ALTERED PATTERNS OF AMYGDALA ACTIVATION AND FUNCTIONAL CONNECTIVITY IN INDIVIDUALS WITH BORDERLINE PERSONALITY DISORDER DURING NONCONSCIOUS AND CONSCIOUS EMOTION PROCESSING

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BY

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Abstract

Objective: Borderline personality disorder (BPD) is characterized by emotion dysregulation, which underlies symptoms such as suicidality and impulsivity. Neuroimaging provides a method for probing the biological basis of emotion dysregulation. We examined neural activation and connectivity in individuals with BPD and healthy controls (HCs) during nonconscious and conscious emotion processing.

Methods: 21 unmedicated individuals with BPD and 10 healthy controls (HCs) completed an fMRI task viewing masked and unmasked happy and fearful faces. Whole brain and region of interest (ROI) analyses examined between group differences in activation. Differences in amygdala connectivity were assessed using psychophysiological interactions (PPI). Results: During unmasked emotion processing, whole-brain and ROI analyses reveal greater activation in the amygdala and hippocampus and PPI analyses show greater connectivity between the amygdala and subgenual anterior cingulate cortex (sgACC) in participants with BPD. In HCs, greater connectivity was found between the amygdala and areas of the prefrontal cortex. During masked emotion processing, HCs show greater activation in frontal and temporal regions and greater connectivity between the amygdala and temporal regions. Conclusion: Results find altered frontal-limbic activation and connectivity in individuals with BPD relative to HCs, varying depending on whether the emotional stimulus is consciously or nonconsciously perceived. This suggests that there may be more than one neural pathway underlying emotion dysregulation in BPD.
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Introduction

Borderline personality disorder (BPD) is a serious mental illness that affects an estimated 2-5.9% of the general population (Coid et al., 2006; Grant et al., 2008; Lenzenweger et al., 2007). According to DSM-V diagnostic criteria, BPD is characterized by affective instability, impulsivity, stormy interpersonal relationships, and suicidality, with suicide rates much higher than the general population (Paris, 2002; Oldham, 2007). Despite the diversity in clinical presentations of BPD, across a number of theories, emotion dysregulation is understood as core to BPD symptomatology (Siever and Davis, 1991; Conklin et al., 2006; Crowell et al., 2009). Linehan’s Biosocial Theory of BPD (Linehan, 1993) posits that individuals with BPD experience heightened emotional sensitivity, prolonged emotional reactions, and difficulty modulating emotional reactivity. Lacking emotion regulation skills, individuals with BPD often utilize maladaptive behavioral coping strategies, which characterize the clinical presentation of the disorder, such as self-harm, suicidality, dissociation, or behavioral impulsivity (Putnam and Silk, 2005). Targeting this loop between emotion dysregulation and behavioral dyscontrol is often central to treating BPD (Selby and Joiner, 2009); a better understanding of the biological mechanisms underlying emotion dysregulation will help to inform better treatment.

Laboratory studies provide evidence for differences in emotion sensitivity and regulation in individuals with BPD. Research using both cognitive and psychophysiological methods finds evidence suggesting that individuals with BPD have enhanced emotion detection and can perceive increasing intensity in emotional expressions earlier than control subjects (Wagner and Linehan, 1999; Lynch et al., 2006; Domes et al., 2009;
Schulze et al., 2013). Using emotional inductions and an emotional stroop task, studies have shown that individuals with BPD have longer emotional reactions than control subjects (Jacob et al., 2008) and fail to disengage from negative emotional content (Arntz et al., 2000; Domes et al., 2006). Studies using a range of psychophysiological measures find increased emotional sensitivity measured using affective startle modulation (Hazlett et al., 2007; Baskin-Sommers et al., 2012), skin conductance (Kuo and Linehan, 2009), and heart rate in patients with BPD (Limberg et al., 2011).

