

**Regional grey matter volume and concentration in at-risk adolescents: Untangling associations with callous-unemotional traits and conduct disorder symptoms**

**Abbreviated title: VBM in antisocial adolescents**

Moran D. Cohn<sup>a</sup>, Essi Viding<sup>b</sup>, Eamon McCrory<sup>b</sup>, Louise Pape<sup>a,c</sup>, Wim van den Brink<sup>d</sup>, Theo A.H. Doreleijers<sup>a</sup>, Dick J. Veltman<sup>e</sup>, Arne Popma<sup>a,f</sup>

<sup>a</sup> VU University medical center Amsterdam, Child and Adolescent Psychiatry; Amsterdam, The Netherlands

<sup>b</sup> University College London, Developmental Risk and Resilience Unit; London, United Kingdom <sup>c</sup> NYU Langone Medical Center, Department of Radiology/Center for Biomedical Imaging; New York, United States of America

<sup>d</sup> University of Amsterdam - Academic Medical Center, Amsterdam Institute for Addiction Research; Amsterdam, The Netherlands

<sup>e</sup> VU University medical center Amsterdam, Psychiatry; Amsterdam, The Netherlands

<sup>f</sup> Leiden University, Institute of Criminal Law & Criminology; Leiden, The Netherlands

**Correspondence to:**

Moran Daniel Cohn, MD

VU University medical center Amsterdam, Department of Child and Adolescent Psychiatry

P.O. Box 303

1115 ZG Duivendrecht

The Netherlands

Telephone: +31208901545

Fax: +31206952541

E-mail: [m.cohn@debascul.com](mailto:m.cohn@debascul.com)

## **Abstract**

Structural Magnetic Resonance Imaging studies have reported volume reductions in several brain regions implicated in social cognition and emotion recognition in juvenile antisocial populations. However, it is unclear whether these structural abnormalities are specifically related to antisocial features, or to co-occurring callous-unemotional (CU) traits. The present study employed voxel-based morphometry to assess both grey matter volume (GMV) and grey matter concentration (GMC) in a large representative at-risk sample of adolescents (n=134; mean age 17.7y), characterised by a broad range of CU trait and conduct disorder (CD) symptom scores. There was a significant interaction between CD symptom and CU trait scores in the prediction of GMV in the anterior insula, with a significant positive association between CU traits and GMV in youth low on CD symptoms only. In addition, we found a significant unique positive association between CD symptoms and GMC in the amygdala, and unique negative associations between CU traits and GMC in the amygdala and insula. These findings are in line with accumulating evidence of distinct associations of CD symptoms and CU traits with amygdala and insula GMC in juvenile antisocial populations.

## **Keywords:**

Voxel-based morphometry / brain structure / conduct disorder / callous-unemotional traits / antisocial personality disorder / limited prosocial emotions / psychopathy

## 1. Introduction

Children who exhibit antisocial behaviour are at risk of persistent criminality, as well as a wide range of physical and mental health problems (Odgers et al. 2007). However, they are not all the same. For example, some children with antisocial behaviour also have high levels of callous-unemotional traits (CU traits; i.e. they lack empathy and remorse) and this differentiates them from other antisocial children with respect to several important etiological, clinical and criminological factors: children with these traits have severe and persistent conduct problems, display resistance to some conduct problem interventions, seem to be characterized by distinct neurobiological correlates and appear more genetically vulnerable to antisocial behaviour (Frick et al. 2014; Viding et al. 2012). The importance of this distinction is also reflected in the fact that the latest edition of the Diagnostic Statistical Manual of Mental Disorders (DSM-5; American Psychiatric Association 2013) includes CU traits as a specifier for conduct disorder: 'low prosocial emotions'.

While much remains unknown about the pathogenesis of antisocial behaviour and CU traits, it is increasingly clear that children and juveniles showing these traits are characterized by severe disturbances in emotion processing (see Blair 2013 for a thorough review). An increasing number of functional MRI studies have reported atypical brain function in several core emotion processing regions in antisocial youths, when compared to typically developing children, including in the amygdala (Marsh et al. 2008; Jones et al. 2009; Viding et al. 2012; White et al. 2012), insula (Rubia et al. 2009; Lockwood et al. 2013; Sebastian et al. 2012) and orbitofrontal cortex (OFC; Finger et al. 2011; Finger et al. 2008; Rubia et al. 2009). Structural brain differences are also seen in these areas, with a number of studies in children and adolescents with conduct disorder (CD) as well as adults with antisocial personality disorder (ASPD), reporting decreased grey matter volume (GMV) compared to healthy controls in the amygdala (Huebner et al. 2008; Wallace et al. 2014; Sterzer et al. 2007; Fairchild et al. 2011; Yang et al. 2009), insula (de Oliveira-Souza et al. 2008; Tiihonen et al. 2008; Sterzer et al. 2007; Fairchild 2011; Fairchild et al. 2013), and OFC (Yang et al. 2010; Tiihonen et al. 2008; Huebner et al. 2008; Sebastian et al. 2015; although see De Brito et al. 2009, Michalska et al., 2015 for opposite or null findings). Two recent meta-analyses confirm insula GMV reductions in antisocial individuals of all ages (Aoki et al. 2013), and insula (extending into ventrolateral prefrontal cortex) and amygdala GMV reductions in antisocial children and adolescents (Rogers & De Brito, 2015). Many studies have assessed the unique associations between GMV and either CU traits or antisocial behaviour (i.e. variance of one dimension not shared with the other

dimension), in recognition of the partially distinct etiology of these dimensions (Viding & McCrory, 2012). Fairchild et al. (2011) reported a unique negative association between CD symptoms and insula GMV. With regard to GMV in the OFC, there have been reports of both positive (Fairchild et al. 2013) and negative (Ermer et al. 2013; Sebastian et al. 2015) unique associations with CU traits and positive (Ermer et al. 2013) and negative (Ermer et al. 2012) unique associations with antisocial behaviour. The dimensional analyses have yielded consistent null results in the amygdala for both CD symptoms and CU traits (Fairchild et al. 2013; Fairchild et al. 2011; Ermer et al. 2012; Ermer et al. 2013; Sebastian et al. 2015)<sup>1</sup>.

Although these initial findings are exciting, the heterogeneity of the samples that have been studied (in terms of measures used to chart CD and CU symptoms, age and sex of participants, sample size and the range of behavioural scores represented), as well as the dearth of replication analyses, mean that we do not yet have a clear picture of the respective contributions of CD and CU symptoms on brain structure. Furthermore, most of these studies only report associations with GMV, and not Grey Matter Concentration (GMC). GMV and GMC measures tend to be correlated, but they are thought to reflect different aspects of brain structure (Good et al. 2001; Mechelli et al. 2005): absolute regional volume (GMV) versus regional grey matter density (GMC). While GMV has been the mainstay of VBM research, recent evidence suggests that GMC may be more sensitive and specific at detecting volumetric differences than GMV (Radua et al. 2014). Indeed, one study reported differential association patterns of antisocial behaviour and CU traits with GMV and GMC, respectively (Ermer et al. 2012).

