Callous and uncaring traits are associated with reductions in amygdala volume among youths with varying levels of conduct problems

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Abstract

Background. The emergence of callous unemotional (CU) traits, and associated externalizing behaviors, is believed to reflect underlying dysfunction in the amygdala. Studies of adults with CU traits or psychopathy have linked characteristic patterns of amygdala dysfunction to reduced amygdala volume, but studies in youths have not thus far found evidence of similar amygdala volume reductions. The current study examined the association between CU traits and amygdala volume by modeling CU traits and externalizing behavior as independent continuous variables, and explored the relative contributions of callous, uncaring, and unemotional traits.

Methods. CU traits and externalizing behavior problems were assessed in 148 youths using the Inventory of Callous Unemotional Traits (ICU) and the Child Behavior Checklist (CBCL). For a subset of participants (n = 93), high-resolution T1-weighted images were collected and volume estimates for the amygdala were extracted.

Results. Analyses revealed that CU traits were associated with increased externalizing behaviors and decreased bilateral amygdala volume. These results were driven by the callous and uncaring sub-factors of CU traits, with unemotional traits unrelated to either externalizing behaviors or amygdala volume. Results persisted after accounting for covariation between CU traits and externalizing behaviors. Bootstrap mediation analyses indicated that CU traits mediated the relationship between reduced amygdala volume and externalizing severity.

Conclusions. These findings provide evidence that callous-uncaring traits account for reduced amygdala volume among youths with conduct problems. These findings provide a framework for further investigation of abnormal amygdala development as a key causal pathway for the development of callous-uncaring traits and conduct problems.
signaling in the amygdala, CU youths fail to develop appropriate guilt and empathy in response to others’ distress, and so persist in behaviors like aggression and violence that would normally be inhibited by these emotional responses (Kochanska, 1993; Frick and Morris, 2004; Blair, 2005, 2013; Marsh, 2016; Seara-Cardoso et al., 2016). These theories are reinforced by consistent findings of reduced amygdala responsivity to fearful facial expressions in high CU youths (Marsh et al., 2008; Jones et al., 2009; Viding et al., 2012; White et al., 2012; Lozier et al., 2014) as well as aberrant functional connectivity between the amygdala and other regions implicated in emotion processing (Marsh et al., 2011a; Finger et al., 2012; Aghajani et al., 2016).

Despite a robust literature examining amygdala activity in CU youths, relatively few structural magnetic resonance imaging (MRI) studies have examined the association between CU traits and brain structure. Reduced amygdala volume has repeatedly been found in both studies of youths with conduct disorder (Sterzer et al., 2007; Huebner et al., 2008; Fairchild et al., 2013; Wallace et al., 2014; Rogers and De Brito, 2016) and studies of adults with psychopathic traits, who are distinguished from other antisocial populations primarily by their elevated CU traits (Yang et al., 2009; Cope et al., 2014; Pardini et al., 2014). These findings strongly implicate reduced amygdala volume in the development of antisociality and CU traits, but four studies of children and adolescents have not yet found an association between amygdala volume and CU traits (De Brito et al., 2009; Wallace et al., 2014; Cohn et al., 2016; Sebastian et al., 2016) (although Cohn et al. found that increased CU traits were associated with reduced amygdala gray matter concentration). This could be interpreted to mean that CU traits are associated with amygdala volume only in adulthood, but not childhood or adolescence. Alternately, methodological considerations may have concealed a relationship between amygdala volume and CU traits in youths. For example, although CU traits are continuously distributed and more accurately assessed using continuous analyses (Guay et al., 2007; Moffitt et al., 2008; Lozier et al., 2014), three of the four studies of volumetric differences in CU youths employed primarily group-based approaches (De Brito et al., 2009; Wallace et al., 2014; Sebastian et al., 2016).

In addition, Wallace et al. and De Brito et al. compared youths with both CU traits and conduct problems to healthy control youths, an approach that hinders the dissociation of correlates of CU traits and conduct problems more generally (De Brito et al., 2009; Wallace et al., 2014). Sebastian et al. compared groups of youths with conduct problems and both low and high levels of CU traits to healthy controls (Sebastian et al., 2016). However, similar to the prior two studies, their use of a primarily group-based approach lacked the power of a continuous analysis, and may have been affected by suppressor effects (Sebastian et al., 2012).