Neuroimaging studies allow probing of the neural mechanisms that support heightened sensitivity and prolonged emotional reactions in individuals with BPD. Recent meta-analyses have found converging evidence for altered patterns of limbic and frontal brain activation in individuals with BPD relative to controls during emotion processing (Krause-Utz, Winter, Niedtfeld, & Schmahl, 2014; Mitchell, Dickens, & Picchioni, 2014; van Zutphen, Siep, Jacob, Goebel, & Arntz, 2015). These studies suggest overactive amygdala functioning in individuals with BPD, and altered activation in certain frontal regions, such as the dorsolateral prefrontal cortex (dlPFC), insula, ACC anterior cingulate cortex (ACC), orbital frontal cortex (OFC) and medial prefrontal cortex (mPFC) across a number of emotion processing task paradigms. Such a pattern of activation suggests overactivity in regions involved in emotion generation and inefficient processing of inhibitory control regions involved in emotion regulation. An activation likelihood estimation (ALE) study of negative emotionality across emotion processing tasks found reduced amygdala activation in individuals with BPD relative to controls, but greater activation in the insula during negative emotion processing (Ruocco et al., 2013),
suggesting that hyperarousal in socio-emotional brain areas in response to negative emotional stimuli is characteristic of the pathophysiology of BPD.

Research has shown that effective treatments of BPD may be targeting these neural pathways. Dialectical behavioral therapy (DBT) specifically treats symptoms of emotion dysregulation in BPD; improvements in emotion regulation following DBT may also explain post-treatment reductions in impulsive behaviors, such as substance abuse (Axelrod et al., 2011). Pre- and post-treatment neuroimaging studies find that after DBT, individuals with BPD have decreased amygdala activation to repeated emotional picture viewing (Schnell and Herpertz, 2007; Goodman et al., 2014). Goodman and colleagues (2014) found that increased emotion regulation following DBT treatment was also associated with improved amygdala habituation during repeated viewings of emotional imagery. Potentiated amygdala response and delayed habituation to repeated emotional images in individuals with BPD suggests not only heightened sensitivity, but also failures in modulating longer emotional reactions (Hazlett et al., 2012).

Using various measures of functional connectivity, emerging studies have identified altered limbic-prefrontal connectivity as well as altered ACC connectivity in BPD relative to controls (Cullen et al., 2011; Kluetsch et al., 2012; Koeningsberg et al., 2014; Salvador et al., in press). Research in non-clinical populations has identified the strength of the limbic-frontal connectivity as predictive of effective emotion regulation (Banks et al., 2007; Ochsner et al., 2012). Findings from clinical studies on BPD suggest that frontal networks, traditionally understood to regulate emotional responses, may not
effectively synchronize with limbic structures in individuals with BPD. Connectivity studies provide evidence for disturbances in a neural network, which can explain heightened emotional reactivity as well as altered emotion regulation mechanisms in individuals with BPD. Growing research points to the importance of assessing neural connectivity and adopting a neural systems approach for better understanding psychopathology (Fornito and Bullmore, 2012).

Research on emotion processing has found neural differences between conscious and nonconscious emotional perception, wherein fMRI paradigms that utilize a masking technique enable probing of emotional responses to stimuli that do not reach conscious awareness (Whalen et al., 1998). Studies find differences in activation and connectivity in individuals with BPD and other related psychiatric disorders, such as PTSD and anxiety, (Rauch et al., 2000; Etkin et al., 2004; Cullen et al., 2011) depending on whether the stimuli are consciously or non-consciously perceived. To date though, no studies have reported both activation and connectivity in a sample of individuals with BPD during nonconscious and conscious emotion processing. We examine group differences in a sample of 21 individuals with BPD and 10 HCs using a paradigm in which the stimuli of emotional faces are masked and unmasked. We hypothesized differences in activation and connectivity in individuals with BPD relative to HCs during both consciously perceived and non-consciously perceived emotional stimuli, and that differences in these neural mechanisms may shed light on the clinical presentation of BPD, in which emotional reactivity to subtle internal and external cues characterizes the disorder.
Methods

Participants

21 unmedicated participants with BPD (7 males; mean age: 28.86, standard deviation: 7.11) and 10 HC (4 males; mean age: 27.07, standard deviation: 7.46) participants were included in this study. Participants were recruited for impulsivity, distrustfulness, and difficult relationships through referral, advertisements, and word of mouth. Participants were screened with the Structured Clinical Interview for DSM-IV (Spitzer et al., 1994), the Revised Diagnostic Interview for Borderline Personality Disorder (DIB-R; Zanarini et al., 1989) and the Zanarini Rating Scale for Borderline Personality Disorder (ZAN-BPD) (Zanarini, 2003). For inclusion in the BPD group, participants had to meet criteria on both the DIB-R and the ZAN-BPD. Assessments were conducted by a registered nurse or trained graduate student and were overseen by a psychiatrist.

