CHAPTER 5

Distinct courses of CU-traits in relation to HPA-axis activity in adolescents with conduct problems during closed treatment

Tijs Jambroes, Arne Popma, Peter M. van de Ven, Evelien Platje, Robert R.J.M. Vermeiren, Theo A.H. Doreleijers, Lucres M.C. Jansen

Under review
ABSTRACT

Callous-Unemotional (CU)-traits and decreased Hypothalamic-Pituitary-Adrenal (HPA)-axis activity have been related to antisocial behavior and reduced treatment-outcome in adolescents. In this study, the relation between courses of CU-traits and HPA-axis activity was investigated during treatment. In a closed treatment facility, 84 adolescents (48% male, 15.0 ± 1.3 years) were assessed before and after intervention, and at dismissal. CU-traits were measured with the Youth Psychopathic Traits-Inventory and HPA-axis activity with the cortisol awakening response (CAR). Latent-Class-Growth-Analysis resulted in a stable low and a stable high CU-group. For the total sample, CAR development did not differ between CU-groups. For participants that completed treatment and all CAR-assessments, CAR-levels in the high CU-group increased, reaching similar levels as in the low CU-group. Based on current findings it appears that neurobiological changes in adolescents with antisocial behavior may occur during an intervention, whereas important phenotypical characteristics associated with persistent antisocial behavior remain stable.

Keywords. HPA-axis functioning, callous-unemotional, treatment, alteration
INTRODUCTION

Callous Unemotional traits (CU-traits) have increasingly been acknowledged as an important characteristic in the development of antisocial behavior (see Frick et al. (2014b) for a comprehensive review). Specifically youth with stable high CU-traits are at risk of persistent conduct problems (Fanti, Colins, Andershed, & Sikki, 2016; Fontaine, McCrory, Boivin, Moffitt, & Viding, 2011; Klingzell et al., 2016; Pardini & Loeber, 2008). Moreover, they have been found to respond less well to treatment compared to stable low or decreasing levels of CU-traits (Hawes & Dadds, 2007). Furthermore, high levels of CU-traits have been associated with hypoactivity of the Hypothalamic-Pituitary-Adrenal axis (HPA-axis), and it is thought that this combination may particularly associate with persistent antisocial behavior (Hawes et al., 2009). Current knowledge on the development of CU-traits, and its relation with HPA-axis functioning, is predominantly based on studies that identified subgroups of CU-trajectories in community settings and children’s samples. However, studying a clinical sample of youth with severe behavioral problems during treatment is important, as this may bear direct clinical relevance. Therefore, the aim of this study is to identify subgroups of adolescents with distinct courses of CU-traits during treatment in a closed facility for youth with conduct problems. Subsequently, it is tested whether these subgroups show different alterations in HPA-axis activity.

Callous-Unemotional traits, characterized by a shallow affect, lack of empathy and absence of guilt, are increasingly recognized as a core feature in the development of antisocial behavior (Frick, Ray, Thornton, & Kahn, 2014a). Prior research has shown the stability of elevated CU-traits across childhood and adolescence into adulthood (Burke et al., 2007; Munoz & Frick, 2007). More recently, subgroups of youth with different CU-traits trajectories have been identified (Fanti et al., 2016; Fontaine, Rijsdijk, McCrory, & Viding, 2010; Fontaine, Hanscombe, Berg, McCrory, & Viding, 2016; Klingzell et al., 2016). Stable high CU-trajectories were in particular characterized by negative outcomes, such as increased conduct problems and more fearlessness (Fanti et al., 2016; Klingzell et al., 2016). However, most studies on CU-trajectories have been performed in young children in community settings with follow-up periods of several years to identify differences in normal development, even though one specific study examined distinct courses of CU-traits during and shortly after an intervention (Hawes & Dadds, 2007). In their study, subgroups based on CU-traits were
Chapter 5

identified during a 10-week parent training intervention for young children with conduct problems. They reported a stable high, stable low and decreasing CU-trajectory subgroup. The high CU-subgroup showed to have poorer treatment outcomes regarding antisocial and oppositional behavior, as compared to the other two subgroups. As most research on this topic is performed in child-samples, the relevance of different CU-trajectories for adolescents with persistent antisocial behavior, specifically during interventions, is less known. To increase the understanding of the persistence of antisocial behavior into adulthood it is important to fill this gap.

