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Dynamic changes in amygdala activation and functional connectivity in children and adolescents with anxiety disorders

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Abstract

Anxiety disorders are associated with abnormalities in amygdala function and prefrontal cortex–amygdala connectivity. The majority of functional magnetic resonance imaging studies have examined mean group differences in amygdala activation or connectivity in children and adolescents with anxiety disorders relative to controls, but emerging evidence suggests that abnormalities in amygdala function are dependent on the timing of the task and may vary across the course of a scanning session. The goal of the present study was to extend our knowledge of the dynamics of amygdala dysfunction by examining whether changes in amygdala activation and connectivity over scanning differ in pediatric anxiety disorder patients relative to typically developing controls during an emotion processing task. Examining changes in activation over time allows for a comparison of how brain function differs during initial exposure to novel stimuli versus more prolonged exposure. Participants included 34 anxiety disorder patients and 19 controls 7 to 19 years old. Participants performed an emotional face-matching task during functional magnetic resonance imaging scanning, and the task was divided into thirds in order to examine change in activation over time. Results demonstrated that patients exhibited an abnormal pattern of amygdala activation characterized by an initially heightened amygdala response relative to controls at the beginning of scanning, followed by significant decreases in activation over time. In addition, controls evidenced greater context-modulated prefrontal cortex–amygdala connectivity during the beginning of scanning relative to patients. These results indicate that differences in emotion processing between the groups vary from initial exposure to novel stimuli relative to more prolonged exposure. Implications are discussed regarding how this pattern of neural activation may relate to altered early-occurring or anticipatory emotion-regulation strategies and maladaptive later-occurring strategies in children and adolescents with anxiety disorders.

Characterization of alterations in neural function in children and adolescents with anxiety disorders has the potential to inform our understanding of the development and treatment of anxiety disorders (Hyde, Bogdan, & Hariri, 2011; Paulus & Stein, 2007; Swartz & Monk, 2014a, 2014b; Viding, Williamson, & Hariri, 2006). In particular, altered amygdala function has received a great deal of attention as a potential neural correlate of anxiety disorder development, given its role in socioemotional processing (Adolphs, 2010). In adult anxiety disorder patients, meta-analytic studies of functional MRI (fMRI) indicate heightened amygdala activation during the processing of threatening or emotion-related stimuli (Etkin & Wager, 2007; Hattingh et al., 2013).

Identifying alterations in neural function observable during childhood and adolescence, the developmental stages when anxiety disorders most frequently onset (Kessler et al., 2005), will be critical for advancing our knowledge of the development of these disorders. Including the brain as a level of analysis in research on children and adolescents with anxiety disorders will help to elucidate the alterations in socioemotional processing involved in the development of these disorders and may lead to the development of novel empirically based treatments that impact the function of altered neural circuitry. In order to be useful for these purposes, it is first necessary to understand the specific task contexts in which abnormal neural processing occurs and why it occurs under these conditions, in order to be able to achieve reliable differences in brain-based measures in patient groups that can be replicated across studies. In addition, because development involves the fine-tuning of connections between different regions, it will be important to consider functional connectivity in addition to activation in order to gain a more comprehensive view of abnormalities in brain function in anxiety disorder patients. Finally, a critical step in this program of research is linking brain function to symptoms in order to understand how it contributes to individual variability

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in psychological outcomes of interest. Therefore, the goal of this paper is to examine four levels of analysis (neural activation, functional connectivity, symptoms, and disorder) in children and adolescents. With this approach, we aim to demonstrate how including the brain as a level of analysis can provide unique insights into anxiety disorder development that would otherwise not be obtained by measuring behavior alone.

Several studies have provided evidence of amygdala hyperactivation during emotion processing in children and adolescents with anxiety disorders. For this paper, we focus on studies of generalized anxiety disorder (GAD), social phobia (SP), or separation anxiety disorder (SAD) because they share overlap in cognitive and neural abnormalities in pediatric patients (Pine, 2007). Direct emotion processing tasks requiring participants to rate how afraid they felt while viewing fearful faces (Beesdo et al., 2009; McClure et al., 2007), or how they would be evaluated by disliked peers (Guyer, Lau, et al., 2008), have elicited heightened amygdala activation in patients relative to controls. Likewise, implicit emotion processing tasks, including identifying the gender of threatening faces (Battaglia et al., 2012; Blair et al., 2011), have also provided evidence for amygdala hyperactivation in pediatric anxiety disorder patients. However, some studies conducted in youth and adults have reported no differences in amygdala activation between patients and controls (Monk et al., 2006; Ziv, Goldin, Jazaieri, Hahn, & Gross, 2013), suggesting that differences across tasks contribute to variability in neuroimaging results.

Whereas the studies described above used an approach of examining mean group differences in activation across an entire emotion processing task, emerging evidence suggests that amygdala activation in anxiety disorder patients may vary depending on the timing of a task. In particular, Sladky et al. (2012) used a direct emotion processing task (i.e., emotional face matching) to show that adult anxiety disorder patients, relative to healthy controls, exhibit initial heightened amygdala response to faces during the first blocks of the scanning session but then subsequently demonstrate decreases in amygdala activation over the course of scanning. These results suggest that amygdala activation in anxiety disorder patients may not be stably high over the entire course of scanning, and differences in activation relative to controls may vary at different points of the task (e.g., during initial exposure to novel stimuli versus more prolonged exposure). However, because this task was performed in adults, it is unclear whether children and adolescents with anxiety disorders would also evidence changes in amygdala activation over time during a direct emotion processing task.

Another line of study using an implicit emotion processing task, the probe detection task, in pediatric anxiety disorder patients suggests amygdala activation may vary depending on the length of presentation of stimuli. In the probe detection task, participants view a pair of faces followed by a nonface probe, and they are required to indicate the location of the probe with a button press. When threatening faces are presented very briefly (17 ms) and then masked, followed by the appearance of the probe, pediatric anxiety disorder patients evidence amygdala hyperactivation relative to controls, although there is no behavioral difference in attention bias measured during the task (Monk et al., 2008). In contrast, when threatening faces are presented for relatively longer presentation times (500 ms), followed by the appearance of the probe, anxiety disorder patients do not evidence heightened amygdala activation (Monk et al., 2006). Instead, they evidence an attentional bias away from threatening faces and increased activation in the ventrolateral prefrontal cortex (Monk et al., 2006). These results suggest that differences in the dynamics of processing and attending to emotional faces in pediatric anxiety disorder patients may lead to distinct patterns of amygdala activation at different points in time relative to controls. However, this suggestion is based on the observation of differences in activation during tasks that varied in length of presentation of emotional stimuli at the trial level (between 17 and 500 ms) during implicit emotion processing. To date, no study in pediatric anxiety disorder patients has examined change in amygdala activation during a direct emotion processing task over the course of a scanning session, which would allow a more direct comparison of changes in activation during initial exposure to emotional stimuli relative to more prolonged exposure.

Overall, prior research suggests that changes in neural activation may occur throughout the duration of an emotion processing task, but analyses using a traditional group mean difference approach are not able to detect whether patient and control groups differ in the dynamics of these changes. Thus, an investigation of changes over scanning in amygdala activation in pediatric anxiety disorder patients is warranted for several reasons. The results will have implications for understanding how factors associated with task design, such as the length of the scanning session, may affect findings of amygdala activation. In addition, such an investigation will contribute to our knowledge of the dynamics of amygdala dysfunction in pediatric anxiety disorder patients. For example, a finding of changes in amygdala activation over time could indicate that patients evidence different emotion processing styles during first exposure to novel emotional stimuli relative to prolonged exposure. In contrast, a pattern of consistent amygdala hyperactivation over time would suggest a more stable abnormality in emotion processing.