Exclusion criteria included: present or past psychotic disorder; a primary neurological condition; cognitive impairment; current substance dependence or recent abuse of opiates, amphetamines, barbiturates, cocaine, or hallucinogens; current medical instability; a history of lack of response to an atypical antipsychotic; pregnancy or lactation; acute suicidality; or current major depressive disorder, posttraumatic stress disorder, panic disorder, or obsessive compulsive disorder though individuals with lifetime histories of these disorders were included. BPD Participants were selected from a larger longitudinal, multi-site clinical drug trial and inclusion and exclusion criteria were the same (Black et al., 2014), with an additional assessment for MRI contraindications for both BPD and HC participants. The data from the current study are from a pre-treatment
visit at one of the sites (University of Minnesota). Subjects gave consent to participate in the additional MRI component of the larger clinical trial.

Emotion processing fMRI task

We used an emotional face viewing task paradigm to evoke non-conscious and conscious emotion processing using masked and unmasked emotion conditions. Versions of this task have been used in previous studies to measure brain activation in response to socio-emotional cues. Meta-analytic work indicates that such tasks activate limbic, prefrontal, visual and tempoparietal brain regions (Fusar-Poli et al., 2009). Participants completed two, 5.2-minute runs of a task in which a series of grayscale images of adult emotional (fearful, happy) faces (Ekman and Friesen, 1976) were presented in a block design format, and were contrasted with blocks of a standard fixation. Participants viewed the task, which was rear projected to a screen at the back of the scanner, through a mirror attached to the head coil. Both runs of the task were identical in terms of block order, consisting of 13 24-second blocks (4 happy (H), 4 fear (F), 5 fixation (C)) presented in a counterbalanced order (CFHCHFCHFCFHC) (Figure 1). During the first run of the task, participants saw emotional faces (fearful, happy) for 20 ms, which were then masked for 180 ms by a neutral face from the same individual. During most trials, this was followed by a standard fixation (+) for 1300 ms, which required no response. During a small number of randomly distributed trials, a target stimulus (O) was presented for 1300 ms, which participants were asked to monitor and respond to with a button press, in order to ensure ongoing attention to stimuli. During the second task run, participants saw the same emotional stimuli (fearful, happy) unmasked for 200 ms, using the same counterbalanced
order as in the first run of the task, followed by either a standard fixation or target stimulus.

MRI image acquisition
The MRI scan procedure was conducted at the Center for Magnetic Resonance Imaging at the University of Minnesota on a Siemens 3T Tim Trio scanner using an 12 channel head coil and a vacuum pillow to reduce head motion. A 5-minute structural scan was acquired using a T1-weighted, high-resolution, magnetization-prepared gradient-echo sequence: repetition time, 2530 milliseconds; echo time, 3.65 milliseconds; inversion time, 1100 milliseconds; flip angle, 7°; field of view, 256 × 176 mm; voxel size, 1-mm isotropic; 224 slices; and generalized, auto-calibrating, partially parallel acquisition acceleration factor, 2. These images were used for registration of functional data. Functional MRI data were acquired during the face viewing task using a BOLD echo planar imaging sequence with 156 volumes with 34 oblique axial slices acquired without gap and an interleaved and TE = 30ms, TR = 2000ms, matrix = 64x64, voxel size = 3.44mm x 3.44mm x 4mm.