Clinical features of fearlessness, insensitivity to punishment, and deficits in reactivity to signs of fear or distress in others have been linked with CU-traits (Dadds & Rhodes, 2008; Frick & White, 2008). Particularly these features are reflected in the low arousal theory (Raine, 1993), in which antisocial behavior is thought to relate with decreased levels of arousal and can be reflected in low reactivity of the HPA-axis (Van Goozen et al., 2007). As such, decreased HPA-axis activity may be an underlying neurobiological factor associated with stable high CU-trajectories. The relation between decreased HPA-axis functioning and antisocial behavior has been reported in several studies with cross-sectional designs (e.g., McBurnett et al., 2000; Popma et al., 2006; Van Goozen et al., 2007). Furthermore, several longitudinal studies showed that decreased levels of cortisol, as a measure of HPA-axis functioning, are consistent over time in persistently aggressive children and adolescents (McBurnett et al., 2000; Platje et al., 2013). However, inconsistent findings have been reported also (see Alink et al. (2008) for a comprehensive review). It is assumed that these equivocal findings occurred as a consequence of the phenotypic heterogeneity within the study samples, and that altered HPA-axis functioning may particularly be present in the samples with significantly more fearlessness, reward dominance and insensitivity to punishment, as described in youth with CU-traits (Dadds & Rhodes, 2008; Hawes et al., 2009). In this line, it could be suggested that the combination of high CU-traits and low HPA-axis activity is an underlying mechanism related to a high risk of persistent antisocial behavior. Consequently, several studies in different samples and settings have tested the relation between HPA-axis functioning and CU-traits. Although not all studies could attest a relation (Feilhauer, Cima, Korebrits, & Nicolson, 2013; Poustka et al., 2010; Stoppelbein, Greening, Luebbe, Fite, & Becker, 2014), most studies endorsed an inverse relation between CU-traits and cortisol levels and thereby corroborate the low arousal theory (e.g.,
Courses of CU-traits and their relation with HPA-axis activity

Burke et al., 2007; Loney et al., 2006; Stadler et al., 2011; Von Polier et al., 2013). However, all previous research used cross-sectional approaches while their relation over time has not been investigated yet. Furthermore, elevated levels of CU-traits and reduced HPA-axis activity at pretreatment have been related to reduced treatment effect (Manders et al., 2013; Motamedi et al., 2008; Van De Wiel et al., 2004), though a decrease in CU-traits and an increase in HPA-axis functioning during treatment have been reported also (Dorn, Kolko, Shenk, Susman, & Bukstein, 2011; Nickel et al., 2006; White et al., 2013). This may suggest that specifically their course, and their relationship during and after interventions can be relevant in the persistence of antisocial behavior. Therefore, the relation between CU-traits and HPA-axis functioning during an intervention may reveal important underlying neurobiological mechanisms in phenotypic changes due to interventions, and thus increase knowledge on subgroups of youth with persistent antisocial behavior (Dadds & Rhodes, 2008; Hawes et al., 2009; Stadler et al., 2010).

The aim of this study is twofold. First, it investigates the presence of subgroups of adolescents based on their course of CU-traits during closed treatment. Second, it tests whether distinct courses of CU-traits differ in their HPA-axis functioning over the same period. In accordance with previous research (Hawes & Dadds, 2007), it is hypothesized that a stable high, a stable low, and a decreasing course of CU-traits can be detected and, subsequently, that HPA-axis activity shows an inverse relation with the course of CU-traits.

METHOD

Participants
Participants were recruited from De Koppeling, a closed treatment facility in Amsterdam for adolescents with severe conduct problems. In the Netherlands, adolescents with severe behavioral problems can be sent to compulsory treatment in a closed facility by civil law. For the current study, between 2010 and 2013, all adolescents that started a group training to reduce aggressive and antisocial behavior, the Training Aggression Control (TACt), were asked to participate. Of the initial 159 adolescents admitted to the training, 87 (55%) agreed to participate. Three adolescents were excluded due to refusal to answer the questionnaire to measure CU-traits, resulting in a total sample of 84 adolescents (mean age 14.96 (SD 1.26) year, 47.62% male). Slightly less than half
(44%) were of non-western origin, predominantly Moroccan or Surinamese, which is representative for the inhabitants in this region of the Netherlands. There were no significant differences between participants and non-participants on the basis of gender, age and origin.