Moreover, these studies all examined CU traits as a unitary construct, although, increasingly, assessments of CU traits have focused on the three subfactors comprising CU traits (callous, uncaring, and unemotional traits) (Kimonis et al., 2008a, b). Examining the independent associations between callous, uncaring, and unemotional traits with aberrant amygdala volume may clarify the relevance of amygdala development to the emergence of CU traits. Given the key role of the amygdala in emotional processing, it may be primarily the unemotional component of CU traits that is associated with reduced amygdala volume, a finding that would implicate the amygdala in global affective deficits in CU youths. By contrast, associations with the callous and/or uncaring components of CU traits would suggest that the amygdala may play a more complex role in interpersonal empathy and caring. Examination of associations between unemotional traits and amygdala volume may also provide important insight into the validity of assessments of unemotional traits, the nature of which have been subject to recent concerns (Henry et al., 2016; Cardinale and Marsh, 2017).

The current study therefore examined associations between amygdala gray matter volume and externalizing behaviors as well as callous, uncaring, and unemotional traits in a sample of youths with varying levels of conduct problems and CU traits. Through a series of multiple linear regression analyses, we investigated how total ICU scores and the three subfactor scores correspond to both aberrant structural development of the amygdala and the emergence of externalizing behaviors, and whether CU traits mediate the relationship between reduced amygdala volume and externalizing behavior severity. We hypothesized that CU traits would be associated with increased externalizing behavior problems and decreased bilateral amygdala volume. Furthermore, we predicted that these relationships would be driven by the callous and uncaring subscales of the ICU.

Methods

Participants

One hundred forty-eight children, aged 9–18 (M = 13.96, s.d. = 2.44, % male = 59.46), were recruited from Washington, DC and surrounding regions through referrals, advertisements, and fliers seeking both healthy children and children with conduct problems. All participants and their parents first completed an initial visit during which demographic and clinical measures were completed along with IQ testing using the Kaufman Brief Intelligence Test (Kaufman and Kaufman, 2004). Participants reported a wide range of scores on our clinical measures, confirming that our sample included both healthy youths and youths with elevated conduct problems and varying CU traits, as well as psychiatric symptoms including externalizing behaviors, internalizing behaviors, and attentional difficulties (Table 1). Consistent with our recruitment effort to specifically target both healthy children and children with elevated conduct problems, 77 participants reported clinical levels of externalizing behavior as assessed by an age and gender standardized externalizing symptomology score on the Child Behavior Checklist (CBCL) that placed them above the 98th percentile (Achenbach, 1991).

Of participants who completed the initial visit, 93 were eligible for and consented to participate in an MRI scan. Participants were excluded from MRI scanning for: history of head trauma or neurological disorder, symptoms of pervasive developmental disorder, IQ <80, or MRI contraindications such as claustrophobia or metallic implants including braces or permanent retainers. The MRI sample consisted of children aged 10–17 (M = 13.98, s.d. = 2.36, % male = 59.14) and varied widely in externalizing behavior, including 46 participants with clinically significant externalizing scores. The MRI sample did not differ from the full sample in terms of externalizing and CU scores or any other clinical or demographic measures, with the exception of a trend-level difference in age between the full sample and the scanned sample (Table 1). All participants were native English speakers. Written informed assent and consent were obtained from children and parents before testing. Approval for all procedures was obtained from the Georgetown University Institutional Review Board. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant
### Table 1. Descriptive statistics