Image Processing
The FMRIB software library (FSL; http://fsl.fmrib.ox.ac.uk/fsl/) was used to conduct the pre-processing and data analysis of the MRI data. Functional data were registered to the anatomical data set using a rigid-body linear transformation. These data were then registered to standard space (Montreal Neurological Institute’s MNI 152 2mm volume)
using a full affine transformation, to allow cross-subject comparisons in standard space. The functional data was preprocessed using the following steps: 1) motion correction using the first volume as the reference and six motion predictors of non-interest 2) unwarping using a field-map 3) slice timing correction 4) skull stripping of the functional data 5) spatial smoothing using a 7.0 mm FWHM Gaussian Filter 6) High-pass temporal filtering of 100 seconds. Next the three linear and three rotational motion parameters produced using MCFLIRT were collapsed into a single metric, root mean square (rms). Data were evaluated for excessive motion. Volumes that had greater than 1 voxel absolute displacement (3.4 mm) relative to the reference volume or relative displacement of greater than half a voxel (1.7 mm) from one volume to the next were considered as having excessive motion. We removed participants with greater than 25% volumes with above threshold motion on either run of the task. One HC was removed from the final data set due to excessive motion. Additionally, one BPD participant was removed due to knowledge of a neurological abnormality. Our final sample consisted of 20 BPD and 9 HC subjects.

Processed data was next submitted to a subject-level GLM for each run of the task consisting of two predictors of interest: fear and happy. Task predictors were convolved with a gamma-function approximation of the hemodynamic response. An additional nuisance predictor was included for volumes that exceeded the motion criteria. Emotion predictors were time locked to the onset of fear and happy faces. Contrasts of interest included 1) fear greater than (or “minus”) fixation, 2) happy greater than fixation, 3) fear greater than happy, and 4) happy greater than fear.
Whole Brain Analyses

Whole-brain analyses were conducted for both the masked and unmasked run of the task to examine group differences in activation by conducting a general linear model (GLM) on the pre-processed subject-level data. Two regressors of interest related to group status (BPD and HC) were included in the model. A between-groups t-test was conducted using the FSL tool FLAME for examining mixed effects. Age and sex were included as predictors of non-interest in the model. These analyses were thresholded with an uncorrected voxel p-value of < .005 and a cluster criterion of p < .05, where minimum cluster size was determined using the 3dClustSim tool found in AFNI (http://afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html). Pre-processed data was submitted to an additional whole group (BPD and HC) GLM for each run of the task, in order to determine amygdala activation for ROI analyses (reported below).

ROI Analyses

Many studies of BPD report altered amygdala activation during emotion processing, though fewer studies have examined this during non-conscious emotion processing. In the current study, ROI analyses were conducted to look at group differences in amygdala activation in order to assess the nature of amygdala differences during conscious and non-conscious fear processing. A mask was created for the right and left amygdala for both runs of the task based on the overlap of functional activation across both groups during the fear compared to fixation contrast and the anatomical amygdala region, as
defined by the Harvard-Oxford Subcortical Atlas. ROIs were constructed in this fashion to ensure that they were located in the anatomical amygdala and focused on the region within the amygdala that was activated by this task in this sample. No whole group activation was found in the right amygdala during the masked fear condition compared to fixation and thus no mask was created. Additionally, no whole group activation was found in either the left or right amygdala during the masked or unmasked conditions for happy compared to fixation, so ROI analyses were only conducted for fear processing. This resulted in three ROIs: left amygdala during masked fear compared to fixation and left and right amygdala during unmasked fear compared to fixation. Using these masks, subject level beta-values in the left amygdala (masked fear compared to fixation) and right and left amygdala (unmasked fear compared to fixation) were extracted. To determine if there was a significant group by condition (masked compared to unmasked) interaction we conducted a repeated-measures ANOVA for activation in the left amygdala. Additionally, one-tailed t-tests were conducted to compare activation in BPD to HCs during the fear compared to fixation contrast of the task during both task runs in the three ROIs.

