ORIGINAL ARTICLE

Amygdala and Ventrolateral Prefrontal Cortex Activation to Masked Angry Faces in Children and Adolescents With Generalized Anxiety Disorder

Christopher S. Monk, PhD; Eva H. Telzer, MA; Karin Mogg, PhD; Brendan P. Bradley, PhD; Xiaoqin Mai, PhD; Hugo M. C. Louro, MSE; Gang Chen, PhD; Erin B. McClure-Tone, PhD; Monique Ernst, MD, PhD; Daniel S. Pine, MD

Context: Vigilance for threat is a key feature of generalized anxiety disorder (GAD). The amygdala and the ventrolateral prefrontal cortex constitute a neural circuit that is responsible for detection of threats. Disturbed interactions between these structures may underlie pediatric anxiety. To date, no study has selectively examined responses to briefly presented threats in GAD or in pediatric anxiety.

Objective: To investigate amygdala and ventrolateral prefrontal cortex activation during processing of briefly presented threats in pediatric GAD.

Design: Case-control study.

Setting: Government clinical research institute.

Participants: Youth volunteers, 17 with GAD and 12 without a psychiatric diagnosis.

Main Outcome Measures: We used functional magnetic resonance imaging to measure blood oxygenation level–dependent signal. During imaging, subjects performed an attention-orienting task with rapidly presented (17 milliseconds) masked emotional (angry or happy) and neutral faces.

Results: When viewing masked angry faces, youth with GAD relative to comparison subjects showed greater right amygdala activation that positively correlated with anxiety disorder severity. Moreover, in a functional connectivity (psychophysiological interaction) analysis, the right amygdala and the right ventrolateral prefrontal cortex showed strong negative coupling specifically to masked angry faces. This negative coupling tended to be weaker in youth with GAD than in comparison subjects.

Conclusions: Youth with GAD have hyperactivation of the amygdala to briefly presented masked threats. The presence of threat-related negative connectivity between the right ventrolateral prefrontal cortex and the amygdala suggests that the prefrontal cortex modulates the amygdala response to threat. In pediatric GAD, amygdala hyperresponse occurs in the absence of a compensatory increase in modulation by the ventrolateral prefrontal cortex.

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Author Affiliations:
Departments of Psychology (Dr Monk and Mr Louro) and Psychiatry (Dr Monk) and Center for Human Growth and Development (Drs Monk and Mai), University of Michigan, Ann Arbor; Department of Psychology, University of California, Los Angeles (Ms Telzer); School of Psychology, University of Southampton, Southampton, England (Drs Mogg and Bradley); Scientific and Statistical Computing Core (Dr Chen) and Mood and Anxiety Disorders Program (Drs Ernst and Pine), National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland; and Department of Psychology, Georgia State University, Atlanta (Dr McClure-Tone).

VIGILANCE FOR THREAT REPresents a prominent feature of generalized anxiety disorder (GAD).[1-4] Neuroimaging research implicates a neural circuit that includes the amygdala and the ventrolateral prefrontal cortex in vigilance for threat.[4-7] Within this circuit, the amygdala is thought to support vigilance through immediate threat processing,[8,9] whereas the ventrolateral prefrontal cortex facilitates later processes related to emotion regulation.[5,10] Disturbed amygdala–ventrolateral prefrontal cortex interactions are thought to influence anxiety.[10]

Developmental work in this area is important because most adult anxiety disorders arise in adolescence. Adolescent GAD shows particularly strong ties to adult anxiety.[11] Findings from studies[12,13] in animal models suggest that early-life amygdala–ventral prefrontal cortex circuit dysfunction lays a foundation for persistent anxiety. Translational work has begun to extend these findings to humans through brain imaging. Such studies consistently find that adults with various anxiety disorders exhibit altered activation in the amygdala and the prefrontal cortex,[14-19] with positive correlations between amygdala activation and anxiety severity.[15]

These studies typically present threats under prolonged viewing conditions in which the nature of the threat can be readily discerned. Prior research implicates the amygdala and associated circuitry in processing rapidly presented threats.[5,7] Therefore, studies using brief rather than prolonged viewing conditions of threat may better clarify the nature of amygdala–ventrolateral prefrontal cortex interactions in adult and pediatric anxiety. Behavioral investigations used spatial-orienting paradigms with briefly presented (17 milliseconds) threat

and nonthreat cues to reveal anxiety-related attentional biases.[20] Such paradigms might be used in the context of brain imaging research to engage regions involved in evaluating threat under conditions that afford limited opportunities for elaborative processing.

Consistent with data implicating the amygdala in processing of briefly presented threats, neuroimaging studies demonstrate amygdala engagement to masked threats,[5,7,21] particularly among adults with elevated trait anxiety.[22] The only published studies[23,24] to date using masked threatening stimuli in anxiety disorders found heightened right amygdala activation to masked fear faces in adult posttraumatic stress disorder. It remains unclear if these findings apply to other anxiety disorders or to youth.

A recent study[4] examined neural responses in pediatric GAD to 500-millisecond threat cues (angry faces). Youth with GAD exhibited greater right ventrolateral prefrontal cortex activation than healthy peers, with no between-group differences in the amygdala. Ventrolateral prefrontal cortex activation was greater in youth having GAD with mild anxiety relative to those with severe anxiety, consistent with studies[15,25-27] implicating the ventrolateral prefrontal cortex in emotion regulation through effects on the amygdala. However, as with most prior reports, this study[4] involved events containing prolonged presentation of threats. Events with briefly presented masked threats may reveal between-group differences in the amygdala and associated brain regions that are engaged by events affording limited opportunities for elaborative, strategic, or regulatory processing. No prior imaging study in healthy or anxious youth has examined neural responses to such events, to our knowledge.