<table>
<thead>
<tr>
<th></th>
<th>Total sample (n = 148)</th>
<th></th>
<th>Scanned participants (n = 84)</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Range</td>
<td>Skew</td>
<td>Kurt</td>
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<tr>
<td>Demographic variables</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Age</td>
<td>13.96</td>
<td>2.44</td>
<td>9.56–18.07</td>
<td>0.01</td>
<td>1.75</td>
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<td>IQ</td>
<td>101.59</td>
<td>15.84</td>
<td>60–136</td>
<td>0.05</td>
<td>2.64</td>
</tr>
<tr>
<td>Gender, % male</td>
<td>58.78%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race/ethnicity, n</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, non-Hispanic</td>
<td>40</td>
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<td></td>
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<td>African-American, non-Hispanic</td>
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<td>Hispanic</td>
<td>14</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>13</td>
<td></td>
<td></td>
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<td></td>
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<td>Clinical measures</td>
<td></td>
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<tr>
<td>CU</td>
<td>36.64</td>
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<td>13–63</td>
<td>0.05</td>
<td>2.19</td>
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<td>Callous</td>
<td>11.33</td>
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<td>0–29</td>
<td>0.57</td>
<td>2.67</td>
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<td>Uncaring</td>
<td>14.83</td>
<td>5.25</td>
<td>2–24</td>
<td>−0.30</td>
<td>2.14</td>
</tr>
<tr>
<td>Unemotional</td>
<td>8.98</td>
<td>2.54</td>
<td>3–15</td>
<td>0.01</td>
<td>2.69</td>
</tr>
<tr>
<td>Externalizing</td>
<td>17.99</td>
<td>15.64</td>
<td>0–57</td>
<td>0.50</td>
<td>2.07</td>
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<td>Internalizing</td>
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<td>10.82</td>
<td>0–48</td>
<td>1.43</td>
<td>4.69</td>
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<tr>
<td>Attentional difficulties</td>
<td>7.05</td>
<td>5.67</td>
<td>0–19</td>
<td>0.33</td>
<td>1.89</td>
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<td>Brain volume estimates (mm³)</td>
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<td></td>
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<td>Left amygdala</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>Right amygdala</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Intracranial volume</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</tbody>
</table>

*p* Values are reported for comparisons of total sample v. scanned participants.
national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

**Clinical measures**

**Inventory of Callous Unemotional Traits**
The ICU was used to assess CU traits (Kimonis et al., 2008a, b). The ICU was completed separately by parents and participants. Scores on the ICU were calculated by summing the highest item response from either the child or parent version (Jones et al., 2009; Sebastian et al., 2012; Viding et al., 2012; Lozier et al., 2014; Breeden et al., 2015). This scoring approach follows the recommended scoring practices for the parent scale of the ICU (Frick and Hare, 2001), and has been shown to reduce susceptibility to social desirability biases and optimize accuracy across multiple contexts (Piacentini et al., 1992; Frick et al., 2003).

**Child Behavior Checklist**
The CBCL is a parent report-based assessment of behavioral and emotional problems in children and adolescents (Achenbach, 1991). Externalizing and internalizing syndrome scales were calculated for each participant. Attentional difficulties were also measured using the attention difficulties syndrome scale. The use of the CBCL to assess the severity of various clinical symptoms in community samples has been demonstrated to be reliable and valid (Biederman et al., 1993; Warnick et al., 2008).

**Image acquisition and analysis**

Three-dimensional anatomical images were acquired using a 3.0 Tesla Siemens (Erlangen, Germany) TIM Trio. High-resolution T1-weighted images were collected for each participant (TR = 1900 ms, TE = 2.52 ms, TI = 900.0 ms, 1.0 mm³ voxels, 176 slices, matrix = 246 × 256, field of view = 250 mm²). Prior to analyses, all images were visually inspected for motion artifacts. Any potential motion artifacts were examined by three independent evaluators and only scans for which all three evaluators reached agreement were included in the dataset. Thirty-nine participants completed more than one anatomical scan; the scan with the fewest motion artifacts and clearest contrast was selected for these participants. Data from nine participants could not be analyzed due to excessive motion artifacts in all completed anatomical scans, resulting in a final sample size of 84 participants. Images were collected using an eight-channel phased-array head coil for 16 participants and using a 12-channel phased-array head coil for the remaining 68 participants. For all analyses investigating neural volume, a dummy coded variable for use of the 8 v. 12-channel was included as a covariate (Breeden et al., 2015). Because participants completed the scan during a separate visit, we also included age at the time of the scan in addition to age at the time of the initial visit for all analyses investigating neural volume.

