baseline, stress and recovery phases for the ODD/CD+ANX, ODD/CD-ANX and NC group.

ODD/CD-ANX group had a significantly lower baseline cortisol level than both the ODD/CD+ANX and NC group, $F=4.45$, $p=.014$.

The ODD/CD-ANX group had a marginally significant lower cortisol stress reactivity (AUCi) than the ODD/CD+ANX group, $p=.066$.
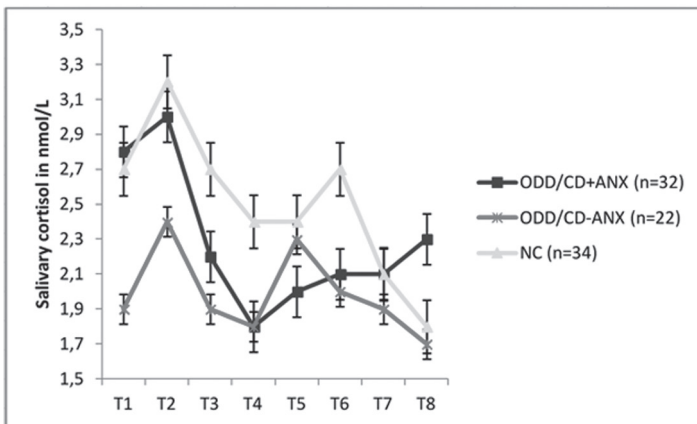

Fig. 3. Salivary cortisol levels during baseline, stress and recovery phases for the ODD/CD+ANX, ODD/CD-ANX and NC group. Means and standard errors are indicated.

Finally, for the recovery phase it was found that the ODD/CD+ANX showed significantly less cortisol recovery than the ODD/CD-ANX and NC group, $F=9.44$, $p<.001$. Furthermore, paired samples t-test revealed that the cortisol levels of the

43

ODD/CD+ANX group did not decline in the recovery phase, $t=-1.19$, $p=.245$, whereas cortisol levels declined in the ODD/CD-ANX group, $t=2.28$, $p=.034$, and NC group, $t=4.59$, $p<.001$.

Predictive value of clinical symptoms for cortisol levels during baseline, stress and recovery

The correlation matrix shows that anxiety correlated positively with baseline cortisol and negatively with cortisol stress reactivity (AUCi) and cortisol recovery (see Table 2). CU traits correlated positively with baseline cortisol. CD symptoms correlated negatively with cortisol stress reactivity; no correlations were found with ODD symptoms and anxiety, CD and CU traits did not correlate with each other.

Table 2. *Correlation matrix of predictors and cortisol (r) within ODD/CD.*

	Baseline	Stress reactivity (AUCi)	Recovery
Anxiety	.38**	-.28*	-.27*
CU traits	.29*	-.09	-.03
ODD	.13	-.02	.02
CD	.21	-.35*	.04

CU, callous-unemotional; ODD, oppositional defiant disorder; CD, conduct disorder; AUCi, area under the curve with respect to increase

*: correlation is significant at the .05 level (two-tailed)

**: correlation is significant at the .01 level (two-tailed)

Backward regression analyses were done to predict the three phases of cortisol from the three predictors: anxiety and CD symptoms. Baseline cortisol was best predicted by a model that included anxiety and CU traits, $F=8.25$, $p=.001$, $R=.50$ (see Table 3); together they explained 25% of the variance in baseline cortisol. High levels of anxiety and high levels of CU traits were related to higher levels of baseline cortisol.

Cortisol stress reactivity (AUCi) was best predicted by a model that included only CD symptoms, $F=7.03$, $p=.011$, $R=.35$. In this model CD symptoms significantly inversely predicted cortisol stress reactivity (see Table 3) and explained 12% of the variance in cortisol stress reactivity.

Finally, cortisol recovery was best predicted by a model that had anxiety as the only predictor, $F=4.23$, $p=.045$, $R=.27$ (see Table 3). High levels of anxiety were related to high levels of cortisol recovery. Eight percent of the variance in cortisol recovery was explained by anxiety.