**Procedure**
Within the first 6 weeks of admission, information regarding psychiatric problems was collected as part of the standard diagnostic process. All participants and their legal guardians were informed of the nature of the study and the potential risks and benefits. All participants voluntarily agreed to participate and both the adolescents and their legal guardians signed an informed consent form. Participants were informed that all research data would be processed anonymously and separated from the normal diagnostic procedure in the institution, so that their participation had no influence on their treatment or duration of stay. The study was approved by the Medical Ethical Committee of VU University Medical Center (Protocol number 2009/309). At three time points during the participants’ stay; the week before the intervention (T0; mean 91 (sd 42) days after admission); the week after the intervention (T1; 191 (sd 44) days after admission); and in the last week before leaving the facility (T2; 268 (sd 40) days after admission), saliva was gathered to measure the Cortisol Awakening Response (CAR), as a measure of HPA-axis (re)activity. All participants were rewarded with a €5 gift voucher at each saliva sampling. At these same time points, CU-traits were assessed with the Youth Psychopathic traits Inventory (YPI).

**Training Aggression Control (TACt)**
The TACt is a 12 week standardized group training for adolescents to reduce aggressive and delinquent behavior based on the principals of the Aggression Replacement Training (ART), originally developed by (Glick & Goldstein, 1987). The ART has shown its effectiveness in adolescent and forensic samples in reducing aggression (Hornsved & Kraaimaat, 2011; Nugent et al., 1999). Like ART, TACt contain three main components; social skills training, anger control training, and moral reasoning. The effective principles of TACt are based on the role of social learning in the acquisition of social and behavioral skills, and moral beliefs. Each week, the three components of the TACt were included at separate days in a one-hour long group session.
Callous-Unemotional traits
The Dutch version of the Youth Psychopathic Traits Inventory (YPI), a self-report questionnaire for psychopathic traits, was used to measure CU-traits (Hillege et al., 2010). The YPI has been shown to enable a reliable and valid assessment of psychopathic traits in detained adolescents (Skeem & Cauffman, 2003; Vahl et al., 2014). It consists of 50 items, organized into three dimensions in line with the three-factor model of psychopathy (Cooke & Michie, 2001). For the current study, only the YPI CU-dimension (e.g., “I seldom regret things I do, even if other people feel that they are wrong”; “When other people have problems, it is often their own fault, therefore, one should not help them”; “What scares others usually doesn’t scare me”) was used. All items are scored on a 4-point Likert scale, with scores ranging from 1 (does not apply at all) to 4 (applies very well). Cronbach’s alpha for the overall CU-dimension in the current study was .84.

Hypothalamic-Pituitary-Adrenal axis functioning
HPA-axis functioning was assessed by measuring cortisol levels in saliva. Cortisol is the most important stress hormone and an end product of the HPA-axis. For this study HPA-axis functioning was measured by means of the Cortisol Awakening Response (CAR). The CAR is the steep increase in cortisol levels approximately 30 minutes after awakening and is a rough measure of the sensitivity of the HPA-axis. The CAR is a widely used and reliable measure for HPA-axis (re)activity (Stalder et al., 2016). To assess the CAR, sampling of saliva was done at the institution on a regular school day after forced awakening up; at 7:30 (T0), and subsequently at 8:00 (T1) and 8:30 (T2) A.M.. Participants were instructed not to sleep, eat, drink or smoke until after T2 sampling. Trainees from the research team were present at the ward during the saliva sampling to assist the adolescent during sampling, as well as to assure that correct sampling took place at the prescribed intervals. For each measurement at least 0.1 ml saliva was collected in a Sarstedt Salivette. The samples were stored in a freezer at -20 degrees Celsius until final analysis. Analyses were performed at the Endocrinology Laboratories of the University Medical Center Utrecht. Cortisol in saliva was measured without extraction using an in-house competitive radio-immunoassay, and employing a polyclonal anticortisol-antibody (K7348) and [1,2-3H(N)]-Hydrocortisone (PerkinElmer NET396250UC) as a tracer. The lower limit of detection was 1.0 nmol/l. The Area Under the Curve with respect to the ground (AUCg), and the Area Under the Curve with respect to the increase (AUCi) were calculated on
the basis of the three consecutive cortisol measurements (Pruessner et al., 2003). The AUCg reflects the total cortisol secretion in the first hour after awakening and is calculated as $\text{AUCg} = ((\text{CortisolT1} + \text{CortisolT0}) / 2 * 30) + ((\text{CortisolT2} + \text{CortisolT1}) / 2 * 30))$. The AUCi reflects the increase in cortisol secretion in response to awakening and is computed as; 

$$\text{AUCi} = ((\text{CortisolT1} + \text{CortisolT0}) / 2 * 30) + ((\text{CortisolT2} + \text{CortisolT1}) / 2 * 30) - (\text{CortisolT0} * (30 + 30)).$$

The AUCg indicates the basal cortisol levels of an individual, while the AUCi shows the reaction at awakening and is a sign of the sensitivity of the HPA-axis (Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010).