The current study used VBM to investigate the unique associations of CD and CU symptoms with GMC, as well as GMV, in a large sample of well-characterized at-risk youths with a broad symptom severity range. All participants had at least one recorded offence that took place before age 12. Based on findings from previous functional and structural MRI studies comparing groups of antisocial individuals with healthy controls, we selected the amygdala, insula, and OFC as *a priori* regions of interest (ROIs), and hypothesized that variance uniquely attributable to CU traits and CD symptoms would be negatively and positively associated, respectively, with amygdala GMC and GMV – given previous reports of such association patterns with function in this region

---

<sup>1</sup> Walters and Kiehl (2015) do report a negative association between amygdala GMV and a 'fearlessness' measure derived from items from Factors 1 and 2 of the Psychopathy Checklist: Youth Version, controlling for a 'disinhibition' measure derived from Factor 3.

(Sebastian et al. 2012; Lozier et al. 2014), while investigation of their relation with structure in the insula and OFC was more explorative.

## 2. Methods

### 2.1 Participants

Participants were recruited from a Dutch national cohort of 364 adolescents who were all childhood arrestees with an index crime before age 12, including petty theft, arson, vandalism, trespassing, burglary, assault, sexual abuse and robbery. They had already participated in three previous waves of this longitudinal study (Domburgh et al. 2009): mean age at study entrance was 10.9 (SD 1.4) years and mean age at wave three was 13.1 (SD 1.5) years. For the current neuroimaging study (wave four; mean age 17.7 (SD 1.6) years), a subsample (total n=150) was recruited, ensuring participants to cover the complete severity spectrum of antisocial behaviour and psychopathic traits (see Table 1). This was accomplished by recruiting participants with a low risk, a medium risk and a high risk for antisocial behaviour and psychopathic traits. Low risk participants were those without a DSM-IV Disruptive Behaviour Disorder [DBD; Oppositional Defiant Disorder, ODD, or CD] diagnosis in the three previous waves according to the Diagnostic Interview Schedule for Children version IV [DISC-IV], and with aggression scores [Reactive Proactive Aggression Questionnaire; RPQ] and CU trait scores [Youth Psychopathic Traits Inventory; YPI, CU subscale] below the median during all previous waves (n=37 out of the original n=110). Medium-risk participants were those with above-median scores on aggression [RPQ] and CU traits [YPI] during previous waves, but no previous DSM-IV diagnosis of DBD (n=57 out of the original n=174). High-risk participants were those with a DSM-IV DBD diagnosis on at least one of the previous waves (n=56 out of the original n=80). A total of 16 participants were excluded from the analyses because of missing data (n=1), intracerebral cysts (n=6) or missing MRI data due to artifacts (n=9). All analyses were performed continuously across the entire final sample (n=134).

< Table 1 here >

### 2.2 Procedure and image acquisition

The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2008, and was approved by the IRB of the VU University Medical Center Amsterdam (VUmc). All participants (and their parents/custodians, if age of the participant was below 18) signed informed consent and were visited at home for a structured psychiatric interview (Diagnostic Interview Schedule for Children version IV; DISC-IV) and self-report questionnaires, including the Youth Psychopathic Traits Inventory (YPI), Reactive Proactive Aggression

Questionnaire (RPQ), Child-Behaviour Checklist (CBCL) and Youth Self-Report (YSR). On a second occasion, participants were scanned using a Philips 3T Intera MRI-scanner at the Academic Medical Center in Amsterdam, The Netherlands. High resolution T1-weighted anatomical scans, consisting of 180 sagittal 1mm thickness slices, with an in-plane resolution of 1x1mm (FOV 256x256 mm, TR 9.0 ms, TE 3.5 ms), were acquired using an 8-channel SENSE head-coil.

### *2.3 Assessment*

Both the parent and youth versions of the National Institute of Mental Health DISC-IV (Shaffer et al. 2000) were used to assess DSM-IV criteria for CD, ODD, Attention Deficit/Hyperactivity Disorder (ADHD) and Post-traumatic Stress Disorder (PTSD). CD symptoms were considered present if reported by either the parent or youth report. These symptoms, which are identical in DSM-IV and DSM-5, were summed to calculate the CD symptom score (internal consistency: Cronbach's  $\alpha=.78$ ).

The CU subscale of the YPI (Andershed et al. 2002) was used to assess CU traits. To ensure that all participants would understand the questions, the Dutch child version of the YPI was used (Baardewijk et al. 2008). This version uses the same questions as the adolescent YPI in simplified wordings. The CU traits scale consists of 15 items (internal consistency in the current study: Cronbach's  $\alpha=.86$ ) and has been shown to exhibit good criterion validity and 6-month test-retest reliability (ICC.61; Baardewijk et al. 2008).

CD symptoms scores and CU traits scores were modestly correlated ( $r=.44$ ,  $p<.001$ ) with sufficient unique variance to allow for multiple regression modeling (i.e. without risking multicollinearity; variance inflation factor=1.23).

Externalizing and internalizing problem scores were reported by both parent (Child Behaviour Checklist: CBCL; Achenbach 1991) and child (Youth Self Report: YSR; Achenbach 1991). Substance use frequency was assessed using a standardized questionnaire (based on Zwart et al. 2000).

### *2.4 Voxel-based morphometry*

Structural MRI data were processed using the typical SPM8 VBM pipeline. Preprocessing included manual checking and reorienting, segmentation, creation of a 'Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra' (DARTEL) template, normalization to Montreal Neurological Institute (MNI) space

based on this template and 8 mm FWHM smoothing. The size of the smoothing kernel is consistent with previous VBM studies in children (e.g. Sterzer et al. 2007; De Brito et al. 2009) and allows assessment of relatively small subcortical structures (e.g. amygdala). Normalization was implemented both with and without modulation (i.e. scaling the normalized grey matter maps by the Jacobian transformation parameters, which preserves the original absolute regional volumes, while not scaling yields regional grey matter density maps) to allow investigation of GMV and GMC, respectively. GMV analyses additionally included the total intracranial volume (TIV) estimates as covariates in second-level models to obtain a measure of relative regional GMV (cf. Ashburner 2010).

### 2.5 Statistical analysis

SPM8 was used to conduct the primary analyses, consisting of two multiple regression models (for GMV and GMC) including (z-standardized) CU traits and CD symptom scores as independent variables. Interaction-terms were tested and dropped from models when insignificant. Small-volume correction at family wise error (FWE) corrected  $p_{FWE} < .05$  was used in anatomically defined *a priori* regions of interest (ROI; i.e. amygdala, insula, and orbitofrontal cortex), based on the automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer et al. 2002).

