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Amygdala Functional Connectivity in Young Women with Borderline Personality Disorder

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Abstract

Borderline personality disorder (BPD) is a complex psychiatric disorder that involves the core feature of affect dysregulation. Prior neuroimaging studies have indicated that BPD patients have (1) excessive amygdala activation to negative emotion and (2) diminished frontal regulation. This study examined amygdala functional connectivity in 12 women with BPD and 12 matched healthy comparison volunteers. We explored how connectivity patterns would change in the context of processing neutral, overt fear, or masked fear face expressions. Each participant underwent three 5-min fMRI scans in which they primarily viewed: (1) neutral, (2) overt fear, and (3) masked fear faces. In comparison to their healthy counterparts, young women with BPD showed (1) lower connectivity between bilateral amygdala and mid-cingulate cortex during the neutral scan; (2) higher connectivity between bilateral amygdala and rostral anterior cingulate cortex during the overt fear scan; and (3) higher right amygdala connectivity with bilateral thalamus and right caudate during the masked fear scan. Exploratory analyses revealed interesting correlations between amygdala connectivity in these conditions with multiple clinical measures. Results from the neutral scan add to the few prior connectivity studies in BPD that have been suggestive of lower fronto-limbic connectivity in BPD. However, the connectivity findings during fear processing are novel, and map onto basic research models for amygdala connectivity, that is, connections to frontal areas for overt fear processing versus connections to thalamus for automatic fear processing. Further, results suggest that BPD subjects tap into both pathways more strongly than healthy comparisons.

Key words: amygdala; borderline personality disorder; functional connectivity; functional neuroimaging; masked fear; overt fear

Introduction

Borderline personality disorder (BPD) is a complex psychiatric disorder characterized by pervasive disturbances in affect regulation, interpersonal relations, and self-image (Skodol et al., 2002). Neuroimaging studies have begun to elucidate the biological underpinnings of affect dysregulation in BPD by examining fronto-limbic neural networks, which mediate affect regulation (Phillips, 2003). Early studies have indicated that BPD patients may have (1) excessive amygdala activation to negative emotion (Donegan et al., 2003; Koenigsberg et al., 2009) and (2) diminished frontal (presumably regulatory) responses (Goyer et al., 1994; Koenigsberg et al., 2009; Minzenberg et al., 2007; Silbersweig et al., 2007). These findings suggest a potential neurobiological mechanism to the hypotheses that BPD involves excessive reactivity to negative emotion, and diminished capacity to regulate this response (Linehan, 1987).

The initial studies in BPD that identified critical areas implicated in emotion processing have provided the groundwork for more sophisticated approaches to understanding neural circuitry. A systems-based approach that examines the integrity of neural connections within fronto-limbic circuits or "connectivity" is necessary to better understand the neural deficits underlying affect dysregulation in BPD.

Emerging studies have begun to provide evidence supporting the hypothesis that affect dysregulation results from aberrant connectivity within fronto-limbic neural networks.

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An initial study that examined brain connectivity in BPD using 18-fluorodeoxyglucose positron emissions tomography found reduced coupling of metabolic activity between the amygdala and the orbitofrontal cortex in patients with BPD (New et al., 2007). Other approaches have used diffusion tensor imaging (DTI) to examine white matter connectivity; boadly, these initial DTI efforts have revealed reduced integrity of white matter connections relevant to fronto-limbic circuitry (Grant et al., 2007; Rusch et al., 2007, 2010).

Another, noninvasive technique is to use functional magnetic resonance imaging (fMRI) to measure the correlation of blood oxygen level-dependent (BOLD) signal fluctuations between brain regions as an index of functional connectivity (Biswal et al., 1995). Acquisition of fMRI data to examine functional connectivity can be conducted both at rest (Biswal et al., 1995; Fox and Raichle, 2007) and also during a specific task (Pezawas et al., 2005; Rich et al., 2008). The task of viewing standardized emotional expressions evokes similar emotions in human subjects (Wild et al., 2001) and has been used in numerous fMRI studies that have investigated the neural basis for emotion processing [see review by Vuilleumier and Pourtois (2007)]. In particular, viewing faces with fearful expressions is associated with a robust amygdala response and has been used extensively to understand amygdala reactivity (Phan et al., 2002) and also amygdala connectivity (Stein et al., 2007). In an effort to focus in on circuitry relevant to affect processing, in the current study we examine functional connectivity of amygdala using an fMRI paradigm that involves viewing of emotional facial expressions.

Examination of amygdala function and its associated pathways has led to rapid advancement in neuroscience for understanding the neural basis of emotion-related behavior (Ressler, 2010). An extension of this research has been to delineate distinct amygdala circuits that mediate conscious versus subconscious processing of fear by using a masking technique to present fear stimuli that are not consciously perceived. Studies using this approach have identified that in healthy adults, amygdala response may be greater in response to covert (masked) than overt fear stimuli (Whalen et al., 1998), and that regions connected to the amygdala vary depending on the level of conscious processing (Morris et al., 1999; Williams et al., 2006). Prior work has noted the importance of subliminal processing of fear and its relation to hypervigilance in BPD (Sieswerda et al., 2007), but no prior studies have used masked fear approaches with fMRI to examine neural circuitry in BPD.

The goal of this study was to examine functional connectivity of amygdala neural circuitry in adults with BPD in comparison to matched healthy volunteers. To minimize confounds due to potential medication effects and sample heterogeneity, we chose to examine young women with limited diagnostic comorbidity who were not taking psychiatric medications. Based on current models for BPD, we predicted a general pattern in which individuals with BPD would demonstrate impaired connectivity between amygdala and prefrontal regulatory areas. We also aimed to explore how these connectivity patterns would change in the context of processing neutral, overt fear, or masked fear facial expressions, and predicted that individuals with BPD would show particular abnormalities in the automatic pathway during processing of masked fear.

