Journal of Clinical Child & Adolescent Psychology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/hcap20

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Published online: 11 Oct 2013.

To cite this article: Whitney A. Brammer & Steve S. Lee (2013) Prosociality and Negative Emotionality Mediate the Association of Serotonin Transporter Genotype With Childhood ADHD and ODD, Journal of Clinical Child & Adolescent Psychology, 42:6, 809-819, DOI: 10.1080/15374416.2013.840638

To link to this article: http://dx.doi.org/10.1080/15374416.2013.840638

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Prosociality and Negative Emotionality Mediate the Association of Serotonin Transporter Genotype With Childhood ADHD and ODD

Whitney A. Brammer and Steve S. Lee

Department of Psychology, University of California, Los Angeles

Although there is evidence that the promoter polymorphism of the serotonin transporter (5-HTTLPR) gene is associated with attention-deficit/hyperactivity disorder (ADHD) and oppositional defiant disorder (ODD), the pathways underlying these associations are largely unknown. Given their theoretical and biological plausibility, we tested whether individual differences in key temperament dimensions (i.e., prosociality, negative emotionality, daring) constituted potential pathways from 5-HTTLPR to ADHD and ODD. Using a well-characterized sample of 194 six to nine-year-old children with and without ADHD, we utilized multiple mediation procedures with bootstrapping to evaluate prosociality, negative emotionality, and daring as independent mediators of 5-HTTLPR with separate parent and teacher ratings of ADHD and ODD. Controlling for ODD, prosociality and negative emotionality significantly mediated the association of 5-HTTLPR and parent-reported ADHD. Similarly, controlling for ADHD, prosociality and negative emotionality each uniquely mediated the association of 5-HTTLPR and parent-reported ODD. For teacher-reported ADHD, prosociality significantly mediated the association of 5-HTTLPR (controlling for ODD) whereas controlling for ODD, negative emotionality significantly mediated the prediction of teacher-reported ODD from 5-HTTLPR. Specifically, the number of 5-HTTLPR long alleles was inversely associated with prosociality and positively associated with negative emotionality; prosociality was inversely associated and negative emotionality was positively associated with ADHD and ODD. We consider the role of temperament in genetically sensitive designs as well as its potential value in the development and delivery of effective interventions.
Influences are more salient to ODD whereas shared environmental influences whereas shared environmental influences are more salient to ODD/CD (Burton, 2009). In sum, because ADHD and ODD reflect distinct causal influences, tests of their underlying genetic influences must properly consider their frequent comorbidity.

One biomarker implicated in the etiology of ADHD and ODD is the promoter polymorphism of the serotonin transporter (5-HTTLPR) gene. A comprehensive review found that 5-HTTLPR was significantly associated with ADHD (Faraone et al., 2005), and a recent meta-analysis specified that the long allele increased susceptibility to ADHD (Gizer et al., 2002; Munafo, Brown, & Hariri, 2008). Although 5-HTTLPR is a biologically plausible biomarker for ADHD and ODD, the factors that potentiate genetic variation into these disorders are poorly understood. That is, theoretically and biologically plausible mediating factors underlying the association of 5-HTTLPR and psychopathology are crucial and largely unknown (Rutter, 2006). Further, the use of putative endophenotypes (i.e., heritable intermediate phenotypes more proximal to the biological basis of the syndrome) may improve statistical power because they are less complex (i.e., fewer genetic and environmental influences) than explicit disorders (Doyle et al., 2005; Gottesman & Gould, 2003).

Dimensions of temperament may be an appropriate endophenotype for ADHD and ODD given that they are heritable, developmentally stable, and prospectively predict ADHD, ODD, and many dimensions of child psychopathology (Bates et al., 1998; Kelvin et al., 1996; Lahey et al., 2008; Saudino, 2005). Temperament refers to individual differences in reactivity and self-regulation that are expressed via emotionality, motor activity, and attention (Rothbart & Derryberry, 1981). Latent variable modeling revealed that early novelty-seeking presaged a lifetime diagnosis of ADHD (Lynn et al., 2005), and teacher-rated negative emotionality predicted parent-rated ADHD severity in preschool children (Healey et al., 2011); moreover, age 3 fear inhibition inversely predicted adult psychopathic personality (Glenn et al., 2007). Temperament models vary widely, but three key facets were identified as part of a developmental propensity model of child psychopathology: prosociality (i.e., “sympathetic concern of others, helping and sharing, respect for social rules, and guilt over misdeeds”), negative emotionality (i.e., “easily and intensely upset by frustrations, threats, and losses”), and daring (i.e., “daring, brave, and adventurous,” and “sensation-seeking”; Lahey et al., 2008, p. 795). Notably, these dimensions were developed and validated for explicit use in studies of psychopathology based on items that excluded clear synonyms and antonyms of psychopathology symptoms. For example, parent ratings of early individual differences in children’s prosociality (low), negative emotionality (high), and daring (high) prospectively predicted parent-reported ODD and CD (Lahey et al., 2008). In a later cross-validation study, youth selfreported prosociality, negative emotionality, and daring similarly predicted parent- and youth-reported CD (Lahey et al., 2008). Moreover, all three dimensions were associated with a latent antisocial factor derived from official court records, CD symptoms, and self-reported delinquency, controlling for initial conduct problems (Trentacosta et al., 2009). Given their favorable psychometric properties and robust associations with ADHD and ODD, there is a strong rationale for using these temperament facets as mediators of psychopathology.