The present study uses an orienting task in pediatric GAD to monitor attentional bias for rapidly presented masked emotional (angry or happy) facial displays. Angry faces were chosen as stimuli for 2 reasons. First, behavioral findings in adults studied using this exact task show that anxious individuals relative to nonanxious individuals exhibit an attentional bias toward masked angry faces.[20] These behavioral data are consistent with other findings on related tasks that demonstrate the capacity of angry faces to disrupt attention in pediatric anxiety disorders[4,28-30] and to elicit attentional biases in anxious adults.[31] Second, the previous functional magnetic resonance (fMR) imaging study[4] among youth with GAD also used angry faces. Therefore, to most effectively build on this previous behavioral and imaging work, we used angry faces. As in the previous study,[4] we included happy faces as a comparison condition to determine whether the effects were selective to the negative emotion (anger).

Our study uses the orienting task with angry and happy faces to test 2 hypotheses. First, as in prior studies[23,24] using rapidly presented threats, we hypothesized that youth with GAD show increased right amygdala activation relative to healthy youth in response to briefly presented masked angry faces. Second, prior research with healthy adults shows that right ventrolateral prefrontal cortex activation was inversely related to right amygdala activation in response to masked angry faces.[5] Therefore, we hypothesized that the right amygdala shows negative connectivity with the right ventrolateral prefrontal cortex in response to threat, particularly among healthy youth.

Table 1. Demographics of the Comparison Group and of Patients With Generalized Anxiety Disorder (GAD)

Variable	Comparison Group (n = 12)	Patients With GAD (n = 17)	Statistical Comparison
Female to male ratio	6:6	6:11	$\chi^2_1 = 0.63$, $P > .2$
Age, mean (SD), y	14.33 (1.67)	13.12 (2.09)	$t_{27} = 1.67$, $P > .1$
IQ, mean (SD)	110.92 (14.24)	105.06 (14.34)	$t_{27} = 1.09$, $P > .2$

METHODS

PARTICIPANTS

The study sample comprised 29 children and adolescents (**Table 1**). The National Institute of Mental Health Institutional Review Board approved the procedures. Parents signed consent forms, and youths signed assents. All participants were evaluated with a physical examination and IQ measurement. The Kiddie Schedule for Affective Disorders and Schizophrenia was administered to participants by trained clinicians.[32] Two patients and 1 comparison subject were left-handed; all other subjects were right-handed.

Inclusion and exclusion criteria followed those of a prior study.[4] Seventeen participants met criteria for GAD based on the following 5 requirements: (1) criteria for GAD were met based on the Kiddie Schedule for Affective Disorders and Schizophrenia, (2) GAD was the primary focus of treatment, (3) clinically significant symptoms were present (Pediatric Anxiety Rating Scale[33] score ≥9 and Children's Global Assessment Scale score >60), (4) families desired treatment, and (5) anxiety as measured by the Pediatric Anxiety Rating Scale persisted during a 3-week period when patients received supportive psychoeducational therapy. As in prior biological and therapeutic studies[4,34,35] of pediatric anxiety, supportive psychoeducational therapy was provided to eliminate patients whose GAD symptoms were transient or responsive to nonspecific supportive intervention. Stability of symptoms during the 3-week period was confirmed by the patient's clinician immediately before fMR imaging.

There were 12 healthy comparison participants who were free of current and past psychiatric disorders based on the Kiddie Schedule for Affective Disorders and Schizophrenia. Control subjects were matched with patients on age, sex, and IQ.

Exclusion criteria followed those of previous work.[4] Specifically, we excluded subjects with psychosis, IQ less than 70, conduct disorder, suicidal ideation, lifetime history of mania, exposure to severe trauma, current Tourette syndrome, posttraumatic stress disorder, obsessive-compulsive disorder, and pervasive developmental disorder. We also excluded those with current use of any psychoactive substance (for GAD, the use of any such substance since the onset of the condition). As in the earlier study,[4] subjects with comorbid GAD and major depressive disorder (MDD) were included because the presence of MDD did not affect prior findings in GAD. This decision was initially based on the fact that family-based and longitudinal investigations documented strong relationships between GAD in youth and MDD. In the present study, this decision reflected the desire to compare present and prior findings. To

