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Research report

Where, when, how high, and how long? The hemodynamics of emotional response in psychotropic-naïve patients with adolescent bipolar disorder.

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ABSTRACT

Background: In response to emotional faces, patients with adolescent bipolar disorder (ABD) exhibit increased neural activity in subcortical emotional processing regions (e.g., amygdala, ventral striatum) and variable prefrontal activity. We extend previous research by identifying cortical and subcortical regions showing altered hemodynamic response shapes in ABD relative to healthy controls (HC).
Methods: ABD ($N=65$) and matched HC ($N=79$) completed a slow event-related affective hemodynamic probe task that required indicating the gender of fearful and neutral faces. An informed basis set in SPM8 evaluated shape variations of the hemodynamic responses to these faces.
Results: Patients with ABD showed higher activity for fearful relative to neutral faces in the amygdala and prefrontal cortex and a delayed hemodynamic response to fearful faces in dorsolateral and ventrolateral prefrontal cortices (PFC), as well as bilateral amygdala and caudate. Furthermore, the ABD group, relative to HC, showed a prolonged response to fearful faces in right dorsolateral PFC. Clinical measures of mania and depression severity correlated with increased processing delays in the amygdala and striatum.
Limitations: By design, the task contained fewer, more widely-spaced stimuli, possibly reducing its power to detect group differences. The use of fearful faces makes comparisons with prior literature in ABD somewhat more difficult.
Conclusions: The ABD group engaged in enhanced neural processing of the fearful faces which was associated with increasingly severe manic/mixed mood states. These exploratory findings could help elucidate a "biosignature" of emotion–attention interactions in ABD and present a potential target for reversal with medication treatment.

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1. Introduction

Adolescent bipolar disorder (ABD) is a severe, early-onset illness marked by episodic affective instability and cognitive abnormalities (Pavuluri et al., 2005). Patients with ABD often experience difficulty in accurately recognizing emotional facial expressions in others, which may lead to socially inappropriate responding (Brotman et al., 2008; Rich et al., 2008; Schenkel et al., 2007). Along with behavioral deficits in recognizing facial emotions, patients with ABD show disrupted patterns of neural activity relative to healthy controls (HC). Across a wide variety of facial emotion response paradigms, patients with bipolar disorder exhibit hyperactivity in the amygdala (Brotman et al., 2008; Kalmar et al., 2009; Pavuluri et al., 2009; Yurgelun-Todd et al., 2000), known to be activated in response to fearful faces and other emotionally salient stimuli (Hariri et al., 2002; LeDoux, 2000), and ventral striatum (Chen et al., 2006; Hassel et al., 2009, 2008; Lawrence et al., 2004; Rich et al., 2006; Surguladze et al., 2010), thought to be involved in attaching motivational significance to stimuli (Cardinal et al., 2002; Surguladze et al., 2003).

In contrast, patients with bipolar disorder show variable frontal activity during emotional face processing, with many reports of activity decreases in frontal regulatory regions such as the dorsolateral prefrontal cortex (DLPFC) (Hassel et al., 2009, 2008; Pavuluri et al., 2009; Yurgelun-Todd et al., 2000) and ventrolateral prefrontal cortex (VLPFC) (Altshuler et al., 2005; Pavuluri et al., 2007, 2009), but also some reports of increased

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activity in the ventral and medial prefrontal cortex (Lawrence et al., 2004; Rich et al., 2006; Surguladze et al., 2010).

Although prior studies have provided important information about the amplitude of the neural response to emotional faces in ABD, studies employing event-related fMRI facial emotion recognition paradigms have not employed the techniques necessary to detect changes in the shape of the blood oxygen level dependent (BOLD) responses to individual faces. These changes in shape include how immediate the responses are and their duration in terms of brain blood flow changes. Beyond simple increases and decreases in regional blood flow, improved information about regional variations in the time course of neural processing in ABD, as indexed by the shape of the hemodynamic response, could help researchers better understand the source of dysfunction and its likely causes, rather than solely learning that dysfunction exists in a particular region. Such characterization offers insight into models of the hemodynamic BOLD responses underlying affective reactivity in mania that could be reversed with interventions.

The current study employs a slow event-related design using an affective hemodynamic probe task with the necessary spacing between trials to fully resolve the BOLD response, thought to be approximately ten seconds for a single brief stimulus such as a face (Logothetis et al., 2001). In conjunction with this design, "informed" basis set functions (Friston et al., 1998) are used to distinguish between regions that primarily show a canonical BOLD response (i.e., how high), regions that primarily show BOLD responses occurring earlier vs. later (i.e., when), and regions primarily showing prolonged vs. abbreviated BOLD responses (i.e., how long).