Furthermore, altered patterns of amygdala activation may be associated with abnormal patterns of connectivity between the amygdala and prefrontal cortex, which has connections to the amygdala and can play a regulatory role in inhibiting amygdala activation (Phillips, Ladouceur, & Drevets, 2008; Ray & Zald, 2012). In adult SP patients, performance of an emotional face-matching task is associated with reduced connectivity between the amygdala and rostral anterior cingulate cortex and dorsolateral prefrontal cortex relative to controls (Prater, Hosanagar, Klumpp, Angstadt, & Phan, 2013). In pediatric anxiety disorder patients, decreased amygdala–ventral prefrontal cortex connectivity is observed when threatening stimuli are presented briefly (Monk et al., 2008), but

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when stimuli are presented for longer presentation times, anxiety disorder patients evidence increased ventral prefrontal cortex activation relative to controls (Monk et al., 2006). Therefore, abnormal amygdala–prefrontal cortex connectivity may contribute to different alterations in amygdala function at different points in time. However, no study to date has examined whether there are changes in amygdala–prefrontal cortex connectivity across the course of scanning and whether these differ in anxiety disorder patients relative to controls.

The goal of the present study was to further characterize alterations in amygdala response in pediatric anxiety disorder patients by examining whether amygdala activation and functional connectivity changes over the course of a scanning session. In order to do so, an emotional face-matching task was chosen to tap direct emotion processing during fMRI scanning. To our knowledge, this type of emotion matching task has not been used within a pediatric anxiety disorder sample. We hypothesized that we would observe one of two patterns within pediatric anxiety disorder patients: either they would evidence overall amygdala hyperactivation over the course of scanning or they would evidence an initial heightened amygdala response followed by decreases over time, similar to previous findings in adult anxiety disorder patients performing an emotional face-matching task (Sladky et al., 2012). Moreover, we hypothesized that changes in amygdala–prefrontal cortex connectivity over the course of scanning would differ between anxiety disorder patients and controls.

We also examined whether changes in amygdala response predicted anxiety symptom severity within the patient group and conducted preliminary analyses in order to determine whether differences in amygdala activation existed across the diagnostic categories of pure GAD, pure SP, or comorbid anxiety disorders. Finally, given the large age range of the sample, we investigated whether the pattern of amygdala response observed in patients varied with age.

Methods

Participants

Participants with anxiety disorders were recruited through university psychiatry outpatient clinics and the community, and controls were recruited via fliers and postings throughout the community. Primary diagnosis was based on structured clinical interview with the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children Present and Lifetime Version (Kaufman et al., 1997) for patients 17 years and younger and with the Structured Clinical Interview for DSM-IV Axis I Disorders (First, Gibbon, & Williams, 1997) for patients 18 years and older. A structured clinical interview was also used to confirm a lack of psychiatric diagnosis within the control group. Inclusion criteria for the anxiety disorder group included having a primary diagnosis of GAD, SP, or SAD, and exclusion criteria included a lifetime history of bipolar disorder, schizophrenia, mental retardation, or developmental disorders. In line with previous work (Beesdo et al., 2009; Guyer, Lau, et al., 2008; McClure et al., 2007; Monk et al., 2006), we included participants with GAD, SP, and/or SAD because these disorders are highly comorbid during development (Verduin & Kendall, 2003). Participants with secondary diagnoses (obsessive–compulsive disorder, tics, panic disorder, posttraumatic stress disorder, specific phobia, depression, attention-deficit/hyperactivity disorder) were included if it was determined by the clinician that SP, GAD, or SAD was the primary diagnosis. None of the anxiety disorder patients were currently taking psychotropic medications or undergoing psychotherapy treatment.

A total of 45 participants with a primary diagnosis of GAD, SP, or SAD and 26 controls completed the emotional face-matching task during fMRI scanning. Three patients dropped out during scanning, 12 participants were removed for having >3 mm maximum movement from the reference image or >3 mm maximum Euclidean distance for translation or rotation during scanning (7 patients and 5 controls), 2 participants were removed due to $<60\%$ accuracy on the behavioral task (1 patient and 1 control), and 1 control was removed due to poor normalization, leaving 34 participants in the anxiety disorder group and 19 controls between 7 and 19 years old available for analysis (Table 1). There were 9 participants with a primary diagnosis of GAD, 7 with SP, and 18 with comorbid diagnoses involving a combination of GAD, SP, and/or SAD. Excluded anxiety disorder patients were significantly younger ($M = 11.2$ years, $SD = 3.5$) than included patients ($M = 13.9$, $SD = 3.2$), $t\ (43) = -2.4$, $p = .02$. Excluded patients did not differ from included patients in anxiety symptom severity based on the anxiety measures described in the following section.

Procedure

Experimental task. Participants performed the emotional face-matching task during scanning. This task is based on a well-established emotional face-matching paradigm (Hariri, Tessitore, Mattay, Fera, & Weinberger, 2002), and a similar version of this task has been shown to elicit amygdala activation in typically developing adolescents (Forbes, Phillips, Silk, Ryan, & Dahl, 2011). Face-matching trials of the emotional face-matching task consisted of three faces in a triangular configuration with a target face on the top row and an emotional and a neutral face on the bottom row (Figure 1). Participants were instructed to select which of the two faces on the bottom row matched the emotion of the face on the top row. The nonmatching emotional face on the bottom row was always neutral. Faces were selected from a validated set of emotional face stimuli (Gur et al., 2002). For the baseline comparison, participants viewed a trio of shapes and were required to select which of two shapes on the bottom row matched a target shape on the top row (Figure 1). Participants responded with a button box; accuracy and reaction times (RT) were recorded.

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Table 1. *Participant characteristics*

	Group		
	Anxiety Disorder ($n = 34$)	Control ($n = 19$)	Difference
Age			$t\ (51) = 1.13, p = .26$
Mean (*SD*)	13.94 (3.2)	15.07 (4.0)	
Min–max	8–19	7–19	
Gender (female)	71%	63%	$\chi^2\ (1, N = 53) = 0.31, p = .58$
Pubertal status			$t\ (46) = 0.61, p = .55$
Mean (*SD*)	3.3 (1.4)	3.5 (1.6)	
Min–max	1–5	1–5	
MASC total scores			**$t\ (51) = -7.26, p < .001$**
Mean (*SD*)	64.5 (17.6)	31.0 (12.9)	
Min–max	27–96	12–53	
LSAS total scores			**$t\ (47) = -7.34, p < .001$**
Mean (*SD*)	68.6 (30.6)	12.1 (10.9)	
Min–max	9–132	0–37	

Note: Bold indicates a significant group difference. Pubertal status was measured using the Pubertal Development Scale. MASC, Multidimensional Anxiety Scale for Children; LSAS, Liebowitz Social Anxiety Scale. Data on pubertal status were missing for three anxiety disorder patients and two control participants; LSAS scores were missing for two anxiety disorder patients and two control participants.

The task consisted of 18 faces blocks, with 6 blocks each of the following types of emotional faces: fearful, angry, and happy. These blocks were interleaved with 18 shape-matching blocks. The order of emotional face blocks was counterbalanced across participants. Each block was 20 s long and consisted of four trials lasting 5 s each. The task was performed across two runs.

fMRI data acquisition. MRI images were acquired on a 3.0 Tesla GE Signa. A high-resolution T1-weighted spoiled-gradient echo image (repetition time = 9 ms, echo time = 1.8 ms, flip angle = 15°, slice thickness = 1.2 mm, 124 slices, field of view = 256 × 256 mm) was acquired for anatomical reference and T2*-weighted blood oxygen level dependent images were acquired using a reverse spiral sequence (repetition time = 2000 ms, echo time = 30 ms, slice thickness = 3 mm, 43 slices collected parallel to the AC-PC line, 64 × 64 matrix, 220 × 220 mm field of view, flip angle = 90°) for the functional data.