DISCUSSION

The aim of our study was to understand individual differences in cortisol patterns

in ODD/CD, by focusing on child factors in terms of comorbid anxiety, and by

Table 3. *Regressions of predictors on baseline cortisol, cortisol reactivity and cortisol recovery.*

	b	SE b	β
Baseline cortisol			
(Constant)	.72	.50	
Anxiety	.90	.27	.41***
CU traits	.17	.06	.33*
Stress reactivity (AUCi)			
(Constant)	.62	.72	
CD	-.79	.30	-.35*
Recovery			
(Constant)	.33	.22	
Anxiety	-.58	.28	-.27*

CU, callous-unemotional; CD, conduct disorder; AUCi, area under the curve with respect to increase

*: $p < .05$ ***: $p < .001$

distinguishing various phases of stress hormone (i.e. cortisol) responses which may be impacted differentially within the group of boys with ODD/CD. To this end, we exposed boys to an established and ecologically valid psychosocial stressor that involved provocation, frustration and competition. Cortisol levels were examined before the stressor (baseline), during the stressor (reactivity), and after the stressor ended (recovery).

Boys with ODD/CD with anxiety (+ANX) and without anxiety (-ANX) reported similar levels of negative mood over the course of baseline, stress and recovery, but showed different cortisol patterns than controls. Overall, i.e. irrespective of the phase, the ODD/CD-ANX group had lower cortisol levels than controls. Because there was a significant interaction between Group and Time we examined cortisol into more detail by looking at the three phases separately. During baseline, the ODD/CD-ANX group, but not the ODD/CD+ANX group, had lower baseline cortisol levels than controls. Both ODD/CD groups did not differ in stress cortisol reactivity levels compared to controls. During recovery the ODD/CD+ANX group showed less cortisol recovery than controls. Furthermore, the ODD/CD-ANX group had lower baseline cortisol levels, marginally lower cortisol stress reactivity and showed more cortisol recovery compared to the ODD/CD+ANX group. While cortisol levels reduced during the recovery phase in the ODD/CD-ANX and NC group, cortisol levels of the ODD/CD+ANX group did not decline. Interestingly, within boys with ODD/CD, CD symptoms were inversely associated with reduced cortisol stress reactivity, whereas anxiety was positively associated with baseline cortisol and inversely associated with reduced cortisol recovery levels. Also, CU

traits were positively associated with baseline cortisol. In other studies CU traits have been associated with low baseline cortisol and low stress cortisol levels (Loney et al., 2006; Stadler et al., 2011), although this was not always found (Feilhauer et al., 2013; Poustka et al., 2010) with recent findings of hyperactivity (rather than hypoactivity) of the HPA axis in children with high levels of CU traits (Mills Koonce et al., 2015). Northover and colleagues (2016) found no correlation between baseline and stress cortisol levels and CU traits in male adolescents with ADHD with and without CD. Interestingly, they found that CD symptoms were predictive of cortisol stress reactivity, just like we found. Literature on CU traits distinguishes between primary and secondary CU traits; secondary CU traits are proposed to be associated with higher levels of anxiety and emotional problems, trauma and maltreatment, whereas primary CU traits are associated with low anxiety, high heritability and low levels of trauma (Kimonis et al., 2012; Sharf et al., 2014). We did not distinguish our ODD/CD sample into boys with primary or secondary CU traits. Our findings that CU traits were only related to baseline cortisol levels, whereas anxiety symptoms were related to cortisol levels during baseline, stress as well as recovery, and CD symptoms were related to stress reactivity suggest that it may be interesting to also include dimensional measures of anxiety and CD symptoms in studies focusing on CU traits.

Our findings of hypoarousal during stress in boys with ODD/CD-ANX and the relation between hyporeactivity and high levels of CD symptoms are in line with earlier clinical studies (Fairchild et al., 2008; Feilhauer et al., 2013; Popma et al., 2006; Snoek et al., 2004; Van Goozen et al., 1998; Van Goozen et al., 2000). In these earlier studies baseline cortisol was not found to be lower in ODD/CD samples. We, however, found that boys with ODD/CD-ANX had lower cortisol levels at baseline too, whereas those with ODD/CD+ANX did not. So distinguishing between those with and without anxiety might help understanding different findings concerning baseline cortisol. The low baseline and stress levels and their relations with a higher number of CD symptoms might be explained by the hypothesis that these children are motivated to seek stimulating activities due to low arousal (*sensation seeking theory*; Zuckerman, 1979) and do not fear consequences of their behaviour (*fearlessness theory*; Raine, 1993). However, these arousal based theories fit boys with ODD/CD+ANX to a lesser extent. They did not differ from controls in baseline cortisol levels and cortisol stress reactivity. Moreover, they showed a significantly impaired cortisol recovery.