Anatomical images were analyzed using FreeSurfer version 5.3.0. Automated segmentation of subcortical regions occurred during the first stage of the FreeSurfer cortical reconstruction process (Fischl et al., 2002, 2004; Fischl, 2012). During this stage, neuroanatomical labels are automatically assigned to each voxel based on probabilistic information acquired through an a priori knowledge of spatial relationships acquired through a manually labeled training set. This classification technique is robust to anatomical variation typical in pediatric populations through the use of a non-linear registration procedure. Segmentation occurs following three automated strategies to disambiguate voxel labels, which assess the prior probability of the tissue class occurring at an atlas location, and given the tissue class, the likelihood of the image and the probability of the local spatial configuration. The resulting subcortical segmentation, has been shown to be reliable (Morey et al., 2010) and comparable to manual segmentation (Fischl et al., 2002; Morey et al., 2009; Grimm et al., 2015; Schoemaker et al., 2016). Following segmentation, 42 subcortical regions were identified and labeled using both subject-independent probabilistic atlases and subject-specific measured variables for each subject. All images were visually inspected following segmentation. Volume estimates for all subcortical regions, including the left amygdala and right amygdala, as well as total intracranial volume were extracted and exported for analysis in STATA (Table 1; online Supplementary Table S2).

**Results**

For all analyses, variables were mean centered, and known correlates of amygdala volume and/or externalizing behaviors were entered as covariates. For analyses of clinical symptomology, gender, IQ, and age at initial visit were included as covariates. For analyses of brain volume, we included total intracranial volume, age at time of scan, and headcoil as additional covariates. Robust standard errors were used to account for heteroscedasticity of the experimental variables and to control for sibling effects. All analyses were repeated with dummy variables coding for the 18 sibling groups present in these data (n = 41). Findings were not affected by the inclusion of these covariates. Therefore, for ease of interpretation of results, we report the findings from analyses excluding sibling status as a covariate.

**ICU scores and clinical symptomology**

The internal consistency of total ICU, α = 0.90, callous subscale, α = 0.84, and uncaring subscale, α = 0.87 were acceptable, whereas the unemotional subscale showed relatively low internal consistency, α = 0.58. Intercorrelations among scores on the ICU were all large (online Supplementary Table S1).

Results of a multiple linear regression analysis across all participants (n = 148) predicting externalizing behaviors from ICU scores confirmed that as total ICU scores increased, externalizing behaviors increased, $\beta = 0.73$, $t(143) = 11.73$, $p < 0.001$ (Fig. 1). This association remained significant after controlling for attentional difficulty and internalizing behavior scores, $\beta = 0.31$, $t(141) = 4.55$, $p < 0.001$. Next, a multiple regression with all three ICU subscales predicting externalizing behavior problems found that scores on the callous, $\beta = 0.53$, $t(141) = 4.56$, $p < 0.001$, and uncaring, $\beta = 0.34$, $t(141) = 3.30$, $p = 0.001$, subscales were independently associated with increased externalizing, whereas unemotional subscale scores were not, $\beta = -0.07$, $t(141) = -1.13$, $p = 0.26$ (Fig. 1). Associations between externalizing and the callous, $\beta = 0.21$, $t(139) = 3.03$, $p = 0.003$, and uncaring, $\beta = 0.20$, $t(139) = 3.19$, $p = 0.002$, subscales persisted when controlling for attentional difficulties and internalizing behaviors, whereas the unemotional subscale scores remained non-significant, $\beta = -0.08$, $t(139) = -1.37$, $p = 0.17$.

We repeated all of the above analyses examining the relationship between ICU scores and clinical symptomologies restricted to only those participants who qualified for inclusion in MRI scanning (n = 84). All patterns of significant findings persisted when analyses were limited to this sample (online Supplementary Text S1), supporting the reliability of the identified patterns.
We next investigated associations between amygdala volume, scores on the ICU, and externalizing symptoms using a region of interest (ROI) approach, consistent with the approaches used in previous studies of neural correlates of CU traits and psychopathy (Sebastian et al., 2012; Lozier et al., 2014; Pardini et al., 2014; Wallace et al., 2014; Breeden et al., 2015; Vieira et al., 2015; Sebastian et al., 2016). Again, analyses included age at scanning, headcoil type, and total intracranial volume as covariates in addition to age at time of initial visit, gender, and IQ. Results of separate multiple linear regression analyses revealed that total ICU scores were associated with decreased left, $\beta = -0.36, t(76) = -3.21, p = 0.002$, and right, $\beta = -0.27, t(76) = -2.64, p = 0.01$, amygdala volume (Fig. 2).