Measures. Anxiety symptoms were measured with the Multidimensional Anxiety Scale for Children (MASC; March, Parker, Sullivan, Stallings, & Conners, 1997) and the Liebowitz Social Anxiety Scale Child—Adolescent version (LSAS; Masia-Warner et al., 2003). The former was chosen as a measure of total anxiety symptoms across a range of dimensions (social anxiety, separation anxiety, etc.), whereas the latter provides a more specific measure of social anxiety symptoms, which are particularly relevant to the developmental stages under investigation (Kessler et al., 2005). The MASC consists of 39 items falling under the following subscales: physical symptoms, social anxiety, harm avoidance, and separation anxiety. Using a 4-point Likert scale, participants rate how often they experience each item (e.g., feeling tense), ranging from 0 (*never true about me*) to 3 (*often true about me*). Prior research indicates that this scale has good internal reliability, with a Cronbach α value of 0.90 for the total score (March et al., 1997). The LSAS is a 24-item scale assessing symptoms of anxiety falling under two subscales: social (e.g., meeting new people or strangers) and performance (e.g., giving a presentation in class). For each item, participants respond with a Likert scale to indicate how much fear or anxiety they feel in that situation (0 = *none*, 3 = *severe*) and how often they avoid that situation (0 = *never*, 3 = *usually*). Prior research indicates this scale has good internal reliability, with a Cronbach α value of 0.97 for the total score (total anxiety + total avoidance; Masia-Warner et al., 2003).

Pubertal status was assessed with the Pubertal Development Scale (Petersen, Crockett, Richards, & Boxer, 1988) or an adapted version of this scale from the Youth-Nominated Support Team study (King et al., 2009) and then converted to Tanner stages. Prior research has shown that pubertal development contributes to changes in limbic processing over and above the effects of age (Forbes et al., 2011; Moore et al., 2012), suggesting that pubertal status may be associated with variation in amygdala function. However, because age was highly correlated with pubertal development ($r = .87$, $p < .001$), for analyses of cross-sectional associations we focused on age as the variable of interest because it would be difficult to disentangle the effects of age and puberty within this sample.

Analyses

Behavioral data analysis. Mean accuracy and RT were obtained for each condition and for each third of the task. Group

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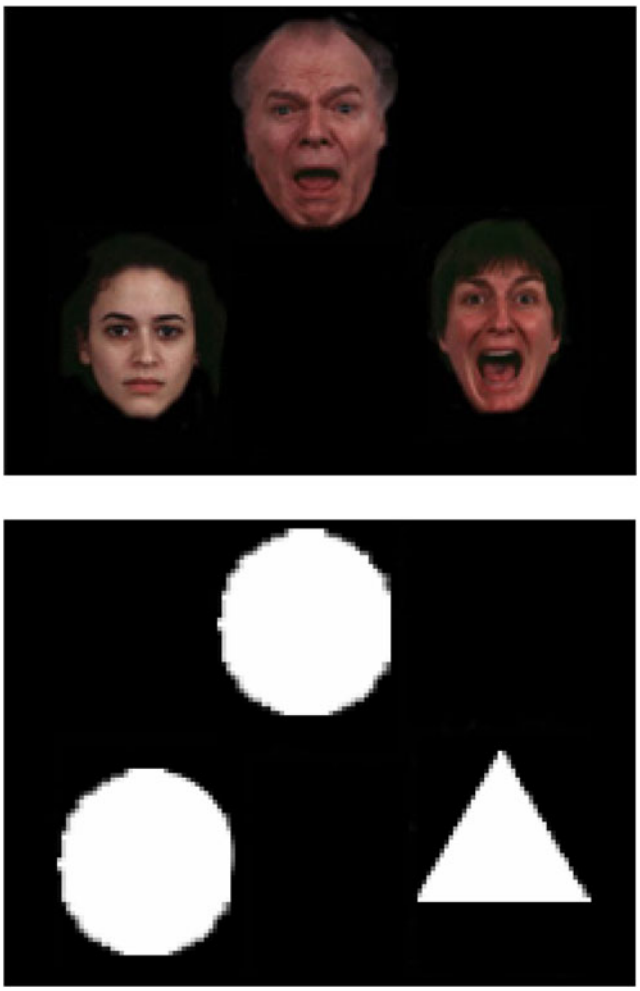


Figure 1. (Color online) Example trials of the emotional face assessment task. (Top) An example trial of face matching with fearful faces. Participants used a button box to indicate which of two faces on the bottom row matched the expression of the target face on the top row. (Bottom) The baseline comparison task was shape matching.

differences in behavior were examined using repeated-measures analyses of variance in SPSS v20. The interaction of Group (anxiety disorder or controls) × Condition (angry, fearful, happy, or shapes) × Time (first third, middle third, or last third) was examined. Age was entered as a covariate.

fMRI data analysis. Data underwent a standard preprocessing procedure in SPM8. Large spikes in the *k*-space data were filtered out, and data were reconstructed into images using field map correction to decrease distortions. Functional images were slice-timing corrected and realigned to the first volume of the first run. The T1-overlay was coregistered to the realigned functional images. Then the high-resolution T1 was coregistered to the (coregistered) T1 overlay. The high-resolution T1 was then segmented using voxel-based morphometry and normalized to a template in Montreal Neurological Institute space using DARTEL (Ashburner, 2007), and the resulting deformation field was applied to the time-series data. Finally, images were smoothed with a 6-mm full width at half-maximum Gaussian kernel.

Condition effects were modeled at the individual subject level. In order to examine changes in activation across scanning, each block was modeled as a separate regressor. The six parameters from the realignment procedure were entered as nuisance covariates in the individual models. The following contrasts were then created in order to divide the task into thirds: Faces1 > Shapes1 (all faces blocks within the first third of the run vs. all shapes blocks within the first third of the run), Faces2 > Shapes2 (all faces within the second third of the run vs. all shapes within the second third of the run), and Faces3 > Shapes3 (all faces within the last third of the run vs. all shapes within the last third). This was done in order to minimize the effects of signal drift across scanning; if signal drift occurred, it should exert equivalent effects on the blocks of interest (the faces blocks) and the comparison condition (the shapes blocks) within each third of the run, and thus the effect of signal drift should be subtracted out through this method. The task was divided into thirds so that each contrast (e.g., Faces1 > Shapes1) contained one face block for each emotion (angry, fearful, and happy). Thus, each third of the task included three faces blocks and three shapes blocks, and combined across two runs, this provided a total of 240 s each for the Faces1 > Shapes1, Faces2 > Shapes2, and Faces3 > Shapes3 conditions. Emotion-specific contrasts (e.g., Angry Faces1 > Shapes1) were also created in order to examine effects for each type of emotional face block separately.

Psychophysiological interaction (PPI) analysis. PPI was performed in order to examine differences in connectivity between the groups (Friston et al., 1997). PPI was conducted in SPM8 by extracting the time course from the left or right amygdala seed. For the PPI, seeds were based on a functional mask of left or right amygdala activation to the contrast of all faces > all shapes. This approach was chosen in order to ensure that only voxels significantly activated during the viewing of faces were included in the seed. Separate PPI models were created for each third of scanning, thus conditions for the three PPIs were Faces1 > Shapes1, Faces2 > Shapes2, and Faces3 > Shapes3. The PPI model included a regressor for the time course of amygdala activation (the physiological variable), the condition of interest (e.g., Faces1 > Shapes1; the psychological variable), and the interaction of these (the psychophysiological interaction). Thus, the PPI indicates regions where connectivity with the amygdala was modulated by task condition (matching faces relative to matching shapes).

Several measures were taken to ensure that movement did not influence the connectivity results. First, the six motion parameters from realignment were entered as nuisance covariates into the individual level models created for the PPI. Second, mean volume–volume displacement was calculated for each participant and entered as a nuisance covariate in any second-level analyses conducted with the PPI.

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Hypothesis 1: Differences in amygdala response in anxiety disorder patients relative to controls

Because the task was performed across two runs and changes in amygdala activation could occur across the runs, we examined a Group (anxiety disorder or controls) × Run (Run 1 or Run 2), × Time (Faces1 > Shapes1, Faces2 > Shapes2, or Faces3 > Shapes3) interaction in SPM8 in order to determine whether effects varied by run. We also examined a Group × Run interaction to test whether the groups differed in changes in activation across the runs. Age was entered as a covariate for this analysis and for all following analyses.