Consistent with its hypothesized mediational role, individual differences in temperament are sensitive to...
null mutation (–/–). First, infants homozygous for the 5-HTTLPR short allele exhibited elevated negative emotionality and distress at 2 months of age (Auerbach et al., 1999). Elevated negative emotionality and diminished positive emotionality were observed in children with at least one short allele of 5-HTTLPR relative to children without the short allele or children with high positive emotionality (Hayden et al., 2010) and healthy adults homozygous for the 5-HTTLPR short allele exhibited more prosocial empathic responding as well as higher sympathetic nervous system activation (i.e., cardiovascular and electrodermal activity) when they viewed others in distress relative to adults homozygous for the 5-HTTLPR long allele (Gyurak et al., 2013). The moderating role of 5-HTTLPR was identified, as positive maternal expressed emotions predicted youth conduct problems but only in youth with one or more positive maternal expressed emotions predicted youth prosociality and negatively predicted ADHD and ODD, whereas negative emotionality and daring would inversely predict prosociality and positively predict ADHD and ODD.

METHOD

Participants

One hundred ninety-four 6- to 9-year-old (M = 7.4, SD = 1.2) children with (n = 101) and without ADHD (n = 93) were recruited using advertisements at local schools and in public locations as well as referrals from local mental health service providers and pediatric offices (Table 1). The sample was ethnically diverse: 55.7% Caucasian (n = 108), 8.8% Hispanic (n = 17), 8.8% African American (n = 17), 3.1% Asian (n = 6), and 23.7% (n = 46) Mixed/Other. Participants were required to live with at least one biological parent at least half time, be enrolled in school full-time, and be fluent in English. Exclusion criteria for all participants included a Full Scale IQ less than 70 or an autism spectrum, seizure, or any neurological disorder. ADHD proband status was based on a positive diagnosis according to a structured diagnostic interview that ascertained all relevant diagnostic criteria (see below for details on clinical assessment procedures). To avoid recruiting a sample of improbably high-functioning youth, non-ADHD comparison children were allowed to meet diagnostic criteria for any disorder other than ADHD. All participants were recruited, screened, and assessed using identical procedures.

Procedures

Families who contacted the study completed a telephone screener to determine their eligibility based on the inclusion and exclusion criteria just listed. Families and teachers received rating scales through the mail, and families were invited to the research laboratory for in-person assessments. Following signed consent and assent procedures for the parent and child, respectively,

TABLE 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>ADHDa</th>
<th>Non-ADHDb</th>
<th>F/v²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (SD)</td>
<td>7.32 (1.13)</td>
<td>7.44 (1.07)</td>
<td>0.61</td>
<td>.43</td>
</tr>
<tr>
<td>% Male</td>
<td>72.00</td>
<td>65.59</td>
<td>0.92</td>
<td>.34</td>
</tr>
<tr>
<td>% Caucasian</td>
<td>54.46</td>
<td>56.99</td>
<td>0.13</td>
<td>.72</td>
</tr>
<tr>
<td>% Household</td>
<td>33.66</td>
<td>30.10</td>
<td>1.09</td>
<td>.30</td>
</tr>
<tr>
<td>Income &lt; $70,000</td>
<td>105.02 (14.61)</td>
<td>108.76 (16.04)</td>
<td>2.82</td>
<td>.10</td>
</tr>
<tr>
<td>WISC-IV FSIQ (SD)</td>
<td>6.24 (5.23)</td>
<td>4.36 (5.16)</td>
<td>5.25</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Total Teacher-Rated</td>
<td>46.53</td>
<td>9.68</td>
<td>37.97</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>ADHD Symptoms</td>
<td>3.25 (2.40)</td>
<td>.96 (1.72)</td>
<td>67.91</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>% ODD Diagnosis</td>
<td>1.34 (2.34)</td>
<td>.79 (1.76)</td>
<td>2.82</td>
<td>.10</td>
</tr>
<tr>
<td>% CD Diagnosis</td>
<td>6.93</td>
<td>0</td>
<td>6.85</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