Similar results were obtained for callous [left: $\beta = -0.32, t(76) = -3.00, p = 0.004$; right: $\beta = -0.24, t(76) = -2.40, p = 0.02$] and uncaring [left: $\beta = -0.35, t(76) = -3.07, p = 0.003$; right: $\beta = -0.24, t(76) = -2.34, p = 0.02$] subscale scores. Unemotional subscale scores were associated with right amygdala volume at a trend level, $\beta = -0.17, t(76) = -1.97, p = 0.05$, but not left amygdala volume, $\beta = -0.18, t(76) = -1.64, p = 0.11$ (Fig. 1).

To assess the specificity of these findings, we conducted parallel analyses examining associations between CU traits and subcortical volume estimates for the nucleus accumbens, caudate, hippocampus, pallidum, putamen, thalamus, and ventral diencephalon (DC). Across all of these subcortical regions, no significant associations (all $p > 0.10$) were found with ICU total (online Supplementary Table S3) or subscale scores (online Supplementary Table S4), or with externalizing behaviors (online Supplementary Table S5), with one exception: decreased right ventral DC volume was associated with increased externalizing behaviors, $\beta = -0.18, t(76) = -2.18, p = 0.03$, and uncaring traits.
of CU traits and amygdala volumes, we created a composite callous-uncaring score by summing the unemotional and social competence subscales. This composite score was moderately correlated with increased externalizing behaviors (r = 0.49) and showed a trend toward significance with increased amygdala volume (r = 0.39). In contrast, the emotional subscale was not closely associated with either externalizing behaviors or amygdala volumes, suggesting that the observed direct statistical effects of amygdala volume on externalizing behaviors were explained by the statistical mediation of CU traits.

**Table 2. Multiple regression models predicting left and right amygdala volumes**

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Left amygdala</th>
<th>Right amygdala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Externalizing behaviors</td>
<td>-0.36 (0.11)**</td>
<td>-0.36 (0.11)**</td>
</tr>
<tr>
<td>CALLous-UNCaring Traits</td>
<td>-0.36 (0.11)**</td>
<td>-0.36 (0.11)**</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.38 (0.11)**</td>
<td>-0.38 (0.11)**</td>
</tr>
<tr>
<td>IQ</td>
<td>-0.36 (0.11)**</td>
<td>-0.36 (0.11)**</td>
</tr>
<tr>
<td>Headcoil</td>
<td>-0.28 (0.11)**</td>
<td>-0.28 (0.11)**</td>
</tr>
<tr>
<td>Age at Time of Screen</td>
<td>-0.22 (0.11)**</td>
<td>-0.22 (0.11)**</td>
</tr>
<tr>
<td>Age at Time of Scan</td>
<td>-0.22 (0.11)**</td>
<td>-0.22 (0.11)**</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.17 (0.09)**</td>
<td>-0.17 (0.09)**</td>
</tr>
<tr>
<td>IQ</td>
<td>-0.15 (0.09)**</td>
<td>-0.15 (0.09)**</td>
</tr>
<tr>
<td>Headcoil</td>
<td>-0.14 (0.09)**</td>
<td>-0.14 (0.09)**</td>
</tr>
</tbody>
</table>

*Note: Standardized betas with standard error in parentheses. Significant effects in bold.*

**Discussion**

Extending previous findings, our mixed-gender sample of children with varying levels of CU traits showed smaller amygdala volumes in youths. Across all participants, amygdala volume was negatively associated with total intracranial volume, all covariates, and IQ. Consistent with this pattern of findings, the conditional effect of CU traits on right amygdala volume is greater at younger ages and in male participants (Fig. 3).

Mediation analyses that included age or gender as moderators of the relationship between CU traits and amygdala volume revealed that age and gender significantly predicted total intracranial volume, all covariates, and IQ. However, age was unrelated to IQ, gender, or CU traits, and IQ, age, externalizing behaviors, and CU traits were not significantly different across male and female participants.