Finally, we performed post-hoc analyses to assess the stability of results (1) after excluding statistical outliers ( $|z_T| > 3$ ) and cases with high leverage (Mahalanobis' distance  $> 16.45$ , cf. Barnett and Lewis 1978) and influence (Cook's distance  $> (4/n)$ , cf. Bollen and Jackman 1990), (2) after excluding all female participants and (3) after controlling for i) socio-demographic covariates (age, neighborhood socio-economic status, gender, ethnicity) and ii) mental health covariates: YSR internalizing problems, IQ, PTSD or ADHD diagnosis, or substance abuse. Peak voxel parameter estimates were extracted and were entered into stepwise regression models in SPSS21, using forward selection procedures to assess the effects of covariates.

### 3. Results

#### 3.1 Whole-brain analyses

Neither CD symptoms, CU traits nor their interaction-terms were significantly associated with GMC or GMV in whole-brain analyses.

#### 3.2 ROI analyses: GMV

No significant unique main effects of the CD symptom score or CU trait score on GMV were found in ROI analyses (Table 2). However, there was a significant interaction between CD symptom score and CU traits in the prediction of left insula GMV. Post-hoc analyses, using parameter estimates from this insula GMV voxel, indicated that there was a positive correlation between CU traits and left insula GMV ( $r=.36$ ,  $p<.001$ ) in youths with conduct problem scores below the median, and no significant correlation in those with conduct problems scores above the median ( $r=-.11$ ,  $p=.43$ ; see Figure 1 and 2A). Although there were no statistical outliers, removing one case with substantial leverage and influence reduced the interaction-term to non-significance ( $p_{FWE}=.82$ ), leaving a positive main effect of CU traits in this voxel that did not reach statistical significance ( $t_{128}=2.8$ ,  $z=2.7$ ,  $p_{FWE}=.32$ ).

< Figure 1 here > < Figure 2 here > < Table 2 here >

#### 3.3 ROI analyses: GMC

ROI analyses revealed a significant unique negative association between CU traits and right insula GMC (see Table 2 and Figure 2). In addition, CU traits were uniquely negatively associated with left amygdala GMC, whereas CD symptom score were uniquely positively associated with left amygdala GMC (see Figures 2 and appendix F1). There was no significant interaction between variables of interest in the prediction of GMC.

Post-hoc analyses using stepwise linear regression modeling of peak voxel parameter estimates showed that the reported findings were not explained by socio-demographic (SES, sex, ethnicity or age) or mental health covariates (IQ, YSR internalizing, PTSD, ADHD or substance use; see appendix T1 for full details). Excluding all female participants ( $n=29$ ) did not affect the significance of either GMV or GMC findings. Results were not confounded by statistical outliers or influential cases.

#### 4. Discussion

This study reports on the unique associations of CU traits and CD symptoms, as well as their interaction, with grey matter volume (GMV) and grey matter concentration (GMC) in a sample of 134 adolescent childhood arrestees. There was no main effect of either variable on GMV, but there was a significant interaction effect with anterior insula GMV. This interaction reflected a significant positive correlation between callous-unemotional traits and GMV only in subjects scoring low on CD symptoms, but was not significant anymore after excluding participants with extreme scores. However, callous-unemotional traits were negatively correlated with insula and amygdala GMC and CD symptoms were uniquely positively correlated with amygdala GMC. There was no evidence for unique contributions of callous-unemotional traits and CD symptoms or their interaction to GMC or GMV in the orbitofrontal cortex. Findings were not explained by socio-demographic or mental health covariates.

It is noteworthy that we did not find any significant associations between variables of interest and GMV. It is possible that some of the participants with low levels of current conduct problems and CU traits have had significant levels of such traits in the past, and in fact represent childhood-limited antisocial individuals. Such youths may resemble other antisocial youths in many ways (Barker and Maughan 2009) and may therefore obscure true differences between youths who have never had serious conduct problems and those who do. However, our findings with respect to GMC suggest that our CD symptoms and CU traits scales do capture variance relevant to understanding the neurobiology of antisocial development in this at risk group. First, our findings regarding the amygdala are similar to a study by Yang and colleagues (2009), who found that amygdala volume was negatively correlated with psychopathic traits in adult psychopaths, with the association being strongest for affective-interpersonal features. We found that the unique variance associated with CU traits was related to lower left amygdala GMC. We also found a unique positive association between CD symptoms and left amygdala GMC. Although translation of our structural findings to regional brain function is not straightforward, our results are in line with evidence from functional MRI studies that have reported differential patterns of amygdala activity related to CU traits and CD symptoms. Specifically, these studies have shown negative correlations between CU traits and amygdala activity to affective stimuli, but positive associations of CD symptoms and impulsive-antisocial scores with amygdala activity in response to affective

stimuli (Cohn et al. 2013; Viding et al. 2012; Sebastian et al. 2012; Lozier et al. 2014; although see e.g. Passamonti et al. 2010). As such, our findings are consistent with a theoretical framework implicating atypical amygdala development in relation to both CU and antisocial features, and the presence of a number of risk processes associated with CU traits and CD symptoms in at-risk youths. Clinically, the opposite patterns of amygdala dysfunction associated with these traits may be related to the low reactivity to negative stimuli and low empathy in children with high levels of CU traits, and to a hostile attribution bias in children with CD who do not display such traits (Frick and Viding 2009; Blair 2013).

The negative correlation between CU traits and insula GMV in the current study is consistent with a previous GMV study by Fairchild and colleagues (2013), although the association in the latter study was not significant after correction for CD symptoms. Moreover, these findings complement a body of functional MRI literature showing hyporesponsiveness of the insula in individuals high on psychopathic traits (Sterzer et al. 2005; Sebastian et al. 2012; Cohn et al. 2013; Birbaumer et al. 2005) and may provide a possible structural basis for such differences in reactivity to emotional stimuli. As the insula has been implicated in the detection of salient and emotional events (Menon and Uddin 2010) through awareness of bodily states (Craig 2009), such dysfunction may relate to some of the emotional deficits associated with CU features. However, conflicting evidence, such as our finding of a positive correlation between insula GMV and CU traits in youths exhibiting low levels of antisocial behaviour, and their positive zero-order correlation in females (Fairchild et al. 2013), suggests that the relation is likely to be more complex. Further investigation, taking into account the complexity of the development of the structure-function relationship of the brain (Burnett et al. 2011) with respect to both GMV and GMC, is warranted.