Furthermore, within boys with ODD/CD, high anxiety predicted high baseline cortisol and less cortisol recovery, whereas CD symptoms could not predict baseline and cortisol recovery levels. Higher cortisol levels in children with ODD/CD with comorbid anxiety (McBurnett et al., 1991) or higher levels of anxiety (Van Goozen et al., 1998; Van Goozen et al., 2000) are in line with earlier studies. In another study higher cortisol stress reactivity was found in boys with ADHD and comorbid

anxiety, whereas those with comorbid ODD/CD had diminished cortisol reactivity (Hastings et al., 2009). Similarly, we also found that the ODD/CD+ANX group had marginally higher cortisol stress reactivity than the ODD/CD-ANX group. We add to this literature that those with anxiety also have higher cortisol recovery levels compared to non-anxious boys with ODD/CD or controls.

This hyperarousal during recovery of the ODD/CD+ANX group may be explained by an overly responsive 'basic threat circuit' (Blair, 2013) that continues to be activated after the stressor has ended. This circuit runs from the amygdala to the hypothalamus to the periaqueductal gray and is activated when a threat is experienced as impossible to escape. The behaviour that follows is defensive or reactively aggressive. Indeed, in some boys with CD increased amygdala response to fearful expressions have been found (Viding et al., 2012). This 'basic threat circuit' becomes overly responsive by prior priming or inadequate regulation. Our results indicate that boys with ODD/CD with anxiety problems may continue to react to stressors after the stressful event is gone. Thus this subgroup might be better characterized as having impaired recovery or regulation instead of tonically low arousal. Self-regulation abilities are needed to manage stress levels and return to baseline states. It is known that individuals with high anxiety have reduced self-regulation and emotion regulation abilities. For example, individuals may have increased rumination, excessive worrying and decreased re-appraisal abilities (Meuwly et al., 2012; Stewart et al., 2013; Verstraeten et al., 2011), reflecting a lack of control over emotions and a continuation of emotional states even though the events that triggered these emotions have already subsided. This may also characterize children with ODD/CD scoring high on anxiety. Although a pure deficit in recovery or down regulation of the HPA axis would be reflected in cortisol levels that stay continuously high from T2 (when the stress induction began) onwards till the recovery phase, the cortisol pattern of the ODD/CD+ANX group showed a drop from T2 to T4 (see Fig. 3) and then a deflection upwards during the recovery phase. Apparently there was some regulation during the stress phase but not during the recovery phase. Interestingly, the ODD/CD group with anxiety reported improved mood once the stressor had terminated, just like the other two groups. We could speculate that this may suggest a discrepancy between subjective experience and physiological state, and that they are not aware of the physiological state of their body. However, further research is warranted to test this hypothesis.

The current study investigated cortisol recovery, besides baseline and reactivity, in a clinical sample of school-aged boys with ODD/CD. In this study cortisol recovery was investigated separate form cortisol stress reactivity. We used a highly controlled experiment involving provocation, frustration and competition to evoke psychosocial stress and collected multiple saliva samples to measure reactivity as well as recovery up till one hour after stress. Our sample consisted of boys only. Although gender differences in cortisol response in community children

have not been found (Kudielka et al., 2004) and low baseline cortisol levels have been obtained in girls with CD as well (Pajer et al., 2001), we are hesitant to generalize our results to girls. Future studies should first examine cortisol stress reactivity and cortisol recovery in girls with ODD/CD. We did not include a group of boys with anxiety disorders without aggression. It would have been interesting to examine their response to the provocation, frustration and competition of our experiment, since such a stress situation has not been tested in anxious children yet and literature on their HPA axis activity is mixed (Dietrich et al., 2013). This study did not include puberty status of the boys. This might be an interesting topic for future studies to include in their analyses since puberty status might influence baseline and stress cortisol levels (Gunnar and Quevedo, 2007).

Taken together, although both ODD/CD groups may have abnormal cortisol patterns, they are of a different nature; those without anxiety have low baseline cortisol levels, whereas those with high anxiety have a normal baseline cortisol level, but an impaired cortisol recovery. So different subtypes of children with ODD/CD experience different types of difficulties in adaptation to the environment. In line with this, within the ODD/CD group, those with more severe CD problems had more impaired stress responsivity. The aggressive and antisocial behaviour of boys with ODD/CD may thus result from different underlying mechanisms. These results provide further evidence to the notion that boys with ODD/CD are a heterogeneous group (Stadler, 2010) and may ask for different interventions (Van Goozen and Fairchild, 2008). For example Van de Wiel et al. (2004) demonstrated that children with ODD/CD who showed elevated cortisol stress reactivity profited more from an intervention than those with low cortisol stress reactivity. The neurobiological profile of a child could thus provide information that can help to optimize treatment outcome.