Methods

Subjects

Participants included 12 women with BPD aged 18–33 (mean age 24.1, standard deviation [SD] 4.7), and 12 healthy women aged 19–34 (mean age 25.1, SD 4.7). Participants were matched one-to-one between groups for age, ethnicity, and handedness. Diagnostic assessment included structured interviews conducted by a trained graduate student (N.V.) and/or a registered nurse (A.R.), supervised by a psychiatrist (S.C.S). The diagnostic assessments included two versions of the Schedule Clinical for DSM-IV (SCID). The SCID-I was used to screen for major Axis I psychiatric disorders, and the SCID-II was used to confirm the diagnosis of BPD. Additional scales included the Symptom Checklist-90-R (SCL-R) (Derogatis, 1994) and the State-Trait Anxiety Inventory (Speielberger, 1983). (The STAI measure was collected directly after the scanning session, whereas all other measures were collected during the diagnostic assessment.)

As noted above, a study goal was to reduce confounds associated with diagnostic comorbidity or medication effects. Thus, for the BPD group, exclusion criteria included a history of any psychotic disorder, bipolar disorder, major depressive disorder (MDD) with psychotic features, obsessive-compulsive disorder, generalized anxiety disorder (GAD), social phobia, and post-traumatic stress disorder (PTSD). The rationale for these diagnostic exclusions is that previous research has reported differential amygdala findings in patients with PTSD (Rauch et al., 2003; St Jacques et al., 2010), GAD (McClure et al., 2007), and social phobia (Birbaumer et al., 1998; Yoon et al., 2007), relative to healthy controls, in response to neutral and emotional face stimuli. Inclusion of these particular anxiety disorders would thus introduce a potential confound in the study design. However, a history of PTSD was allowed, as was a history of MDD without psychotic features.

These past comorbidities were included due to high rates of lifetime MDD (Gunderson et al., 2008; Shea et al., 2004) and PTSD (Shea et al., 2004) among individuals with BPD. Similarly, substance use disorders are very common in BPD (Zanarini et al., 2011). If participants met criteria for substance abuse or dependence, it was required that they be in at least partial remission. Participants were instructed to refrain from abusing substances for before the scanning session (1 week of abstinence for illicit substances and 24 h for alcohol). Compliance was assessed at the scanning session by self-report. It was determined by the study team that exclusion of all subjects with a history of MDD, PTSD, and substance use disorders would result in a sample that was not representative of BPD. Finally, we excluded all subjects who were taking medications prescribed for a psychiatric diagnosis.

For the control group, participants had to demonstrate fewer than two BPD criteria as assessed by the SCID-II, and could not meet criteria for any axis I disorder as assessed by the SCID I.

MRI data acquisition

All MRI data were acquired at the University of Minnesota's Center for Magnetic Resonance Research. Structural and functional MRI data were acquired on a Siemens 3T Trio scanner using an 8-channel parallel imaging coil. Scout images were obtained for purposes of slice prescription (TE = 5 ms, TR = 20

ms, FOV = 256, matrix = 256 × 256, slice thickness = 3 mm, 20% gap, flip angle = 40°, 7 sagittal slices). Whole-brain anatomical images were acquired using a high-resolution FLASH sequence (TE: 4.7 ms, TR: 20 ms, field of view: 256, slice thickness: 1 mm, with a 20% gap, flip angle: 22°, number of slices: 176, matrix: 256 × 256, and slice orientation: sagittal). Functional images were obtained during the face viewing task using an echo-planar imaging (EPI) sequence (TE = 28 ms, TR = 2000 ms, field of view = 200 mm, slice thickness = 3.1 mm, no gap, flip angle = 90°, matrix 64 × 64, 34 oblique slices, 316 sec). Slices were acquired at a 30° tilt toward coronal to reduce signal loss in amygdala and orbito-frontal cortex.

FMRI paradigm

In the current approach, we included an emotional face-viewing task in our fMRI paradigm to assess functional connectivity in the context of affect processing. The task has been used extensively in the clinical imaging literature and by our own collaborators (Breiter et al., 1996; Thomas et al., 2001; Whalen et al., 1998). Each participant completed three separate scans, each focusing on a specific emotion condition: (1) neutral (N), (2) overt fear (OF), and (3) Masked Fear (MF). Stimuli selected from the Pictures of Facial Affect (Eckman and Friesenm, 1976) were presented in alternating 24-sec blocks of fearful or neutral expressions. Face stimuli were presented for 184 ms with an interstimulus interval between 3500 and 500 ms. A standard fixation point (+) was presented for 1292 ms between faces. The task included blocks of fixation trials interspersed among the emotion conditions, allowing a nonface baseline condition. To ensure attention to the stimuli, participants were asked to monitor the fixation point for the occurrence of a rare stimulus (0 rather than +). Every block of stimuli included an equal and randomly distributed number of fixation changes.

For the masked fear condition, a probe of a fearful face is presented for 24 ms, followed by a mask of a neutral face for 174 ms. Each of the three scans lasted 5 min, and each was made up of 13 blocks, as follows: Neutral—(+N N + N N + N N + N N+); Overt Fear—(+OF N + OF N + OF N + OF N+); and Masked Fear—(+MF N + MF N + MF N F + MF N+). To avoid confounds due to potential ordering effects of the scans, the experiment included a randomization of the masked versus overt fear. All subjects experienced the neutral scan first, followed by a randomly assigned masked fear scan or overt fear scan. In a final step of the matching process, control subjects were matched to BPD subjects that had the same order of scans for group comparison.

fMRI preprocessing procedures

The FMRIB software package (FSL; Oxford, England; www.fmrib.ox.ac.uk/fsl/) was used to conduct the majority of data processing steps and data analyses. Preprocessing procedures on the raw functional images included slice scan time correction, high-pass temporal filtering to remove non-linear drifts, spatial data-smoothing with a Gaussian kernel (6 mm FWHM), and three-dimensional motion correction. Functional data were transformed into standard space (Montreal Neuroimaging Institute; MNI) using FLIRT (FSL).