In summary, our findings provide the first evidence linking CU traits to reduced amygdala gray matter volume in youths. Across a mixed-gender sample of children with varying levels of CU traits, we observed a significant negative partial correlation between CU traits and amygdala volume, even after accounting for externalizing scores (Table 2). Mediation analyses that included age or gender as moderators of the relationship between CU traits and amygdala volume revealed that age and gender significantly predicted total intracranial volume, all covariates, and IQ. However, age was unrelated to IQ, gender, or CU traits, and IQ, age, externalizing behaviors, and CU traits were not significantly different across male and female participants. Whereas age was unrelated to IQ, gender, or CU traits, IQ, age, externalizing behaviors, and CU traits were not significantly different across male and female participants. In accordance with previous research, we found that the observed direct statistical effects of amygdala volume on externalizing behaviors were explained by the statistical mediation of CU traits.
externalizing behavior and CU traits, we found that variation in amygdala volume is associated with levels of both callous-uncaring traits and antisocial and externalizing behaviors, even after accounting for variation in children’s age, sex, cognitive abilities, and total intracranial volume. In our sample, the volume of left amygdala was associated with engagement in externalizing behaviors (e.g. aggression, theft, rule-breaking) and the volume of both left and right amygdala was associated with CU traits including limited empathy, remorse, and guilt. Multiple regression analyses revealed, however, that when externalizing behaviors and CU traits were modeled simultaneously, only CU traits remained associated with amygdala volume. Moreover, the relationship between amygdala volume and CU traits primarily reflected callous and uncaring subscale scores (both of which were also robustly associated with externalizing behavior), supporting a role for the amygdala in interpersonal empathy and caring. Alone, unemotional traits as measured by the ICU were unrelated to externalizing behaviors or amygdala volume. Comparable patterns were not observed in parallel analyses of subcortical volume, suggesting that findings were specific to the amygdala rather than limbic structures generally.

Together, these findings suggest that aberrant development of the amygdala, particularly relative reductions in bilateral amygdala volume as a proportion of total intracranial volume, may play a role in the emergence of CU traits and subsequent externalizing behavior. Structural amygdala abnormalities in high CU youths may lead to functional impairments in stimulus reinforcement learning and empathy, two processes that typically promote the avoidance of externalizing behaviors that cause distress in others (Marsh, 2016; Seara-Cardoso et al., 2016). Among callous and uncaring youths, developmental deficits in the amygdala may stunt the development of empathy and guilt, leading to engagement in increased externalizing behaviors such as aggression (Kochanska, 1993; Frick and Morris, 2004; White et al., 2009). The results of our moderator analyses suggest a developmental trajectory for the association between amygdala volume and CU traits such that the association between reduced amygdala volume with elevated CU traits was greatest at younger ages within our sample. This is consistent with the hypothesis that the amygdala plays a key role in moral development in childhood and early adolescence, such that anatomical abnormalities during this period are particularly important. Gender also emerged as a significant moderator. While we observe similar patterns in both genders, the association between CU traits and amygdala volume was stronger and more consistent in males. Future studies should investigate younger developmental periods, as the associations between amygdala volume and CU traits in female children may be stronger at even younger ages given evidence that brain maturation occurs earlier in females in comparison to males (Giedd et al., 1999; Lenroot et al., 2007).

Our findings contrast with those of four previous investigations that have observed no correspondence between amygdala gray matter volume and CU traits (De Brito et al., 2009; Wallace et al., 2014; Cohn et al., 2016; Sebastian et al., 2016). However, the present investigation benefited from several alternative analytical approaches that may explain this disparity. We employed continuous analyses of CU traits rather than group-based analyses, in keeping with emerging trends in evaluating CU traits (and psychopathology more generally) (Guay et al., 2007; Moffitt et al., 2008; Lozier et al., 2014). Given that CU traits are highly correlated with externalizing behaviors, groups defined by CU traits may also be characterized by high levels of externalizing behaviors, making it more difficult to isolate variables that are specifically associated with CU traits. Furthermore, lack of group differences in amygdala volume could result from suppressor effects arising from the strong positive correlation between externalizing behaviors and CU traits but inverse associations of amygdala volume with these behaviors, making it more difficult to characterize variables that are specifically associated with CU traits. Additionally, group-based studies using different analytic techniques can produce different results for the examination of subcortical gray matter structures (Heinen et al., 2016; Katuwal et al., 2016; Popescu et al., 2016), which could be due to fundamental methodological differences or their varied statistical requirements. The main aim of VBM...
is to characterize differences in the local composition of brain tissues (at the voxel level) while discounting gross anatomical and positional differences (Mechelli et al., 2005) accomplished through spatial normalization to a template space. Each voxel is then assigned a value indicating the concentration of a given tissue class (i.e. gray matter), which is then statistically analyzed using mass-univariate testing. By contrast, FreeSurfer’s sub cortical segmentation pipeline labels each voxel as being part of a particular brain region based on anatomical priors. The resulting metrics are not at the voxel level but rather the volume of the segmented subcortical structure. While VBM is highly applicable for data-driven analyses, variations in local gray matter (i.e. within the medial temporal lobe) may be anatomically imprecise and difficult to interpret. FreeSurfer was chosen for the current study given our anatomically specific hypotheses, desire for strong interpretability and detailed statistical modeling procedure. One previous study employed similar analyses in FreeSurfer as the current study (Wallace et al., 2014). However, the group-level statistical models primarily employed a group-based approach and did not account for covariation between CU traits and conduct problems.