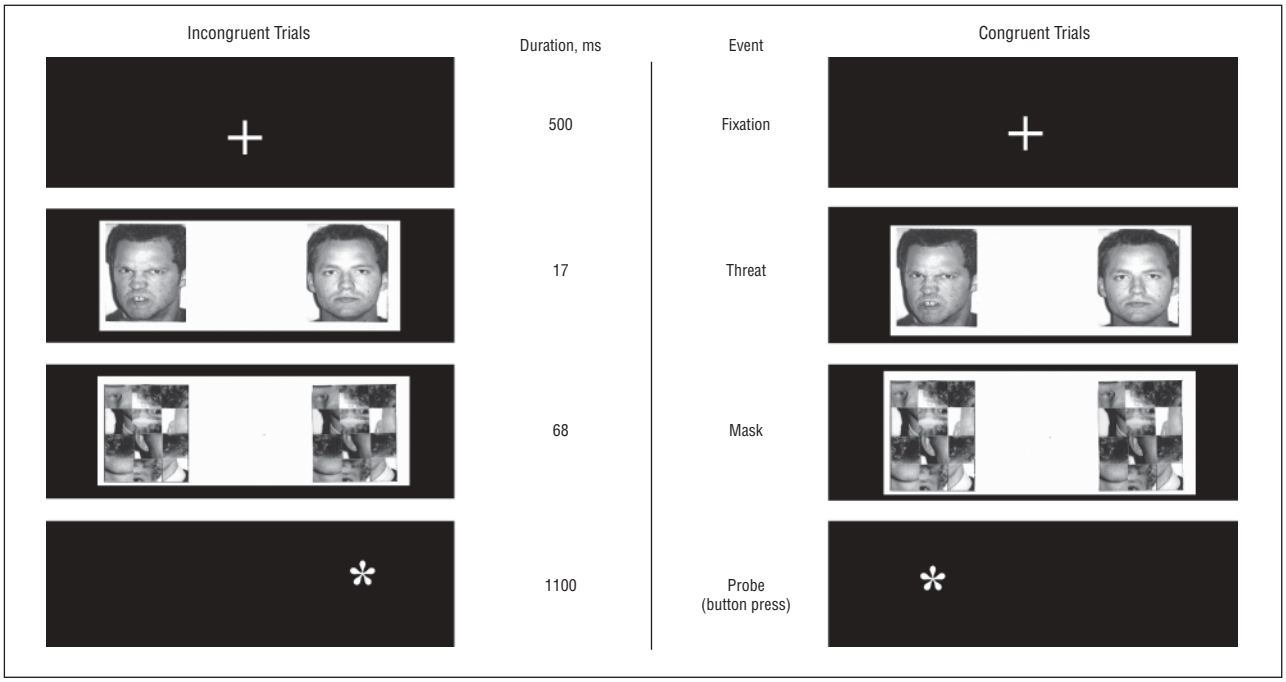

Figure 1. The 2 main trial types used to assess attentional bias for masked angry faces. The columns on the far left and on the far right show (from top to bottom) the screens that appear in 2 types of trials. The same model always displays the 2 expressions in a given trial. The middle 2 columns display the duration of each event and the event name for both trial types. In the sample trial on the left, the angry face and probe are displayed on different sides of the screen (incongruent). In the sample trial on the right, the angry face and probe are displayed on the same side (congruent). Happy/neutral and neutral/neutral trials (not shown) were also presented to subjects.

evaluate the effects of MDD and of social phobia on our results, we conducted secondary fMR imaging analyses between patients who had comorbidity and patients who did not have comorbidity.

TASK

The task closely followed established procedures.[20] Trials started with a 500-millisecond fixation point in the center of the screen (**Figure 1**). Two pictures of an actor's face then appeared simultaneously for 17 milliseconds. In some trials, one picture showed a neutral expression and the other an emotional expression; in other trials, both pictures showed the actor with a neutral expression. Immediately after this brief presentation, 2 scrambled faces (the mask) appeared for 68 milliseconds in the same locations as the 2 faces. The mask was replaced by an asterisk in 1 hemifield for 1100 milliseconds. Subjects were instructed to press one button with their thumb when the asterisk appeared on the left and to press another button with their index finger when the asterisk appeared on the right. The duration of the intertrial interval was 2300 milliseconds. A previous study using these parameters show that subjects report minimal awareness of details of the briefly presented face stimuli. Each participant was trained to perform the task before fMR imaging. These procedures are similar to previous work.[20] The key difference is that the faces were presented for 17 milliseconds and were masked for this study, whereas in the previous study unmasked faces were presented for 500 milliseconds. Eighty actors were each presented twice to participants, for a total of 160 trials. Forty blank trials were included to facilitate fMR imaging analysis.

There were 5 trial types, including the following 2 primary conditions of interest for the behavioral measure of attentional bias: congruent trials (wherein a masked angry/neutral face pair was followed by an asterisk on the same side of the screen as the angry face) and incongruent trials (wherein a masked angry/neutral face pair was followed by an asterisk on the opposite side from the angry face). Other control conditions included masked happy/neutral face trials (congruent and incongruent) and masked neutral/neutral face pairs. There were 32 trials for each of the 5 conditions. For each participant, the order of trial presentation was randomly determined. Emotional faces and asterisks were displayed an equal number of times on each hemifield.

BEHAVIORAL DATA ANALYSIS

The same criteria for determining acceptability of trials were applied to behavioral and fMR imaging data. Specifically, trials with incorrect responses and responses with reaction times less than 200 milliseconds and greater than 1000 milliseconds were excluded. The behavioral measure of attentional bias for masked angry faces was calculated for each subject by subtracting the mean reaction time on congruent trials (asterisk in the same position as the masked angry face) from the mean reaction time on incongruent trials (asterisk in a different position from the masked angry face). Positive values indicate an attentional bias toward the spatial location of the masked threat. Bias scores were similarly calculated for masked happy faces.

fMR IMAGING ANALYSIS

Images were acquired using a 3-T system (GE Medical Systems, Milwaukee, Wisconsin) with 29 contiguous 3.3-mm axial sections using echoplanar single-shot gradient-echo T2 weighting (repetition time, 2300 milliseconds; echo time, 23 milliseconds; field of view, 240 mm; acquisition matrix, 64×64 pixels; and voxel size, $3.3 \times 3.75 \times 3.75$ mm). Sections were parallel to the anterior commissure or posterior commissure line. Ramp sampling was used to correct possible distortion. For the T1-weighted volumetric images, we used a magnetization-prepared gradient-echo sequence (180 one-millimeter axial sec-

tions; 256-mm field of view; 1 signal acquired; repetition time, 11.4 milliseconds; echo time, 4.4 milliseconds; acquisition matrix, 256×256 pixels; inversion time, 300 milliseconds; 130 Hz/pixel bandwidth (33 kHz for 256 pixels); and 1-mm^3 in-plane resolution).

