Importance
Psychiatric comorbidity complicates clinical care and confounds efforts to elucidate the pathophysiology of commonly occurring symptoms in youths. To our knowledge, few studies have simultaneously assessed the effect of 2 continuously distributed traits on brain-behavior relationships in children with psychopathology.

Objective
To determine shared and unique effects of 2 major dimensions of child psychopathology, irritability and anxiety, on neural responses to facial emotions during functional magnetic resonance imaging.

Design, Setting, and Participants
Cross-sectional functional magnetic resonance imaging study in a large, well-characterized clinical sample at a research clinic at the National Institute of Mental Health. The referred sample included youths ages 8 to 17 years, 93 youths with anxiety, disruptive mood dysregulation, and/or attention-deficit/hyperactivity disorders and 22 healthy youths.

Main Outcomes and Measures
The child's irritability and anxiety were rated by both parent and child on the Affective Reactivity Index and Screen for Child Anxiety Related Disorders, respectively. Using functional magnetic resonance imaging, neural response was measured across the brain during gender labeling of varying intensities of angry, happy, or fearful face emotions. In mixed-effects analyses, the shared and unique effects of irritability and anxiety were tested on amygdala functional connectivity and activation to face emotions.

Results
The mean (SD) age of participants was 13.2 (2.6) years; of the 115 included, 64 were male. Irritability and/or anxiety influenced amygdala connectivity to the prefrontal and temporal cortex. Specifically, irritability and anxiety jointly influenced left amygdala to left medial prefrontal cortex connectivity during face emotion viewing ($F_{2.888} = 9.20; P < .001$ for mixed model term). During viewing of intensely angry faces, decreased connectivity was associated with high levels of both anxiety and irritability, whereas increased connectivity was associated with high levels of anxiety but low levels of irritability ($\chi^2 = 21.3; P < .001$ for contrast). Irritability was associated with differences in neural response to face emotions in several areas ($F_{2.888} = 13.45; all P < .001$). This primarily occurred in the ventral visual areas, with a positive association to angry and happy faces relative to fearful faces.

Conclusions and Relevance
These data extend prior work conducted in youths with irritability or anxiety alone and suggest that research may miss important findings if the pathophysiology of irritability and anxiety are studied in isolation. Decreased amygdala-medial prefrontal cortex connectivity may mediate emotion dysregulation when very anxious and irritable youth process threat-related faces. Activation in the ventral visual circuitry suggests a mechanism through which signals of social approach (ie, happy and angry expressions) may capture attention in irritable youth.

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The Research Domain Criteria (RDoC) framework calls for studies examining the neural circuitry of dimensional traits across diagnoses. Such studies are particularly important in children, who typically present with impairment due to symptoms spanning multiple diagnoses and dimensions. Thus, while previous studies have examined the neural circuitry mediating single symptom dimensions, it is important to extend this work by examining interactions among 2 or more commonly co-occurring traits. Here, we test the hypothesis that dimensional variation in irritability and anxiety jointly influence the neural circuitry of face emotion processing.

Irritability and anxiety are 2 of the most common, frequently co-occurring problems of youth seeking psychiatric care. Research reveals strong clinical and pathophysiological associations between them. Longitudinal studies have shown that childhood irritability predicts the risk for anxiety in adulthood, whereas cognitive studies have found similar attention biases in youths with irritability and anxiety. However, virtually no research has considered how anxiety and irritability independently and mutually predict brain function.

Independent lines of research have linked irritability and anxiety to perturbed amygdala–prefrontal cortex (PFC) circuitry function during face emotion processing. Here, we used a common face emotion viewing paradigm to examine amygdala–PFC engagement to graded levels of specific face emotions. In addition to examining regional changes in neural activity, we assessed task-associated changes in amygdala connectivity. Irritability-related constructs have been associated with reduced functional connectivity between the amygdala and regulatory regions of the prefrontal cortex at rest in adults with high trait anger and during angry face emotion processing in adults with intermittent explosive disorder. Childhood generalized anxiety disorder, social phobia, separation anxiety disorder, and behavioral inhibition have been associated with disrupted amygdala–PFC functional connectivity, assessed while participants process face emotions. Indeed, one study in adult men found that high trait anxiety and anger interact to predict amygdala response to angry faces. However, to our knowledge, no study in youths has examined the effect on amygdala circuitry of anxiety and irritability, as either independent or interacting variables, although these symptoms often present together.