Amygdala Connectivity Analyses

To determine amygdala connectivity during the task, PPI (Psychophysiological interactions) analyses were conducted using FSL to investigate amygdala functional connectivity. FLIRT was used to transform the three group-level amygdala masks that were described in the previous section (right and left amygdala during unmasked fear and left amygdala during masked fear) into each individual’s functional space. Mean time
series (using the fslmeants tool) were extracted for each individual from the three ROIs. Subject level connectivity analyses were conducted using the FEAT tool in FSL. The preprocessed, unwarped, and motion corrected functional data from activation analyses were included in these analyses, with the regressor of non-interest for excessive motion. In these analyses, five regressors of interest were included: 1) fear time course, 2) happy time course, 3) the time course of the average BOLD signal extracted from voxels within the amygdala mask 4) the interaction between the amygdala time course and the fear time course, and 5) the interaction between the amygdala time course and the happy time course. Fear and happy time courses were convolved with the prototypical gamma-function approximation of the hemodynamic response and included temporal filtering and a temporal derivative. Contrasts of interest for these analyses included: 1) fear compared to fixation, 2) fear greater than happy 3) amygdala time course 4) amygdala time course interaction with fear compared to fixation, 5) amygdala time course interaction with fear greater than amygdala time course interaction with happy. These functional analyses were registered to the anatomical data set and then to the standard space (MNI 152 brain) in the same way as described above.

Subject level analyses were included in a whole-group GLM using the FLAME algorithm in FSL for mixed-effects analysis. Regressors of interest were group status and age and sex were included as covariates of non-interest. These analyses were cluster thresholded using the same threshold reported above for group activation (voxel-wise uncorrected p <.005, cluster significance p < .05).
**Results**

**fMRI Whole Brain Analyses**

Whole brain analyses were conducted to look at differences in activation between BPD and HCs during masked fear processing (Table 1). During fear compared to fixation condition, HCs showed greater activation than individuals with BPD in the cerebellum, middle temporal gyrus (MTG), insula, precuneous, lingual gyrus and lateral occipital cortex (Figure 2a). During happy compared to fixation, HCs showed greater activation in the intracalcarine cortex, lingual gyrus, and lateral occipital cortex relative to BPD subjects (Figure 2b). During fear greater than happy contrast, HCs showed increased activation in the superior frontal gyrus and the orbital frontal cortex (Figure 2c). The BPD group showed greater activation in the hippocampus relative to the HCs during the happy compared to fixation contrast (Figure 2d).

Similar whole brain analyses were conducted to look at differences in activation between BPD and HCs during unmasked fear processing (Table 2). During the fear compared to fixation condition, subjects with BPD showed greater activation than controls in the temporal fusiform cortex, supramarginal gyrus, and anterior cingulate cortex (Figure 3a). During the happy compared to fixation condition, BPD subjects showed greater activation in the hippocampus, temporal fusiform cortex, and amygdala, relative to HCs (Figure 3b). During the fear greater than happy contrast, BPD subjects showed greater activation in the precuneous cortex and the supramarginal gyrus, relative to HCs (Figure 3c). There were no significant clusters of activation for HCs greater than BPD.
ROI Analyses

Because of an *a priori* interest in the amygdala during emotion processing in BPD based on previous literature, an ROI approach was used to probe group differences in amygdala activation during masked and unmasked fear processing. The BPD group showed greater amygdala activation in the right (t=1.8986, p=0.035) and left (t=1.8953, p=0.037) amygdala during the unmasked fear compared to fixation condition compared to HCs. There was no significant group difference in the left amygdala activation during the masked fear condition (t=0.7056, p=0.2443). The ANOVA of group x condition (masked, unmasked) in the left amygdala did not reveal a significant interaction (F=2.9191.27, p = .099).

Functional Connectivity

Connectivity analyses reveal differences between BPD and HCs in amygdala connectivity during the task. During the masked condition of fear compared to fixation, greater left amygdala connectivity to the left temporal occipital fusiform gyrus was found in the HCs whereas the BPD group showed greater connectivity to the left superior temporal gyrus and to the right putamen. During the unmasked condition of fear compared to baseline, greater right amygdala connectivity was found bilaterally with the central opercular cortex, inferior and middle frontal gyrus, putamen, insular cortex, and frontal pole in the HCs. Greater right amygdala connectivity to the anterior cingulate cortex was found for the BPD group. No group differences were found during either the masked or unmasked contrasts of fear compared to happy and no between-groups
differences in connectivity were found in the left amygdala during unmasked fear compared to fixation. Tables 3 and 4 report brain regions associated with peak voxel of connectivity (based on the Harvard-Oxford Subcortical Atlas) as well as the number of voxels in significant clusters. These findings are depicted visually in Figures 3 and 4.