We used free imaging software (Analysis of Functional Neuroimages [AFNI], version 2.56b; http://afni.nimh.nih.gov/afni/).[36] Subjects were excluded from the analysis if they moved more than 2.5 mm in any direction. For movement that was 2.5 mm or less, effects were reduced by registering images to 1 volume in each run. Participant data were smoothed using a 6-mm full width at half-maximum isotropic gaussian filter. Trials with incorrect behavioral responses or responses that were less than 200 milliseconds or greater than 1000 milliseconds were removed from the fMR imaging analysis. Patients had a mean (SD) of 8.6% (8.1%) of trials removed, and comparison subjects had a mean (SD) of 6.0% (4.8%) of trials removed. Groups did not differ in the number of incorrect trials.

Using a 2-level procedure, a random-effects fMR imaging data analysis was conducted. At the subject level, we submitted each subject's data separately to a multiple regression analysis using the 3dDeconvolve module from AFNI. Vectors were created for each of 5 masked conditions (angry/neutral congruent, angry/neutral incongruent, happy/neutral congruent, happy/neutral incongruent, and neutral/neutral) with the onset time of each trial for each condition. Blank trials were modeled as an implicit baseline. An additional vector modeled nuisance trials (ie, trials that contained incorrect responses), responses that were too fast or slow, and null responses. Vectors were transformed into waveforms using a gamma variate,[37] and coefficients were created for each subject and condition. Contrast values were derived from comparisons of coefficients for specific conditions.

For the second level of analysis, individual data sets were converted to Talairach space, and group-level analyses were performed using the 3dttest module from ANFI comparing youth with GAD and comparison subjects. The principal effect of interest was the amygdala response to masked angry faces. The masked neutral/neutral face pairs were the comparison for examining group differences in activation to masked angry faces. Therefore, the main hypothesis concerned group differences in the contrast of masked angry/neutral vs neutral/neutral face pairs. The only difference between these trial types was the presence of a 17-millisecond angry face in the angry/neutral trials. We also examined responses to happy/neutral pairs relative to neutral/neutral pairs. To evaluate the fMR imaging data, we used AlphaSim from AFNI with 1000 Monte Carlo simulations[4,38] to control for multiple comparisons within the amygdala.

CONNECTIVITY ANALYSIS

We performed 2 connectivity analytic procedures. First, because we were primarily interested in group differences in brain interactions in response to threat, we implemented a psychophysiological interaction analysis to examine connectivity between the right amygdala and the ventrolateral prefrontal cortex during angry trials relative to neutral trials. To accomplish this, we adapted established procedures[39,40] for use with AFNI. We deconvolved the blood oxygenation level–dependent signal with an assumed form of hemodynamic response function before the interaction term was created.[40] Each participant's echoplanar imaging time series was placed in Talairach space. The first eigenvariate time series from the amygdala cluster (derived from the main contrast of masked angry/neutral pair vs neutral/neutral pair) was the "seed." To selectively examine activation related to the conditions of interest, we entered the masked angry/neutral pair vs neutral/neutral pair conditions as covariates. The results of this procedure show condition-related changes in the interaction of the right amygdala cluster and the ventrolateral prefrontal cortex. The threshold for the ventrolateral prefrontal cortex activation was set to $P<.005$ based on similar paradigms with rapidly presented emotional faces.[5]

Second, to be consistent with previous work,[34] we performed a standard connectivity analysis to examine interactions across all trials.[41,42] The first eigenvariate time series from the amygdala cluster was the seed, and the time series within it was extracted. For each subject, we performed a voxelwise correlation analysis between each individual voxel's time series and the seed's time series. The threshold was $P<.005$.

The psychophysiological interaction connectivity analysis selectively focuses on threat-related conditions, and the standard connectivity analysis examines interactions across the entire task. Therefore, it is to be expected that the approaches would yield different results. The focus of this article is on group differences in the brain response to threat; therefore, psychophysiological interaction analysis is particularly important. Nevertheless, the connectivity analysis across all conditions is informative because it documents group differences in all trials.

RESULTS

BEHAVIORAL ANALYSIS

Youth with GAD showed a mean (SD) attentional bias of 8.0 (24.8) milliseconds to masked angry faces, and comparison subjects showed a mean (SD) attentional bias of 11.6 (18.6) milliseconds to masked angry faces. No group difference was found for attentional bias to masked angry faces ($t_{27}=0.42$, $P=.68$). Considered together, subjects manifested an attentional bias toward masked angry faces ($t_{28}=2.27$, $P=.03$). For patients with GAD, the mean (SD) reaction times were 578.7 (74.2) milliseconds for masked angry/neutral congruent trials and 586.7 (74.6) milliseconds for masked angry/neutral incongruent trials. For the comparison group, the mean (SD) reaction times were 539.1 (113.8) milliseconds for masked angry/neutral congruent trials and 550.7 (116.7) milliseconds for masked angry/neutral incongruent trials. There was no group difference in reaction times to trials containing masked angry faces ($t_{27}=1.08$, $P=.29$). Moreover, we found no group difference in attentional bias to masked happy faces ($t_{27}=0.67$, $P=.51$), and the groups together did not show an attentional bias toward or away from masked happy faces ($t_{28}=0.77$, $P=.45$). Finally, there was no group difference in reaction time overall to trials containing masked happy faces ($t_{27}=0.95$, $P=.35$).