A further preprocessing step was to use a denoising procedure using a combination of FSL and AFNI (Cox, 1996) software. FSL was used to conduct an exploratory independent component analysis on the processed functional data for each individual. Following the methods Kelly and colleagues (Kelly et al., 2010), components were inspected with regard to spatial clusters, time series, and power spectra. Those components that were considered most likely to represent noise such as artifact due to heart rate, respiration, or movement; white matter; or cerebrospinal fluid (CSF) were removed (Kelly et al., 2010). Briefly, the decision to label components as noise was made when the clusters were primarily in the periphery or in nongray matter (either CSF or white matter), or when they formed a spotty pattern, scattered over the brain without regard for functional anatomical boundaries.

Components were considered to represent real networks when small (roughly 25 voxels in 4 × 4 cm resolution) to medium clusters were localized to gray matter regions of the brain. Secondary criteria for determining noise components included (a) high frequencies (more than 50% of the power in the Fourier frequency spectrum of the component's time course lies above 0.1 Hz); (b) saw-tooth pattern time course; (c) sinus coactivation (roughly 10 or more thresholded voxels present in the superior sagittal sinus); (d) spikes (one or more large, abrupt changes in the normalized time course) (Kelly et al., 2010). We also used AFNI (afni.nimh.gov/afni) using the 3dtoutcount program to identify outliers of BOLD signal, and the time points of these outliers were also used as a guide in identifying components that likely represented noise. Removal of components was conducted using fsl_regfilt (www.fmrib.ox.ac.ul/fsl/melodic/index.html). Table 1 summarizes the number of average number of components generated and removed for the groups overall and for each scan. After the denoising procedure, the outlier computation was repeated in AFNI to confirm that outliers were removed for each individual as a result of the denoising process.

Functional connectivity analysis

Extraction of BOLD timeseries from Amygdala regions of interest. Spherical regions of interest (ROIs) with radius = 6 mm were created for left amygdala in standard MNI around published locations from previous work in healthy adults

Table 1. Summary of Average Number of Components Yielded by Each Independent Components Analysis Conducted for the Denoising Procedure, and the Number of Components Removed for Each Group and Each Scan Type

Group	*Total components (mean, SD)*	*Good components (mean, SD)*	*Noise components removed (mean, SD)*
All	23.0, 3.4	7.3, 1.6	15.8, 2.9
BPD	23.7, 3.1	7.0, 1.6	16.7, 2.7
Neutral	23.4, 3.8	8.7, 3.3	14.8, 3.5
Overt fear	24.3, 3.2	6.3, 2.3	18.0, 3.0
Masked fear	23.3, 3.3	6.0, 2.1	17.3, 3.3
Control	22.4, 3.7	7.5, 1.7	14.9, 3.0
Neutral	21.5, 4.1	8.1, 2.7	13.4, 3.9
Overt fear	23.1, 2.9	6.1, 1.1	17.0, 2.7
Masked fear	22.6, 4.4	8.4, 2.2	14.2, 3.3

BPD, borderline personality disorder.

that documented amygdala connectivity during emotional face processing with fMRI (MNI coordinates −26, 0, −20) (Stein et al., 2007). A standard-space ROI was also created for right amygdala using the mirror-image MNI coordinates (26, 0, −20). These standard ROIs were then transformed to individual fMRI space using FLIRT (FSL), and mean time series of all voxels within the individual's ROI were extracted using *fslmeants*.

Amygdala connectivity-individual level analysis. For each individual and each scan (neutral, overt fear, masked fear), we used FEAT (FSL) to perform regression analyses to generate amygdala connectivity maps. For this procedure, the time series from the amygdala ROI is used as the main regressor against the preprocessed fMRI and denoised data across the 5-min scan. We also included signal intensity normalization to further reduce noise. Correction for multiple comparisons was conducted using Gaussian Random Field Theory to set significance threshold across the brain at $z > 2.3$, $p < 0.05$, corrected.

Amygdala connectivity-group level analysis. FLAME (FSL) was used to compare amygdala connectivity maps between groups for the neutral, overt, and masked fear scans. Correction for multiple comparisons was conducted again using Gaussian Random Field Theory to set significance threshold across the brain at $z > 2.3$, $p < 0.05$, corrected. Separate group analyses were conducted for amygdala connectivity maps from right and left amygdala seeds for each scan.

Clinical correlations of amygdala connectivity

To further investigate the clinical meaning of group differences in amygdala connectivity, we sought to measure correlation between clinical measures and amygdala connectivity with the region of group difference. The resulting clusters from group comparison analysis were used to create three masks (one for each scan type). We then applied that mask to the z statistic map that resulted from each individual's amygdala connectivity analysis. The z statistic map includes a z statistic for each voxel, which represents the strength of connectivity between that voxel and the amygdala seed. After applying the group-difference cluster mask to the z statistic map, we extracted the average of the z statistic values for all the voxels in that mask region. This result provided us with a value representing connectivity between amygdala and the group difference region for each individual. These values were then correlated to clinical measures. Correlations were conducted initially for the entire sample, and then for each group separately.

Results

Subjects

Mean ages for the BPD and healthy groups, respectively, were 25.17 (SD 4.67) and 24.17 (SD 4.63). Past comorbid diagnoses and treatments for all participants are listed in Table 2. Although current medications taken for a psychiatric indication were exclusionary, one BPD participant was taking a low dose of gabapentin for a medical condition (neuropathic pain). Since gabapentin has psychoactive properties, that person is listed as having a current medication in the table. When examining symptomatology, BPD patients scored higher than controls on all clinical measures, with significant differences in most cases (Table 3). Overall, the BPD group demonstrated moderate levels of symptomatology.