Our approach to sampling differs from most prior brain imaging work on anxiety or irritability. Specifically, we operationalized these symptoms dimensionally and studied them in children receiving psychiatric care. This differs from 2 common approaches in the literature. Some prior studies examined children with anxiety disorders, defined categorically, or 2 categories of irritability-related disorders, bipolar disorder and severe mood dysregulation or disruptive mood dysregulation disorder. These studies did not consider how symptoms of anxiety or irritability, occurring across disorders, relate to brain function. Other studies adopted continuous approaches, typically in community-based samples. These studies rarely included large numbers of youths surpassing clinical thresholds for a disorder. To address the limitations of these prior approaches, we studied the neural correlates of dimensional measures of irritability and anxiety in youths undergoing treatment for clinically significant disorders.

In sum, we examined 115 youths with varying diagnoses and levels of anxiety and irritability using a common face emotion processing task. Based on prior studies that included either anxious or irritable youth (see also the eAppendix in the Supplement), we hypothesized that irritability and anxiety exhibit independent and interacting associations with perturbed amygdala–PFC circuitry function in response to specific face emotion displays.

Methods

Participants

The study included 115 youths aged 8 to 17 years with primary diagnoses of disruptive mood dysregulation disorder (DMDD; n = 37), anxiety disorder (ANX; n = 32), attention-deficit/hyperactivity disorder (ADHD; n = 24), or no psychopathology (healthy volunteers; n = 22) (Table 1; eTable 1 in the Supplement). Primary diagnosis reflected the chief symptom for which patients were seeking or receiving treatment. Consistent with an RDoC approach, the study recruited samples with diverse diagnoses and rich variability in symptom levels, particularly irritability and anxiety. While the chief symptom of youths with DMDD was severe irritability, they also had high rates of ANX (49%) and ADHD (84%). Because DMDD was exclusionary for the ANX or ADHD groups, patients in the latter 2 groups had low to moderate irritability. Data were obtained between November 2011 and July 2015. The National Institutes of Health institutional review board approved this study. Written consent/assent from parents/children was obtained, and youth were paid for participation.

The Affective Reactivity Index (ARI)23 and the Screen for Child Anxiety Related Disorders (SCARED)24 were used to measure irritability and anxiety, respectively. Data were collected within 60 days of scan and total scores for children and parents were averaged (see Figure 1 for distributions). See eMethods 1 in the Supplement for participant assessment and...
Table 1. Characteristics of 115 Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Descriptive Statistics</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>13.2 (2.6)</td>
<td>NA</td>
</tr>
<tr>
<td>Range</td>
<td>8-17</td>
<td>NA</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>64</td>
<td>NA</td>
</tr>
<tr>
<td>Female</td>
<td>51</td>
<td>NA</td>
</tr>
<tr>
<td>IQ, mean (SD)*</td>
<td>110.2 (13.4)</td>
<td>NA</td>
</tr>
<tr>
<td>SES, mean (SD)*</td>
<td>35.5 (18.5)</td>
<td>NA</td>
</tr>
<tr>
<td>ARI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>3.5 (2.9)</td>
<td>NA</td>
</tr>
<tr>
<td>Range</td>
<td>0-12</td>
<td>NA</td>
</tr>
<tr>
<td>SCARED</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>18.6 (12.5)</td>
<td>NA</td>
</tr>
<tr>
<td>Range</td>
<td>0-53.5</td>
<td>NA</td>
</tr>
<tr>
<td>Presenting diagnosis, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>22 (19)</td>
<td>NA</td>
</tr>
<tr>
<td>Any anxiety</td>
<td>32 (28)</td>
<td>NA</td>
</tr>
<tr>
<td>ADHD</td>
<td>24 (21)</td>
<td>NA</td>
</tr>
<tr>
<td>DMDD</td>
<td>37 (32)</td>
<td>NA</td>
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<tr>
<td>Lifetime diagnoses, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any anxiety</td>
<td>52 (45)</td>
<td>NA</td>
</tr>
<tr>
<td>ADHD</td>
<td>58 (50)</td>
<td>NA</td>
</tr>
<tr>
<td>MDD</td>
<td>7 (6)</td>
<td>NA</td>
</tr>
<tr>
<td>Medications, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSRI</td>
<td>11 (10)</td>
<td>NA</td>
</tr>
<tr>
<td>Stimulants</td>
<td>40 (35)</td>
<td>NA</td>
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<tr>
<td>SGA</td>
<td>14 (12)</td>
<td>NA</td>
</tr>
<tr>
<td>AED</td>
<td>7 (6)</td>
<td>NA</td>
</tr>
<tr>
<td>Image quality, mean (SD)</td>
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<td></td>
</tr>
<tr>
<td>Motion</td>
<td>0.077 (0.045)</td>
<td>NA</td>
</tr>
<tr>
<td>Censor fraction</td>
<td>0.029 (0.032)</td>
<td>NA</td>
</tr>
<tr>
<td>Associations*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARI and SCARED</td>
<td>r = 0.43</td>
<td>.001</td>
</tr>
<tr>
<td>ARI and age</td>
<td>r = −0.26</td>
<td>.004</td>
</tr>
<tr>
<td>ARI and IQ</td>
<td>r = 0.06</td>
<td>.56</td>
</tr>
<tr>
<td>ARI and gender</td>
<td>t = −0.19</td>
<td>.85</td>
</tr>
<tr>
<td>ARI and SES</td>
<td>r = 0.04</td>
<td>.73</td>
</tr>
<tr>
<td>ARI and motion</td>
<td>r = 0.25</td>
<td>.008</td>
</tr>
<tr>
<td>SCARED and age</td>
<td>r = −0.22</td>
<td>.02</td>
</tr>
<tr>
<td>SCARED and IQ</td>
<td>r = −0.04</td>
<td>.67</td>
</tr>
<tr>
<td>SCARED and SES</td>
<td>r = 0.10</td>
<td>.35</td>
</tr>
<tr>
<td>SCARED and gender</td>
<td>t = −3.17</td>
<td>.002</td>
</tr>
<tr>
<td>SCARED and motion</td>
<td>r = 0.01</td>
<td>.91</td>
</tr>
</tbody>
</table>