Overall, this study adds to an increasing body of literature supporting the notion that CU traits and antisocial behaviour have distinct clinical and neurobiological correlates. While there is clear evidence for a common latent genetic factor, which partly explains the co-occurrence of CU traits and antisocial behaviours in juveniles (Larsson et al. 2007), the current findings suggest that there are also unique etiological factors. Specifically, the current findings highlight the differential associations between CU traits, antisocial behaviour and amygdala structure, a brain region hypothesized to be of key importance in the pathogenesis of both constructs (Blair 2013). Our findings also provide evidence for the notion that GMV and GMC can provide complementary information. Although the histological correlates of these indices are currently unclear (Mechelli et al. 2005),

our study suggests that they represent different features of the structure of the brain and can be used as distinct biomarkers with relevance for the study of antisocial behaviour, CU traits and other forms of psychopathology.

Our findings should be interpreted in light of several limitations. First, we used a self-report measure to assess psychopathic traits. While the YPI is a well-established instrument in psychopathy research, and has a criterion validity that is comparable to several other psychopathy measures, it is likely that it captures variance that is partially non-overlapping with other-reported psychopathy scales (Caffungman et al. 2009). Replication of these results using different psychopathy scales and reports by caregivers, parents or teachers is therefore warranted. Second, while voxel-based morphometry provides an unbiased automated method for assessing brain structure, its histological underpinnings are still poorly understood, and more methodological work is needed to enhance its interpretation (although see Radua et al. 2014). Third, although we excluded scans with clear artefacts, we can not rule out the possibility of residual head motion effects confounding the current effects to some extent, warranting their replication in independent samples. Fourth, while the interaction effect detected in the GMV analyses may aid in understanding the divergence of previous research findings, we caution against too much speculation in this respect until independent replication has taken place, given the instability of this finding when several participants with extreme scores on independent variables were excluded. Furthermore, while including TIV in GMV analyses yields estimates of relative regional GMV, it removes potentially relevant variance associated with overall brain size from analyses. Additionally, we can not assume that the relation between local and total brain volume is necessarily linear (cf. Ashburner 2009). While we think that the current analytic strategy is warranted given the substantial variation in TIV in our sample (1.2-1.8L) and our primary interest in regional GMV, we advise future studies with smaller age ranges to additionally run analyses without such covariates. Finally, the complexity of the brain's structure-function relation (Young and Scannell 2000) is amplified by its non-linear developmental trajectory during adolescence (Burnett et al. 2011), and structural abnormalities cannot be assumed to necessarily translate to functional abnormalities in the same direction. However, the current findings are consistent with a large body of literature implicating atypical amygdala and insula structure and function in the development of antisocial behaviour and CU traits. Moreover, our results emphasize the heterogeneity of antisocial populations at the neural level, as evidenced by the novel finding that the characteristic differential relations and suppressor effects between antisocial

behaviour and CU traits in the functional neuroimaging and behavioural domain also exist with respect to grey matter structure.

#### *4. 1 Conclusion*

This study provides evidence for differential unique associations of CU traits and antisocial behaviours with grey matter concentration in the amygdala and insula, as well as for their interaction in the prediction of grey matter volume in the insula. These findings support the notion of neurobiological heterogeneity in individuals displaying antisocial behaviour, and emphasize the need to use these distinct dimensions to enhance specificity of research findings and, ultimately, to improve treatment outcomes through personalized treatment.

#### **Acknowledgements**

The authors would like to thank all the participants and their parents. This project was funded by a Netherlands Organisation for Scientific Research (NWO) Mosaic grant (017.007.022) and a NWO Brain & Cognition grant (056-23-010). Moran Cohn was supported by an International Brain Research Organization InEurope Short Stay grant during the analysis and writing of this paper. Essi Viding is a Royal Society Wolfson Research Merit Award holder.

#### **Conflicts of interest**

None.

## Reference List

- Achenbach, T.M., 1991. *Manual for the Child Behavior Checklist/4-18 and 1991 Profile* University of Vermont, Burlington, VT.
- American Psychiatric Association, 2013. *Diagnostic and Statistical Manual of Mental Disorders (DSM-5- $\alpha$ )* American Psychiatric Pub.
- Andershed, H., Kerr, M., Stattin, H., 2002. Understanding the abnormal by studying the normal. *Acta Psychiatr Scand. Suppl* 75-80.
- Aoki, Y., Inokuchi, R., Nakao, T., Yamasue, H., 2013. Neural bases of antisocial behavior: a voxel-based meta-analysis. *Social Cognitive and Affective Neuroscience* nst104.
- Ashburner, J., 2010. VBM tutorial.
- Ashburner, J., 2009. Computational anatomy with the SPM software. *Magnetic Resonance Imaging* 27, 1163-1174.
- Baardewijk, Y.v., Stegge, H., Andershed, H., Thomaes, S., Scholte, E., Vermeiren, R., 2008. Measuring psychopathic traits in children through self-report. The development of the Youth Psychopathic Traits Inventory-Child Version. *Int J Law Psychiatry* 31, 199-209.
- Barker, E.D., Maughan, B., 2009. Differentiating early-onset persistent versus childhood-limited conduct problem youth.
- Barnett, V., Lewis, T., 1978. *Outliers on statistical data*. John Wiley and sons, New York.
- Birbaumer, N., Veit, R., Lotze, M., Erb, M., Hermann, C., Grodd, W., Flor, H., 2005. Deficient fear conditioning in psychopathy: a functional magnetic resonance imaging study. *Arch Gen Psychiatry* 62, 799-805.
- Blair, R.J.R., 2001. Neurocognitive models of aggression, the antisocial personality disorders, and psychopathy. *J Neurol. Neurosurg. Psychiatry* 71, 727-731.
- Blair, R.J.R., 2013. The neurobiology of psychopathic traits in youths. *Nat Rev Neurosci* 14, 786-799.
- Bollen, K.A., Jackman, R.W., 1990. Regression diagnostics: an expository treatment of outliers and influential cases. In: Fox, J. and Long, J.S. (Eds.), *Modern Methods of Data Analysis*. Sage, Newbury Park
- Burnett, S., Sebastian, C., Cohen, K.K., Blakemore, S.J., 2011. The social brain in adolescence: evidence from functional magnetic resonance imaging and behavioural studies. *Neurosci Biobehav. Rev* 35, 1654-1664.
- Cauffman, E., Kimonis, E.R., Dmitrieva, J., Monahan, K.C., 2009. A multimethod assessment of juvenile psychopathy: comparing the predictive utility of the PCL:YV, YPI, and NEO PRI. *Psychol Assess.* 21, 528-542.
- Cohn, M.D., Popma, A., van den Brink W., Pape, L.E., Kindt, M., Van Domburgh, L., Doreleijers, T.A., Veltman, D.J., 2013. Fear conditioning, persistence of disruptive behavior and psychopathic traits: an fMRI study. *Transl Psychiatry* 3, e319.
- Craig, A.D., 2009. How do you feel--now? The anterior insula and human awareness. *Nat Rev Neurosci* 10, 59-70.
- De Brito, S.A., Mechelli, A., Wilke, M., Laurens, K.R., Jones, A.P., Barker, G.J., Hodgins, S., Viding, E., 2009. Size matters: increased grey matter in boys with conduct problems and callous-unemotional traits. *Brain* 132, 843-852.

de Oliveira-Souza, R., Hare, R.D., Bramati, I.E., Garrido, G.J., Azevedo, I.F., Tovar-Moll, F., Moll, J., 2008. Psychopathy as a disorder of the moral brain: fronto-temporo-limbic grey matter reductions demonstrated by voxel-based morphometry. *NeuroImage* 40, 1202-1213.