Note: Mean differences evaluated by one-way analysis of variance or chi-square. ADHD = attention deficit/hyperactivity disorder; WISC-IV = Wechsler Intelligence Scale for Children-Fourth Edition; FSIQ = Full-Scale IQ; ODD = oppositional defiant disorder; CD = conduct disorder.

a n = 101.

b n = 93.
clinical psychology graduate students or B.A.-level trained staff assessed children using tests of cognitive ability and academic achievement, whereas a second trained member of our research staff interviewed parents about their child’s psychopathology. All interviewers were initially blind to the child’s diagnostic status, but the blind could not always be maintained given the extensive information collected about the child. Parents were asked to rate each child based on his or her undemicated behavior. Approximately 85% of children were evaluated without their medication during the in-person assessment. The Institutional Review Board approved all study procedures.

Measures

Genotype. DNA was extracted from saliva using DNA Genotek Oragene Self-Collection Kits (DNA Genotek, Inc., Ottawa, CA). The 48-base pair (bp) insertion/deletion polymorphism in the promoter region of 5-HTTLPR was genotyped using standard primers, which produced fragments of either 484 or 528 bp (Heils et al., 1996). The long variant (528 bp) has approximately 3 times the activity of the shorter promoter (484 bp) with the deletion (Lesch et al., 1996). This was the only genetic variant tested in this study. Because the precise mode of transmission for 5-HTTLPR is unknown (i.e., dominant, recessive, heterosis), we separately compared all three genotypes with the following distribution: long/long (30%, n = 60), short/long (45%, n = 88), and short/short (25%, n = 46). These frequencies did not deviate from Hardy-Weinberg equilibrium, \( \chi^2(1) = 1.5, p = .22 \).

Temperament. The Child and Adolescent Dispositions Scale is a parent interview of children’s temperament with items that were explicitly developed for studies of psychopathology by excluding clear synonyms or antonyms of psychiatric symptoms (Lahey et al., 2008). Parents used a 4-point Likert scale to rate 48 items that yielded three factors: prosociality, negative emotionality, and daring. Previous studies reported good internal consistency, high test–retest reliability, construct validity, and external validity (Lahey et al., 2008; Trentacosta et al., 2009). In this sample, the Cronbach alpha was .87, .77, and .79 for the prosociality, negative emotionality, and daring factors, respectively.

ADHD and ODD. We administered ADHD and ODD modules from the Diagnostic Interview Schedule for Children, fourth edition (DISC-IV) to each child’s parent (Shaffer, Fisher, Lucas, Dulcan & Schwab-Stone, 2000). The DISC-IV is extensively validated and psychometrically sound. In the DSM-IV Field Trials, test–retest reliability for ADHD from the DISC ranged from .51 to .64 (Lahey et al., 1994). Forty-two percent (n = 51) of ADHD probands were diagnosed with the Inattentive type, 11% (n = 13) with the Hyperactive type, and 47% (n = 57) as Combined type. However, given that the predictive validity of dimensionally rated ADHD and ODD was superior to diagnoses (Fergusson & Horwood, 1995), we used the total number of ADHD and ODD symptoms from the DISC-IV as separate dependent variables. The number of ADHD and ODD symptoms were significantly correlated \( r = .58, p < .001 \) as were the number of inattention and hyperactivity symptoms \( r = .58, p < .001 \). The Cronbach alpha was .91 and .84 for the total number of ADHD and ODD symptoms, respectively.

Teachers completed the Disruptive Behavior Disorder Rating Scale (Pelham, Gnagy, Greenslade, & Milich, 1992) and rated 45 items keyed to DSM-IV ADHD, ODD, and CD from “not at all” to “very much.” Items rated as “pretty much” or “very much” were scored as a symptom (Lahey et al., 1994). The Disruptive Behavior Disorder has good psychometric properties (e.g., predictive validity; Lahey et al., 2004). Cronbach alphas were .93 for ADHD and .90 for ODD in this study.