**Statistical analysis**

To optimize the data from the YPI, values from missing items were imputed, and co-variances were used to see if the missing items in the questionnaire were completely random (MCAR). The Little’s MCAR test assured that they were ($\chi^2(341) = 362.26, p < .206$), which is necessary to perform Expectation-Maximization (EM) to impute missing data (Allison, 2001). Then Latent Class Growth Analysis (LCGA; Reinecke, 2006) in Mplus version 6.11 was used to identify subgroups of adolescents with distinct courses of CU-traits during the intervention and at follow-up. LCGA classifies subgroups of adolescents with distinct patterns of CU-traits over the three consecutive CU measurements (T0, T1, T2). Four criteria were used to decide on the number of distinct classes. The first criterion was parsimony of the model assessed using fit indices and statistical tests, meaning that if an additional does not improve the model as judged by both a decrease in the Sample Size Adjusted Bayesian Information Criterion (SSA BIC) and a significant Lo-Mendell-Rubin adjusted likelihood ratio test (LMR-adj.LRT), the model with the smaller number of subgroup is preferred. The second criterion was that the entropy, a standardized measure of classification quality, had to be acceptable. Entropy values range from 0 to 1, and values around 0.70 or higher indicate clear classification and greater power to predict class membership (Nagin, 2009; Reinecke, 2006). The third criterion was the interpretability of the subgroups. If an additional group was found to be a slight variation of a group already found in a lower class solution, the most parsimonious model was chosen. A final criterion was related to the size of the subgroups, which meant we required that every group had to cover at least 5% of the sample for meaningful interpretation and further analysis.
Differences in means between the subgroups identified were tested using independent sample t-tests. Bivariate Pearson correlations were computed to quantify associations between pairs of variables. Next, to analyze differential relations between the groups with distinct courses of their CU-traits, and the alterations in AUCg and AUCi during treatment, Linear Mixed Model Analyses (LMM) were used. Separate analyses were performed with AUCg and AUCi as dependent variables. The CU-group, together with time of measurement (pretreatment, post-treatment, follow-up), was the main predictor. Confounding by age and gender were assessed by adding their main-effects and interactions with time to the model. Interaction terms with time were consecutively dropped from the model until all remaining interaction terms with time and candidate confounders were statistically significant. If different courses in AUCg or AUCi between distinctive CU-traits groups were present, post-hoc t-tests analyses with Bonferroni corrections were performed to analyze differences in the levels of AUCg and AUCi at all measurements. As a consequence of the rather large amount of participants that did not complete all measurements, the results may have been influenced by the missing data. The LMM analysis was therefore performed for the total study sample, as well as for the group that completed all measurements.

RESULTS

Latent Class Growth Analysis
In the LCGA analysis we compared models with two, three and four subgroups. The criteria to determine the best model fit are presented in Table 1. Although the model with three classes had an acceptable model fit, the smallest class contained only four participants, which was slightly less than 5% of the total group and therefore too small for meaningful interpretations and further analysis. Consequently, the model with two classes showed to be the best applicable model, and was therefore selected for further analysis.
Chapter 5

Table 1 Model fit statistics for the latent class growth analysis (LCGA)

<table>
<thead>
<tr>
<th>Classes</th>
<th>Sample size</th>
<th>adj. BIC</th>
<th>LMR adj. LRT</th>
<th>Entropy</th>
<th>N (%) Smallest Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1112</td>
<td>N/A</td>
<td>N/A</td>
<td>84 (100)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1081</td>
<td>&lt; .05</td>
<td>0.67</td>
<td>29 (35)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1072</td>
<td>&lt; .14</td>
<td>0.71</td>
<td>4 (4.8)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1066</td>
<td>&lt; .24</td>
<td>0.61</td>
<td>3 (3.5)</td>
<td></td>
</tr>
</tbody>
</table>

CU: Callous-Unemotional

The two-classes model displayed two distinctive groups with a distinct course of CU-traits: one group with stable high CU-traits, and one with stable low CU-traits. As compared with the model with three classes, the stable high CU-traits group in the two classes model contained all participants that were classified in the dropped third class. Table 2 shows sample sizes and mean scores with standard deviations for CU-traits, and the AUCg and AUCi of cortisol at pre, post and follow-up measurements for the two distinctive groups of CU-traits. The stable high CU-group consisted of 29 (35%) participants and the stable low CU-group of 55 (65%) participants.