Discussion

In the current study, we identify group differences between individuals with BPD and HCs in task-related brain activation and amygdala connectivity during unmasked and masked emotion processing. These results support previous studies, which have found differences in activation and connectivity in individuals with BPD relative to control subjects during task and rest. These findings suggest that altered emotion processing in individuals with BPD results from both localized disturbances, as well as altered interactions between brain regions. Altered neural mechanisms in BPD during emotion processing may vary depending on whether or not emotional stimuli are consciously perceptible.

During unmasked processing of happy faces, greater activation was found in the right hippocampus and amygdala, as well as in the temporal fusiform cortex in subjects with BPD relative to HCs. Previous studies have found evidence for greater amygdala activation in individuals with BPD during both positive and negative emotion processing (Hazlett et al., 2012). Studies using measures such as skin conductance and emotion discrimination show greater arousal and sensitivity to both positive and negative emotional prompts (Lynch et al., 2006; Limberg et al., 2011). The current findings, as well as previous studies supporting similar results, may help explain a phenomenon
which is observable clinically, where many patients with BPD experience both positive and negative emotions as emotionally dysregulating.

ROI analysis of amygdala activation during unmasked fear processing as well as whole brain analyses during unmasked emotion processing (fear, happy) are in line with previous studies, which have shown greater activation in limbic and temporal regions during various emotion processing paradigms in individuals with BPD (Herpertz et al., 2001; Donegan et al., 2003; Minzenberg et al., 2007; Koenigsberg et al., 2009). Greater activation in the amygdala, hippocampus, and temporal gyrus, which together are involved in emotion processing, emotional memory, and facial encoding (Canli et al., 2000; Gur et al., 2002), provide a possible neurobiological explanation for findings that patients with BPD have greater sensitivity to and discrimination of negative emotional faces (Wagner and Linehan, 1999; Lynch et al., 2006) as well as evidence for greater memory for negative emotional content (Winter et al., 2014).

Contrary to predictions, the groups did not differ with respect to amygdala activation during the masked fear processing condition and there was no significant group by condition interaction for amygdala activation. In the whole brain analysis, greater activation was found in the HCs relative to participants with BPD during masked fear processing in the vIPFC and MTG, regions which have been associated with perceiving and regulating subliminal but salient socio-emotional content (Liddell et al., 2005). Previous studies have found reduced metabolic activity in these regions in BPD patients (Soloff et al., 1999; Schmahl et al., 2003). In the absence of conscious awareness, regions involved in emotion regulation are active in HCs during the masked fear condition. The current study adds to our prior work (in revision) showing differences between subjects
with BPD and HCs in task-related brain activation in these regions during non-consciously perceived socio-emotional content. These findings suggest that during non-conscious emotional processing, individuals with BPD may not be exhibiting limbic hyperactivity, supporting the findings of Ruocco and colleagues (2011); rather, they may fail to automatically activate brain regions associated with higher order control of emotional processing.

To date, few studies have examined neural connectivity in individuals with BPD and the current study adds to this small literature. In the examination of connectivity during masked fear processing, greater connectivity was found between the left amygdala to the right putamen and left STG in the BPD subjects relative to HCs. Similar to the finding of Cullen and colleagues (2011) during masked fear processing, the current study finds greater sub-cortical connectivity in BPD patients during masked fear conditions. A recent study found increased amplitude of low frequency fluctuation, a measure of spontaneous brain activity, in the left putamen in a large sample of BPD patients (Salvador et al., 2014). Greater sub-cortical connectivity, particularly with dopamine-rich striatal regions, may serve to strengthen fear related memories in individuals with BPD in the absence of unmasked fearful cues (Wittmann et al., 2005). The current findings, in light of previous literature, suggest that during non-conscious fear processing, individuals with BPD may have more automatic connectivity between brain regions involved in encoding and reinforcing the undetected socio-emotional content and reduced activation of regions associated with higher order control of emotional processes.