Amygdala connectivity

Group comparisons revealed significant differences for each scan. First, during the neutral scan, from both hemisphere amygdala seeds, the BPD group showed lower connectivity to bilateral mid-cingulate cortex in comparison to the healthy group (Fig. 1). During the overt fear scan, also from both hemispheres amygdala seeds, patients had greater connectivity to bilateral rostral anterior cingulate than the healthy group (Fig. 2). Finally, during the masked fear scan, patients had higher right amygdala connectivity with bilateral thalamus and right caudate (Fig. 3). No significant group differences were noted for left amygdala connectivity during the masked fear scan.

Clinical correlations of amygdala connectivity

For the neutral scan, in both hemispheres, connectivity between amygdala and the mid-cingulate cortex region in the group difference mask was strongly related to several clinical symptoms (Table 4). Interestingly, the pattern of relationships varied by group. For the whole sample, SCL-R measures of depression, hostility, anxiety, paranoia, global severity, and positive symptoms were all negatively correlated with this connectivity. However, for the control group alone, only positive correlations were observed, including obsessive compulsive, interpersonal sensitivity, global severity, positive symptom distress, and positive symptom total (more correlations observed for the right hemisphere amygdala ROI than the left). For the BPD group only, only negative correlations were seen, which included total hostility and phobic anxiety.

For the overt fear scan, connectivity between left amygdala and the anterior cingulate regions in the group difference mask was positively correlated with state and trait anxiety and total interpersonal sensitivity in the whole sample. These relationships were not present when examining the groups separately. When examining the control group alone, left amygdala-cingulate connectivity was negatively correlated with state anxiety and with total interpersonal sensitivity. In the BPD group alone, no correlations were significant (Table 5).

For the masked fear scan, among the sample as a whole, both STAI scores and Total Interpersonal Sensitivity on the SCL were positively correlated with amygdala connectivity to the thalamus/caudate derived from the group difference mask (Table 6). However, when the groups were examined separately, only negative correlations were seen. Multiple SCL-90 scales were negatively related to amygdala-thalamus/caudate connectivity for controls, whereas as listed in Table 6, only Total Somatization showed a significant negative relationship for the BPD group.

Discussion

We report significant differences between young women with BPD and healthy comparison volunteers in amygdala connectivity patterns during processing of emotion expressions. A strength of this study is that the participants were

Table 2. Demographic and Clinical Characteristics of Participants with Borderline Personality Disorder and Healthy Control Participants

Characteristic	*BPD,* n = *12*	*Healthy,* n = *12*
Age (mean years ± SD)	25.17 (4.67)	24.17 (4.63)
Ethnicity, *n* (%)		
Caucasian	10 (84)	11 (92)
African American	1 (8)	1 (8)
Hispanic	1 (8)	0
Asian	0	0
Other	0	0
Abuse history, *n* (%)		
Physical abuse	2 (17)	0
Sexual abuse	6 (50)	0
Emotional abuse	5 (42)	2 (17)
Past comorbidity, *n* (%)		
Post-traumatic stress disorder	4 (33)	0
Major depressive disorder	10 (83)	0
Attention-deficit/hyperactivity disorder	0	1 (8)
Substance use disorder, early partial remission	6 (50)	2 (17)
Substance use disorder, sustained full remission	5 (42)	1 (8)
Current medication treatment, *n* (%)		
Gabapentin[a]	1 (8)	0
Prior history of treatment, *n* (%)		
Any medication	8 (67)	1 (8)
Antidepressants[b]		0
Mood stabilizers[c]		0
Atypical antipsychotics[d]		0
Stimulants[e]	1 (8)	1 (8)
Unknown	2 (16)	0
Hospitalizations	5 (42)	0
Therapy[f]	8 (67)	6 (50)

[a]Participants taking medications prescribed for medical indications were not excluded. One BPD participant was taking gabapentin for neuropathic pain.
[b]Prior antidepressants included fluoxetine ($n=3$), sertraline ($n=3$), citalopram ($n=1$), escitalopram ($n=2$), venlafaxine ($n=4$), and bupropion ($n=3$).
[c]Prior mood stabilizers included lamotrigine ($n=1$), valproic acid ($n=1$), and gabapentin ($n=1$).
[d]Prior atypical antipsychotics included ziprasidone ($n=1$) and quetiapine ($n=2$).
[e]Prior stimulant medication was amphetamine/dextroamphetamine (Adderall) in both cases.
[f]Therapies for the BPD group included dialectical behavioral therapy ($n=3$), family therapy ($n=1$), unspecified therapy ($n=7$), and unknown ($n=2$). Therapies for the control group included cognitive behavioral therapy ($n=1$) and unspecified therapy ($n=5$).