Given that fearful faces served as a robust probe to activate subcortical emotional regions in previous studies (Hariri et al., 2002; Johnstone et al., 2005; Surguladze et al., 2003) and because fear, as opposed to anger, has been underexplored in fMRI studies of patients with ABD (Pavuluri et al., 2007, 2009), we were interested in examining the fronto-limbic responses to fear faces in ABD with a hypothesis-driven ROI-based analysis using planned comparisons within and between groups. Patients with bipolar disorder could potentially show a reduced ability to exert executive control to regulate their emotions and behavior as they take more time to engage regulatory brain regions (Leibenluft et al., 2007), which could manifest as a slowed BOLD response. Similarly, the overreaction of ABD to emotional material could be expressed as a rapid, uncontrolled engagement of affective systems, in addition to the previously detected overactivation (Chang et al., 2004; Pavuluri et al., 2007; Rich et al., 2006). Thus, we expected to observe reduced amplitudes and delays in processing in prefrontal regions, along with increased amplitudes and earlier processing in subcortical regions such as the amygdala and ventral striatum.

2. Methods and materials

2.1. Design

We conducted a cross-sectional study of the neural response to emotional faces of psychotropic-naïve patients with adolescent mania and HC. Informed consent was obtained from at least one parent, and assent was obtained from all participants. The study was approved by the institutional review board at the University of Illinois at Chicago.

2.2. Sample

Inclusion criteria for patients were a DSM-IV (APA, 1994) diagnosis of mixed or manic type 1 bipolar disorder, 10–18 years old, and never having taken psychotropic medications. Exclusion criteria included: active substance abuse within three months prior to scanning; serious medical problems or history of head trauma; autism and non-affective psychotic disorders. In addition, 25 participants (13 ABD and 12 HC) were unable to successfully complete the scan because they showed very large amounts of motion in the scanner, and these participants were excluded at the preprocessing step as their data were not suitable for inclusion in analyses. Participants who were able to scan but had motion artifacts (HC: $n=1$; ABD: $n=2$) exceeding our criteria (described below) were also excluded, so the final sample consisted of 65 ABD and 79 HC. No differences across groups for age, race, gender, handedness (Annett, 1970), or SES (Hollingshead, 1983), were found, and despite a statistically marginal difference in the gender distributions between groups, gender had no effect on the results. Demographic and clinical characteristics of our final samples are summarized in Table 1.

2.3. Clinical assessment

Each child and their parent or legal guardian were interviewed by doctoral-level clinicians with established inter-rater reliability using the Washington University in St. Louis Kiddie Schedule for Affective Disorders and Schizophrenia (WASH-U KSADS; Geller et al., 1998) supplemented by the episode characterization of bipolar disorder from the KSADS—Present and Lifetime versions (Kaufman et al., 2000). Three independent masters-level clinical raters with established reliability (Cohen's kappa=0.94) administered the Young Mania Rating Scale (YMRS; Young et al., 1978), as well as the Child Depression Rating Scale-Revised (CDRS-R; Poznanski et al., 1984) as indicators of clinical status.

2.4. Affective hemodynamic probe task

After training in a realistic mock scanner, participants performed the affective hemodynamic probe task for approximately eight minutes in the fMRI scanner. Participants viewed images of 16 fearful and 16 neutral faces from the Gur facial stimulus set (Gur et al., 2002) presented for 250 milliseconds. Each face was followed by a prolonged period of crosshair fixation (15 s), which allowed adequate time to visualize the BOLD response to each face without any intervening external stimuli. Half of the faces were female, and the participants' task was to indicate the gender of each face. In order to assess participants' automatic reactions to the stimuli, participants were not told anything about the emotionality of the faces.

Table 1
Clinical and demographic information for ABD and HC.

Variable	ABD ($N=65$) Mean (SD)	HC ($N=79$) Mean (SD)	t, p
Age in years	13.5 (2.2)	14.1 (2.3)	$t=1.73$, $p=.086$
SES	2.9 (1.2)	2.8 (1.2)	$t=.421$, $p=.67$
YMRS	23.4 (6.0)	2.3 (2.8)	$t=27.9$, $p<.001$
CDRS-R	41.9 (11.2)	21.0 (3.9)	$t=15.5$, $p<.001$
Variable	N (%)	N (%)	df, X^2
Sex			$df=2$, $X^2=3.28$, $p=.094$
Male	37 (56.9%)	33 (41.8%)	
Female	28 (43.1%)	46 (58.2%)	
Handedness			df=2, $X^2=.438$, $p=.51$
Left	7 (10.8%)	6 (7.6%)	
Right	58 (89.2%)	73 (92.4%)	
Race Composition			df=2, $X^2=2.93$, $p=.23$
Caucasian	45 (69.2%)	45 (57.0%)	
African American	14 (21.5%)	20 (25.3%)	
Other	6 (9.2%)	14 (21.5%)	

Note: YMRS=Young Mania Rating Scale; CDRS-R=Child Depression Rating Scale-Revised; SES=socioeconomic status; ABD=Adolescent Bipolar Disorder; HC=Healthy Control.