Next, the runs were collapsed together, and change in activation across each third of the task was examined. The interaction of Time (first third, second third, or last third) × Emotion (angry, fearful, or happy) × Group (anxiety disorder or controls) was examined in order to determine whether there were differences in changes within runs between the groups that varied by emotion. Because this interaction was not significant, the interaction of Time (Faces1 > Shapes1, Faces2 > Shapes2, or Faces3 > Shapes3) × Group was examined to assess group differences in changes in amygdala activation across all emotion types. Finally, the main effect of group was examined in order to determine whether there were overall group differences in activation averaged across the entire task (similar to the traditional mean group differences approach).

To assess significance, family-wise error (FWE) small-volume correction was applied within the bilateral amygdala region of interest (ROI), structurally defined using the Wake Forest University Pickatlas (Maldjian, Laurienti, Kraft, & Burdette, 2003). The significance threshold was set at $p <$ .05 FWE-corrected for ROI analyses. Whole-brain effects outside of the a priori ROI were subsequently examined using a threshold of $p < .001$ uncorrected and a cluster threshold of 10 voxels.

Because prior research points specifically to amygdala hyperactivation to threatening faces in anxiety disorder patients (Beesdo et al., 2009; Blair et al., 2011; McClure et al., 2007; Monk et al., 2008), we also performed a planned post hoc analysis to examine differences in amygdala activation for the contrasts of angry versus happy faces and fear versus happy faces, in order to detect whether there was a threat-specific effect in the present study.

Hypothesis 2: Differences in changes in connectivity across scanning in anxiety disorder patients relative to controls

A similar approach as for the first hypothesis was used in order to examine differences in connectivity. Based on the results for the first hypothesis, we collapsed all three emotional face types (angry, fearful, and happy) into a faces condition rather than examine emotional stimuli separately for this analysis. A full factorial model was created using the PPI images for each third of the run. A Time (PPI for Faces1 > Shapes1, Faces2 > Shapes2, or Faces3 > Shapes3) × Group (anxiety disorder or controls) interaction was examined in order to determine whether changes in context-modulated functional connectivity across scanning differed by group. The main effect of group was examined in order to test whether there were overall group differences in connectivity across the entire scanning session. Significance was tested with ROIs created using the Automated Anatomical Labeling Atlas in the Wake Forest University Pickatlas to cover dorsolateral, dorsomedial, ventrolateral, and ventromedial prefrontal cortical regions.

Secondary analyses: Amygdala response and its relation to anxiety symptoms, diagnosis, and age

Based on the results obtained for the first hypothesis, we calculated the difference between amygdala activation to faces during the first third of scanning and amygdala activation to faces during the last third of scanning in order to create a difference score representing the change in amygdala activation from beginning to end of scanning. We then used partial correlations in SPSS v20 to examine the relation between MASC and LSAS scores and amygdala change, controlling for age, within the anxiety disorder group.

Differences across diagnostic categories were examined by performing a Group (pure GAD, pure SP, or comorbid diagnoses) × Time interaction similar to that used to examine differences in amygdala response with controls.

The relation between amygdala response and age was examined by conducting a Group × Age interaction in SPSS using the difference score calculated as described above as the dependent variable. Because there was a significant correlation with age and mean RT on the matching task ($r = –.71$, $p < .001$), mean RT was entered as a covariate in this analysis.

Results

Group differences in behavioral performance

There were no differences in accuracy between groups or changes in accuracy across the scanning session. There was a main effect of condition, $F\,(3, 48) = 2.82$, $p = .049$. Accuracy was highest for fearful and happy face matching, followed by shape matching, and then angry face matching (Table 2). Similarly, there was no group difference in RT, but there was a main effect of condition, $F\,(3, 48) = 21.3$, $p < .001$. Participants responded most quickly for shape matching, followed by happy face matching, then fearful face matching, and were slowest for angry face matching (Table 3). There was also a main effect of time, $F\,(2, 49) = 3.21$, $p = .049$, and age, $F\,(1, 50) = 47.7$, $p < .001$. The effect of time was due to participants being slowest to respond in the first third of scanning and then becoming faster to respond to the next two-thirds. The effect of age was due to older participants being faster to respond.

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Table 2. *Accuracy by group, time, and condition*

Time	Condition	Group: Anxiety Disorder Mean (*SD*)	Group: Control Mean (*SD*)
First third	Angry face	78.3% (19.3)	77.6% (18.4)
	Fearful face	92.3% (16.3)	95.4% (7.5)
	Happy face	92.6% (16.9)	96.1% (7.3)
	Shape	89.3% (12.9)	90.8% (4.9)
	All conditions	88.1% (14.2)	90.0% (5.7)
Middle third	Angry face	88.2% (16.8)	90.8% (11.7)
	Fearful face	93.8% (14.2)	98.0% (4.7)
	Happy face	94.9% (15.7)	98.0% (6.3)
	Shape	87.5% (15.1)	92.5% (3.6)
	All conditions	91.1% (14.0)	94.8% (3.4)
Last third	Angry face	82.0% (20.2)	86.2% (10.1)
	Fearful face	94.5% (16.6)	98.0% (4.7)
	Happy face	95.6% (15.4)	97.4% (5.2)
	Shape	88.0% (15.0)	91.2% (3.9)
	All conditions	90.0% (15.7)	93.2% (3.3)
Mean accuracy		89.7% (13.6)	92.7% (3.1)

Note: Mean accuracy represents the accuracy averaged across all portions of the task for all conditions.

Hypothesis 1: Differences in amygdala response in anxiety disorder patients relative to controls

The Group × Run × Time and Group × Run interactions were not significant, and thus we performed all following analyses collapsing across the two runs. The Group × Emotion × Time interaction was not significant within the amygdala, indicating that differences across groups did not differ by emotion. However, in support of the hypothesis of differences in changes in amygdala activation with time in anxiety disorder patients, the Group × Time interaction was significant in the left amygdala, F (2, 152) = 8.09, FWE-corrected p = .023, size = 19 voxels (–28, 0, –20). As shown in Figure 2, this interaction was driven by differences in patterns of activation across the groups. The control group maintained a steady level of activation across scanning, whereas the anxiety disorder group had a heightened initial amygdala response followed by decreases in amygdala activation across the following blocks. As shown in Figure 3, and as suggested by the lack of a Group × Run × Time interaction, patients evidenced this pattern of response across both runs.

The main effect of group was not significant within the amygdala, indicating that the groups did not differ in overall amygdala activation averaged across the course of scanning; however, a t test for the contrast of anxiety disorder patients > controls for all faces > all shapes approached significance, t (152) = 2.92, p = .07, corrected for the bilateral amygdala ROI. Whole-brain results for these tests are presented in Table 4. As seen in the table, the only main effect of group on activation (averaging across time) was a difference in right fusiform activation, with controls demonstrating greater activation relative to the anxiety disorder group for all faces. We did not find support for threat-specific effects in the amygdala in the planned post hoc analyses, because there were no differences in amygdala activation between groups for the contrasts of angry versus happy or fearful versus happy face matching.

Table 3. *Reaction time by group, time, and condition*

Time	Condition	Group: Anxiety Disorder Mean (*SD*)	Group: Control Mean (*SD*)
First third	Angry face	1916.0 (498)	1766.0 (470)
	Fearful face	1699.0 (389)	1612.7 (482)
	Happy face	1627.9 (465)	1520.2 (463)
	Shape	1123.4 (308)	1048.2 (307)
	All conditions	1591.6 (343)	1486.8 (371)
Middle third	Angry face	1791.3 (348)	1639.5 (477)
	Fearful face	1709.1 (421)	1589.4 (505)
	Happy face	1533.9 (455)	1425.4 (545)
	Shape	1058.9 (272)	950.1 (263)
	All conditions	1523.3 (311)	1401.1 (402)
Last third	Angry face	1663.7 (363)	1689.1 (589)
	Fearful face	1591.2 (530)	1425.0 (509)
	Happy face	1594.5 (415)	1504.9 (482)
	Shape	1131.0 (310)	1019.1 (277)
	All conditions	1495.1 (351)	1409.6 (433)
Mean RT		1536.7 (325)	1432.5 (394)

Note: Mean RT represents the reaction time (ms) averaged across all portions of the task for all conditions.