Table 3. Symptomatology of Study Participants

	*BPD (*n = *12), mean (SD)*	*Control (*n = *12), mean (SD)*	*Comparison BPD vs. Control*
State and Trait Anxiety Inventory[a]			
State anxiety[a]	45.50 (11.94)	28.00 (6.12)	$t(16.40)=4.52$, $p<0.001$
Trait anxiety[a]	56.42 (8.65)	31.08 (6.19)	$t(22)=8.25$, $p<0.001$
Symptom Checklist-90[b]			
Somatization	52.17 (5.89)	48.83 (5.98)	ns
Obsessive compulsive	55.33 (4.44)	51.42 (9.20)	ns
Interpersonal sensitivity	57.17 (8.49)	46.17 (6.98)	$t(22)=3.47$, $p<0.01$
Depression	53.08 (9.06)	47.58 (5.37)	ns
Anxiety	49.17 (8.93)	47.17 (6.34)	ns
Hostility	55.58 (9.13)	47.50 (6.54)	$t(22)=2.49$, $p<0.05$
Phobic anxiety	51.92 (8.54)	45.42 (4.91)	$t(22)=2.29$, $p<0.05$
Paranoid ideation	51.67 (7.18)	43.00 (4.97)	$t(22)=3.44$, $p<0.01$
Psychoticism	52.83 (8.88)	46.58 (6.47)	ns
Global severity index	53.67 (7.88)	46.33 (7.38)	$t(22)=2.35$, $p<0.05$
Positive symptom	50.42 (8.52)	46.75 (6.38)	ns
Distress index			
Positive symptom total	55.33 (7.30)	46.08 (6.57)	$t(22)=3.26$, $p<0.01$

[a]Spielberger et al., 1983.
[b]Derogatis, 1994.
SD, standard deviation.

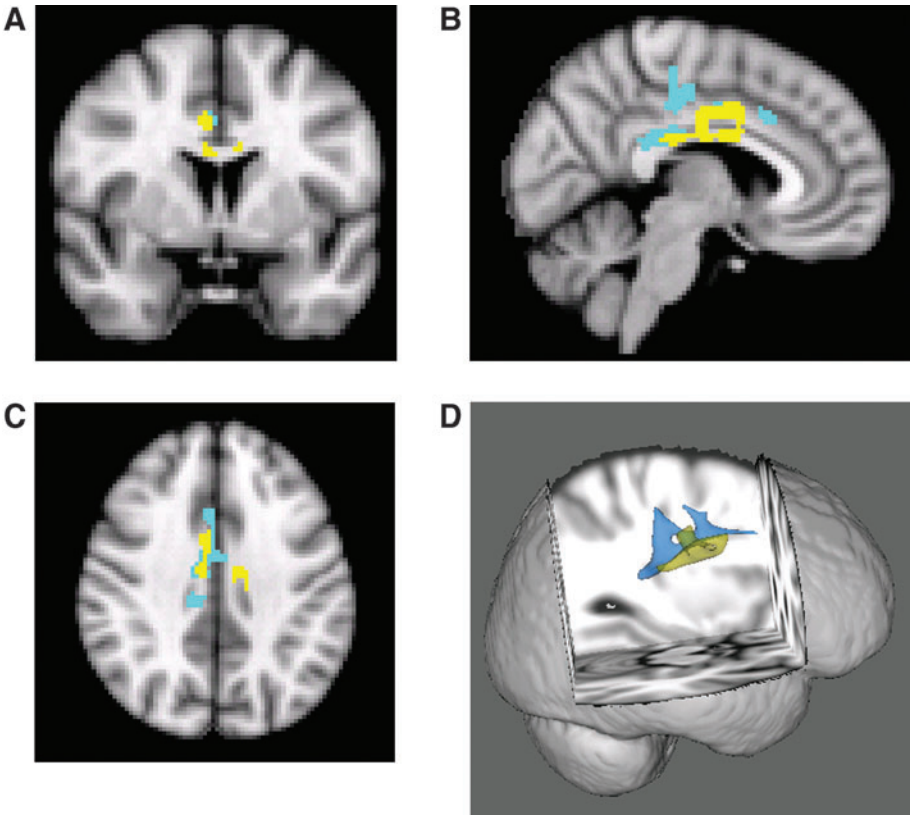

FIG. 1. Amygdala Connectivity during the Neutral Scan. Group difference in connectivity was observed in the control > borderline personality disorder (BPD) comparison. Group difference results in connectivity from right amygdala regions of interest (ROIs) are shown in yellow; group difference results in connectivity from the left amygdala seed are shown in blue. **(A)**, **(B)**, and **(C)** are views at location X = 42, Y = 64, Z = 54. **(D)** is a 3D rendering of both masks (yellow-right amygdala connectivity, blue = left amygdala connectivity).

largely unmedicated with limited diagnostic comorbidity. In this study, in comparison to their healthy counterparts, young women with BPD showed (1) lower connectivity between bilateral amygdala and mid-cingulate cortex during the scan that included only neutral faces; (2) higher connectivity between bilateral amygdala and rostral anterior cingulate cortex during a scan that included both overt fear and neutral faces; and (3) increased right amygdala connectivity with bilateral thalamus and right caudate during the scan that included both masked fear and neutral faces.