2.5. MRI protocols

T2*-weighted functional images were acquired with a gradient-echo echo-planar sequence (TR=1200 ms, TE=25.4 ms, flip angle=82°, FOV 20 × 20 cm^2, 64 × 64 matrix, 3.125 × 3.125 mm in-plane resolution, 23 slices, 4-mm slice thickness, 1-mm gap) using the 3.0 T magnet (Signa, General Electric Medical System, Milwaukee, WI). Anatomical images were also acquired in the axial plane (3-D SPGR, 1.5 mm thick contiguous axial slices) and were co-registered with the functional data. The experiment run consisted of 426 time points.

2.6. Image preprocessing

Participants' functional images were preprocessed in SPM8 (Wellcome Dept. of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk/spm). Slice timing correction was applied on the data to remove variation in BOLD signal intensity due to slice acquisition temporal onset differences. Each participant's first functional image volume was used to determine parameters for spatial normalization into Montreal Neurological Institute standardized space employed in SPM8 using non-linear transformation. The normalization parameters determined for the first functional volume were applied to all 426 functional image volumes for each participant, and the normalized images were smoothed with an 8-mm full-width-at-half-maximum Gaussian filter. Artifact Detection Tools (ART, http://www.nitrc.org/projects/artifact_detect) software was used for automatic detection of the global mean and motion outliers in the functional data (z-threshold=6, and movement threshold=1.5 mm). All participants included in the analysis had at least 85% of volumes retained (M=98.7%, SD=2.1%), and there were no differences in the average number of volumes retained between ABD (M=98.5%, SD=2.4%) and HC (M=98.9%, SD=1.9%), $t(142)=1.33$, $p=.19$, $d=0.03$, 95% CI [−.012, .002].

2.7. Temporal modeling of the BOLD response

The "informed basis" set (Friston et al., 1998), as implemented in SPM 8, for each participant's first-level model consisted of the canonical BOLD response model, and derivatives for "time" (modeling temporal peak onset) and "dispersion" (modeling the persistence of the BOLD response). This step produced separate sets of beta weights for each regressor (canonical, temporal peak, and dispersion) for each emotion type. Specific regions of interest (ROIs) for each hemisphere were constructed from the Wake Forest University PickAtlas (version 3.03; Maldjian et al., 2003) for VLPFC (Brodmann's Area (BA) 47), DLPFC (BA 9), BA 46 to represent the DLPFC/VLPFC junction area detected in previous studies as responding abnormally to emotional stimuli in ABD (Pavuluri et al., 2008), amygdala, and caudate. All regional maps from the PickAtlas were dilated by one voxel in three dimensions to ensure adequate coverage of functional regions and to account for individual differences in neuroanatomy.

Next, all within-subjects second-level contrasts (e.g., Fear Canonical vs. Neutral Canonical for ABD) and between-subjects second-level contrasts (e.g., ABD vs. HC for Fear Canonical) were evaluated in SPM at $p<.001$, uncorrected, and contrasts without any suprathreshold voxels at $p<.001$ uncorrected were not considered further. Subsequently, small volume correction (SVC) in SPM 8 was applied to each of the remaining contrasts for all ROIs. Clusters within each ROI were removed from consideration if they showed a p value greater than .05 family-wise error (FWE), applying SVC for the ROI, or were smaller than four voxels, regardless of p value. Peak coordinates, cluster sizes and their corresponding contrasts are presented in Table 3.

In order to visualize and quantify these results, event time course eigenvalues, which represent the mean weighted response in a given cluster, were directly extracted from the clusters identified by each regressor. Example time courses of areas that showed differences in the canonical response, its peak, or dispersion are displayed in Figs. 1–3, respectively. These examples were chosen because they showed highly visually striking differences between groups. Other time courses were also statistically significant but were more subtle visually. Time courses for all of the other clusters in Table 3 can be found in the online supplementary material.

2.8. Clinical and behavioral analyses

Participants' YMRS and CDRS-R ratings were compared between groups with independent-samples t tests (Table 1). Participants'