Hypothesis 2: Differences in changes in connectivity across scanning in anxiety disorder patients relative to controls

We focused connectivity analyses on the left amygdala seed based on the results for the first hypothesis. There was a significant Group × Time interaction in the dorsomedial prefrontal cortex, F (2, 151) = 10.96, FWE-corrected p = .035, size = 31 voxels (–6, 60, 28). As seen in Figure 4, post hoc analyses indicated this interaction was due to controls demonstrating greater context-modulated dorsomedial prefrontal cortex–amygdala connectivity during the first third of scanning whereas the anxiety disorder group evidenced increased context-modulated connectivity during the middle third of scanning. There was also a main effect of group within the dorsolateral prefrontal cortex, F (1, 151) = 19.89, FWE-corrected p = .031, size = 15 voxels (32, 28, 50). Post hoc analyses indicated that the group difference was strongest during the first third of scanning, with the control group evidencing greater context-modulated functional connectivity relative to anxiety disorder patients (Figure 5).

Secondary analyses: Amygdala response and its relation to anxiety symptoms, diagnosis, and age

There was no relation between total MASC scores and amygdala response, but the relation between LSAS scores and change in amygdala activation approached significance, ad-

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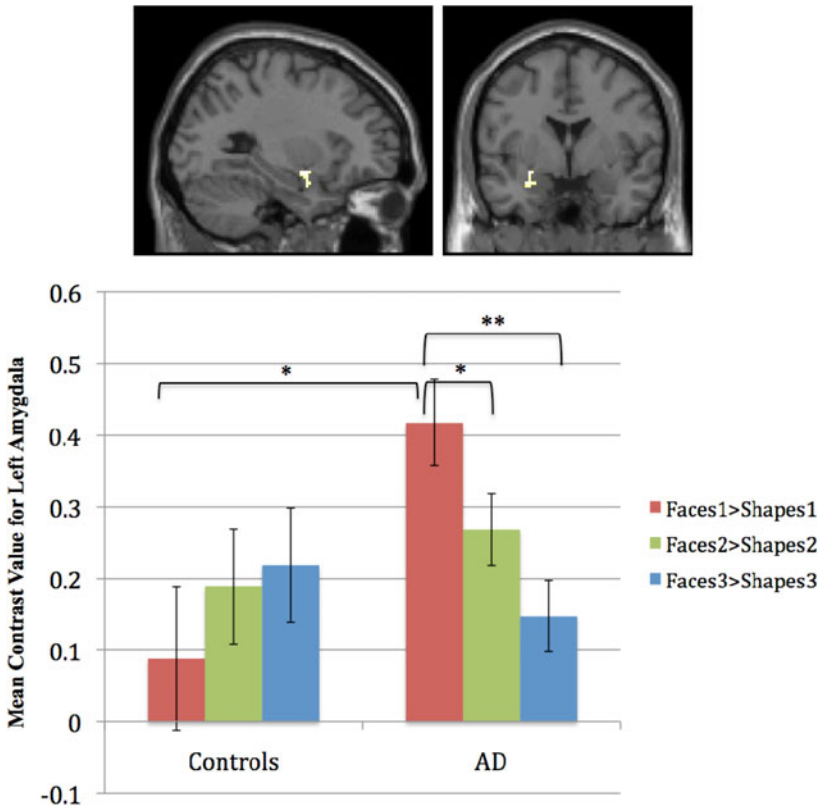


Figure 2. (Color online) There is a significant interaction of Group × Time within the left amygdala. SPM figure is thresholded at $p < .001$ uncorrected and demonstrates the effect of Group × Time. Bar graph displays mean contrast values extracted from the anatomically defined left amygdala for the following contrasts: Faces1 > Shapes1, Faces2 > Shapes2, and Faces3 > Shapes3. AD, Anxiety disorder group. Error bars represent 1 *SE*. *$p < .05$, **$p < .001$.

justing for age, within the anxiety disorder group ($r = .34$, $p = .06$). The positive correlation suggests that greater social anxiety symptom severity is associated with a greater decrease from the first to the third portions of the task. In terms of diagnosis, there was no Group × Time interaction or main effect of group for different diagnoses within the amygdala. All groups showed a general pattern of decreased amygdala activation within the final third of scanning (Figure 6). The

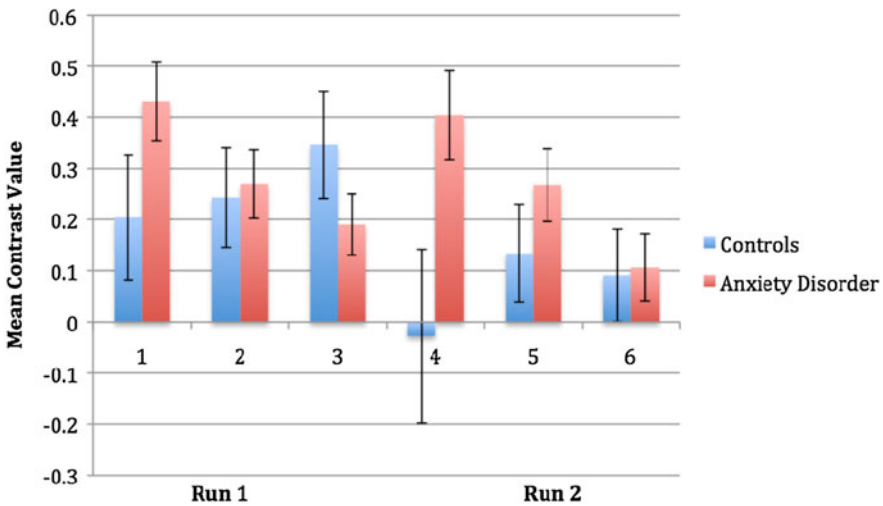


Figure 3. (Color online) Changes in left amygdala activation viewed separately for each run. Bar graph displays mean contrast values extracted from the anatomically defined left amygdala for the following contrasts: Faces1 > Shapes1, Faces2 > Shapes2, and Faces3 > Shapes3 within the first run (bars 1–3) and within the second run (bars 4–6). The significances of paired and independent group *t* tests are not displayed for clarity of viewing.

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Table 4. *Whole-brain activation results for group differences in changes in activation and overall activation*