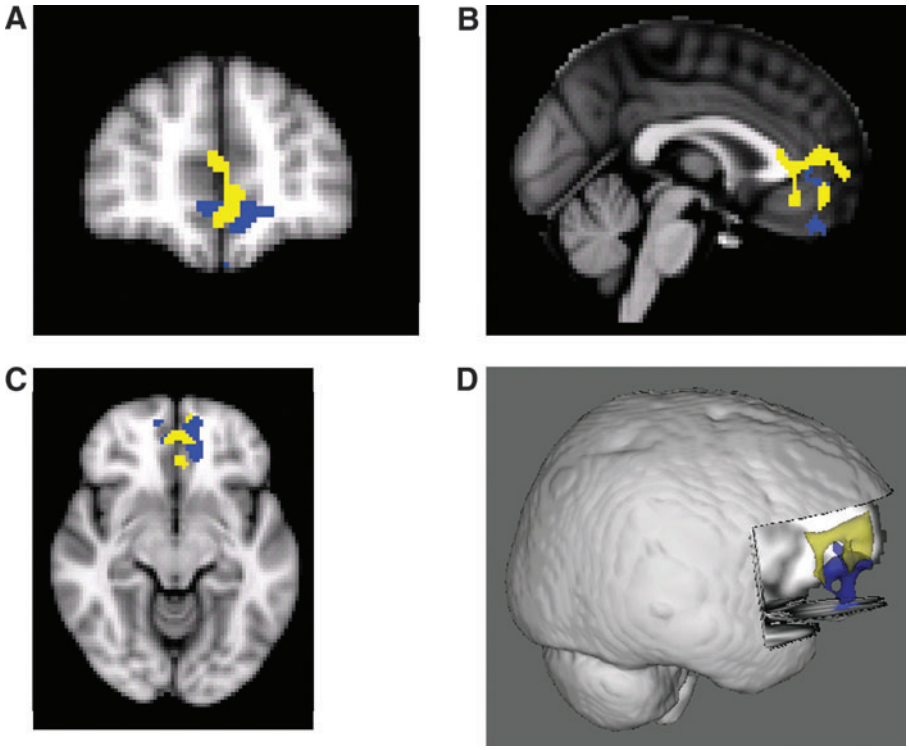

FIG. 2. Amygdala Connectivity during the Overt Fear Scan. Group difference in connectivity was observed in the BPD > control comparison. Regions that were different between groups in amygdala connectivity for the right amygdala ROIs are shown in yellow; group difference in connectivity results from the left amygdala seed are shown in blue. **(A)**, **(B)**, and **(C)** are views at location X = 45, Y = 88, Z = 31. **(D)** is a 3D rendering of both masks (yellow = right amygdala connectivity, blue = left amygdala connectivity).

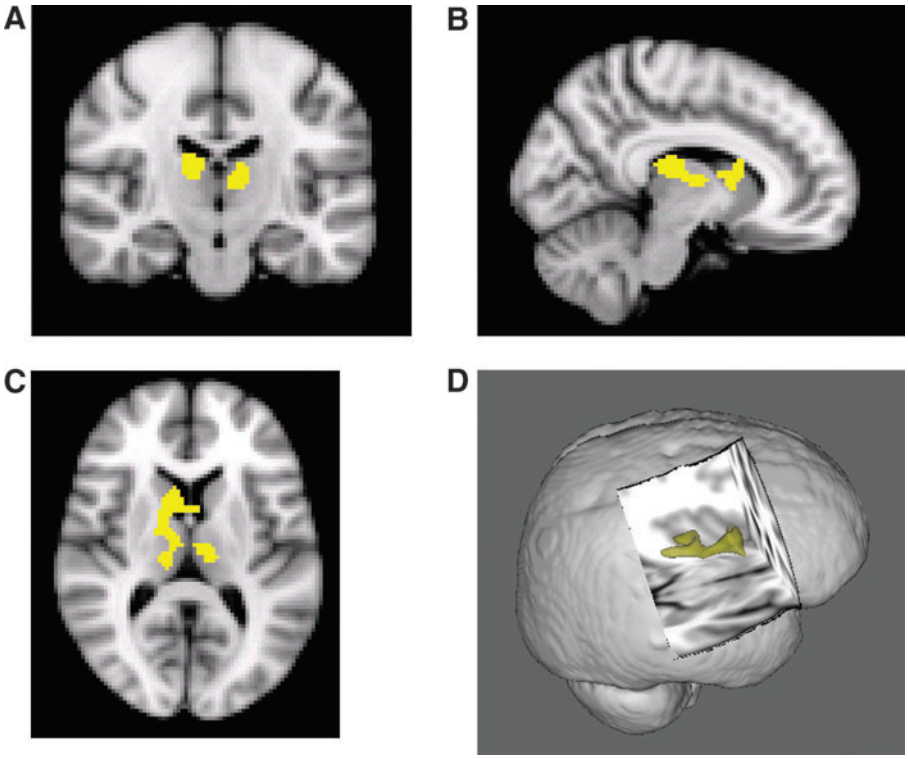

FIG. 3. Right Amygdala Connectivity during the masked fear scan. Group difference in connectivity was observed in the BPD > Control comparison, and only for the right amygdala ROIs. Connectivity results from the right amygdala ROIs are shown in yellow; connectivity results from the left amygdala seed are shown in blue. **(A)**, **(B)**, and **(C)** coronal, sagittal, and axial views, respectively, at location X = 40, Y = 54, Z = 41. **(D)** is a 3D rendering of the right amygdala connectivity group difference mask.

The present findings add to a small but growing literature examining the neural circuitry of BPD by applying measures to assess brain connectivity. The first study that examined functional connectivity in BPD used 18-fluorodeoxyglucose positron emissions tomography found reduced coupling of metabolic activity between the amygdala and the orbitofrontal cortex in the patient group (New et al., 2007). Other initial efforts have looked at structural connectivity using diffusion tensor imaging (DTI). The first two DTI studies in patients with BPD both found reduced integrity of white matter within frontal white matter, suggesting lower connectivity in BPD in areas that would be important for fronto-limbic circuitry (Grant et al., 2007; Rusch et al., 2007).

More recently, Rusch et al. (2010) reported impaired interhemispheric structural connectivity between left and right anterior cingulate cortices in adult women with BPD and comorbid attention-deficit/hyperactivity disorder, in comparison to healthy controls (Rusch et al., 2010). Together with our findings, these data resulting from varying imaging techniques are in support of the notion that during a resting condition (or when not processing fear), individuals with BPD demonstrate impaired circuitry that may underlie their

Table 4. Association Between Clinical Symptoms and Amygdala Functional Connectivity to Region in Group Difference Mask (Mid-Cingulate Cortex) During the Neutral Scan