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; AED, antiepileptic drug; ARI, Affective Reactivity Index; DMDD, disruptive mood dysregulation disorder; MDD, major depressive disorder; NA, not applicable; SCARED, Screen for Child Anxiety Related Disorders; SES, socioeconomic status; SGA, second-generation antipsychotic; SSRI, selective serotonin reuptake inhibitor.

Table 2 in the Supplement for participants excluded owing to poor or incomplete imaging data.

**Task**
An implicit face emotion processing task was adapted from Kim et al. Participants labeled the gender of 10 actors’ happy, angry, and fearful face emotion pictures. Expressions at 50%, 100%, and 150% intensities were presented randomly for 2000 milliseconds followed by jittered fixation (mean, 1400 milliseconds; range, 500-6000 milliseconds). Trials appeared in 3 blocks, generating 30 trials of each emotion at each intensity and 90 neutral face emotion trials.

**Imaging Procedures**
Magnetic resonance images (MRI) were acquired on a General Electric 3-T scanner with a 32-channel head coil. Blood oxygen level-dependent signal was measured by echoplanar imaging at 2.5 × 2.5 × 3.0-mm voxel resolution. Standard pre-
Statistical Analyses
Analyses conducted between August 2015 and August 2016 used AFNI and R (R Foundation for Statistical Computing). Omnibus analyses used mixed-effects models in AFNI’s 3dLME for images and the R package lme4 for behavior and post hoc analyses of imaging results. The mixed model tested effects of emotion, intensity, ARI, and SCARED, with age and gender as covariates and participant as a random effect. Motion was an additional covariate in all imaging analyses. Motion and intensity were modeled as within-participant factors, each with 3 levels (emotion: happy, angry, and fearful; intensity: 50%, 100%, and 150%). Continuous variables were mean centered. Table 1 shows the associations among variables. Dependent variables were accuracy (percentage correct), response time, ARI, and SCARED, with age and gender as covariates and participant as a random effect. Motion was an additional covariate in all imaging analyses. Motion and intensity were modeled as within-participant factors, each with 3 levels (emotion: happy, angry, and fearful; intensity: 50%, 100%, and 150%). Continuous variables were mean centered. Table 1 shows the associations among variables. Dependent variables were accuracy (percentage correct), response time, ARI, and SCARED, with age and gender as covariates and participant as a random effect.