Our findings are also consistent with recent concerns about the validity of the unemotional subscale of the ICU (Roose et al., 2010; Byrd et al., 2013; Kimonis et al., 2013; Hawes et al., 2014; Waller et al., 2015; Henry et al., 2016). Among the studies that have investigated associations between externalizing behaviors and subfactors of CU traits, callous and uncaring traits generally demonstrate stronger associations with externalizing behaviors than do unemotional traits (Essau et al., 2006; Kimonis et al., 2008b; Ciucci et al., 2014; Gluckman et al., 2016) and may emerge from distinct etiologies (Henry et al., 2016), which, as our findings suggest, may influence the growth of the amygdala during adolescence. We found no association between the unemotional subscale and either externalizing behaviors or amygdala volume, suggesting that the unemotional subscale of the ICU may fail to capture the affective deficits underlying CU traits. This could be due to poor psychometric properties of the scale, such as poor internal reliability (α = 0.58 in our sample) and small correlations with total ICU scores. Alternatively, unemotionality as a construct may fail to capture the nature of affective deficits underlying CU traits, which are not uniformly associated with deficits in all aspects of emotion (Cardinale et al., 2018). Whereas high CU youths frequently report and exhibit decreased experience of fear (Kimonis et al., 2008a; Muñoz et al., 2008; Jones et al., 2010; Marsh et al., 2011b), reports and experiences of, for example, disgust and happiness may be relatively unaffected (Marsh and Blair, 2008; Marsh et al., 2011b; Dawel et al., 2012).

The current study is limited in its ability to draw causal conclusions regarding reduced amygdala volume and the emergence of CU traits and externalizing behavior problems due to the cross-sectional design of this study. Future longitudinal work assessing amygdala volume at various stages in childhood, as well as the trajectory of CU traits and externalizing behavior problems across childhood, adolescence, and into adulthood, would better allow for a more direct investigation of the causal role of the amygdala in the development of CU traits and externalizing behavior. In addition, the current study is limited in that it only investigated associations with subcortical structure. Previous work has linked CU traits to structural abnormalities in cortical regions such as the ventromedial prefrontal cortex, insula, and anterior cingulate cortex (Wallace et al., 2014; Sebastian et al., 2016). As such, future investigation of the association between dimensionally assessed CU traits and subsequent externalizing behaviors with measures of cortical thickness, surface area, and curvature using surface-based methods is necessary to acquire a full understanding of neuroanatomical deficits underlying the development of CU traits.

Despite this limitation, these findings provide the first evidence for volumetric abnormalities in the amygdala associated with CU traits in childhood and adolescence. Our study, along with findings linking psychopathy and decreased amygdala volume in adulthood (Pardini et al., 2014), supports theories that CU traits reflect underlying neuroanatomical deficits during development (Blair et al., 2006; Blair, 2013) and provides the framework for further investigation of abnormal amygdala growth as a key causal pathway for the development of CU traits and conduct problems. This has implications for the potential identification of biomarkers for CU traits early in development and suggests that early interventions aimed at fostering healthy amygdala development may reduce the emergence of CU traits and conduct problems in youths.

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