Data Analytic Procedures

We assessed whether individual differences in prosociality, negative emotionality, and daring mediated the association of 5-HTTLPR with separate parent and teacher ratings of the number of ADHD and ODD symptoms. We used a multiple mediator macro (Preacher & Hayes, 2008) with bootstrapping, a statistically powerful nonparametric resampling procedure (MacKinnon, Krull, & Lockwood, 2000). Bootstrapping samples from a data set \( k \) number of times and uses percentages of those distributions to calculate confidence intervals and estimate the indirect effect of mediators. Unlike traditional mediation (Baron & Kenny, 1986), recent developments illustrate that mediation does not require a significant direct effect of the predictor on the outcome (MacKinnon et al., 2000; Preacher & Hayes, 2008). Reflecting a “stage sequence” framework (Collins, Graham, & Flaherty, 1998), the predictor first influences the mediator, followed by an effect on the outcome; thus, evaluation of causal mediation through bootstrapping is not contingent upon a significant direct effect (Zhao, Lynch, & Chen, 2010), especially when the effect size of the predictor is modest, as in most genetic association studies (Shrout & Bolger, 2002). Finally, multiple mediation based on bootstrapping is more powerful than traditional approaches (e.g., Sobel test; Zhao et al., 2010).
5-HTTLPR, was coded 0, 1, and 2 for the long/long, short/long, and short/short genotypes, respectively, whereas prosociality, negative emotionality, and daring were entered simultaneously to evaluate their independent contribution. The number of ADHD and ODD symptoms constituted separate dependent variables in separate models. Because bootstrapping does not assume normality (Preacher & Hayes, 2008), the dependent variables did not require transformation. Also, the bootstrap distribution is nonsymmetrical; thus, the reported betas for the indirect effect, divided by the standard error (SE), is not equivalent to the traditional t statistic. Next, given the case-control design, and to improve specificity, we controlled ADHD diagnostic status (i.e., ADHD vs. non-ADHD control) when predicting ODD and controlled ODD diagnostic status (i.e., ODD vs. non-ODD control) when predicting ADHD. Race-ethnicity was a covariate in all models. We first present the total effect of 5-HTTLPR on ADHD and ODD excluding prosociality, negative emotionality, and daring, followed by the associations of 5-HTTLPR with temperament dimensions, ADHD, and ODD. Third, we report the direct effect of 5-HTTLPR (i.e., the effect of 5-HTTLPR with mediators included in the model) of 5-HTTLPR on ADHD and ODD, concluding with the indirect effects from the bootstrapping analyses.

RESULTS

Population Stratification

Given evidence of racial-ethnic differences in allele frequencies of 5-HTTLPR (Gelernter, Cubells, Kidd, Pakstis, & Kidd, 1999), population stratification is a threat to the internal validity of studies with multiple racial-ethnic groups. Population stratification requires that race-ethnicity is significantly associated with 5-HTTLPR, ADHD, and ODD (Hutchison, Stallings, McGearry, & Bryan, 2004). Youth race-ethnicity was significantly related to 5-HTTLPR, $\chi^2(10) = 29.004, p < .01$, but was not related to ADHD, $\chi^2(4) = 2.73, p = .61$, or ODD, $\chi^2(4) = 2.53, p = .64$. Although population stratification was not a threat in this study, race-ethnicity was controlled in all models.

Temperament as Mediators of 5-HTTLPR and ADHD

To review, we predicted that prosociality, negative emotionality, and daring would mediate the association of 5-HTTLPR with ADHD and ODD symptoms. First, controlling for ODD (Figure 1) and excluding temperament from the model, the total effect of 5-HTTLPR was unrelated to the total number of parent-rated ADHD symptoms ($B = -0.26, SE = 0.48, p = .60$) and daring

![Figure 1](https://via.placeholder.com/150)

**FIGURE 1** Multiple mediator model of the effect of 5-HTTLPR on parent-reported ADHD via temperament dimensions (beta coefficients). Note: "p ≤ .05. "*p ≤ .01. "p ≤ .10. (B) Indirect Effects

($B = -0.14, SE = 0.33, p = .67$). The number of long alleles inversely predicted prosociality ($B = 1.36, SE = 0.58, p = .02$) and marginally predicted negative emotionality ($B = -0.80, SE = 0.43, p = .07$). Second, prosociality inversely predicted ($B = -0.17, SE = 0.06, p < .01$), whereas negative emotionality positively predicted the number of ADHD symptoms ($B = 0.28, SE = 0.08, p < .001$); daring was unrelated to ADHD ($B = 0.08, SE = 0.10, p = .41$). Third, there was no significant direct effect of 5-HTTLPR on ADHD when prosociality, negative emotionality, and daring were included ($B = 0.21, SE = 0.46, p = .65$). We calculated the total and specific indirect effects of 5-HTTLPR on ADHD through negative emotionality, prosociality, and daring by using 1,000 bootstrap simulation samples, yielding point estimates and the $95\%$ bias corrected and accelerated (BCa) confidence intervals for each indirect effect. The total indirect effect (i.e., point estimate of the difference between the total effect and direct effect through the three mediators) significantly differed from zero (Table 2), such that prosociality and negative emotionality, but not daring, significantly mediated the association of 5-HTTLPR and the total number of ADHD (Table 2).