Behavior
Accuracy was associated with irritability as a function of emotion and intensity (ARI by emotion by intensity interaction; $F_{3,888} = 2.77; P = .03$; eFigure 2 in the Supplement). Specifically, increasing irritability was associated with decreasing accuracy when labeling the gender of angry faces at 100% (Wald $\chi^2_1 = 7.58; P = .05$; age- and sex-adjusted ARI and accuracy, $r = -0.27; P = .004$) and 150% (Wald $\chi^2_1 = 11.94; P = .005$; age- and gender adjusted ARI and accuracy, $r = -0.31; P < .001$). Imaging analyses controlled for this potential confound by including only correct trials. There were no associations between accuracy and SCARED or between mean correct reaction time and either irritability or anxiety.

Amygdala Functional Connectivity
Connectivity between the left amygdala and left medial PFC interacted with all modeled terms of interest (ARI by SCARED by emotion) by SCARED by emotion; $F_{4,888} = 9.20; P < .001; k = 61$; CoM = $-7.5, 58.1, 10.1$ (Figure 2). Post hoc general linear tests revealed a relatively clear pattern of results. The association between ARI scores and change in connectivity when viewing high-intensity (150%) angry faces varied significantly with SCARED score (at 150% angry; Wald $\chi^2_1 = 21.3; P < .001$). Figure 2B and C illustrate the interaction, showing a decrease in connectivity in participants who are highly anxious and irritable (blue quadrant; Figure 2B), but an increase in connectivity in those who are highly anxious but not irritable (red quadrant; Figure 2B).

In addition, a lower-level interaction between the left amygdala and the left lateral orbitofrontal cortex emerged (ARI by SCARED by emotion; $F_{2,888} = 15.28; P < .001; k = 52$; CoM = $-32.1, 33.1, -5$). Relative to angry expressions, connectivity to fearful expressions decreased in highly irritable, low-anxious individuals (eFigure 3 in the Supplement). Finally, a main effect of SCARED on left amygdala connectivity was present in the subgenual anterior cingulate/orbitofrontal cortex ($F_{1,108} = 25.48; P < .001; k = 43$; CoM = $-15.9, 33.1, -3.8$), where coordinates in Talairach space. Additional event-specific analyses relied on mean connectivity or activity extracted via AFNI’s 3dROIstat.

For post hoc analyses, we fit mixed-effects models using the same formula as the functional MRI group analysis mixed model. From these, we used general linear tests (Wald $\chi^2$) of specific contrasts or fixed effects of any variables while adjusting for all others (R package phia). We used Holm-Bonferroni corrections for multiple comparisons. Participants with influential observations were identified by their Cook’s distance using R package influence.ME. Influen
t observations were participants with a Cook’s distance greater than 0.053, a threshold defined by sample size and number of mixed-model parameters ($n = 39$). Iterative post hoc analyses leaving out individuals taking each class of medication, or who were influential, were done to ensure findings were robust to their exclusion (medication classes are listed in Table 1).
SCARED was positively associated with connectivity (age-, gender-, and motion-adjusted $r = 0.37; P < .001$; eFigure 3 in the Supplement). Connectivity to the right amygdala was modulated by SCARED and intensity in the bilateral superior temporal gyri (SCARED by intensity; right: $F_{2,888} = 15.03; P < .001; k = 95$; CoM $= 61.2, -6.2, 3.8$; and left: $F_{2,888} = 13.00; P < .001; k = 69$; CoM $= -53.8, -23.8, 8.8$). In both areas, SCARED was associated with the difference in connectivity between 50% and both 100% and 150% intensities across emotions (Wald $\chi^2 \geq 18$; all $P < .001$). Generally, this difference increased with increasing SCARED (age-, gender-, and motion-adjusted $r > 0.28$; all $P < .003$).

Activation
Activation was associated with irritability rather than anxiety. Across intensities, 7 regions exhibited an ARI-by-emotion interaction (Table 2; eFigure 4 in the Supplement). This generally reflected increasing activity with increasing irritability to happy or angry, relative to fearful, faces (Table 2). No associations manifested between SCARED and neural activity.

Post Hoc Analyses
In leave-out analyses, we evaluated confounding by medication status (eTable 3 in the Supplement). We iteratively excluded individuals by medication class in analyses of mean connectivity or activity. The 4 participants whose medication status was unknown were excluded from these analyses. All $F$ tests of the effects we found in the whole sample remained statistically significant, with a similar pattern of significant post hoc contrasts, except in the right fusiform gyrus where, when patients receiving antipsychotics were excluded, the $F$ test became a trend ($F_{2,744} = 2.3; P = .10$). To facilitate comparisons...
with prior research, categorical analyses of diagnosis are presented (eResults 1, eResults 2, and eResults 3 in the Supplement).