Effect	Direction of Effect	Statistic	Number of Voxels	Coordinates	Region
Group × Run × Time		F (2, 305) = 9.83	35	64, –22, 42	Right supramarginal gyrus
		F (2, 305) = 9.65	13	–58, –70, –4	Left inferior temporal gyrus
		F (2, 305) = 9.36	46	56, –40, 4	Right superior temporal gyrus
		F (2, 305) = 8.15	18	–38, –36, 32	Right inferior parietal lobule
		F (2, 305) = 8.08	10	–48, –50, 30	Left supramarginal gyrus
Group × Run		No significant clusters			
Group × Time × Emotion		F (4, 458) = 5.48	25	–24, –22, –16	Left hippocampus
		F (4, 458) = 5.21	12	–34, –10, –20	Left hippocampus
Group × Time		F (2, 152) = 8.49	41	–28, –2, –12	Left amygdala
		F (2, 152) = 8.49	17	50, –66, 44	Right angular gyrus
		F (2, 152) = 7.70	11	–42, –70, 42	Left angular gyrus
Main effect of group	Controls > AD	F (1, 152) = 21.00	70	22, –76, –14	Right fusiform
Group × Emotion		No significant clusters			
Main effect of emotion	Threat > happy	F (2, 458) = 19.48	940	–38, 20, 0	Left insula
	Threat > happy	F (2, 458) = 17.77	639	34, 24, –4	Right insula
	Threat > happy	F (2, 458) = 16.02	615	2, 28, 42	Right medial frontal gyrus
	Angry > happy	F (2, 458) = 13.14	99	–48, 40, 8	Left inferior frontal gyrus
	Threat > happy	F (2, 458) = 13.12	302	46, 32, 40	Right middle frontal gyrus
	Threat > happy	F (2, 458) = 13.09	44	–44, 50, –14	Left inferior frontal gyrus
	Happy > angry	F (2, 458) = 12.87	133	18, –48, 62	Right superior parietal region
	Threat > happy	F (2, 458) = 12.15	164	–28, –66, –14	Left fusiform gyrus
	Threat > happy	F (2, 458) = 11.56	223	40, 44, 6	Right middle frontal region
	Threat > happy	F (2, 458) = 11.51	100	44, 56, –10	Right inferior frontal gyrus
	Angry > happy	F (2, 458) = 11.42	62	–50, –50, 10	Left middle temporal gyrus
	Threat > happy	F (2, 458) = 10.34	39	–8, –74, –36	Left cerebellum
	Threat > happy	F (2, 458) = 10.16	154	–30, –54, 38	Left superior parietal lobule
	Angry > happy	F (2, 458) = 9.49	21	46, –58, 54	Right angular gyrus
	Threat > happy	F (2, 458) = 8.80	16	–36, –64, –32	Left cerebellum
	Threat > happy	F (2, 458) = 8.57	15	4, –84, –6	Right lingual gyrus
	Threat > happy	F (2, 458) = 8.46	29	10, –10, 12	Right thalamus
	Threat > happy	F (2, 458) = 8.33	13	–44, –74, –6	Lateral occipitotemporal gyrus
	Threat > happy	F (2, 458) = 8.00	20	–40, –92, 12	Left occipital pole
	Happy > angry	F (2, 458) = 7.86	11	–10, –56, 62	Precuneus

Note: Threshold for whole-brain analyses is set at $p < .001$ uncorrected and a cluster threshold of 10. The results were examined first using F tests and then followed up with t tests in order to determine the direction of effect for main effects. Threat > happy = angry and fearful faces > happy faces; AD, anxiety disorder group.

Group × Age interaction and main effect of age were not significant for change in amygdala activation, suggesting this effect did not vary with age in this sample.

Discussion

The aim of the present study was to investigate differences in the pattern of amygdala response and connectivity over time in pediatric anxiety disorder patients relative to controls during an emotional face-matching task. We found that children and adolescents with anxiety disorders exhibit an altered pattern of amygdala response relative to controls across the course of scanning. This pattern was characterized by initial heightened amygdala response to emotional faces during the first third of the task, followed by a decline in amygdala activation across the session. In addition, we found differences in dorsomedial and dorsolateral prefrontal cortex–amygdala context-modulated connectivity, which indicated altered connectivity in anxiety disorder patients during the first third of scanning.

The results of this study identified two important alterations in amygdala activation in pediatric anxiety disorder patients: first, it showed a heightened initial amygdala response during the first third of the task; and second, it revealed an abnormal pattern (relative to controls) of decreases in activation during the second and third portions. Further research is necessary in order to elucidate the mechanisms underlying heightened amygdala activation during the first third of scanning in anxiety disorder patients. A study conducted in spider-phobic adults found that spider-phobic participants evidenced a faster onset and time to peak of the blood oxygen level dependent response within the amygdala to spider-related pictures relative to controls (Larson et al., 2006). Exam-

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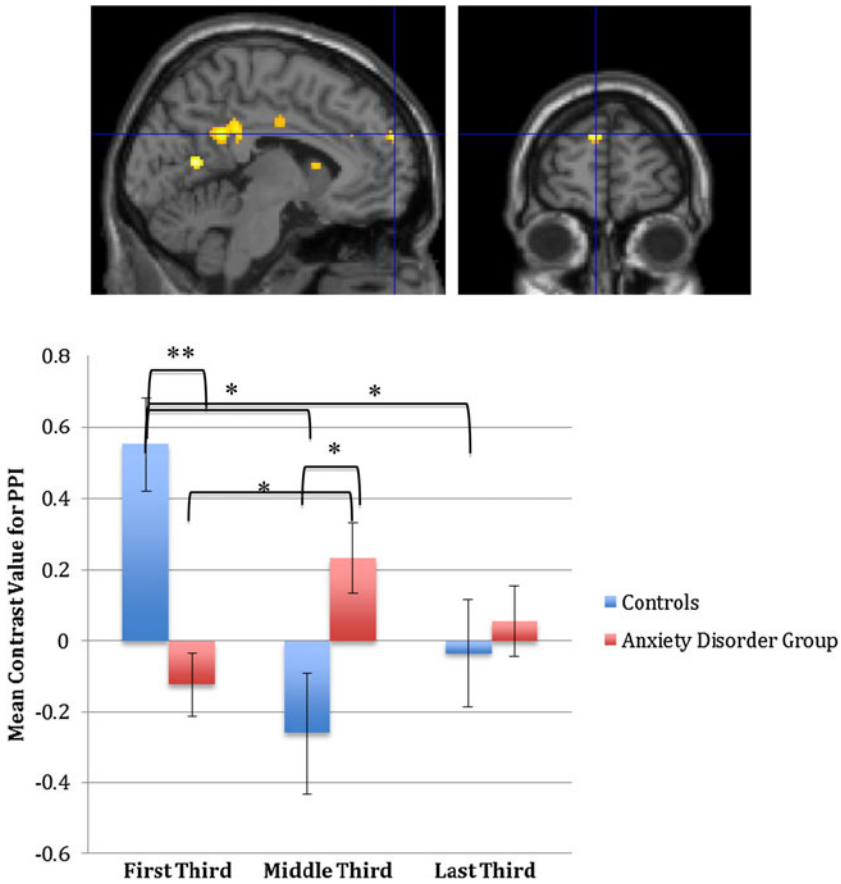


Figure 4. (Color online) Group × Time interaction for amygdala–dorsomedial prefrontal cortex context-modulated connectivity during face versus shape matching. SPM image demonstrates the Group × Time interaction for the psychophysiological interaction of face versus shape matching seeded with the left amygdala, thresholded at $p < .001$ uncorrected. Bar graph shows contrast values for the psychophysiological interaction extracted from a 4-mm sphere around the peak voxel of activation for the interaction. *$p < .05$, **$p < .001$.

ining differences in amygdala response at the trial level may therefore be informative regarding the results obtained here at the block level.

In addition, the results of the PPI analysis showed that controls evidence greater context-modulated left amygdala connectivity with dorsal regions of the prefrontal cortex during scanning, particularly during the first third. Thus, controls may recruit prefrontal regions in order to regulate amygdala response to novel emotional stimuli at the beginning of a scanning session, whereas failure to do so may result in the heightened initial amygdala response observed in patients. These findings provide preliminary evidence that the ability to modulate prefrontal cortex–amygdala connectivity at the beginning of a scanning session, when novel stimuli are first presented, may differentiate children and adolescents with anxiety disorders from typically developing controls. In a study of healthy adults, Goldin, McRae, Ramel, and Gross (2008) found that instructing participants to cognitively reappraise negative emotional stimuli was associated with early-occurring prefrontal cortex activation (within the first 5 s of a trial), whereas instructing participants to suppress negative affect while viewing emotional stimuli was associated with later occurring prefrontal cortex activation (10–15 s into a trial). Moreover, during a task requiring the cognitive reappraisal of negative self-beliefs, adults with social anxiety disorder evidenced reduced early-occurring activation (within the first 3 s of a trial) of regulatory prefrontal cortical regions, including the dorsolateral and dorsomedial prefrontal cortex, relative to controls (Goldin, Manber-Ball, Werner, Heimberg, & Gross, 2009). Therefore, the results of the present study could reflect the possibility that controls and anxiety disorder patients differ in the types and timing of emotion processing strategies used when first exposed to novel stimuli, but this needs to be tested with further research before drawing strong conclusions. Directly manipulating participants' strategies as was done in the study by Goldin et al. (2008) could help to determine whether differences between the groups are seen for early-occurring prefrontal activation or connectivity during cognitive reappraisal.