Domburgh, L.v., Vermeiren, R., Blokland, A.A., Doreleijers, T.A., 2009. Delinquent development in Dutch childhood arrestees: developmental trajectories, risk factors and co-morbidity with adverse outcomes during adolescence. *J Abnorm Child Psychol* 37, 93-105.

Ermer, E., Cope, L.M., Nyalakanti, P.K., Calhoun, V.D., Kiehl, K.A., 2012. Aberrant paralimbic gray matter in criminal psychopathy. *J Abnorm Psychol* 121, 649-658.

Ermer, E., Cope, L. M., Nyalakanti, P. K., Calhoun, V. D., Kiehl, K. A. (2013), 2013. Aberrant paralimbic gray matter in incarcerated male adolescents with psychopathic traits. *Journal of the American Academy of Child and Adolescent Psychiatry* 52, 94-103.

Fairchild, G., 2011. Commentary: I don't second that emotion: subjective experience of fear in adolescents with psychopathic traits - reflections on Marsh et al. (2011). *J Child Psychol Psychiatry* 52, 842-843.

Fairchild, G., Hagan, C.C., Walsh, N.D., Passamonti, L., Calder, A.J., Goodyer, I.M., 2013. Brain structure abnormalities in adolescent girls with conduct disorder. *J Child Psychol Psychiatry* 54, 86-95.

Fairchild, G., Passamonti, L., Hurford, G., Hagan, C.C., von dem Hagen, E.A., van Goozen, S.H., Goodyer, I.M., Calder, A.J., 2011. Brain structure abnormalities in early-onset and adolescent-onset conduct disorder. *The American journal of psychiatry* 168, 624-633.

Finger, E.C., Marsh, A.A., Blair, K.S., Reid, M.E., Sims, C., Ng, P., Pine, D.S., Blair, R.J., 2011. Disrupted reinforcement signaling in the orbitofrontal cortex and caudate in youths with conduct disorder or oppositional defiant disorder and a high level of psychopathic traits. *The American journal of psychiatry* 168, 152-162.

Finger, E.C., Marsh, A.A., Mitchell, D.G., Reid, M.E., Sims, C., Budhani, S., Kosson, D.S., Chen, G., Towbin, K.E., Leibenluft, E., Pine, D.S., Blair, J.R., 2008. Abnormal ventromedial prefrontal cortex function in children with psychopathic traits during reversal learning. *Arch Gen Psychiatry* 65, 586-594.

Frick, P.J., Ray, J.V., Thornton, L.C., Kahn, R.E., 2014. Can callous-unemotional traits enhance the understanding, diagnosis, and treatment of serious conduct problems in children and adolescents? A comprehensive review. *Psychol Bull.* 140, 1-57.

Frick, P.J., Viding, E., 2009. Antisocial behavior from a developmental psychopathology perspective. *Dev.Psychopathol.* 21, 1111-1131.

Gao, Y., Raine, A., 2010. Successful and unsuccessful psychopaths: a neurobiological model. *Behav Sci Law* 28, 194-210.

Good, C.D., Johnsrude, I.S., Ashburner, J., Henson, R.N., Friston, K.J., Frackowiak, R.S., 2001. A voxel-based morphometric study of ageing in 465 normal adult human brains. *NeuroImage* 14, 21-36.

Hodgins, S., 2007. Persistent violent offending: what do we know? *The British Journal of Psychiatry* 190, s12-s14.

Huebner, T., Vloet, T.D., Marx, I., Konrad, K., Fink, G.R., Herpertz, S.C., Herpertz-Dahlmann, B., 2008. Morphometric brain abnormalities in boys with conduct disorder. *Journal of the American Academy of Child and Adolescent Psychiatry* 47, 540-547.

Jones, A.P., Laurens, K.R., Herba, C.M., Barker, G.J., Viding, E., 2009. Amygdala hypoactivity to fearful faces in boys with conduct problems and callous-unemotional traits. *Am J.Psychiatry* 166, 95-102.

Larsson, H., Tuvblad, C., Rijdsdijk, F.V., Andershed, H., Grann, M., Lichtenstein, P., 2007. A common genetic factor explains the association between psychopathic personality and antisocial behavior. *Psychol Med* 37, 15-26.

Lockwood, P.L., Sebastian, C.L., McCrory, E.J., Hyde, Z.H., Gu, X., De Brito, S.A., Viding, E., 2013. Association of callous traits with reduced neural response to others' pain in children with conduct problems. *Curr.Biol* 23, 901-905.

Lozier, L.M., Cardinale, E.M., Vanmeter, J.W., Marsh, A.A., 2014. Mediation of the Relationship Between Callous-Unemotional Traits and Proactive Aggression by Amygdala Response to Fear Among Children With Conduct Problems. *JAMA Psychiatry*.

Marsh, A.A., Finger, E.C., Mitchell, D.G., Reid, M.E., Sims, C., Kosson, D.S., Towbin, K.E., Leibenluft, E., Pine, D.S., Blair, R.J., 2008. Reduced amygdala response to fearful expressions in children and adolescents with callous-unemotional traits and disruptive behavior disorders. *The American journal of psychiatry* 165, 712-720.

Mechelli, A., Price, C.J., Friston, K.J., Ashburner, J., 2005. Voxel-based morphometry of the human brain: methods and applications. *Current Medical Imaging Reviews* 1, 105-113.

Menon, V., Uddin, L.Q., 2010. Saliency, switching, attention and control: a network model of insula function. *Brain Struct.Funct.* 214, 655-667.

Michalska, K.J., Decety, J., Zeffiro, T.A., Lahey, B.B., 2015. Association of regional gray matter volumes in the brain with disruptive behavior disorders in male and female children. *Neuroimage Clin* 7, 252-257.

Odgers, C.L., Caspi, A., Broadbent, J.M., Dickson, N., Hancox, R.J., Harrington, H., Poulton, R., Sears, M.R., Thomson, W.M., Moffitt, T.E., 2007. Prediction of differential adult health burden by conduct problem subtypes in males. *Arch Gen Psychiatry* 64, 476-484.