Table 2 Sample sizes and means with standard deviations of CU-traits, AUCg and AUCi for the two CU-groups

<table>
<thead>
<tr>
<th></th>
<th>Stable low CU-group</th>
<th>Stable high CU-group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>CU n</td>
<td>53</td>
<td>30</td>
</tr>
<tr>
<td>CU</td>
<td>12.0 (5.2)</td>
<td>10.6 (4.3)</td>
</tr>
<tr>
<td>AUC n</td>
<td>50</td>
<td>38</td>
</tr>
<tr>
<td>AUCg</td>
<td>1064.2 (315.8)</td>
<td>996.9 (305.8)</td>
</tr>
<tr>
<td>AUCi</td>
<td>317.6 (326.7)</td>
<td>253.8 (291.1)</td>
</tr>
</tbody>
</table>

CU: Callous-Unemotional traits
AUCg: cortisol awakening response area under the curve with respect to the ground
AUCi: cortisol awakening response area under the curve with respect to the increase
n: sample size
Courses of CU-traits and their relation with HPA-axis activity

Correlations between all study variables at pre, post and follow-up measurements showed significant moderate to strong positive correlations between CU-traits at all the consecutive measurements (pre-post: $r = 0.43, p < .004$; pre-follow-up: $r = 0.44, p < .014$; post-follow-up: $r = 0.75, p < .001$). Further, AUCg at pretreatment showed a significant moderate positive correlation with AUCg at post-treatment ($r = 0.38, p < .007$), and a strong positive correlation with AUG at follow-up ($r = 0.58, p < .002$). AUCi at pretreatment had no significant correlations with the consecutive AUCi values. There was no significant correlation between CU-traits and all AUCg and AUCi measurements.

As presented in Table 2, a substantial part of the total sample was lost for post-treatment or follow-up cortisol measurements. There was no difference in mean CU-traits at either of the three measurements between the group that did, and the group that did not complete all cortisol measurements (T0, $t(80) = -0.371, p = .712$; T1, $t(44) = 1.644, p = .107$; T2, $t(20) = 1.678, p = .109$). Further, both the group with all assessments, and the group with missings were equally distributed between the two distinct courses of CU-traits. As for the CAR measurements, no differences were present for AUCg (T0, $t(74) = 0.575, p = .567$; T1, $t(55) = 0.071, p = .94$; T2, $t(27) = 1.773, p = .087$), or AUCi (T0, $t(74) = 0.032, p = .975$; T1, $t(55) = 1.191, p = .239$; T2, $t(27) = 1.340, p = .192$) between the group with all measurements, and the group with missings. Finally, neither group differed with respect to age ($t(82) = -0.358, p = .721$), gender ($X^2(1) = 0.274, p = .601$) and origin ($X^2(3) = 1.054, p = .590$). Participants that completed all measurements had a significantly longer duration of stay in the facility, with an average of 158 (sd 54) days, compared to participants with missed cortisol measurements (102 (sd 73) days; $t(51)=-3.23, p < .00$). Further, participants with all measurements did finish the intervention more often those with missing’s ($X^2(1, N=84) = 7.29, p < .01$). To take into account the influence of the missed cortisol measurements, and the possible effect of the intervention and the duration of stay, the LMM analysis was performed for the total study sample, as well as for the group that completed all measurements only.

**Linear Mixed Model Analyses**

**LMM analysis of the total study sample**

Linear Mixed Model Analyses (LMM) were used to test differences in the course of mean AUCg and mean AUCi over time between the two groups with distinctive courses of CU-traits. In the analysis of the total study sample, the interactions
with Gender x Time, and Age x Time, did not reach significance, and dropped from the model. The results of this final LMM model are presented in Table 3 with $F$-values, while significant Estimate or $B$-values are reported in the text. Table 3 shows that the interaction between the CU-traits groups, and Time of Measurement did not reach significance for both the AUCg ($F = 2.45, p = .09$) and the AUCi ($F = 1.42, p = .25$) analysis. The final model for AUCg showed that males had significant lower AUCg levels than females at pretreatment, which remained stable at all measurements ($B = -130.58, p = .04$). This gender difference was not present in the level of the AUCi ($F = 3.04, p = .09$).