We then reproduced the identical models just presented but examined teacher ratings of ADHD symptoms. First, controlling for teacher-rated ODD (Figure 2) and excluding temperament from the model, the total effect of 5-HTTLPR was unrelated to the total number of teacher-rated ADHD symptoms ($B = -0.30, SE = 0.55, p = .59$) and daring ($B = -0.18, SE = 0.36, p = .62$). However, the number of long alleles inversely predicted prosociality ($B = 1.86, SE = 0.71, p = .01$) and positively predicted negative emotionality ($B = -1.39, SE = 0.59, p = .02$). Second, prosociality inversely predicted the number of teacher-rated ADHD
TABLE 2
Mediation by Prosociality, Negative Emotionality, and Daring on 5-HTTLPR With Total ADHD Symptoms, Inattention Symptoms, Hyperactivity Symptoms, and ODD Symptoms

<table>
<thead>
<tr>
<th>Point Est.</th>
<th>SE</th>
<th>95% BCa Bootstrap CI Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Parent-Rated ADHD Symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prosociality</td>
<td>-23</td>
<td>.14</td>
<td>-.59</td>
</tr>
<tr>
<td>Negative Emotionality</td>
<td>-22</td>
<td>.14</td>
<td>-.62</td>
</tr>
<tr>
<td>Daring</td>
<td>-01</td>
<td>.04</td>
<td>-.14</td>
</tr>
<tr>
<td>Total</td>
<td>-47</td>
<td>.20</td>
<td>-.92</td>
</tr>
<tr>
<td>Total Teacher-Rated ADHD Symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prosociality</td>
<td>-.35</td>
<td>.19</td>
<td>-.94</td>
</tr>
<tr>
<td>Negative Emotionality</td>
<td>-03</td>
<td>.12</td>
<td>-.32</td>
</tr>
<tr>
<td>Daring</td>
<td>-02</td>
<td>.07</td>
<td>-.30</td>
</tr>
<tr>
<td>Total</td>
<td>-41</td>
<td>.21</td>
<td>-.94</td>
</tr>
<tr>
<td>Parent-Rated Inattention Symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prosociality</td>
<td>-13</td>
<td>.08</td>
<td>-.37</td>
</tr>
<tr>
<td>Negative Emotionality</td>
<td>-10</td>
<td>.07</td>
<td>-.29</td>
</tr>
<tr>
<td>Daring</td>
<td>.003</td>
<td>.02</td>
<td>-.03</td>
</tr>
<tr>
<td>Total</td>
<td>-.24</td>
<td>.11</td>
<td>-.51</td>
</tr>
<tr>
<td>Parent-Rated Hyperactivity Symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prosociality</td>
<td>-10</td>
<td>.07</td>
<td>-.28</td>
</tr>
<tr>
<td>Negative Emotionality</td>
<td>-12</td>
<td>.08</td>
<td>-.30</td>
</tr>
<tr>
<td>Daring</td>
<td>-01</td>
<td>.04</td>
<td>-.11</td>
</tr>
<tr>
<td>Total</td>
<td>-.23</td>
<td>.11</td>
<td>-.46</td>
</tr>
<tr>
<td>Total Parent-Rated ODD Symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prosociality</td>
<td>-.06</td>
<td>.04</td>
<td>-.18</td>
</tr>
<tr>
<td>Negative Emotionality</td>
<td>-31</td>
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<td>-.56</td>
</tr>
<tr>
<td>Daring</td>
<td>-.03</td>
<td>.04</td>
<td>-.12</td>
</tr>
<tr>
<td>Total</td>
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<td>.14</td>
<td>-.70</td>
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<tr>
<td>Total Teacher-Rated ODD Symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prosociality</td>
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<td>.05</td>
<td>-.14</td>
</tr>
<tr>
<td>Negative Emotionality</td>
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<td>-.44</td>
</tr>
<tr>
<td>Daring</td>
<td>-.02</td>
<td>.05</td>
<td>-.16</td>
</tr>
<tr>
<td>Total</td>
<td>-.21</td>
<td>.11</td>
<td>-.50</td>
</tr>
</tbody>
</table>

Note: Models predicting attention-deficit/hyperactivity disorder (ADHD) controlled for ODD diagnostic status whereas models predicting oppositional defiant disorder (ODD) controlled for ADHD diagnostic status. All models controlled for race-ethnicity. BCa bootstrap CI = bias corrected and accelerated confidence intervals; Point est. = point estimate of the indirect effect. Boldface indicates significant mediation.