Passamonti, L., Fairchild, G., Goodyer, I.M., Hurford, G., Hagan, C.C., Rowe, J.B., Calder, A.J., 2010. Neural abnormalities in early-onset and adolescence-onset conduct disorder. *Arch Gen Psychiatry* 67, 729-738.

Radua, J., Canales-Rodriguez, E.J., Pomarol-Clotet, E., Salvador, R., 2014. Validity of modulation and optimal settings for advanced voxel-based morphometry. *NeuroImage* 86, 81-90.

Rogers, J.C., De Brito, S.A., 2015. Cortical and Subcortical Gray Matter Volume in Youths With Conduct Problems: A Meta-analysis. *JAMA Psychiatry* [epub ahead of print].

Rubia, K., Smith, A.B., Halari, R., Matsukura, F., Mohammad, M., Taylor, E., Brammer, M.J., 2009. Disorder-specific dissociation of orbitofrontal dysfunction in boys with pure conduct disorder during reward and ventrolateral prefrontal dysfunction in boys with pure ADHD during sustained attention. *The American journal of psychiatry* 166, 83-94.

Sebastian, C.L., De Brito, S.A., McCrory, E.J., Hyde, Z.H., Lockwood, P.L., Cecil, C.A., Viding, E., 2015. Grey matter volumes in children with conduct problems and varying levels of callous-unemotional traits. *Journal of Abnormal Child Psychology* [epub ahead of print].

Sebastian, C.L., McCrory, E.J., Cecil, C.A., Lockwood, P.L., De Brito, S.A., Fontaine, N.M., Viding, E., 2012. Neural responses to affective and cognitive theory of mind in children with conduct problems and varying levels of callous-unemotional traits. *Arch Gen Psychiatry* 69, 814-822.

Shaffer, D., Fisher, P., Lucas, C.P., Dulcan, M.K., Schwab-Stone, M.E., 2000. NIMH Diagnostic Interview Schedule for Children Version IV (NIMH DISC-IV): description, differences from previous versions, and reliability of some common diagnoses. *J Am Acad.Child Adolesc Psychiatry* 39, 28-38.

Sterzer, P., Stadler, C., Krebs, A., Kleinschmidt, A., Poustka, F., 2005. Abnormal neural responses to emotional visual stimuli in adolescents with conduct disorder. *Biol Psychiatry* 57, 7-15.

Sterzer, P., Stadler, C., Poustka, F., Kleinschmidt, A., 2007. A structural neural deficit in adolescents with conduct disorder and its association with lack of empathy. *NeuroImage* 37, 335-342.

Tiihonen, J., Rossi, R., Laakso, M.P., Hodgins, S., Testa, C., Perez, J., Repo-Tiihonen, E., Vaurio, O., Soininen, H., Aronen, H.J., Kononen, M., Thompson, P.M., Frisoni, G.B., 2008. Brain anatomy of persistent violent offenders: more rather than less. *Psychiatry Res* 163, 201-212.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B., Joliot, M., 2002. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage* 15, 273-289.

Viding, E., Blair, R.J., Moffitt, T.E., Plomin, R., 2005. Evidence for substantial genetic risk for psychopathy in 7-year-olds. *J Child Psychol Psychiatry* 46, 592-597.

Viding, E., Sebastian, C.L., Dadds, M.R., Lockwood, P.L., Cecil, C.A., De Brito, S.A., McCrory, E.J., 2012. Amygdala response to preattentive masked fear in children with conduct problems: the role of callous-unemotional traits. *The American journal of psychiatry* 169, 1109-1116.

Wallace, G.L., White, S.F., Robustelli, B., Sinclair, S., Hwang, S., Martin, A., Blair, R.J., 2014. Cortical and subcortical abnormalities in youths with conduct disorder and elevated callous-unemotional traits. *Journal of the American Academy of Child & Adolescent Psychiatry* 53, 456-465.

Walters, G.D., Kiehl, K.A., 2015. Limbic correlates of fearlessness and disinhibition in incarcerated youth: Exploring the brain-behavior relationship with the Hare Psychopathy Checklist: Youth Version. *Psychiatry Research* 230, 205-210.

White, S.F., Marsh, A.A., Fowler, K.A., Schechter, J.C., Adalio, C., Pope, K., Sinclair, S., Pine, D.S., Blair, R.J., 2012. Reduced amygdala response in youths with disruptive behavior disorders and psychopathic traits: decreased emotional response versus increased top-down attention to nonemotional features. *The American journal of psychiatry* 169, 750-758.

Yang, Y., Raine, A., Colletti, P., Toga, A.W., Narr, K.L., 2010. Morphological alterations in the prefrontal cortex and the amygdala in unsuccessful psychopaths. *J Abnorm Psychol* 119, 546-554.

Yang, Y., Raine, A., Narr, K.L., Colletti, P., Toga, A.W., 2009. Localization of deformations within the amygdala in individuals with psychopathy. *Arch Gen Psychiatry* 66, 986-994.

Young, M.P., Scannell, J.W., 2000. Brain structure-function relationships: advances from neuroinformatics. *Philos.Trans.R Soc Lond B Biol Sci* 355, 3-6.

Zwart, W.M.d., Smit, F., Monshouwer, K., 2000. Youth and risky behaviour 1999. Smoking, drinking drug use and gambling among secondary school students above the age of 10. [Dutch: Jeugd en riskant gedrag 1999. Roken, drinken, drugsgebruik en gokken onder scholieren vanaf tien jaar.]. Trimbo, Utrecht, The Netherlands

## Appendices

**Table 1. Socio-demographic and mental health characteristics of the current sample (n=134)**

CD symptoms, mean (SD), range	1.0 (1.9), 0-9
YPI Callous-Unemotional, mean (SD), range	24.3 (7.1), 15-55
ODD no. (%)	16 (11.9%)
CD no. (%)	18 (13.4%)
Age, mean (SD), range	17.7 (1.5), 12-20
Male gender, no. (%)	114 (85%)
Low SES neighborhood, no. (%)	73 (54.4%)
Non-Western ethnicity, no. (%)	36 (27%)
IQ, mean (SD), range	91.3 (13.5), 62-126
CBCL Internalizing, mean T-score (SD), range	51.1 (10.8), 33-77
YSR Internalizing, mean T-score (SD), range	47.6 (10.0), 30-75
CBCL Externalizing, mean T-score (SD), range	51.8 (11.4), 34-78
YSR Externalizing, mean T-score (SD), range	52.4 (10.1), 34-77
ADHD no. (%)	40 (29.9%)
PTSD no. (%)	2 (1.5%)

Abbreviations: CD: Conduct Disorder, YPI: Youth psychopathic traits inventory, SES: Socio-economic status, CBCL: Child Behaviour Checklist, YSR: Youth Self Report, ADHD: Attention Deficit/Hyperactivity Disorder, ODD: Oppositional Defiant Disorder, PTSD: Post-Traumatic Stress Disorder.