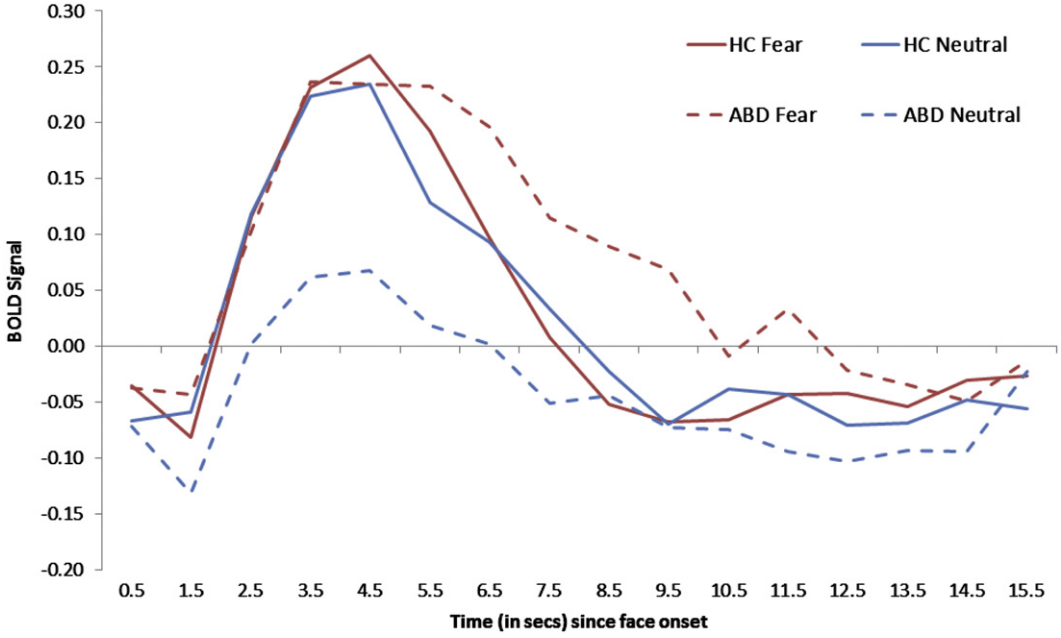

Fig. 1. Left DLPFC/VLPFC junction showed reduced amplitude for ABD in response to neutral faces. Legend: DLPFC=dorsolateral prefrontal cortex; VLPFC=ventrolateral prefrontal cortex; ABD=adolescent bipolar disorder; HC=healthy control; BOLD=blood oxygen-level dependent.

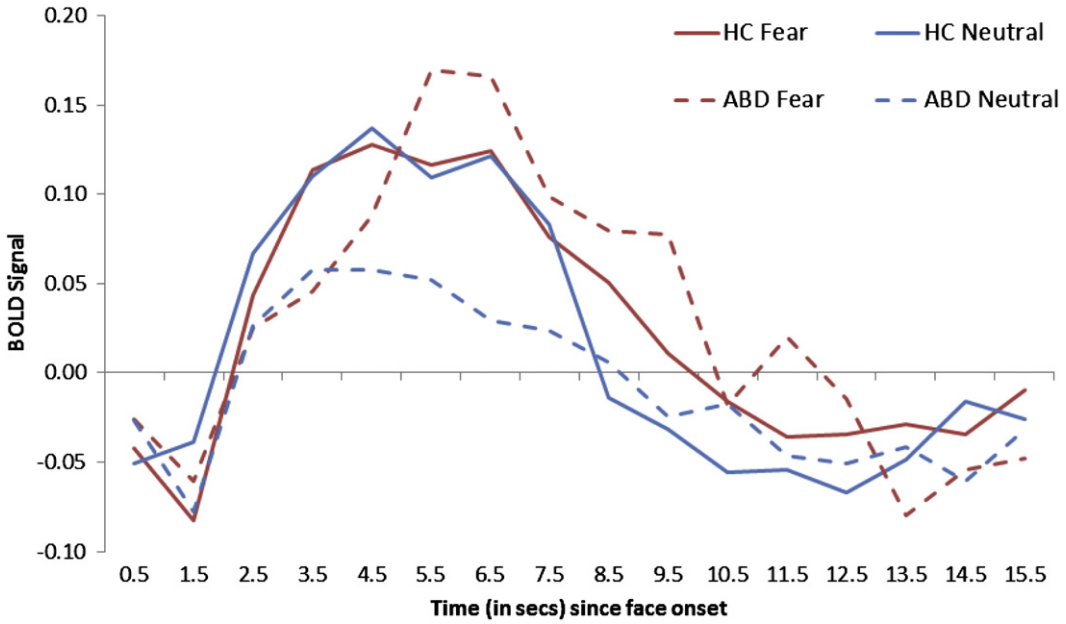

Fig. 2. Right amygdala showed delayed peak for ABD for fearful faces. Legend: ABD=adolescent bipolar disorder; HC=healthy control; BOLD=blood oxygen-level dependent.