fMR IMAGING ACTIVATION

To test our first hypothesis, we examined group differences in activation to trials containing masked angry faces vs trials with masked neutral face pairs. As hypothesized, youth with GAD relative to comparison subjects showed greater right amygdala activation (Talairach x, y, z coordinates of 28, −1, −18; $t_{27}=2.74$, $P<.05$), corrected using a Monte Carlo simulation for multiple comparisons within the amygdala (**Figure 2**). Areas of activation outside the amygdala are given in **Table 2**. To evaluate the association between severity of anxiety symp-

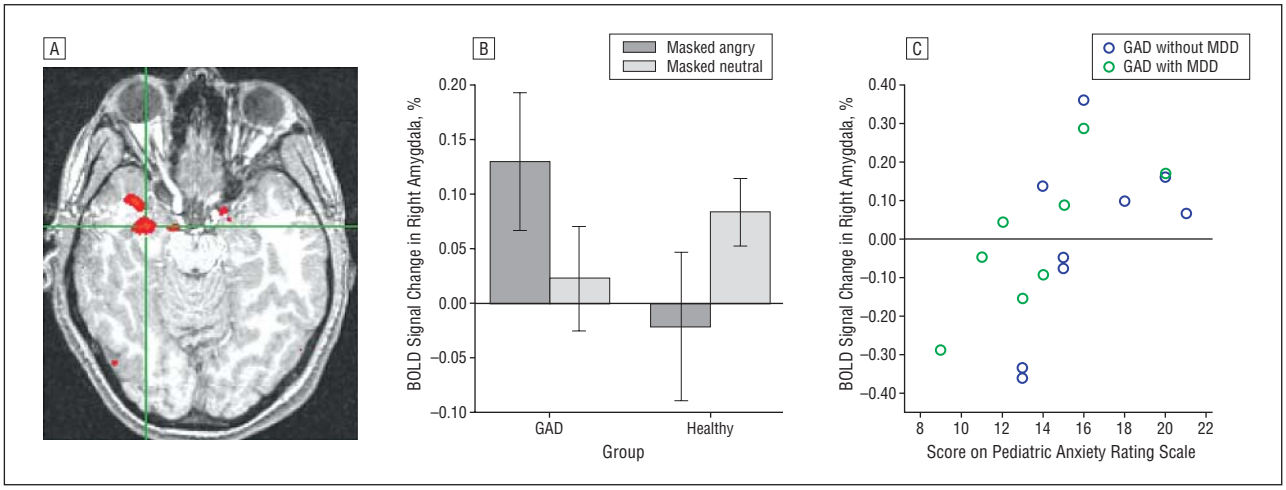

Figure 2. Functional magnetic resonance imaging activation. A, In the comparison of trials in which the angry face appeared relative to trials in which the neutral face appeared, youth with generalized anxiety disorder (GAD) show greater activation than control subjects in the right amygdala (right is left and left is right). The Talairach x, y, z coordinates for peak activation are 28, –1, –18. B, Bar graphs depicting activation to masked angry and neutral faces separately by group (error bars indicate standard errors of the mean). Bar graphs represent the mean activation within the amygdala cluster. Within-subject post hoc *t* tests showed that participants with GAD had statistically significantly greater activation to masked angry faces relative to masked neutral faces (t_{16}=2.47, *P*=.03), and there was no statistically significant difference in healthy comparison subjects between masked angry and masked neutral faces (t_{11}=1.29, *P*=.22). C, Relationship between patients' blood oxygenation level–dependent (BOLD) response in the right amygdala and severity of anxiety symptoms (Pediatric Anxiety Rating Scale) (Pearson product moment *r*=0.60, *P*=.01). The location of the amygdala cluster of activation (Talairach x, y, z coordinates of 18, –5, –10) is distinct from the cluster in A. Patients having GAD with and without major depressive disorder (MDD) are differentiated.

Table 2. Activation Areas Outside the Amygdala (*P*<.001 Uncorrected) in the Primary Contrast (Angry vs Neutral Faces)

Talairach x, y, z Coordinates	Statistical Comparison[a]	Location	Brodmann Area
Patients With GAD vs Comparison Group			
–46, –62, –25	t_{27} = 3.89	Left cerebellum	
Comparison Group vs Patients With GAD			
–58, –32, –16	t_{27} = 4.06	Left inferior temporal gyrus	20
–53, –40, –12	t_{27} = 3.71	Left middle temporal gyrus	20
Patients With GAD			
16, 36, –1	t_{16} = –4.84	Right anterior cingulate	32
Comparison Group			
15, –65, 25	t_{11} = –4.79	Right precuneus	21
8, –2, 30	t_{11} = –5.05	Right cingulate gyrus	24
13, –46, 29	t_{11} = –4.80	Right cingulate gyrus	31

Abbreviation: GAD, generalized anxiety disorder.
[a] Negative *t* values represent greater activation to the neutral faces relative to the angry faces.

toms and amygdala activation, patients' Pediatric Anxiety Rating Scale scores were entered in a covariate analysis using the 3dRegAna module of AFNI.[4] This analysis showed that increased anxiety symptoms were associated with increased activation with the right amygdala (Talairach x, y, z coordinates of 18, –5, –10; t_{15}=3.96, *P*=.001) (Figure 2). Anxiety severity and activation within the cluster derived from this analysis statistically significantly correlated (Pearson product moment *r*=0.60, *P*=.01). (The cluster from this association was in an adjacent but distinct area from that associated with a diagnosis of anxiety.) For masked happy faces relative to masked neutral faces, we found no group difference in the amygdala (t_{27}=2.00, *P*=.15).