**Table 2. Partial correlations between CD symptoms and callous-unemotional traits and grey matter volume and concentration, using small volume correction in a priori regions of interest (n=134)**

<i>Grey matter volume</i>								
	Brain region	MNI coordinates			T-value	Z-value	p <sub>FWE</sub>	Direction
		X	Y	Z				
CD symptoms	-	-	-	-	-	-	-	-
Callous-unemotional traits	-	-	-	-	-	-	-	-
Interaction-term	Insula	-44	20	1	3.63	3.53	.040	Negative†

<i>Grey matter concentration</i>								
	Brain region	MNI coordinates			T-value	Z-value	p <sub>FWE</sub>	Direction
		X	Y	Z				
CD symptoms	Amygdala	-24	-3	-17	3.19	3.12	.043	Positive
Callous-unemotional traits	Amygdala	-24	-1	-15	3.17	3.10	.046	Negative
Interaction-term	Insula	39	-6	-5	4.12	3.99	.018	Negative

Abbreviations: MNI = Montreal Neurological Institute standard anatomical space. p<sub>FWE</sub> = p-value, family wise error

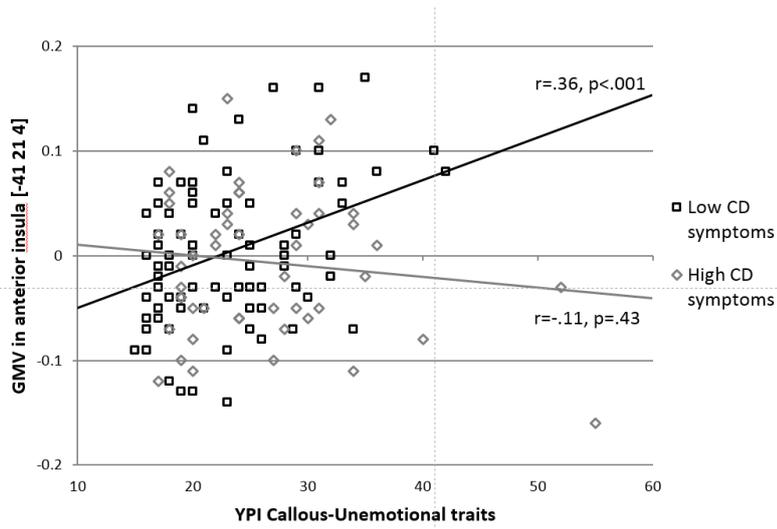
corrected for multiple comparisons. † Post-hoc analyses showed that callous-unemotional traits were positively correlated with anterior insula volume ( $r=.36$ ,  $p<.001$ ) in youths with below-median conduct disorder symptoms, while this relation was negative and non-significant ( $r=-.11$ ,  $p=.43$ ) in those with conduct disorder symptoms above the median.

**Appendix Table 1. Multiple regression models with CD symptom scores and callous-unemotional traits scores predicting GMC /GMV parameter estimates for all main findings , using forward selection to control for socio-demographic and mental health covariates.**

	Unstandardized coefficients		Standardized coefficient	t	P-value
	B	SE	Beta		
<b>Left amygdala [x=-24 y=-1 z=-15] GMC model</b>					
<i>Step 1 (R<sup>2</sup>= .10, F(2,131)=7.2, p&lt;.001)</i>					
Constant	0.77	0.002		349.4	<.001
YPI callous-unemotional traits z-score	-0.008	0.002	-.30	-3.2	.002
CD symptoms z-score	0.008	0.002	.30	3.2	.002
<i>Step 2</i>					
<i>No variables were entered into the equation</i>					
<b>Left amygdala [x=-24 y=-3 z=-17] GMC model</b>					
<i>Step 1 (R<sup>2</sup>= .10, F(2,131)=7.2, p&lt;.001)</i>					
Constant	0.83	0.002		510.7	<.001
YPI callous-unemotional traits z-score	-0.006	0.002	-.29	-3.2	.002
CD symptoms z-score	0.006	0.002	.30	3.2	.002
<i>Step 2</i>					
<i>No variables were entered into the equation</i>					
<b>Right insula [x=39 y=-6 z=-5] GMC model</b>					
<i>Step 1 (R<sup>2</sup>= .09, F(2,132)=13.5, p&lt;.001)</i>					
Constant	0.82	0.002		517.5	<.001
YPI callous-unemotional traits z-score	-0.006	0.002	-.31	-3.7	<.001
<i>Step 2</i>					
<i>No variables were entered into the equation</i>					
<b>Left insula [x=-41 y=21 z=-4] GMV model</b>					
<i>Step 1 (R<sup>2</sup>= .11, F(3,130)=5.4, p=.002)</i>					
Constant	0.62	0.006		97.8	<.001
YPI callous-unemotional traits z-score	0.021	0.007	.28	3.0	.004
CD symptoms z-score	0.004	0.008	.05	0.5	.62
Interaction-term†	-0.013	0.004	-.36	-3.4	.001
<i>Step 2 (R<sup>2</sup>= .16, F(4,129)=6.2, p&lt;.001)</i>					
Constant	0.65	0.012		52.2	<.001
YPI callous-unemotional traits z-score	0.021	0.007	.30	3.1	.002
CD symptoms z-score	0.006	0.008	.09	0.9	.40
Interaction-term†	-0.013	0.004	-.34	-3.3	.001
Ethnicity	-0.019	0.007	-.23	-2.8	.006

Abbreviations: CD = conduct disorder, GMC = grey matter concentration, GMV = grey matter volume, YPI = youth psychopathic traits inventory. †Interaction-term = callous-unemotional traits z-score\*CD symptoms z-score.

**Figure 1. Callous-unemotional traits and anterior insula GMV in low and high antisocial youth. Abbreviations:  
GMV – Grey Matter Volume; YPI – Youth Psychopathic Traits Inventory; CD – conduct disorder.**



**Figure 1. Callous-unemotional traits and anterior insula GMV in low and high antisocial youth. Abbreviations:  
GMV = Grey Matter Volume; YPI = Youth Psychopathic Traits Inventory; CD = conduct disorder.**

Figure 2. Multislice images displaying the interaction between DISC CD symptoms and YPI Callous-Unemotional traits in the prediction of GMV (frame A), the association between YPI Callous-Unemotional traits and GMC (frame B) and the association between DISC CD symptoms and GMC (frame C), overlaid on an anatomical template (thresholded at  $t > 2$  for display purposes and ranging from  $t = 2$  [red] to  $t = 4$  [yellow]). Abbreviations: DISC = Diagnostic Interview Schedule for Children; CD = conduct disorder; YPI = Youth Psychopathic Traits Inventory; GMC = Grey Matter concentration.