Clinical measure[a]	*Connectivity from left amygdala seed*	*Connectivity from right amygdala seed*
Full Sample ($n = 24$)		
Total depression	−0.429 ($p < 0.05$)	−0.482 ($p < 0.05$)
Total anxiety	−0.414 ($p < 0.05$)	NS
Total hostility	−0.596 ($p < 0.01$)	−0.586 ($p < 0.01$)
Total phobic anxiety	−0.573 ($p < 0.01$)	−0.673 ($p < 0.001$)
Total paranoia	−0.445 ($p < 0.05$)	−0.506 ($p < 0.05$)
Global severity index	−0.471 ($p < 0.05$)	−0.420 ($p < 0.05$)
Positive symptom total	−0.479 ($p < 0.05$)	−0.474 ($p < 0.05$)
Control ($n = 12$)		
Total interpersonal sensitivity	0.644 ($p < 0.05$)	0.605 ($p < 0.05$)
Total obsessive compulsive	NS	0.621 ($p < 0.05$)
Global severity index	NS	0.666 ($p < 0.05$)
Positive symptom distress index	NS	0.7000 ($p < 0.05$)
Positive symptom total	NS	0.685 ($p < 0.05$)
BPD ($n = 12$)		
Total hostility	−0.773 ($p < 0.01$)	−0.673 ($p < 0.05$)
Total phobic anxiety	−0.787 ($p < 0.01$)	−0.785 ($p < 0.01$)

[a]All measures are from the Symptom Checklist-90-R (Derogatis 1994).

Table 5. Association Between Clinical Symptoms and Amygdala Functional Connectivity to Region in Group Difference Mask During the Overt Fear Scan

	Connectivity from left amygdala seed	*Connectivity from right amygdala seed*
Full Sample ($n=24$)		
State Anxiety[a]	0.456 ($p<0.05$)	NS
Trait Anxiety[a]	0.547 ($p<0.01$)	0.414 ($p<0.05$)
Controls ($n=12$)		
Total Interpersonal Sensitivity[b]	−0.699 ($p<0.05$)	NS
BPD ($n=12$)	NSC	NSC

[a]Stait and Trait Anxiety measures are provided by the STAI.
[b]Total Personal Sensitivity is a measure from the SCL-90.
NS, nonsignificant; NSC, no significant correlations detected.

diminished capacity to handle stress when it arises. The findings from the present study add to these data with information on how amygdala connectivity in BPD differs from controls in different emotional contexts.

Results from our all-neutral scan fit in best with previous work by documenting a deficit in connectivity within fronto-limbic networks in BPD. In our study, the BPD group demonstrated lower functional connectivity between amygdala and the mid-cingulate cortex, and on an individual basis, this connectivity was strongly related to symptomatology. The mid-cingulate is not frequently a region of focus in emotion fMRI research and requires some comment. The relevance of connectivity between amygdala and mid-cingulate to emotion regulation is unknown, but the deficit of this connection in BPD in our study and its relationship to symptomatology are worth further investigation. In a prior study of resting-state amygdala functional connectivity in healthy adults, similar regions of the mid-cingulate cortex were shown to be connected to amygdala (Roy et al., 2009).

A review of previous fMRI emotion research on the subdivisions of the cingulate cortex noted that the mid-cingulate region tends to show less activation during emotion conditions, and activates primarily under control conditions (Vogt, 2005). The regions represented in our group difference mask (Fig. 1) map onto regions summarized by Vogt as mediating fear avoidance, skeletomotor orientation, visuospatial orientation, and self-relevance assessment (Vogt, 2005). Against the backdrop of these previous papers, our study's finding of connectivity during processing neutral faces fit in with other approaches that did not apply emotion-laden contexts. In that context, the result of lower connectivity between amygdala and cortical regulatory regions in general falls in line with the previous connectivity findings discussed above. When considered with our other findings from the other emotion conditions (discussed below), one interpretation would be that a deficit in connectivity during nonemotion settings may predispose an individual to a compensatory hyper-connectivity of other amygdala connections that is elicited during particular emotion settings.

Table 6. Associations Between Clinical Symptoms and Right Amygdala Functional Connectivity to Region in Group Difference Mask (Bilateral Thalamus and Right Caudate) During the Masked Fear Scan

Clinical measure	*Connectivity from right amygdala seed to group difference mask*
Full Sample ($n=23$)	
State anxiety[a]	0.626 ($p<0.01$)
Trait anxiety[a]	0.598 ($p<0.01$)
Total Interpersonal Sensitivity[b]	0.425 ($p<0.05$)
Controls ($n=12$)	
Total Obsessive Compulsive[b]	−0.786 ($p<0.01$)
Total Depression[b]	−0.703 ($p<0.05$)
Total Hostility[b]	−0.782 ($p<0.01$)
Global Severity Index[b]	−0.729 ($p<0.01$)
Positive Symptom Distress Index[b]	−0.686 ($p<0.05$)
Positive Symptom Total[b]	−0.642 ($p<0.05$)
BPD ($n=12$)	
State anxiety[a]	0.678 ($p<0.05$)

[a]Stait and Trait Anxiety measures are provided by the STAI.
[b]Measures listed are from the SCL-90.

In contrast, our results of functional connectivity in the context of processing covert and overt fear revealed abnormalities in the opposite direction; that is, the BPD group shows higher levels of amygdala connectivity than the healthy group. Further, the pattern of group differences in our results was very intriguing, and fit into prior work that has examined amygdala connections in animals and humans. LeDoux (2000) has described two pathways for amygdala connections: (1) connections to frontal regions—described as a slower, interpretive pathway; (2) connections to the thalamus-described as a faster, automatic pathway, not requiring conscious awareness. Brain imaging research in humans has also delineated these pathways by examining amygdala connectivity in the context of covert versus overt fear presentations (Morris et al., 1999; Whalen et al., 1998; Williams et al., 2006). Mapping onto Ledoux's animal work, these studies have found amygdala connectivity with prefrontal regions, including orbitofrontal cortex and anterior cingulate cortex during overt fear, in contrast to subcortical regions such as the thalamus (the fast, automatic pathway) during masked fear presentations (Morris et al., 1999; Williams et al., 2006). Thus, the results of the current study are consistent with the model laid out by LeDoux, with the added information that these pathways are more strongly present in women with BPD. One interpretation of this result could be that in BPD, hypervigilance to fear, either conscious or unconscious, is expressed by heightened tone of relevant amygdala circuitry. This idea is supported by the positive correlations between amygdala connectivity and clinical measures such as state/trait anxiety and interpersonal sensitivity

during both covert and overt fear, and with interpersonal sensitivity during covert fear.