**Table 3** LMM analysis with AUCi and AUCg for the total study sample

<table>
<thead>
<tr>
<th></th>
<th>AUCg</th>
<th>AUCi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F$</td>
<td>$df$</td>
</tr>
<tr>
<td>Gender</td>
<td>4.37</td>
<td>67.00</td>
</tr>
<tr>
<td>Age</td>
<td>2.25</td>
<td>69.42</td>
</tr>
<tr>
<td>Time of assessment</td>
<td>2.61</td>
<td>85.84</td>
</tr>
<tr>
<td>CU-group</td>
<td>0.20</td>
<td>83.33</td>
</tr>
<tr>
<td>CU-group x Time of assessment</td>
<td>2.45</td>
<td>85.89</td>
</tr>
</tbody>
</table>

CU: Callous Unemotional traits
AUCg: cortisol awakening response area under the curve with respect to the ground
AUCi: cortisol awakening response area under the curve with respect to the increase

**LMM-analysis of participants that completed all measurements**

To analyze a possible influence of participants that missed the post-treatment or follow-up measurement the LMM analysis was repeated with participants that completed all cortisol assessments only (Table 4).
Courses of CU-traits and their relation with HPA-axis activity

Table 4 LMM analysis with AUCi and AUCg for participants that completed all measurements

<table>
<thead>
<tr>
<th></th>
<th>AUCg</th>
<th></th>
<th></th>
<th>AUCi</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>df</td>
<td>p</td>
<td>F</td>
<td>df</td>
<td>p</td>
</tr>
<tr>
<td>Gender</td>
<td>5.10</td>
<td>21</td>
<td>.04</td>
<td>2.80</td>
<td>21</td>
<td>.11</td>
</tr>
<tr>
<td>Age</td>
<td>0.71</td>
<td>21</td>
<td>.41</td>
<td>0.01</td>
<td>21</td>
<td>.94</td>
</tr>
<tr>
<td>Time of assessment</td>
<td>2.60</td>
<td>46</td>
<td>.09</td>
<td>3.67</td>
<td>42</td>
<td>.03</td>
</tr>
<tr>
<td>CU-group</td>
<td>1.22</td>
<td>21</td>
<td>.28</td>
<td>3.81</td>
<td>21</td>
<td>.06</td>
</tr>
<tr>
<td>Gender x Time of assessment</td>
<td>4.25</td>
<td>42</td>
<td>.02</td>
<td>3.56</td>
<td>42</td>
<td>.04</td>
</tr>
<tr>
<td>Age x Time of assessment</td>
<td>5.86</td>
<td>42</td>
<td>.01</td>
<td>5.86</td>
<td>42</td>
<td>.01</td>
</tr>
</tbody>
</table>

AUCg: cortisol awakening response area under the curve with respect to the ground
AUCi: cortisol awakening response area under the curve with respect to the increase

For the AUCg, the LMM revealed a model with a significant interaction between the CU-groups and Time ($F = 3.67, p = .03$). This indicates a difference in the course of AUCg between the CU-groups. There was no significant Gender x Time and Age x Time interaction. Post-hoc t-tests analyses were performed to test differences between the two CU-groups in their level of AUCg at all measurements. This revealed no significant difference (pretreatment: $F (42,12) = 2.90, p = .10$; post-treatment: $F (42,12) = 2.77, p = .10$; follow-up: $F (42,12) = 0.42, p = .52$), illustrating that although the LMM showed a difference in the course of AUCg, the level of AUCg at all three measurements did not. Furthermore, the model also showed a significant effect of gender ($B = -191.30, p = .04$): females proved to have a similar course of the AUCg, though with higher levels than males.

Likewise, the final model for AUCi showed a significant interaction between the CU-groups and Time ($F = 5.86, p < .01$), with $B = -503.36, p < .01$ at post-treatment and $B = -303.94, p < .05$ at follow-up.

For AUCi, the interaction variables Gender x Time ($B = 329.08, p = .01$ at post-treatment and $B = 15.30, p = .91$ at follow-up), and Age x Time ($B = -114.19, p = .09$ at post-treatment and $B = 54.91, p = .40$ at follow-up) reached significance also. This indicates that the two CU-groups differ in their course of AUCi, while there is also a difference in development based on gender and age. Post-hoc testing for differences in the level of AUCi at the pre, post and follow-up
measurements revealed that the stable high CU-group had significantly lower AUCi levels at the pretreatment measurement \((F(56,22) = 11.02, p < .01)\) but not at post-treatment \((F(56,22) = 2.78, p = .10)\) and follow-up \((F(56,22) = 0.73, p = .40)\). Figure 1 schematically shows the results of the model for AUCi.

![Graph showing AUCi levels](image)

**Figure 1.** Calculated AUCi levels at all measurements for the two CU-groups in the total sample, and those without missed assessments only. Gender fixed at 50%; age fixed at 15 years.