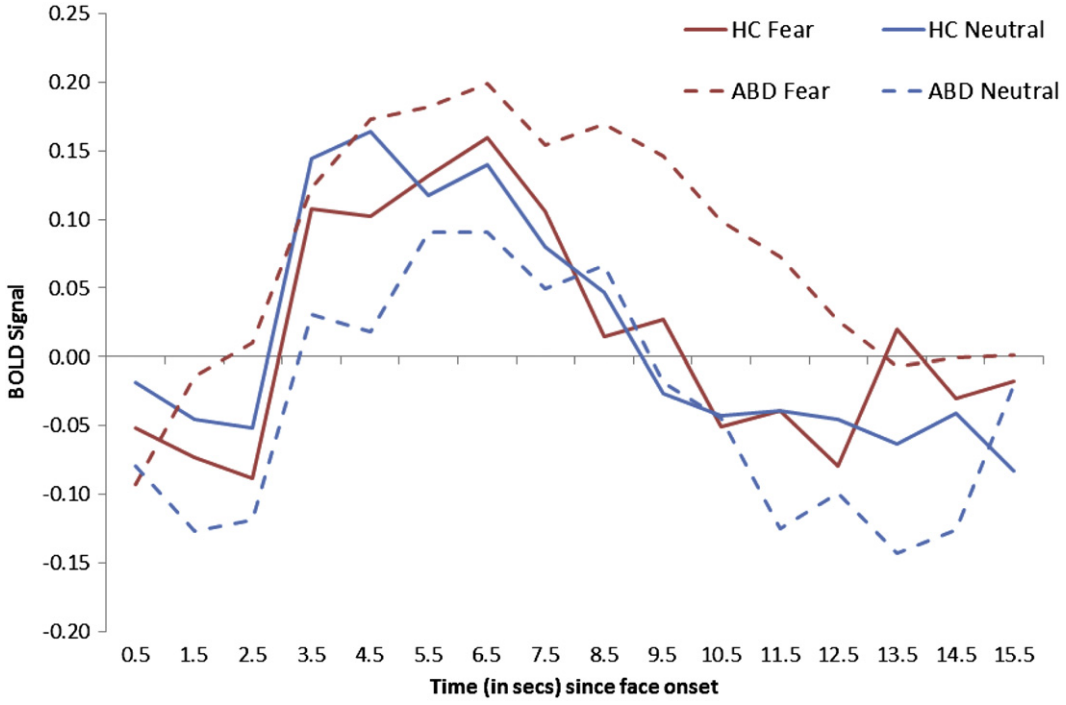

Fig. 3. Right DLPFC showed prolonged time course for ABD for fearful faces. Legend: DLPFC=dorsolateral prefrontal cortex; ABD=adolescent bipolar disorder; HC=healthy control; BOLD=blood oxygen-level dependent.

median reaction times (RTs) and accuracy were examined with 2×2 analyses of variance (ANOVAs) with Valence (fearful vs. neutral faces) and Group (ABD, HC) as factors (Table 2).

2.9. Correlations with clinical data

In order to relate the neuroimaging data to participants' clinical symptoms, Spearman correlations were conducted between participants' YMRS and CDRS-R scores and the eigenvalues for fear and neutral from each of the ROIs and corrected for multiple comparisons.

3. Results

3.1. Clinical and behavioral data

Participants with ABD, as expected, were in a manic and mildly dysphoric state, showing significantly higher scores relative to HC on both the YMRS, $t(142)=24.26$, $p<.001$, $d=10.2$, 95% CI [19.6, 22.6] and the CDRS-R, $t(142)=15.5$, $p<.001$, $d=7.8$, 95% CI [18.2, 23.5]. For the behavioral data (Table 2), HC were significantly more accurate overall than ABD, $F(1, 142)=12.89$, $p<.001$, $\eta_p^2=.083$, fear faces were significantly less likely overall than

neutral faces to be identified correctly, $F(1, 142)=12.05$, $p=.001$, $\eta_p^2=.078$, and there was a trend toward different accuracy rates on the fear and neutral trials for the two groups, as indicated by a marginal two way interaction between Group and Valence, $F(1, 142)=2.76$, $p=.099$, $\eta_p^2=.019$. Follow up paired-samples t tests revealed that the ABD group was significantly less accurate at the gender identification task for the fearful faces than for the neutral faces, $t(64)=3.49$, $p=.001$, $d=0.43$, 95% CI [−1.35, −.37], whereas the HC showed no significant difference in accuracy between fearful and neutral faces, $t(78)=1.34$, $p=.18$, $d=15$, 95% CI [−.76, .15]. Separate comparisons between ABD and HC for fear and neutral faces revealed that ABD were worse than HC at identifying both fearful faces, $t(142)=4.17$, $p<.001$, $d=1.3$, 95% CI [−3.32, −1.19] and neutral faces, $t(142)=2.79$, $p<.001$, $d=.9$, 95% CI [−2.90, −.49].

Similarly, for median reaction time, there was a trend toward an interaction between valence and group, with different RTs in response to fearful and neutral faces across the two groups, $F(1, 142)=3.36$, $p=.069$, $\eta_p^2=.023$. There was also a trend toward fear faces being responded to later than neutral faces, regardless of group, $F(1, 142)=3.51$, $p=.063$, $\eta_p^2=.024$, but no significant main effect of group, $F(1, 142)=2.13$, $p=.15$, $\eta_p^2=.015$. Follow up paired-samples t tests revealed that the ABD group showed a trend toward slower median RTs to the fear faces, relative to neutral faces, $t(64)=1.70$, $p=.095$, $d=0.21$, 95% CI [−235.5, 19.3], whereas the HC showed no significant difference in median RTs between fearful and neutral faces, $t(78)=0.17$, $p=.87$, $d=0.02$, 95% CI [−13.0, 15.5]. However, there were no significant differences in median RT for ABD and HC groups for fearful faces, $t(142)=1.64$, $p=.103$, $d=6.8$, 95% CI [−28.98, 311.66], or neutral faces, $t(142)=0.88$, $p=.38$, $d=2.3$, 95% CI [−42.67, 111.56].