In addition to higher connectivity between right amygdala and thalamus during masked fear, the BPD group also showed higher connectivity between amygdala and the right caudate. Developmental studies have demonstrated that the connection between these areas develops within 30 days after birth (Nieto et al., 1983), but prior connectivity studies have not demonstrated amygdala-caudate connection during unconscious fear processing (Morris et al., 1999; Williams et al., 2006). Additionally, a recent connectivity study using resting-state fMRI demonstrated higher connectivity between amygdala and caudate in patients with schizophrenia (Salvador et al., 2010), suggesting that this may be a more general marker for psychopathology.

The results presented here on functional connectivity should be considered against the backdrop of a growing body of research that has been developing a broad range of strategies for examining and interpreting functional connectivity. While some approaches involve measurement of functional connectivity based on the correlation between brain regions of spontaneous BOLD signal fluctuation during rest (Fox and Raichle, 2007), others have examined the correlation of brain areas during a task. For those that utilize task-based fMRI paradigms, some studies have used a psychophysiological interaction (PPI) analysis to examine functional connectivity specifically during the task (Cremers et al., 2010; Friston et al., 1997).

A common challenge of this approach is that by decreasing the number of observations in the analysis (i.e., only those time points during the "on" part of the task), significant group differences are more difficult to identify due to lost power (www.fmrib.ox.ac.uk). Another approach is to measure functional connectivity across the entire scan, which would include blocks of time with the psychological condition and also times of rest or a control task. For example, Pezawas and colleagues examined the coupling between amygdala and anterior cingulate cortex during processing of negative affect, and demonstrated differences in this coupling that varied by serotonin transporter genotype (Pezawas et al., 2005).

Using a similar approach, another study that examined functional connectivity in the context of face processing revealed that adolescents with bipolar disorder had lower connectivity between left amygdala and right posterior cingulate/precuneus and right fusiform gyrus/hippocampal gyrus than the healthy comparison group (Rich et al., 2008). An advantage of the latter approach is that the larger number of time points under consideration adds power to the analysis. More importantly, inclusion of all the time points in the scan may be more relevant and applicable to understanding the neural basis of psychopathology.

Limitations

Emerging research has revealed that after completing a task, networks do not recover fully to their resting parameters until several minutes after the task (Barnes et al., 2009). Thus, examination of connectivity over larger time frames (i.e., a 5-min scan as opposed to a 30-second block) may be more informative regarding how viewing emotion faces alters relevant circuitry in individuals with and without BPD. Further, examination of connectivity during a task in healthy and diseased populations can reveal how task modulation of functional networks may differ between groups (Sakoglu et al., 2010). A limitation of the current study is the lack of a resting-state scan that could have been used as a comparison. Future studies using multiple approaches will serve to further enhance understanding of functional connectivity and guide researchers in the most useful strategies.

A limitation of the current study is the small sample size. Therefore, these results must be interpreted with caution and viewed as preliminary as we await replication in future studies. The issue of sample size is especially important in light of the heterogeneity of BPD (there are 126 ways for a person to meet five of nine BPD criteria to obtain a BPD diagnosis). Studies with larger samples will have greater power to uncover correlations between neural patterns and specific symptomatology. In our small study, we were able to identify some correlations between specific symptoms and amygdala connectivity; future investigations with larger samples of individuals with BPD and subgroups that have similar predominant symptoms (e.g., impulsivity and aggression) will allow for a better examination of the association between dimensions of clinical presentation with connectivity patterns.

We have highlighted the fact that the young women with BPD in this study were largely unmedicated (with the exception of one participant who was taking gabapentin for neuropathy) as a strength of the study. However, we should also note this as a limitation. Whereas the advantage to studying unmedicated samples is the avoidance of confounds due to medication effects, this strategy introduces two disadvantages. First, the study of participants with BPD with severe symptoms that are not medicated necessarily leads to ethical concerns for the clinical research team. Therefore, for feasibility reasons, the sample becomes less likely to be severe. Our sample of young women with BPD had symptoms that fell into the moderate range.

One would assume that biological abnormalities would be easiest to detect in individuals with the most severe clinical pathology; that is, severe symptoms would be linked with most abnormal neural substrates. Therefore, by selecting a sample with less clinical severity, we are limiting our ability to detect the scope of abnormalities that plausibly underlie severe symptoms in BPD. Another disadvantage to studying unmedicated samples is that the data are less easily generalizeable to clinical samples of BPD, a group of patients who frequently are prescribed multiple medications. These same arguments apply to the question of selecting a sample with limited comorbidity (fewer confounds, but less representative of the clinical phenotype.)

Conclusion

We have identified abnormal functional connectivity of the amygdala in young women with BPD and have observed that the pattern of abnormalities varies with emotional context in which connectivity is measured. These findings have important implications for the underlying neurobiology of BPD, and raise questions for future study. The limitations of this study should be addressed in future studies with larger samples that can examine and compare subgroups that are medicated versus unmedicated, and include varying levels of comorbidity. Further, incorporation of a longitudinal design will be necessary to examine whether biological anomalies would normalize after treatment, and to elucidate the

developmental timing of abnormalities in the fronto-limbic circuitry of individuals with BPD.

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Author Disclosure Statement

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