FUNCTIONAL CONNECTIVITY ANALYSIS

For our second hypothesis, we examined connectivity between the right amygdala cluster (derived from results of the first hypothesis) and the ventrolateral prefrontal cortex during the masked angry face relative to masked neutral face conditions. To be consistent with previous work, we first report connectivity in all subjects together.[34] Activation in the amygdala cluster negatively coupled with activation in the right ventrolateral prefrontal cortex for all subjects (**Table 3** and **Figure 3**). A post hoc *t* test showed weaker negative connectivity in youth with GAD relative to comparison subjects in the same area of activation, but this effect was modest (Talairach x, y, z coordinates of 29, 31, –10; t_{29}=–2.12, *P*<.05). At the peak location for the group difference results (Talairach x, y, z coordinates of 30, 25, –10), youth with GAD considered alone showed modest negative connectivity (t_{16}=–2.21, *P*<.05), whereas healthy youth considered alone showed strong negative connectivity (t_{11}=–4.48, *P*<.001).

In the connectivity analysis for all conditions, there was a positive coupling between the right amygdala cluster and the right ventrolateral prefrontal cortex in all subjects (Talairach x, y, z coordinates of 44, 23, –6; t_{28}=4.84, *P*<.001). Youth with GAD had a statistically significantly stronger positive functional connectivity relative to comparison subjects in a slightly more anterior region (Talairach x, y, z coordinates of 45, 30, –6; t_{27}=3.13, *P*=.004). Youth with GAD considered alone showed positive connectivity (Talairach x, y, z coordinates of 44, 28, –6; t_{16}=3.60, *P*=.002), whereas healthy youth considered alone showed positive connectivity in a slightly more posterior area (Talairach x, y, z coordinates of 47, 23, –6; t_{16}=5.71, *P*<.001). Both groups of subjects showed strong positive connectivity in the right ventrolateral prefron-

Table 3. Activation From the Psychophysiological Connectivity Analysis ($P<.001$ Uncorrected) in the Primary Contrast (Angry vs Neutral Faces)

Talairach x, y, z Coordinates	Statistical Comparison[a]	Location	Brodmann Area
		All Subjects	
29, 25, −10	$t_{28} = -4.08$	Right ventrolateral prefrontal cortex	47
17, −85, 10	$t_{28} = -3.70$	Right cuneus	17
25, −57, 57	$t_{28} = 4.04$	Right superior parietal lobule	7
		Patient With GAD vs Comparison Group	
42, −30, 15	$t_{27} = 3.86$	Right superior temporal gyrus	41
−57, −9, 12	$t_{27} = 3.91$	Left precentral gyrus	43
		Comparison Group vs Patients With GAD	
39, 38, 28	$t_{27} = 4.18$	Right middle frontal gyrus	9
		Patients With GAD	
−14, −49, −1	$t_{16} = -4.02$	Left lingual gyrus	19
		Comparison Group	
29, 25, −10	$t_{11} = -4.52$	Right ventrolateral prefrontal cortex	47
−37, 21, −11	$t_{11} = -5.07$	Left ventrolateral prefrontal cortex	47
−28, 25, −14	$t_{11} = -4.52$	Left ventrolateral prefrontal cortex	47
−43, −16, 8	$t_{11} = -4.90$	Left insula	13
−43, 34, 31	$t_{11} = 5.31$	Left middle frontal gyrus	9
39, −27, 41	$t_{11} = 4.44$	Right postcentral gyrus	2

Abbreviation: GAD, generalized anxiety disorder.
[a] Positive *t* values represent positive connectivity with the seed region (the amygdala cluster); negative *t* values, negative connectivity with the seed region.

tal cortex, but youth with GAD had greater positive connectivity in a slightly more anterior area.

EXAMINATION OF COMORBID CONDITIONS

Eight of 17 subjects with GAD also had MDD. To evaluate whether MDD accounted for the amygdala findings, we conducted analyses within the right amygdala using uncorrected *t* tests. For the comparison of GAD with MDD vs GAD without MDD, there was no difference ($t_{15}=0.04$, $P=.72$). Relative to the comparison group, GAD with MDD showed greater amygdala activation (Talairach x, y, z coordinates of 25, −1, −18; $t_{18}=2.31$, $P=.04$). Similarly, relative to comparison subjects, youth with GAD without MDD showed greater amygdala activation (Talairach x, y, z coordinates of 30, −1, −18; $t_{19}=2.56$, $P=.02$).

Eight youth with GAD also had social phobia. To evaluate whether social phobia contributed uniquely to the amygdala findings, we followed the same procedures as for MDD. There was no statistically significant difference in amygdala activation between youth having GAD without social phobia and youth having GAD with social phobia ($t_{15}=1.60$, $P=.13$). There was statistically significantly greater amygdala activation in patients having GAD with social phobia relative to healthy controls