Despite these differences in behavioral performance, covarying for participants' median reaction time or accuracy did not alter the fMRI results. Thus, delayed peak times and increased dispersion of the BOLD response, suggestive of slowed and prolonged processing, are likely to be driven by the participants' diagnostic and clinical states, rather than their behavioral performance.

Table 2
Number correct and median reaction time by valence and group.

Variable	Valence	ABD ($N=65$) Mean (*SEM*)	HC ($N=79$) Mean (*SEM*)	t (142), p
Number correct	Fear	10.7 (.47)	13.0 (.30)	$t=4.17$, $p<.001$
	Neutral	11.6 (.55)	13.3 (.32)	$t=2.79$, $p<.001$
Median RT (ms)	Fear	855 (92)	713 (19)	$t=1.64$, $p=.10$
	Neutral	747 (35)	712 (20)	$t=0.88$, $p=.38$

ABD=Adolescent Bipolar disorder; HC=Healthy Control; RT=reaction time.

3.2. How high? (canonical regressor)

The left DLPFC/VLPFC junction, the right DLPFC, the left amygdala, and bilateral VLPFC showed a consistently higher BOLD response for the fearful faces relative to the neutral faces in ABD (Table 3; Fig. 1). As an illustrative example, the time courses for each group in the left DLPFC/VLPFC cluster in response to fearful and neutral faces are displayed in Fig. 1.

3.3. When? (temporal peak regressor)

The temporal peak regressor identified regions in the bilateral amygdala and caudate, as well as the right DLPFC/VLPFC junction and VLPFC (Table 3). The right amygdala, caudate, VLPFC, and DLPFC/VLPFC junction showed a later peak for fearful faces in ABD relative to HC. Furthermore, the DLPFC/VLPFC junction also showed a later peak for neutral faces in ABD relative to HC. In addition, within the ABD group considered alone, the bilateral amygdala and left caudate showed a delayed peak for fearful relative to neutral faces (Fig. 2 illustrates the delayed peak in right amygdala).

Table 3
Clusters identified by each hemodynamic regressor surviving small volume correction.

Effect	Location	Peak (MNI)	Size in voxels	p (FWE)
Canonical differences				
ABD: Fear > neutral	L DLPFC/VLPFC junction*	[−51, 26, 22]	38	.014
(larger response for fearful vs. neutral faces in ABD)	L Amygdala	[−18, −7, −20]	13	.01
	L VLPFC	[−48, 23, −5]	207	.001
	R VLPFC	[27, 32, −20]	43	.019
	R DLPFC	[12, 50, 43]	26	.027
	R DLPFC	[6, 59, 16]	13	.04
Peak time differences				
ABD: Fear < neutral	R Amygdala **	[27, −4, −20]	14	.009
(later peak for fearful vs. neutral faces in ABD)	L Amygdala	[−24, 2, −17]	10	.011
	L Caudate	[−12, 23, −5]	5	.015
ABD < HC for fear	R Amygdala	[18, 2, −20]	8	.012
(ABD show later peak for fear faces than HC)	R Caudate	[15, 11, −2]	9	.012
	R DLPFC/VLPFC junction	[45, 14, 28]	12	.031
	R DLPFC/VLPFC junction	[45, 41, 19]	9	.035
	R VLPFC	[27, 29, −20]	57	.014
ABD < HC for neutral	R DLPFC/VLPFC junction	[48, 17, 28]	12	.031
(ABD show later peak for neutral faces than HC)	R DLPFC/VLPFC junction	[48, 32, 13]	10	.033
Dispersion differences				
ABD < HC for fear (ABD show prolonged response to fearful faces, relative to HC)	R DLPFC ***	[27, 50, 40]	53	.013

Time courses for all other regions depicted in the supplementary material.
Legend: L=left; R=right; DLPFC=dorsolateral prefrontal cortex; VLPFC=ventrolateral prefrontal cortex; ABD=adolescent bipolar disorder; HC=healthy control; MNI=Montreal Neurological Institute; FWE=family-wise error.

* Depicted in Fig. 1.
** Depicted in Fig. 2.
*** Depicted in Fig. 3.

3.4. How long? (diffusion regressor)

The diffusion regressor only identified one cluster, which appeared in the right DLPFC (Table 3), and the time courses for ABD and HC in this cluster in response to fearful and neutral faces are depicted in Fig. 3. As indexed by the eigenvalues for the diffusion regressor, this right DLPFC region exhibited a much more prolonged time course for the ABD group in response to fearful faces than to neutral faces.

3.5. Correlations with clinical data

Although numerous correlations between brain activation and clinical measures had p values $<.05$, only two correlations survived our stringent threshold for multiple comparisons ($p<.0015$). Participants who showed higher YMRS scores also showed a more delayed peak in the right amygdala (i.e., *lower* eigenvalues) in response to fearful faces, Spearman's rho $=-.270$, $p=.0011$, and participants with increased CDRS-R scores showed a more delayed peak in the left caudate, Spearman's rho $=-.279$, $p=.0007$. In addition, participants who showed higher CDRS-R scores also exhibited a delayed peak in the right amygdala, Spearman's rho $=-.258$, $p=.0018$, which fell just short of surviving our correction for multiple comparisons.