Discussion

Two key findings from this study clarify associations among irritability, anxiety, and neural function. First, during implicit processing of emotional faces, connectivity between the amygdala and its prefrontal regulatory areas varied strongly as a function of both irritability and anxiety, across healthy youth and those with at least 1 of 3 diagnoses (anxiety disorder, DMDD, or ADHD). Specifically, when participants viewed intensely angry expressions, high levels of both anxiety and irritability were associated with decreased amygdala–medial prefrontal cortex connectivity, whereas high levels of anxiety but low levels of irritability were associated with increased connectivity. Second, for regional activation, more findings emerged for irritability than for dimensional measures of anxiety or for categorical diagnoses. Specifically, high levels of irritability were associated with brain function as well as task performance, particularly when labeling the gender of intensely angry faces.

Several factors suggest the robust nature of our findings. Our relatively large sample of well-characterized children showed high variability for both anxiety and irritability, with many youths exhibiting symptoms well within the clinical range. This maximized statistical power to examine associations between brain function and clinically meaningful variation in these 2 symptom dimensions. Moreover, we used a relatively conservative analytic strategy, with an omnibus statistical model and appropriate whole-brain–corrected statistical thresholds for tests of high-order interactions. (See eResults 4 in the Supplement for resampling-based tests of robustness.) The use of an event-related design with face-
Irritability, Anxiety, and Neural Processes of Face Emotion Processing in Youth With Psychopathology

Limitations

This study had limitations. The cross-sectional design of this study was a fundamental limitation. These results apply to irritability and anxiety only in the disorders that were well-sampled in this study. They do not apply to other diagnostic groups where high irritability and anxiety are often present (e.g., major depressive disorder or bipolar disorder); such groups should be included in future studies. Inclusion criteria varied somewhat across diagnoses. Thus, all patients with anxiety disorders in the absence of DMDD or ADHD were actively seeking treatment, whereas most patients with DMDD or ADHD were already receiving treatment. The fact that associations with symptom dimensions manifested independent of diagnostic group suggests that this limitation does not account for our findings. Differences in psychotropic medication exposure may have influenced the results, although post hoc analyses suggest that no specific medication class explained the findings. Severely irritable children typically receive complex medication regimens, and the severity of their illness makes it unethical to maintain and study such youths medication-free. Given the stability of the ARI23 and SCARED42 and to include as many participants as possible, we allowed up to 60 days between completion of scales and scan date, although 59% of participants were scanned within 10 days of scale completion. This time lag may have made our measurement of irritability and anxiety less precise. Finally, by using an amygdala seed based on a probabilistic atlas, the findings may reflect signal from surrounding structures in some individuals. However, in post hoc analyses, connectivity results were confirmed using each individual’s FreeSurfer-parcellated amygdala.

Conclusions

We examined associations among neural connectivity, activity, and dimensional measures of 2 commonly co-occurring symptoms in youths, irritability and anxiety, across disorders that often present to clinicians. We found that these 2 prominent dimensions of pediatric psychopathology have interactive, rather than additive, effects on pathophysiology when patients process social threat. This could suggest the need for clinicians to attend to the co-occurrence of anxiety and irritability because the presence of both symptoms might have a unique effect on a child’s response to social threat and/or to treatment, including psychotherapeutic treatments focused on...
social interactions. These findings also have implications for both clinicians and researchers interested in the RDoC framework because they suggest that, like comorbidity among DSM-5 diagnoses, co-occurrence of RDoC traits has important pathophysiological implications that might ultimately affect psychiatric diagnosis.

ARTICLE INFORMATION
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Author Contributions: Drs Stoddard and Leibenluft had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: Kim, Brotman, Pine, Leibenluft. Acquisition, analysis, or interpretation of data: Stoddard, Tseng, Chen, Yi, Donahue, Brotman, Towbin, Pine, Leibenluft. Drafting of the manuscript: Stoddard, Brotman, Pine, Leibenluft. Critical revision of the manuscript for important intellectual content: Tseng, Kim, Chen, Yi, Donahue, Brotman, Towbin, Pine. Administrative, technical, or material support: Donahue, Brotman, Towbin, Pine, Leibenluft. Conflict of Interest Disclosures: None reported.

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