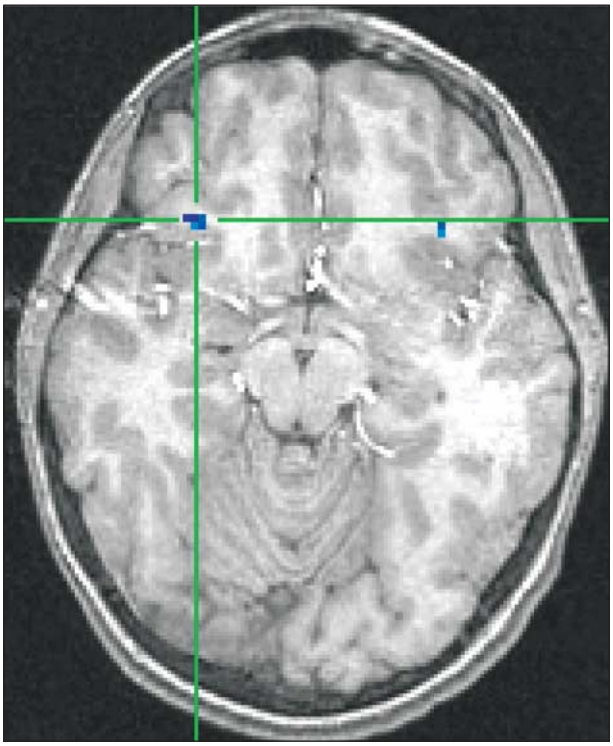

Figure 3. From the psychophysiological interaction analysis with the right amygdala cluster as the seed, subjects show negative coupling in the right ventrolateral prefrontal cortex (right is left and left is right) (Talairach x, y, z coordinates of 29, 25, −10; $t_{28}=-4.08$, $P<.001$).

(Talairach x, y, z coordinates of 26, −1, −18; $t_{18}=3.07$, $P=.007$), with a similar trend between patients having GAD without social phobia vs controls (Talairach x, y, z coordinates of 33, −1, −17; $t_{19}=1.97$, $P=.06$).

BEHAVIORAL PERFORMANCE AND fMR IMAGING ASSOCIATIONS

To evaluate associations between the attentional bias to masked angry faces and the amygdala activation, bias scores to masked angry faces were entered into covariate analyses using 3dRegAna separately for the 2 groups. Youth with GAD displayed a statistically significant positive association between attentional bias for masked angry faces and the strength of activation in the right amygdala (Talairach x, y, z coordinates of 21, −6, −15; $t_{15}=4.96$, $P<.001$) (**Figure 4**). This bias measure correlated with the level of amygdala activation within this cluster (Pearson product moment $r=0.74$, $P=.001$). No statistically significant association was found for comparison subjects. A Fisher exact test *z* score transformation[43] showed that there was a statistically significant difference between the 2 correlations ($z=2.41$, $P=.02$).

ANXIETY SEVERITY AND BEHAVIORAL PERFORMANCE ASSOCIATIONS

There was no association between anxiety severity and attentional bias to angry faces. Anxiety severity and attentional bias to angry faces were each associated with increased activation in nonoverlapping clusters of the amygdala.

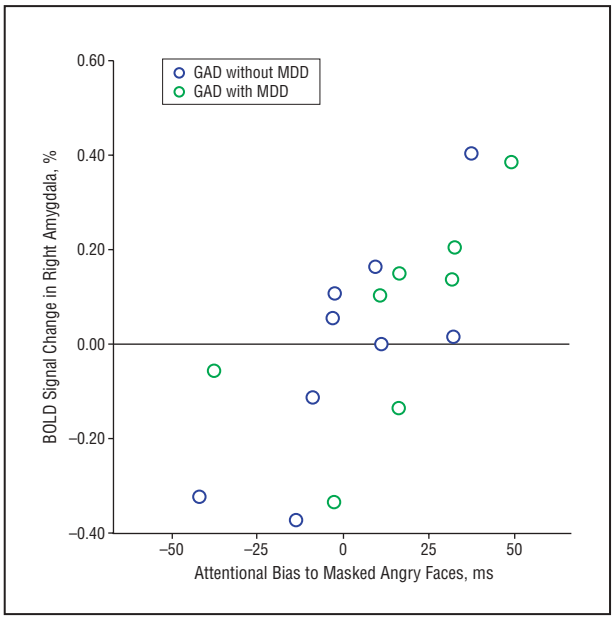

Figure 4. Association between right amygdala activation and attentional bias to masked angry faces in youth with generalized anxiety disorder (GAD) (Talairach x, y, z coordinates for peak activation in this cluster of 21, −6, −15; Pearson product moment $r=0.74$, $P=.001$). BOLD indicates blood oxygenation level–dependent; MDD, major depressive disorder.

COMMENT

In response to rapidly presented threats, youth with GAD show specific disturbances in neural activation. Consistent with our first hypothesis, youth with GAD, when viewing briefly displayed masked angry faces, have greater amygdala activation relative to comparison subjects. Moreover, there is a positive correlation between degree of amygdala activation and anxiety symptom severity. Results also support our second hypothesis. As predicted, right amygdala and right ventrolateral prefrontal cortex activation exhibit negative connectivity during threat trials, which is evident in both groups. Post hoc analysis revealed reduced negative coupling in youth with GAD relative to healthy youth at a liberal statistical threshold. Finally, although both groups show an attentional bias of similar magnitude to masked angry faces, attentional bias correlates with amygdala activation in patients but not in healthy subjects.

The present findings and previous work[4] indicate that youth with GAD process threat faces atypically at behavioral and neural levels. Behaviorally, when angry faces are presented briefly (as in the present study), youth with GAD and comparison subjects show an initial attentional bias toward the spatial location of threat. However, when angry faces were presented for longer periods (500 milliseconds), youth with GAD relative to comparison subjects showed an attentional bias away from threat.[4] Neurally, when threat is presented briefly, youth with GAD show increased amygdala activation, which positively correlates with anxiety severity. In contrast, when angry faces were displayed for 500 milliseconds, youth with GAD showed no difference from healthy peers in the amygdala, but they showed greater right ventrolateral prefrontal cortex activation. Moreover, when using the 500-millisecond threat exposures, patients having GAD with mild symptoms showed greater ventrolateral prefrontal cortex activation than patients having GAD with severe symptoms, suggesting that the right ventrolateral prefrontal cortex compensates for a GAD-related disturbance in functioning elsewhere, potentially in the amygdala.