The role of prosociality, negative emotionality, and daring was sensitive when inattention and hyperactivity symptoms were examined separately. Using the identical data analytic approach just described, with control of ODD and excluding the mediators from the model, 5-HTTLPR was unrelated to inattention symptoms ($B = -0.09, SE = 0.30, p = .77$). The number of 5-HTTLPR long alleles inversely predicted prosociality ($B = 1.36, SE = 0.58, p = .02$), marginally predicted negative emotionality ($B = -0.80, SE = 0.43, p = .07$), but was unrelated to daring ($B = -0.14, SE = 0.33, p = .67$). Second, prosociality inversely predicted ($B = -0.10, SE = 0.04, p = .01$) and negative emotionality positively predicted the number of inattention symptoms ($B = 0.13, SE = 0.05, p < .01$), whereas daring was unrelated to inattention ($B = -0.02, SE = 0.06, p = .75$). Third, the direct effect of 5-HTTLPR on inattention when including mediators in the model was not significant ($B = 0.15, SE = 0.30, p = .63$). Bootstrapping analyses revealed the total indirect effect of 5-HTTLPR on inattention did differ from zero, such that prosociality and negative emotionality were indicated to each significantly mediate the association of 5-HTTLPR and inattention whereas daring did not (Table 2).

We then tested the association of 5-HTTLPR, prosociality, negative emotionality, and daring with the number of hyperactivity symptoms, again controlling for ODD. First, the total effect of 5-HTTLPR was unrelated to hyperactivity with the mediators excluded ($B = -0.17, SE = 0.25, p = .51$). 5-HTTLPR significantly predicted prosociality ($B = 1.36, SE = 0.58, p = .02$) and marginally predicted negative emotionality ($B = -0.80, SE = 0.43, p = .07$) but was unrelated to daring ($B = -0.14, SE = 0.33, p = .67$). Specifically, the number of long alleles inversely predicted prosociality and

![Figure 2](image-url)
positively predicted negative emotionality. Second, whereas prosociality was inversely related to hyperactivity \((B = -0.07, \ SE = 0.03, \ p = 0.2)\), negative emotionality and daring were each positively associated with hyperactivity \((B = 0.15, \ SE = 0.04, \ p < .001 \) and \(B = 0.10, \ SE = 0.05, \ p < .05\), respectively). Third, the direct effect of 5-HTTLPR on hyperactivity with mediators included in the model was not significant \((B = 0.07, \ SE = 0.24, \ p = .78)\). Bootstrapping analyses revealed the point estimate of total indirect effect of 5-HTTLPR on hyperactivity differed significantly from zero. Prosociality and negative emotionality, but not daring, each independently mediated the influence of 5-HTTLPR on hyperactivity (Table 2).

We also tested how temperament mediated the effect of 5-HTTLPR with respect to separate teacher ratings of inattention and hyperactivity. Results indicated that prosociality, but neither negative emotionality nor daring, significantly mediated the effect of 5-HTTLPR on both teacher-rated inattention and hyperactivity. These findings converge with the model predicting teacher-rated ADHD but diverge from the two models predicting parent-rated inattention and hyperactivity, such that these latter two models concluded that prosociality in addition negative emotionality mediated the effect of 5-HTTLPR on inattention and hyperactivity. The results of these analyses are available upon request.

Temperament Mediators of 5-HTTLPR and ODD

To address the specificity of prosociality, negative emotionality, and daring as mediators of 5-HTTLPR and parent ratings of ODD, we used the same data analytic approach just described (Figure 3). First, controlling for ADHD and excluding the temperament dimensions, 5-HTTLPR was significantly related to the number of parent-rated ODD symptoms \((B = -0.45, \ SE = 0.21, \ p = .03)\). The number of long alleles again inversely predicted prosociality \((B = 1.67, \ SE = 0.58, \ p < .01)\) and positively predicted negative emotionality \((B = -1.37, \ SE = 0.51, \ p < .01)\) but was unrelated to daring \((B = -0.24, \ SE = 0.34, \ p = .48)\). Second, both negative emotionality \((B = 0.23, \ SE = 0.02, \ p < .0001)\) and daring \((B = 0.11, \ SE = 0.04, \ p < .01)\) significantly predicted the number of ODD symptoms, whereas prosociality marginally inversely predicted ODD \((B = -0.03, \ SE = 0.02, \ p = .10)\). Third, there was no direct effect of 5-HTTLPR on ODD with temperament facets included \((B = -0.06, \ SE = 0.17, \ p = .73)\). Last, bootstrapping analyses estimated that the total indirect effect of 5-HTTLPR on ODD was significantly mediated by prosociality and negative emotionality but not daring (Table 2).