**DISCUSSION**

The first aim of the present study was to identify subgroups of adolescents based on differences in the course of CU-traits during treatment. Subsequently, it was investigated whether CU subgroups differed in their development of HPA-axis functioning during the same period. As expected, latent class analysis provided subgroups of adolescents with distinctive courses of CU-traits; one subgroup
Courses of CU-traits and their relation with HPA-axis activity

showed stable low CU-traits, and the other stable high CU-traits. However, the present study did not reveal a subgroup with changing levels of CU-traits during treatment. Regarding the second aim, the analysis in the total samples showed no difference in the HPA-axis development between the two CU-groups. In the analysis with only the participants that completed all measurements, however, the two CU-groups differed in their development of the HPA-axis functioning. At pretreatment, the stable high CU-group related to lower HPA-axis activity than the stable low CU-group. This changed at posttreatment and follow-up as the HPA-axis in the stable high CU-group showed increasing activity such that it reached similar levels as in the stable low CU-group. This was not in line with the hypothesis as the stable high CU-group was expected to relate with stable low HPA-axis activity, and the stable low CU-group with stable high HPA-axis activity. This finding may indicate that different arousal levels in subgroups of adolescents with distinct courses of CU-traits may be susceptible to change during and after an intervention.

Distinctive courses of CU-traits
In line with previous research of subgroups with distinct courses of CU-traits the current study revealed two separate groups: one with stable low CU-traits, and one with stable high CU-traits (Fanti et al., 2016; Fontaine et al., 2011; Fontaine et al., 2016; Hawes & Dadds, 2007; Klingzell et al., 2016). In the present high-risk sample, a relatively large percentage fitted in the stable high CU-subgroup (35%), compared to the percentages found in studies with community samples (3 – 10%; Fanti & Centifanti, 2014; Fontaine et al., 2011; Fontaine et al., 2010; Klingzell et al., 2016), or in children with conduct problems (22%; Hawes & Dadds, 2007). The stable low CU-group in the present study contained 65% of the adolescents, which is in line with the percentages found in community studies (58-74%). This study builds on previous research in community and children samples, by showing that distinct courses of CU-traits could also be identified in a closed treatment facility for adolescents with severe conduct problems. Contrary to our hypothesis, we did not demonstrate a subgroup with altering levels of CU-traits. Possibly, alterations in CU-traits may have been less prominent as most previous research followed CU-trajectories for several years, because the follow-up in this study was less than a year. Therefore, probably unobserved subgroups with an altering course may be classified in the stable high CU-group, which consequently leads to the fairly large percentage of this
subgroup in current study. Nevertheless, under the influence of the intervention, a subgroup with decreasing CU-traits was expected, as was observed during a twelve weeks parent training intervention in children with conduct problems (Hawes & Dadds, 2007). The discrepancy with our study may have arisen as a consequence of the sample difference. In childhood, CU-traits seem to be more susceptible to environmental influences such as parental discipline and parent-child communication, making alterations more prominent than in adolescent samples (Fontaine et al., 2011; Fontaine et al., 2010; Pardini & Loeber, 2008). Still, several studies with adolescent samples showed reduced overall CU-traits during and after an intervention (Salekin, Worley, & Grimes, 2010; White et al., 2013). Finally, a fairly large part of the participants in the present study showed incomplete measurements. These participants specifically more frequently did not finish the intervention, than participants that completed all measurements. It could well be that participants that missed assessments benefitted insufficiently from the intervention and therefore failed to show a decrease in CU-traits. In the analysis of the complete sample this may significantly have led to the loss of statistical power, and thus result in only two distinct groups with different courses of CU-traits.

Courses of CU-traits and HPA-axis functioning
To our knowledge, this is the first study that tested the relation between groups with distinct courses of CU-traits with the HPA-axis functioning during the same period. It was expected that, in line with the low arousal theory, a subgroup with stable CU-traits would have a stable inverse relation with HPA-axis functioning. Further, it was hypothesized that a group with decreasing CU-traits would show an inverse development of the HPA-axis functioning, with an increased HPA-axis functioning at post-treatment and follow-up. At pretreatment, this study demonstrated that adolescents in the stable high CU-group had a decreased HPA-axis reactivity, compared to those in the stable low CU-group. This finding is in line with previous cross-sectional studies (e.g., Cima et al., 2008; Loney et al., 2006; Stadler et al., 2008; Von Polier et al., 2013). However, the stable low and the stable high CU-group showed no difference in the course of the HPA-axis activity in analysis with the total sample. This indicates that the inverse relation found in cross sectional studies could not be generalized to a persistent inverse relation over time. Though, as noted before, participants that did not complete all measurements had a significant shorter stay in the treatment
Courses of CU-traits and their relation with HPA-axis activity

setting, and more importantly, did more frequently not finish the intervention successfully than participants that completed all measurements. Therefore, the analysis was repeated with the adolescents that did complete all measurements. In these analyses, the stable high CU-group and low CU-group did show a different course of the HPA-axis. At pretreatment the stable high CU-group had a decreased level of HPA-axis functioning compared to the stable low CU-group, while at post-treatment and at follow-up this relation altered into similar levels of HPA-axis activity. This gives rise to the thought that neurobiological changes in adolescents with antisocial behavior, such as HPA-axis activity, may occur during and after an intervention, whereas important phenotypical characteristics associated with persistent antisocial behavior, such as fearlessness and CU-traits, remain stable in the short-term.