During unmasked fear processing, greater connectivity was found between the right amygdala and the sgACC in BPD subjects, relative to HCs. The sgACC is
associated with error and outcome monitoring, and is more commonly activated by
aversive stimuli, such as fear. The sgACC is involved in meaning giving during unknown
situations, and choosing actions, such as avoidance in aversive situations (Roy et al.,
2012). These findings replicate those of Cullen and Colleagues (2011), in which amygdala
activation during unmasked fearful face viewing was functionally connected with the
sgACC. A study of trait neuroticism found greater connectivity between the amygdala
and regions of the ACC and dmPFC correlated with increasing trait neuroticism (Cremers
et al., 2010). A study assessing global brain connectivity (GBC), a measure quantifying
covariability between brain regions, found greater GBC in the ACC in individuals with
BPD (Salvador et al., 2014). Salvador and Colleagues (2014) suggested that greater GBC
in this region of the ACC in individuals with BPD might relate to its role in self-
referential processing and inward attention. The connectivity between the amygdala and
the sgACC in this study might reflect stronger co-activation of regions involved in
negatively-valenced cognitions such as worry and rumination in this population (Cremers
et al., 2010).

During unmasked fear processing, we show greater right amygdala connectivity
with prefrontal regions including the dLPFC, vLPFC, and the insula in HC relative to
BPD subjects. This finding overlaps with a prior study of individuals with BPD subjects
(Koeningsberg et al., 2014), which found greater connectivity in HCs between the
amygdala and insula. This prefrontal-limbic pathway has been identified as the neural
network involved in down regulating negative emotions during emotional reappraisal, a
method of emotion regulation which involves using cognitive strategies to change the
affective meaning of a stimulus (Ochsner et al., 2012). Both activation and connectivity
analyses during unmasked fear processing find that BPD subjects show hyperactivity of the limbic system coupled with decreased connectivity with brain regions associated with top-down regulation of emotions. Because the task included in the current study was a passive emotion-processing task, there was no relevant behavioral data. Therefore, it was not possible to test whether the HCs were, in fact, engaging in more effective emotion regulation strategies than the participants with BPD. The results of the current study suggest the need for future neuroimaging studies to better parse out whether neural differences in individuals with BPD during emotion processing result from failure to utilize top-down emotion regulation strategies.

This study is limited by its small sample size. This limitation is particularly salient when studying BPD, given the heterogeneity of the diagnosis, as well as the arguably arbitrary cutoff for diagnosis the disorder (Widiger and Trull, 2000). Future studies will benefit from conducting research on this population using a dimensional approach to the BPD diagnosis, in order to capture the range of symptomatology, including that which might be considered sub-threshold according to the current diagnostic cutoff.

Additionally, research suggests that BPD may have different associations with both internalizing and externalizing dimensions of psychopathology (Eaton et al., 2011). Future research assessing whether there are different neural mechanisms associated with different features of the disorder will be important in order to develop our understanding of the biological underpinnings to the disorder.

An additional limitation is that the presented results are primarily from the contrasts of emotional faces compared to fixation. Previous studies have found neural activation in response to neutral faces, which are often used as a fixation for contrasting
emotional faces, in individuals with various psychiatric disorders (Leppänen et al., 2004; Somerville et al., 2004; Cooney et al., 2006; Hall et al., 2008). As a result, we contrasted happy and fear compared to fixation, as well as happy and fear compared to one another. Few significant findings resulted from the happy compared to fear or fear compared to happy contrasts. The current study found altered activation in subjects with BPD during the happy condition as well as the fearful condition, which is in line with previous research (Hazlett et al., 2007). This may explain the dearth of findings for the contrasts comparing happy and fear to one another. Still, this suggests a unique challenge in studying this population, in that there may not be an ideal “neutral” social cue for baseline comparison.