Finally, controlling for teacher-rated ADHD, using the previous data analytic approach, and excluding the mediators from the model (Figure 4), 5-HTTLPR was unrelated to teacher-rated ODD symptoms \((B = -0.04, \ SE = 0.22, \ p = .87)\). The number of 5-HTTLPR long alleles inversely predicted prosociality \((B = 1.85, \ SE = 0.69, \ p < .01)\) and positively predicted negative emotionality \((B = -1.43, \ SE = 0.62, \ p = .02)\), but it was unrelated to daring \((B = -0.20, \ SE = 0.37, \ p = .59)\). Second, negatively emotionality \((B = 0.12, \ SE = 0.03, \ p < .001)\) and daring \((B = 0.12, \ SE = 0.05, \ p = .01)\), but not prosociality \((B = -0.01, \ SE = 0.03, \ p = .84)\), positively predicted the number of teacher-rated ODD symptoms. Third, there was no significant direct effect of 5-HTTLPR on ODD with inclusion of the mediators \((B = 0.17, \ SE = 0.21, \ p = .42)\). Bootstrapping analyses indicated that negative emotionality, but not prosociality.
and daring, significantly mediated the prediction of teacher-reported ODD symptoms from 5-HTTLPR (Table 2).

**DISCUSSION**

There is limited knowledge about the factors through which genetic variation contributes to childhood ADHD and ODD. We evaluated prosociality, negative emotionality, and daring as mediators of the association of 5-HTTLPR with separate parent and teacher ratings of ADHD and ODD using bootstrapping procedures within a multiple mediation framework. Among 6- to 9-year-old children with and without ADHD, the number of long alleles of 5-HTTLPR inversely predicted prosociality and positively predicted negative emotionality, but it was unrelated to daring. Controlling for ODD, prosociality inversely predicted whereas negative emotionality positively predicted parent-reported ADHD symptoms and separate counts of inattention and hyperactivity symptoms; daring positively predicted hyperactivity but not inattention. Prosociality inversely predicted teacher reports of ADHD symptoms, whereas negative emotionality and daring did not. Second, controlling for ADHD, negative emotionality and daring each positively predicted parent and teacher reports of ODD symptoms whereas prosociality marginally inversely predicted parent-reported ODD symptoms. 5-HTTLPR was not significantly associated with ADHD and ODD, which is consistent with expectations of an endophenotype model. Finally, prosociality and negative emotionality significantly mediated the association of 5-HTTLPR with parent ratings of ADHD (controlling for ODD) and with ODD (controlling for ADHD). In addition, prosociality significantly mediated the association of 5-HTTLPR with teacher ratings of ADHD (controlling for ODD), whereas negative emotionality significantly mediated the association of 5-HTTLPR with teacher ratings of ODD (controlling for ADHD). These findings suggest that individual differences in prosociality and negative emotionality are important pathways from 5-HTTLPR to individual differences in ADHD and ODD. Moreover, both parents and teachers agree in reporting prosociality and negative emotionality as significant mediators of the effect of 5-HTTLPR on ADHD and ODD, respectively. These findings are also noteworthy given that the temperament items did not contain language with obvious overlap with ADHD and ODD.

Perhaps reflecting the highly dispersed neurobiological sequelae associated with 5-HTTLPR variation, including elevated amygdala activation, 5-HTTLPR also has pleiotropic effects across many forms of psychopathology (e.g., depression, anxiety; H. Clarke, Flint, Attwood, & Munafò, 2010; Munafò et al., 2008; Munafò et al., 2009). However, the association of 5-HTTLPR with multiple dimensions of psychopathology may be anchored in several shared endophenotypes, including temperament. Prosociality and negative emotionality each predicted impulsivity and conduct problems in an ethnically diverse sample of adolescents, even after controlling for baseline levels (Trentacosta et al., 2009). Temperament dimensions, then, may represent one nonspecific pathway from an underlying common genetic predisposition to ultimate youth externalizing behavior. Given the pleiotropic effects associated with 5-HTTLPR variation, future research should explore whether prosociality, negative emotionality, and daring similarly mediate pathways from 5-HTTLPR to other clinical outcomes across both externalizing and internalizing domains (e.g., depression, anxiety), including their comorbidity.