The limited research that tested alterations in HPA-axis activity during interventions in youth with antisocial behavior has demonstrated that the HPA-axis activity may increase during and after an intervention (Dorn et al., 2011; Motamed et al., 2008; Nickel et al., 2006). Moreover, this increase has even been accompanied with a decrease in conduct behavior (Motamed et al., 2008; Nickel et al., 2006). Similar findings were reported by (Bruce, McDermott, Fisher, & Fox, 2009), who showed in an RCT that foster children receiving Multidimensional Treatment Foster Care improved on electrophysiological responses whereas foster children receiving service as usual did not, while all behavioral measures showed no differences between both groups. The present study extends on these findings by showing neurobiological alterations during an intervention in a subgroup of adolescents with a high probability to persist in their antisocial behavior. Current findings may indicate that normalization in HPA-axis activity could be a first sign of behavioral improvement in the most problematic adolescents. Future studies should therefore use longer follow-up periods, and subsequently add behavioral measures to follow the treatment-effect on the reduction of antisocial behavior in adolescents with stable CU-traits.

Strengths and Limitations

This study has important strengths as it is the first to analyze the relation between CU-traits and HPA-axis activity with a longitudinal design, and specifically tested its alterations during an intervention. However, a number of limitations should also be considered in the context of our results. First, it should be noted that almost half of the adolescents that were assigned to the TACt refused to
participate in the study. Subsequently, a considerable amount of participants were lost for follow-up measurements. Although these numbers of missings can be expected in closed facilities and is comparable with other longitudinal research on HPA-axis activity in youth with conduct problems (Dorn et al., 2011), it will have influenced the power of the statistical methods and thus the model fit of the LCGA, and the LMM analysis in the complete sample. Although all participants showed antisocial behavior, as they were placed in a closed facility because of that, it is possible that participants with the most severe and persistent antisocial behavior terminated the intervention prematurely, precisely because of their misbehavior. As the results of the analysis may have been influenced by the missed assessments, analysis with only those participants that completed all measurements is warranted. However, as the number of participants in this group was clearly diminished, the results should be interpreted with some caution also. Second, participants stayed already for several weeks in the facility before the first assessment. As a consequence, it could be that alterations in CU-traits or HPA-axis activity may have been present already, but remained unnoticed in this study. However, the stable low and high CU-group did show a significant difference in the HPA-axis activity at pretreatment. Third, due to the relatively short follow-up period, changes in CU-traits and HPA-axis functioning may have been small. In addition with the relative modest sample size, slight statistical differences may not have been noticed. Fourth, it must be considered that although all participants followed the same aggression control training, it is not registered if participants received any additional treatment or pharmacotherapy. In this perspective, it is possible that participants received additional effective interventions which contributed to the changes, or whether HPA-axis measurements were confounded by used medication. Therefore, the changes as presented in the present study should be seen as an effect of closed setting treatment overall and not solely caused by the aggression control training. With these limitations in mind, there is a need for further studies on the course of CU-traits and the HPA-axis activity during interventions, specifically with a longer follow-up period, and with special concerns to reduce missing value. This may further clarify the underlying neurobiological mechanism of antisocial behavior, and how treatment influences this mechanism in youth with severe conduct problems.
CONCLUSIONS

In conclusion, this study increases our understanding of the stability of CU-traits in adolescents during and over a short period after an intervention in a closed treatment institution. A subgroup of adolescents with stable high CU-traits was identified and is thought to represent a group with an unfavorable antisocial development. A pretreatment inverse relation between the stable high CU-group and HPA-axis activity changed into HPA-axis activity with similar levels as the stable low CU-group. It could be speculated that a normalization of underlying neurobiological characteristics during an intervention may take place prior to a change in phenotypic features. Alternatively, neurobiological changes might be indicative for alterations in other important clinical characteristics such as fearlessness, aggression or other behavioral symptoms which have not been assessed here. Current findings could encourage future studies to search for longitudinal trajectories of CU-traits and HPA-axis functioning and link them with target symptoms in interventions such as fearlessness and aggression. Future findings may lead to the use of HPA-axis functioning during treatment as an assessment tool to evaluate the response to treatment.