These results also raise questions regarding the mechanisms through which the observed decreases in amygdala activation occur in anxiety disorder patients and whether this is adaptive or related to worse symptom severity. It is possible that the Group × Time interaction found for dorsomedial prefrontal cortex–amygdala connectivity reflects patients recruiting prefrontal regions later in the scanning session than

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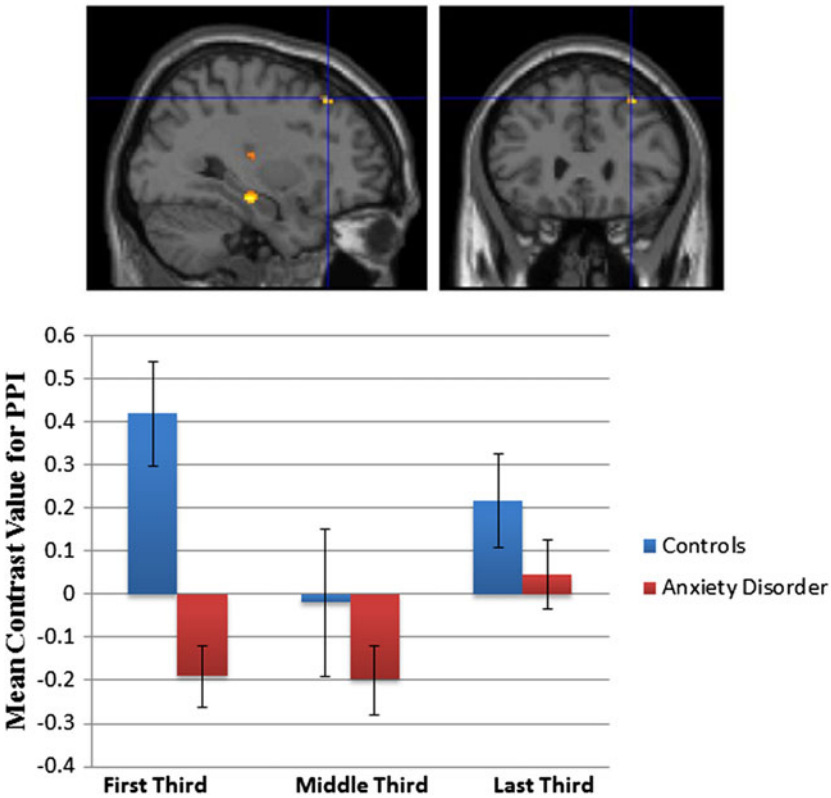


Figure 5. (Color online) Main effect of group for amygdala–dorsolateral prefrontal cortex context-modulated connectivity during face versus shape matching. SPM image demonstrates the main effect of group for the psychophysiological interaction of face versus shape matching seeded with the left amygdala, thresholded at $p < .001$ uncorrected. Bar graph shows contrast values for the psychophysiological interaction extracted from a 4-mm sphere around the peak voxel of activation for the group difference.

controls in order to regulate amygdala activation. Another possibility is that patients begin to avoid attending to the emotional faces across the scanning session, which is consistent with the findings of Monk et al. (2006). This would also be consistent with the finding in the present study of greater overall fusiform activation in controls, which could indicate that the anxiety disorder group was attending less to faces. Given that trials were 5 s each but participants responded

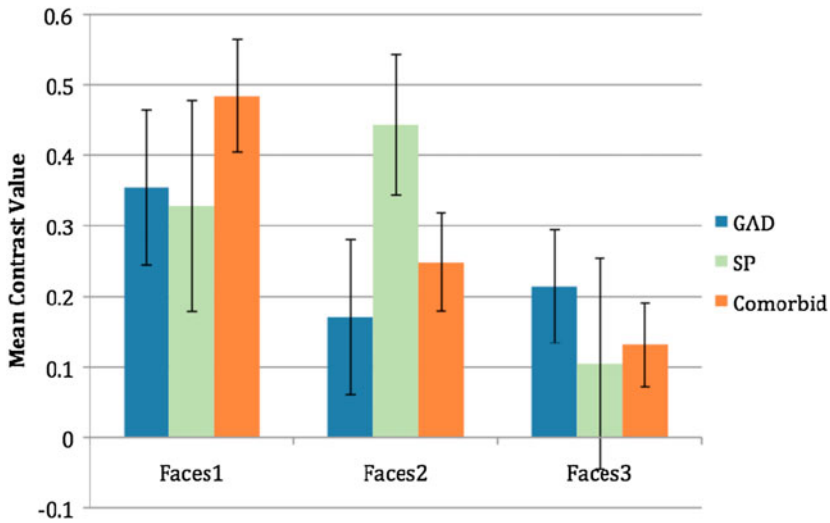


Figure 6. (Color online) Changes in amygdala activation viewed separately for each diagnostic group. Mean contrast values are extracted from the anatomically defined left amygdala region of interest for Faces1 > Shapes1, Faces2 > Shapes2, and Faces3 > Shapes3. GAD, Generalized anxiety disorder; SP, social phobia; comorbid, patients comorbid for GAD, SP, and/or separation anxiety disorder.

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on average within the first 2 s, they could potentially have attended away from faces after responding on each trial without evidencing a decrement in behavioral performance. Future research incorporating eye tracking will be necessary in order to determine whether there are differences in attention to the stimuli during scanning. This could potentially be informative regarding shifts in emotion processing in pediatric anxiety disorder patients when stimuli are first presented at the beginning of a scanning session relative to later, more prolonged exposure toward the end of a session.

The correlation between LSAS scores and change in amygdala activation across scanning approached significance, suggesting that participants who demonstrated a greater drop in amygdala activation had a higher number of social anxiety symptoms. This could suggest that the drop in amygdala activation is maladaptive, but because this result was only marginally significant, this effect should be examined in future research before firm conclusions are drawn. Restriction of range in anxiety symptoms may have reduced the size of correlations observed.

The finding of changes in amygdala activation over scanning in pediatric anxiety disorder patients also has implications for choices of tasks in future research, because it suggests that certain features of the fMRI task will influence whether amygdala hyperactivation is observed in anxiety disorder patients. In the present design, there was no significant overall group difference in amygdala activation due to the significant decreases in activation during the last two thirds of scanning in patients. Neural activation in children and adolescents with anxiety disorders may therefore be highly dependent on the timing and context of the task performed during scanning. There are several potential factors that may affect whether amygdala hyperactivation is observed in patients, including the nature of the task performed and the length of the task. Amygdala hyperactivation has most consistently been shown in tasks that require participants to evaluate their own emotions (e.g., rating how afraid they feel while viewing threatening stimuli) or how they will be judged by peers (Beesdo et al., 2009; Guyer, Lau, et al., 2008; McClure et al., 2007), suggesting that focusing on internal states or evaluations related to emotional stimuli is most likely to produce overall mean group differences in amygdala activation averaged across an entire scanning session, whereas in the current emotion-processing task (matching emotional faces), there was no overall group difference in activation. These results also suggest that amygdala hyperactivation is most likely to be observed during the beginning of a scanning session, thus using tasks with a shorter duration or focusing analyses on amygdala response at the beginning of an emotion-processing task are approaches more likely to result in a finding of amygdala hyperactivation in pediatric anxiety disorder patients.

It is important to note several limitations of the present study. First, as mentioned above, eye-tracking data were not collected, preventing an examination of whether there were differences in eye gaze patterns during performance of the emotional face-matching task. Second, because this was a blocked design, we were unable to separate out effects at the trial level. Future research incorporating a mixed or event-related design could be used to examine whether there are changes in amygdala activation during the first half of a trial (the first 2.5 s, during which participants make a behavioral response) and the second half of a trial. Third, in terms of examining differences across diagnostic groups, there were a relatively small number of participants within each diagnostic category. Therefore, these results should be considered preliminary and require further investigation with a larger sample.