4. Discussion

The present study employed a slow event-related design coupled with an informed basis set to identify regions in ABD and HC that fit the canonical BOLD response model, as well as regions that showed a different time course or duration than the normal hemodynamic response. We found that patients with ABD showed relatively higher neural activity for fearful faces relative to neutral faces in the bilateral VLPFC, right DLPFC, left DLPFC/VLPFC junction and left amygdala. In addition, the ABD group showed delays in the processing of fearful faces in the right DLPFC/VLPFC junction, right VLPFC, bilateral amygdala, and bilateral caudate. They also showed processing delays in the right DLPFC/VLPFC junction in response to neutral faces. Furthermore, the ABD group, relative to the HC group, showed a prolonged response to fearful faces in the right DLPFC.

Behaviorally, we found a slight decrease in accuracy in the ABD patients, relative to HC, that was greater in the fear faces condition and a slight increase in reaction time in the ABD patients for the fearful faces. Although the sparse nature of the behavioral task likely reduced its sensitivity to behavioral differences, the ABD group showed some evidence for a selective impairment for the fear faces on the gender identification task. This finding suggests that the neuroimaging results, which were also largely selective for fearful faces, were not caused by reduced attention by the patients to the task. In contrast, these findings suggest that both the delayed neural and behavioral responses to the faces were modulated by their emotional content. Finally, the delay in right amygdala neural activity was related to participants' clinical symptoms of mania, whereas the delay in left caudate activity was related to their clinical symptoms of depression.

4.1. How high (amplitude)

We expected to find that the ABD group would show increased activity in the limbic regions but not the prefrontal regions such as the DLPFC/VLPFC junction and VLPFC. The only other study to date to investigate the recognition of fearful faces in ABD (Kalmar et al., 2009) used an ROI analysis to focus on amygdala activity and found increased activity in response to fear faces which correlated with smaller amygdala volumes in ABD. The present study is consistent with those findings in that there was increased amygdala activity in response to the fearful faces relative to neutral faces in the ABD group.

Nevertheless, other researchers have found increased activity in bilateral DLPFC in patients with ABD when viewing negative scenes (Chang et al., 2004) and increased activity in the VLPFC when patients rated the hostility of neutral faces or their own subjective fear in response to them (Rich et al., 2006). Furthermore, adults with bipolar disorder exhibited increases in the left amygdala and VLPFC in response to fearful faces (Lawrence et al., 2004), so the relative increase in prefrontal regions could be specific to fear, rather than anger. A fearful face is more ambiguous than an angry face, as a fearful face could be interpreted as either a response to the ones' own anger or as a signal of another environmental threat (Adams et al., 2003; Whalen, 1998), so perhaps this difference between fear and angry expressions explains the difference in findings. Alternatively, fearful faces may simply appear less negative than angry faces to the ABD group, so the patients are able to compensate for it with increased activity in the VLPFC, but they are unable to do so in response to anger.

One caveat remains that the relative activity increases in frontal and limbic regions were solely found *within* the ABD group, rather than between the ABD and HC groups, and the differences appeared to be caused by a reduction in the amplitude of the BOLD response to neutral faces, rather than an increase in the response to fearful faces (Fig. 1). Nevertheless, the ABD group was sensitive to the presence of fearful emotion in these rapidly-presented faces, even when asked to rate a non-emotional characteristic of each face, whereas the HC group did not show evidence of sensitivity to emotion. Thus it is possible that our adolescent patients with mania, like those in previous studies (Rich et al., 2006), were attempting to make sense of the more ambiguous nature of the fearful face stimuli by singling them out for enhanced processing. This interpretation is also consistent with the evidence provided by the behavioral results that ABD showed selectively reduced accuracy and longer reaction times for the gender decision to fearful faces, relative to HC who showed minor differences due to valence.

4.2. When (peak) and how long (dispersion)

Contrary to part of our initial hypothesis, ABD did not show an earlier latency of the BOLD response in the amygdala and ventral prefrontal affective regions in response to fearful faces. Rather, they showed increased engagement, with a later peak response in these regions. These findings of uniformly later peak times for ABD relative to HC for fearful faces across most of our ROIs further support the conclusion that ABD engaged in extended processing of the emotional content. Regions of the DLPFC/VLPFC junction, VLPFC, striatum, and amygdala all showed later peaks for the ABD group in response to fearful faces, relative to HC, and none of the ROIs showed earlier peaks for HC than for ABD. In addition to the delays in frontal and limbic regions for fearful faces in ABD, two small clusters in the right DLPFC/VLPFC junction also showed a delayed response to neutral faces, suggesting either that some general deficits in face processing exist in ABD or that some of the emotionality of the fearful faces spilled over to the neutral faces.