Little is known about the development of the amygdala–ventrolateral prefrontal cortex circuit and how it relates to the emergence of anxiety disorders. Work from animal models indicates that the developmental timing of alterations to the amygdala–prefrontal cortex circuit greatly affects anxiety-related behavior.[44,45] Turning to humans, the question is how do neural disturbances relate to the onset of anxiety during development. It is not known if disturbances in this circuit precede the onset of GAD and are risk markers, or if such disturbances arise with the disorder. Consistent with a risk marker hypothesis, recent findings indicate that amygdala hyperactivation relates to risk for depression and anxiety in youth.[46,47] More work is needed to understand how the development of this circuit relates to the emergence of anxiety and other disorders that increase in prevalence during adolescence.

Work among a nonclinical sample of adults found that the right ventrolateral prefrontal cortex modulates amygdala responses to briefly presented masked threat cues.[5] Extending these findings, our psychophysiological interaction connectivity analysis indicates that the strength of amygdala activation varies as a function of right ventrolateral prefrontal cortex activity in youth and that the negative coupling may be weaker in youth with GAD than in comparison subjects. Consistent with neurobiological models of emotion,[8,48,49] our results suggest that GAD in youth is associated with dysfunction in a threat detection system involving a balance between subcortical and cortical regions (in particular, the amygdala and the ventrolateral prefrontal cortex).

Some research examining the relationship between the amygdala and the right ventrolateral prefrontal cortex in response to threat has emphasized the role of the right ventral prefrontal cortex in modulating amygdala responses in relation to strategic emotion regulation processes likely to be engaged during long periods.[10] Other work has emphasized the amygdala–ventrolateral prefrontal cortex relationship in terms of emotion regulation processes that are engaged even when a threat stimulus is briefly presented.[5,48] The present findings are compatible with the latter view.

Although only detected at a liberal statistical threshold, patients exhibited less negative coupling between the amygdala and the ventrolateral prefrontal cortex relative to comparison subjects. Given that patients show greater amygdala response to threat, the reduced negative coupling in patients relative to comparison subjects may represent a sign of impaired amygdala modulation. From this perspective, GAD may relate more to the balance between amygdala and right ventrolateral prefrontal cortex activation, as opposed to overall increases in amygdala activation. Further research is required to clarify these relationships.

In addition, we performed a connectivity analysis across all task conditions. In contrast to the psychophysiologi-

cal interaction connectivity analysis, this approach showed that both groups had a stronger positive coupling between the same regions and that youth with GAD had greater positive connectivity. Such positive amygdala–ventrolateral prefrontal cortex connectivity has been observed previously in various populations and age groups studied with the standard approach used herein.[34,50] Differences in the approaches of these 2 connectivity procedures may provide insight into the discrepant findings. The goal of the psychophysiological interaction analysis was to examine task-dependent interactions specifically related to threat. In contrast, this connectivity analysis reveals association in activation across the entire course of the task. Therefore, it is not surprising that these procedures yield different results. Further work using both connectivity approaches is necessary to confirm and understand the manner in which threat content modulates amygdala–ventrolateral prefrontal cortex connectivity in healthy and abnormal development.

Another line of research has shown that when stimuli are presented for a long duration in specific attention conditions (eg, participants subjectively evaluate threat-related facial expressions shown individually for several seconds), youth with GAD selectively show greater amygdala activation.[34] Taken together with the present findings, these findings suggest that differential amygdala response profiles are task dependent. The cognitive correlates of amygdala hyperactivation in these 2 studies are likely to differ. The present study may map neural correlates of threat orienting and detection related to vigilance in clinical anxiety. These correlates seem to involve the amygdala and disturbances in the balance between the amygdala and the ventrolateral prefrontal cortex. Other tasks may map neural correlates of psychological processes distinct from threat orienting and detection such as the subjective experience of fear. Work in this area with anxious youth demonstrates that amygdala hyperactivation, in tandem with enhanced ventral prefrontal activation and amygdala-prefrontal coupling, may correlate with subjective fear.[34] Further work is necessary to understand what situations lead to normal and abnormal neural activation in youth with GAD.

Finally, although anxious and comparison groups show an attentional bias toward masked angry faces, amygdala activation correlates with attentional bias only in youth with GAD and not in comparison subjects. This suggests that different neural processes underlie the common behavioral result of attentional bias to masked angry faces. For patients, it may be that the amygdala mediates the processing of rapidly presented threats and that this is part of a profile of cognitive responses to threat that underlies GAD. Because the comparison subjects also show attention toward masked angry faces, the bias toward masked angry faces is not a unique feature of GAD in youth.

There are several limitations to our study. This study of youth with GAD included patients with comorbid MDD and other anxiety disorders, particularly social phobia. However, follow-up analyses showed no group differences in right amygdala activation among patients having different diagnoses. Moreover, each patient subgroup showed greater activation in the same area of the amygdala relative to comparison subjects. These findings indicate that the group differences in amygdala activation were not due to MDD or to social phobia. Another limitation is the small sample. However, because small samples lead to reduced power and the hypothesized findings were confirmed, this limitation is less problematic. A final limitation is that there was a wide age range in both groups, and the small sample size made it unfeasible to examine interactions between age and diagnosis. Future investigators among youth with GAD may wish to select specific age groups to examine how developmental changes relate to anxiety-related influences on brain function.

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Correspondence: Christopher S. Monk, PhD, Department of Psychology, University of Michigan, 530 Church St, Ann Arbor, MI 48109-1043 (csmonk@umich.edu).
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