Conclusions and directions for future research

The results of this study highlight the importance of multilevel research and demonstrate how including brain activation and functional connectivity as levels of analysis help to form a more complete picture of alterations in emotion processing in pediatric anxiety disorder patients than could be obtained with only one level of analysis. Although the two groups did not differ significantly in accuracy or RT during the task, there were significant differences in brain activation revealed over the course of scanning. Therefore, fMRI can help to reveal information-processing abnormalities that may be difficult to measure behaviorally but may still have important functional implications, highlighting the importance of including the brain as a level of analysis. In addition, group differences in amygdala activation mirrored differences in context-modulated functional connectivity. The two groups showed significant differences in activation and connectivity during the first third of scanning, suggesting that anticipatory or early-occurring emotion-regulation processes associated with functional connectivity in the controls may not be occurring in the anxiety disorder group. Thus, these results also demonstrate how including both functional connectivity and activation as levels of analysis within one study can be mutually informative in interpreting results. Including multiple levels of analysis therefore aids in the interpretation of each other level, and can reveal differences in emotion processing that may not be observable by only measuring one level, or behavior alone.

This research signals important future directions for understanding the pathways involved in the development of anxiety disorders. Although we cannot directly compare the present results obtained in youth with anxiety disorders to adults with anxiety disorders, it is notable that the pattern of time-related changes in amygdala activation observed in the present study is similar to the results obtained by Sladky et al. (2012) with adults (mean age 26 years) with social anxiety disorder. This may suggest that the pattern of activation observed in adults has already developed by the adolescent years. We have recently proposed that amygdala hyperactivation in anxiety disorders may reflect altered trajectories of corticolimbic development during childhood and adolescence (Swartz & Monk, 2014a). Specifically, cross-sectional research suggests that amygdala activation to emotional faces shows a linear decline with age, whereas prefrontal

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cortex–amygdala connectivity increases, from childhood through adolescence in typically developing individuals (Gee et al., 2013; Swartz, Carrasco, Wiggins, Thomason, & Monk, 2014). Therefore, deviation from this typical developmental pattern (e.g., not showing a decrease in amygdala activation or an increase in prefrontal cortex–amygdala connectivity with age), could account for the pattern of results observed here (i.e., amygdala hyperactivation during the first third of scanning and altered prefrontal cortex–amygdala connectivity in youth with anxiety disorders). In contrast, or in addition, this pattern could result from increases in amygdala activation with age in individuals who develop anxiety disorders, potentially through a transactional process in which amygdala hyperactivity leads to heightened anxiety and negative emotion, which in turn increases the sensitivity of the amygdala, even in nonthreatening situations.

An important limitation of the present study is that, because it examines youth who have already developed an anxiety disorder, we are not able to disentangle the causal processes through which amygdala hyperactivation and anxiety are linked. It could be that heightened amygdala activation causes the development of an anxiety disorder, or that heightened anxiety results in the development of amygdala hyperactivation, or that these have reciprocal effects on one another that interact across development. This important limitation signals a critical need for prospective, longitudinal examination of individuals at risk for the development of anxiety disorders but before the onset of disorder, in order to be able to disentangle the causal chain of development. If, as we have suggested, adolescence is a critical time for the reorganization of corticolimbic circuitry and potential disruptions in this reorganization, then this will be an important stage to focus on in such prospective research.

Moreover, it would also be of interest to follow adolescents prospectively into adulthood, or perform cross-sectional comparisons of adolescents and adults with anxiety disorders, in order to determine which neural abnormalities observed in adult anxiety disorder patients are the result of adaptation to or years of living with an anxiety disorder and which are present in adolescence. One of the few studies to perform such a comparison found that adults and adolescents with anxiety disorders evidenced amygdala hyperactivation to emotional faces relative to controls but that there was no difference in amygdala hyperactivation between the adult and adolescent patient groups (Blair et al., 2011). This supports the hypothesis that the abnormalities in amygdala activation observed in adulthood would be apparent during adolescence, when altered trajectories of corticolimbic development would give rise to altered functioning. These will all be important directions for future research in order to gain a fuller understanding of how neural development is associated with the development of anxiety disorders and how altered trajectories of development result in the pattern of findings observed in the present study.

As proposed in several frameworks (Hyde et al., 2011; Swartz & Monk, 2014b), we suggest that future research will be needed to determine how genetic and environmental influences on development relate to these observed alterations in neural function. Imaging genetics and imaging gene–environment interaction frameworks emphasize the use of brain-based measures as mediators of the relationship between genetic and environmental influences and the development of psychopathology (Hariri, Drabant, & Weinberger, 2006; Hyde et al., 2011). As a more proximal link to genetic variation, brain function can mediate the relationship between genes and gene–environment interactions with more complex behavioral and psychological phenotypes, such as anxiety symptoms or disorders. There is now substantial evidence for genetic influence on amygdala activation, such as the association between low-expressing alleles of the serotonin transporter-linked polymorphic region and heightened amygdala activation (Munafo, Brown, & Hariri, 2008). The results of the present study indicate a need for further research to examine the relationship between genes and amygdala activation specifically at the beginning of a scanning session (e.g., during initial exposure to novel emotional stimuli), as well as the ability to recruit prefrontal cortex–amygdala functional connectivity when first initiating an emotion regulation task. Research is also needed to examine gene–environment interactions on early-occurring amygdala activation and functional connectivity. Several studies have already demonstrated such interactions on amygdala activation during emotion processing (Bogdan, Williamson, & Hariri, 2012; White et al., 2012), suggesting that the relationship between these genes and brain function varies depending on the presence of environmental stressors. Including the brain as a level of analysis in this research may produce more powerful and replicable effects than using more distal measures such as symptoms or diagnoses.

The present study also has several implications for the development of evidence-based interventions. Amygdala activation may serve as a biomarker for treatment response when testing the effects of novel treatments or when deciding between two types of treatment (e.g., pharmacological treatment vs. psychotherapy) in order to tailor treatments to individuals (Paulus & Stein, 2007). The results of the present study indicate that when using amygdala activation as a biomarker to measure treatment response, it will be important to examine changes in amygdala activation during the initial portion of the scanning session, because this time most clearly differentiates pediatric anxiety disorder patients from controls.

In addition, these results indicate that anxiety disorder patients are capable of decreasing amygdala activation to emotional stimuli over time, although the mechanisms through which this occurs may be maladaptive. Interventions, whether they are administered through psychotherapy or computer-based formats, may therefore take an approach of replacing later occurring emotion regulation strategies such as suppression or attentional avoidance with early-occurring strategies such as cognitive reappraisal, which may be more adaptive and require less effort than later occurring strategies.

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Moreover, initial results of biofeedback studies using real-time fMRI-based measures have been promising, and they represent an exciting area in which fMRI itself may be used as part of a novel empirically based treatment. These studies use real-time fMRI feedback to train participants to regulate neural activity in regions such as the amygdala and the anterior cingulate cortex (deCharms et al., 2005; Johnston, Boehm, Healy, Goebel, & Linden, 2010; Linden et al., 2012). Although fMRI-based biofeedback may be a costly approach to treatment, if performing this type of treatment at earlier developmental stages produces long-lasting effects that prevent persistent disturbances in corticolimbic function or the development of more severe disorders in adulthood, this approach may prove to be more cost-efficient in the long run. Based on the results of the current study, real-time fMRI feedback could be used to guide anxiety disorder patients in recruiting regulatory prefrontal regions or regulating amygdala activation during initial exposure to novel stimuli.

In conclusion, we found that children and adolescents with anxiety disorders evidence an altered pattern of amygdala response over the course of an fMRI session characterized by an initial heightened response, followed by a reduction in amygdala activation. This was coupled with the finding of reduced context-modulated dorsal prefrontal cortex–amygdala connectivity during the first third of scanning in patients. These results demonstrate how the inclusion of measures of brain activation and connectivity as levels of analysis can provide unique insights into alterations in emotion processing in children and adolescents with anxiety disorders. These brain-based measures can be used in future research to link genetic and environmental influences to symptoms and disorders as well as to develop and test novel treatments. In these ways, knowledge of functional alterations in the brain will play an important role in enhancing our knowledge of the development and treatment of anxiety disorders.

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