**Conclusion**

This study suggests that altered emotion regulation in individuals with BPD results from different patterns of neural activation and connectivity between brain regions. These findings further suggest that there may be different markers of altered neural processing, dependent on whether the emotional stimuli are consciously or non-consciously perceived. This provides preliminary evidence for the notion that there may be more than one neural mechanism of emotion dysregulation in this population. Future research dissociating whether these altered patterns relate to specific aspects of the disorder (e.g. whether neural mechanisms of altered nonconscious fear processing predicts hypervigilance in individuals with BPD or whether neural mechanisms of altered conscious fear predict anger-hostility symptoms) will help parse out the underlying biological processes behind this impairing mental illness.
Table 1. Brain Regions Showing Significant Activation Differences Between HCs and individuals with BPD During Masked Emotion Processing

<table>
<thead>
<tr>
<th>Region</th>
<th>Hemisphere</th>
<th>x</th>
<th>y</th>
<th>z</th>
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<td></td>
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Brain regions were identified using the Harvard-Oxford Cortical and Sub-Cortical Atlases. Activations reported were thresholded with an uncorrected voxel p-value of < .005 and a cluster criterion of p < .05.
Table 2. Brain Regions Showing Significant Activation Differences Between HCs and individuals with BPD During Unmasked Emotion Processing

<table>
<thead>
<tr>
<th>Region</th>
<th>Hemisphere</th>
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<th>y</th>
<th>z</th>
<th>Voxels</th>
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<td><strong>Fear greater than happy</strong></td>
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<tr>
<td>HC&gt;BPD</td>
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Brain regions were identified using the Harvard-Oxford Cortical and Sub-Cortical Atlases. Activations reported were cluster thresholded with an uncorrected voxel p-value of < .005 and a cluster criterion of p < .05. There were no significant results from the contrast of HC>BPD.
Table 3. Left Amygdala Connectivity During Masked Fear Processing

<table>
<thead>
<tr>
<th>Region</th>
<th>Hemisphere</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Voxels</th>
</tr>
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</table>

Brain regions were identified using the Harvard-Oxford Cortical and Sub-Cortical Atlases. Activations reported were cluster thresholded with an uncorrected voxel p-value of < .005 and a cluster criterion of p < .05.
Brain regions were identified using the Harvard-Oxford Cortical and Sub-Cortical Atlases.

Activations reported were cluster thresholded with an uncorrected voxel p-value of < .005 and a cluster criterion of p < .05.

### Table 4. Amygdala Connectivity During Unmasked Fear Processing

<table>
<thead>
<tr>
<th>Region</th>
<th>Hemisphere</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Voxels</th>
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</tbody>
</table>

Brain regions were identified using the Harvard-Oxford Cortical and Sub-Cortical Atlases.
Figure 1. Masked and Unmasked Emotion Processing Task. A) Depicts an example of a masked happy trial with a standard fixation, requiring no response by the participant. B) Depicts an example of an unmasked fear trial with one of the randomly distributed (throughout the masked and unmasked runs) target stimuli, requiring a response by the participant in order to ensure ongoing attention to the task stimuli.
**Figure 2.** Masked Whole-Brain Activation. A) HC>BPD activation during masked fear>fixation. B) HC>BPD activation during masked happy>fixation. C) HC>BPD activation during masked fear>happy. D) BPD>HC activation during masked happy>fixation.
Figure 3. Unmasked Whole-Brain Activation. A) BPD>HC activation during unmasked fear>fixation processing. B) BPD>HC activation during unmasked happy>fixation processing. C) BPD>HC activation during unmasked fear>happy processing. There were no significant results from the contrast of HC>BPD.
Figure 4. Left Amygdala Connectivity During Masked Fear Processing. The amygdala ROI (red) represents the overlay of the whole group amygdala activation during masked fear processing compared to fixation and the Harvard-Oxford Subcortical Atlas’s anatomical left amygdala mask.
Figure 5. Right Amygdala Connectivity During Unmasked Fear Processing. The amygdala ROI (red) represents the overlay of the whole group amygdala activation during unmasked fear processing compared to fixation and the Harvard-Oxford Subcortical Atlas’s anatomical right amygdala mask.
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Lieberman MD, Cunningham WA. (2009) Type I and Type II error concerns in fMRI research: Re-balancing the scale. Soc Cogn Affect Neurosci 4:423–428.