In addition, the ABD group exhibited prolonged processing in the right DLPFC, a region previously shown to be hypoactive in ABD relative to HC during the implicit processing of angry faces (Pavuluri et al., 2009). The prolonged activity in this region may actually represent increased and sustained attention to the emotional content of the face, but because of its atypical time course,

activity in this region was detected in previous studies as lower in ABD than HC. Atypical time courses such as these would not show a good fit with the canonical hemodynamic regressor typically employed as the sole measure in conventional fMRI analyses, which could lead to the assumption that there was *less* activity when in reality the activity was just *different*. Thus, techniques such as the informed basis set that investigate the shape of the hemodynamic response, rather than solely its fit to the canonical regressor, can potentially help to clarify ambiguities in the literature (Lindquist et al., 2009).

Finally, clinical measures of mania and depression severity correlated with delays in processing in subcortical emotional processing regions, suggesting that these delays are associated with clinically-relevant emotional instability. Thus, employing the informed basis set in this study provided an improved picture of the facial emotion recognition deficits in ABD, potentially leading toward biosignatures of illness status. Such biosignatures could one day lead to diagnostic tests for bipolar disorder and other mood disorders, and they could provide good criteria with which to evaluate the efficacy of medication and psychological therapies (Mayanil et al., 2011).

4.3. Strengths, limitations, and future directions

The strengths of this study include a large, never medicated, well-characterized sample of patients with ABD in a manic state, paired with a large sample of HC matched on many demographic characteristics (e.g., age, gender, handedness). In addition, the use of a slow event-related design and an informed basis set allowed us the temporal resolution to visualize the BOLD response for each emotional and neutral face without interference from any other external stimuli. This technique provided the capability to investigate whether patients' neural responses were delayed or prolonged in addition to measuring the amplitude of the responses. In addition, the use of an analysis based on standard anatomical ROIs to restrict comparisons to areas already widely known to show dysfunction in patients with bipolar disorder during the processing of emotional faces (Chen et al., 2006; Kalmar et al., 2009; Pavuluri et al., 2007, 2009; Surguladze et al., 2010) provides more confidence that these findings are in fact related to differences in emotional face processing between the two groups.

Limitations of this study include the fact that only a limited number of trials could be presented in the slow event-related design, so the dataset was somewhat sparse. Our design may not have possessed enough trials to be sensitive enough to pick up on the effects of stimulus valence in the HC (Killgore et al., 2008). The effect of stimulus valence may also be more subtle in adolescents, especially for implicit designs such as ours where the emotions are not the focus of attention (Monk et al., 2003). Despite this, we *did* detect meaningful differences between HC and ABD participants and within group differences in the ABD sample using this sparse design.

An additional limitation of this study was that only one type of emotional face was tested, so it remains unclear what patterns of activity would have been detected if angry, sad, or happy faces had been used instead. The sole use of fearful faces was, however, a deliberate decision in study design to probe the affective systems with greater power and with a single emotion. Finally, although the canonical hemodynamic response plus the temporal and dispersion derivatives are capable of modeling delays and dispersions of the neural response of a few seconds before or after the stimulus onset, the informed basis set may not fully capture deviations in stimulus peak or duration beyond a few seconds as well as other techniques (Lindquist et al., 2009). Thus, there may have been additional regions that responded differentially between HC and ABD that we were unable to detect using the informed basis set.

Future studies could employ these informed basis techniques or other advanced hemodynamic modeling techniques (see Lindquist et al., 2009 for a list) to investigate the neural response of participants with ABD to other emotional faces and to explore these responses in patients with other psychiatric disorders. Finally, although this study has revealed more about the time course of emotional face processing in bipolar disorder, we cannot yet say for certain which brain networks were engaged or determine the direction of causality between brain regions. Subsequent studies could be conducted using functional and effective connectivity methods to further elucidate the ebb and flow of emotional reactivity in mood disorders.

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As such, the authors will follow the National Institutes of Health (NIH) revised "Public Access Policy," effective April 7, 2008. We will submit to PubMed Central (PMC), or agree to have submitted on our behalf, our peer-reviewed author manuscripts, to appear on PMC no later than 12 months after final publication.

Conflict of interest

MN Pavuluri's work is currently supported by NIMH 1R01MH85639-01A1, NIMH 5R01MH081019-02, and NIMH 1RC1MH088462-01, and NIH 1K24MH096011-0. She is the current recipient of the Berger-Colbeth Endowed Chair in Child Psychiatry and is currently on the speakers bureau for Bristol-Myers Squibb. AM Passarotti's work is currently supported by a NARSAD Young Investigator's Award #17592 and a grant (#2012-02364-00-00) from the Depression and Bipolar Foundation for Alternative Treatment. No competing financial interests exist for E Wegbreit, JA Ellis, M Wu, N Witowski, JM Fitzgerald, or MC Stevens.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jad.2012.11.025.

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