Although there is replicated evidence that the short allele of 5-HTTLPR affects certain dimensions of temperament (e.g., prosociality, negative and positive emotionality; Gyurak et al., 2013; Hayden et al., 2010), the 5-HTTLPR long allele has also been associated with ADHD and ODD (Gizer et al., 2009; Nobile et al., 2007). Despite the reliable association of temperament with ADHD and ODD (Healey et al., 2011; Lahey et al., 2008), it is unclear which 5-HTTLPR alleles contribute to their development. One possible explanation of the association of the short allele with temperament and the long allele with psychopathology is allele flipping, or positive associations being found between opposite alleles (e.g., short and long alleles of 5-HTTLPR; G. M. Clarke & Cardon, 2010). Allele flipping is increasingly common, leading to investigative efforts to assess which allele is genuinely related to disorders of interest (G. M. Clarke & Cardon, 2010). Another rationale for inconsistent associations of the long versus short allele is the contribution of unmeasured environmental variation that may moderate genetic influences on psychopathology. Differential susceptibility contends that some genetic factors enhance sensitivity to both environmental adversity and enrichment simultaneously, and thus the genotypes that confer plasticity to both positive and negative environments might play a role in the discrepant findings of the long and short alleles with phenotypes (Belsky et al., 2007). As such, although these results support for the role of the long allele of 5-HTTLPR in relation to ADHD and ODD, additional research is needed to clarify the functional consequences of 5-HTTLPR variation. For example, variation in 5-HTTLPR is known to affect the structure, function, and connectivity of neural structures and circuitry affecting negative emotion (e.g., amygdala, perigenual cingulate; Pezawas et al., 2005). Further, the connectivity of this neural circuit was associated with temperamental anxiety (Pezawas et al.,
suggesting that 5-HTTLPR may affect temperament through the neural circuitry thought to underlie emotion regulation. Other environmental factors that influence the pathway from 5-HTTLPR to ADHD and/or ODD through temperament should also be investigated. For example, it is plausible that other environmental conditions (e.g., parental psychopathology, parenting behavior) might interact with 5-HTTLPR in differentiating whether certain youth develop ADHD and/or ODD. Accordingly, exploring these moderators could facilitate the identification of more specific independent pathways from 5-HTTLPR to ADHD versus ODD.

This study also suggests that prosociality and negative emotionality are potentially important influences on ADHD and ODD. Given that temperament is generally stable, reliably measured, and evident early in development, these findings suggest that temperament may usefully facilitate early identification to interrupt pathways to negative outcomes and to promote positive outcomes (Kelvin et al., 1996; Lahey et al., 2010). Specifically, to foster resilience, early assessment of prosociality and negative emotionality may be used to tailor interventions to children. For example, parenting practices (i.e., limit-setting, involvement) inversely predicted child conduct problems only for youth with certain temperament dimensions (i.e., highly resistant to control), controlling for baseline conduct problems, parenting behaviors, gender, and ethnicity (Goodnight, Bates, Pettit, & Dodge, 2008). In a randomized trial, temperament (e.g., activity/flexibility, and mood/sociability) moderated response to psychosocial interventions for adolescent substance use disorders (Kaminer & Burleson, 2008). Thus, further research should identify whether prosociality and negative emotionality moderate the efficacy of available interventions for children with ADHD and ODD to optimize tailored treatments.

Several study limitations should be considered when appraising these findings. First, these are cross-sectional data, which prevents temporal ordering of predictors, mediators, and outcomes, a requirement for inferences of causal mediation (Kraemer, Stice, Kazdin, Offord, & Kupfer, 2001). However, given the scarcity of mediational models in psychiatric genetics and because 5-HTTLPR clearly precedes the development of temperament, ADHD, and ODD, we contend that these preliminary results are temporally sequenced. Future investigations should utilize prospective longitudinal designs to more robustly delineate the timing of key constructs (Maxwell & Cole, 2007). Second, 5-HTTLPR effects in this study assumed linear effects, although alternative models of transmission should be evaluated directly (e.g., heterosis). Third, given that rs25531 was not genotyped in this study, we cannot be certain of the functionality of the long allele (Greenberg et al., 1999; Nobile et al., 1999).

The current study utilized multiple mediation to evaluate whether individual differences in temperament dimensions significantly mediated predictions of parent and teacher ratings of ADHD and ODD from 5-HTTLPR. Prosociality and negative emotionality emerged as important endophenotypes from 5-HTTLPR in the development of ADHD and ODD. Specifically, the number of long alleles was consistently inversely associated with prosociality and positively associated with negative emotionality; in turn, prosociality was inversely associated with whereas negative emotionality was positively associated with parent and teacher ratings of ADHD and ODD, respectively. Moreover, childhood prosociality and negative emotionality mediated trajectories from 5-HTTLPR to parent-reported ADHD (controlling for ODD) and ODD (controlling for ADHD), whereas prosociality and negative emotionality mediated the effect of 5-HTTLPR on teacher-reported ADHD and ODD, respectively. We contend that genetically informative and developmentally sensitive designs (e.g., prospective longitudinal) will provide considerable traction on the roles of other potential endophenotypes (e.g., neural phenotypes) underlying the association of genetic variation and individual differences in child psychopathology.

REFERENCES