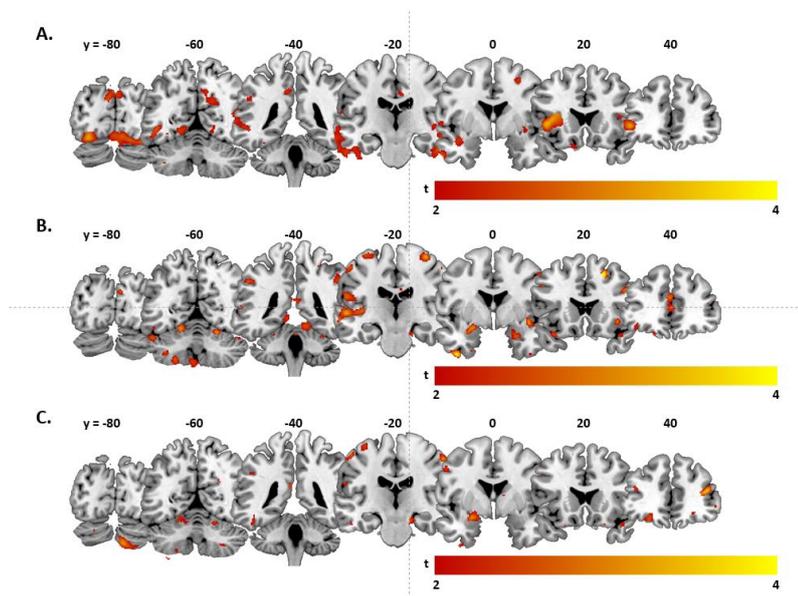
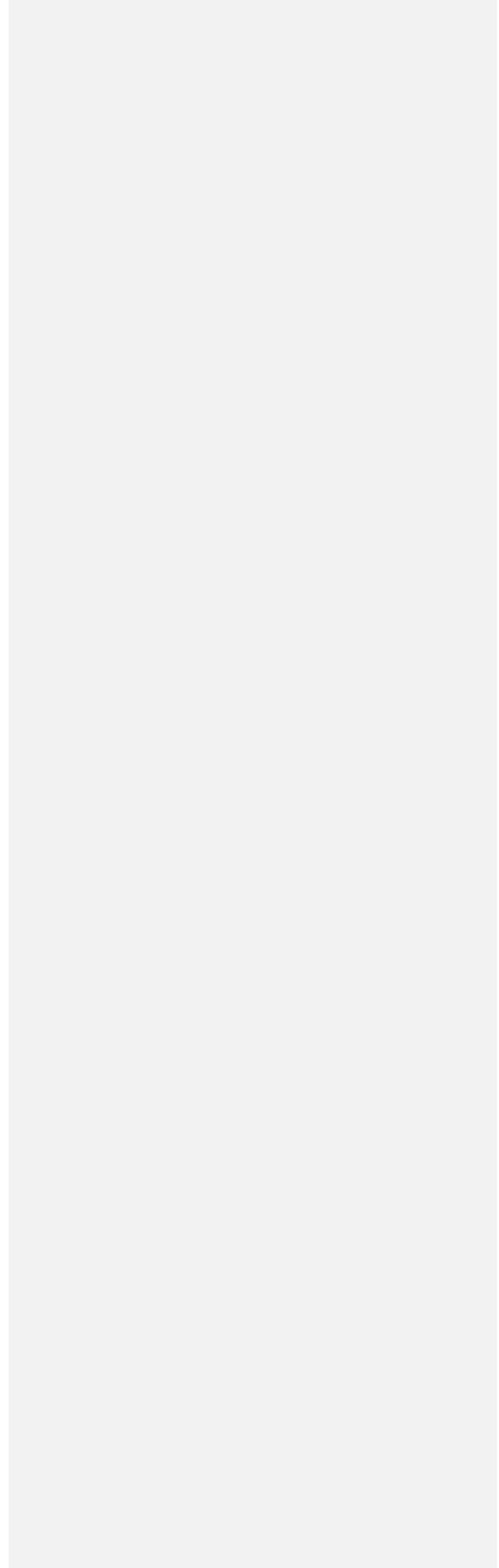
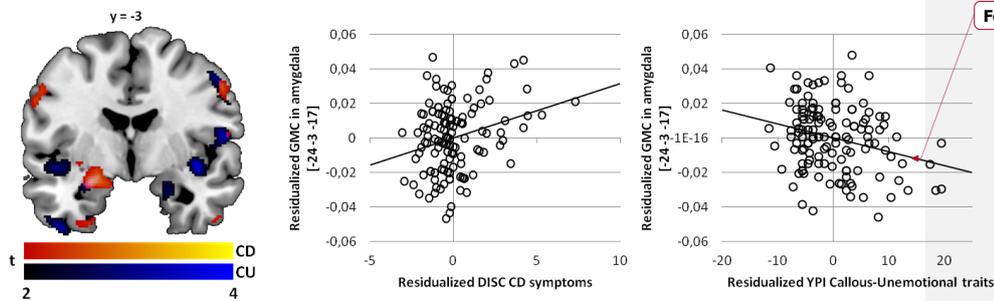


Figure 2. Multislice images displaying the interaction between DISC CD symptoms and YPI Callous-Unemotional traits in the prediction of GMV (frame A), the association between YPI Callous-Unemotional traits and GMC (frame B) and the association between DISC CD symptoms and GMC (frame C), overlaid on an anatomical template (thresholded at  $t > 2$  for display purposes and ranging from  $t = 2$  [red] to  $t = 4$  [yellow]). Abbreviations: DISC = Diagnostic Interview Schedule for Children; CD = conduct disorder; YPI = Youth Psychopathic Traits Inventory; GMC = Grey Matter concentration.

|





Formatted: Centered

**Appendix Figure F1. Unique associations of DISC CD symptoms and YPI callous-unemotional traits with GMC.**  
**Left panel: unique positive association between DISC CD symptoms and GMC (ranging from t=2 [red] to t=4 [yellow]) and unique negative association between YPI Callous-Unemotional traits and GMC (ranging from t=2 [black] to t=4 [blue]) as well as their overlap (pink), thresholded at t>2 for display purposes. Middle panel and right panel: unique associations of DISC CD symptoms and YPI Callous-Unemotional traits, respectively, with GMC in the amygdala peak voxel [-24 -3 -17] (i.e. controlling for the other variable in both graphs) .**  
**Abbreviations: DISC = Diagnostic Interview Schedule for Children; CD (text) = conduct disorder; CD (figure) = CD symptoms score t-value; CU (figure) = YPI callous-unemotional traits t-value; YPI = Youth Psychopathic Traits Inventory; GMC = Grey Matter concentration.**

Formatted: Font: Bold