Resting State Brain Network Disturbances Related to Hypomania and Depression in Medication-Free Bipolar Disorder

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INTRODUCTION
Bipolar disorder (BP) is a chronic and debilitating illness defined by episodes of (hypo)mania and depression, and the neural mechanisms triggering these shifts remain unknown (Blumberg, 2012). Although other disorders include episodes of extreme affect (eg, major depressive disorder (MDD)), BP is uniquely characterized by shifts into two states of seemingly opposing affective valence. Thus, understanding BP-related neural circuitry requires not only identifying disturbances common across mood states but also fractionating disturbances into components characteristic of each state, potentially providing insight into the mechanisms by which each mood state is triggered. In the present study, we examined symptoms continuously, which does not depend on the mood state currently experienced by an individual (eg, manic vs hypomanic). Thus, we refer to symptoms of mania, which occur in both manic and hypomanic episodes, as (hypo)manic symptoms.

Research on resting functional brain networks in bipolar disorder (BP) has been unable to differentiate between disturbances related to mania or depression, which is necessary to understand the mechanisms leading to each state. Past research has also been unable to elucidate the impact of BP-related network disturbances on the organizational properties of the brain (eg, communication efficiency). Thus, the present work sought to isolate network disturbances related to BP, fractionate these into components associated with manic and depressive symptoms, and characterize the impact of disturbances on network function. Graph theory was used to analyze resting functional magnetic resonance imaging data from 60 medication-free patients meeting the criteria for BP and either a current hypomanic (n = 30) or depressed (n = 30) episode and 30 closely age/sex-matched healthy controls. Correction for multiple comparisons was carried out. Compared with controls, BP patients evidenced hyperconnectivity in a network involving right amygdala. Fractionation revealed that (hypo)manic symptoms were associated with hyperconnectivity in an overlapping network and disruptions in the brain’s ‘small-world’ network organization. Depressive symptoms predicted hyperconnectivity in a network involving orbitofrontal cortex along with a less resilient global network organization. Findings provide deeper insight into the differential pathophysiological processes associated with hypomania and depression, along with the particular impact these differential processes have on network function.

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A large body of BP research implicates dysfunction in brain networks supporting emotion regulation (Strakowski et al, 2012; Brady et al, 2014; Wessa et al, 2014). Meta-analytic evidence indicates that structures involved in top-down control (eg, ventrolateral prefrontal cortex (vlPFC)) are hypoactive, whereas structures crucial for bottom-up affective salience (eg, amygdala) are hyperactive (Chen et al, 2011; Houenou et al, 2011; Delvecchio et al, 2012). Imbalance between systems, potentially reflected in disturbed brain network connectivity, could lead to (hypo)manic symptoms.
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default mode network in patients in manic/mixed episodes (Öngür et al., 2010).

Thus, evidence supports the existence of functional network disturbances in BP, but the nature of such disturbances remains poorly understood. Although impressive, several shortcomings in these studies may explain these inconsistencies. For example, the majority did not examine mania and depression simultaneously, making it impossible to parse BP-related disturbances (i) specific to (hypo)mania, (ii) specific to depression, or (iii) present across states. Furthermore, participants were taking psychotropic medications, which can impact fMRI (Anand et al., 2007), making it difficult to disentangle the effects of pathology vs medication.

Finally, the methodology used in these studies has several key limitations. First, each study examined connectivity with only a small set of ‘seed’ regions (and different sets across studies), potentially overlooking important connections. Furthermore, these methods examined only pairwise coupling, ignoring the role of that connection within the greater network. Most importantly, these methods do not provide insight into whether BP is associated with restructuring of the functional organization of networks. For example, do individuals with BP have a network organization with worse communication efficiency? Thus, a comprehensive understanding of network disturbances associated with BP generally, and (hypo)mania and depression in particular, remains unknown.

To address these issues, we examined medication-free patients in hypomanic/manic and depressed episodes using graph theory (only two hypomanic/manic patients were in a full manic episode, the rest in hypomanic episodes). Graph theory can elucidate key organizational properties of the global network and the function of regions therein. Graph property categories include: Functional Segregation—how optimized the network is for specialized processing, Functional Integration—how well the network can combine information across distributed regions, and Resilience—vulnerability of the network to disruption. Thus, graph theory can delineate the functional mechanisms by which altered network structure contributes to BP generally and (hypo)mania and depression specifically.

To date, only one BP study has used the graph theory (Leow et al., 2013). Examination of white matter tracts in medicated euthymic patients revealed decreased global Integration and Segregation of the hippocampus and PCC. Thus, these methods have proven useful in the study of structural networks in euthymic BP, suggesting that they will prove useful in characterizing functional networks in (hypo)manic and depressed BP.

We predicted BP participants (vs healthy controls) would evidence disturbed amygdala–vlPFC connectivity and decreased global Integration. We expected fractionation of BP-related networks to reveal different sets of disturbances for (hypo)manic and depressive symptoms, but did not make specific predictions, given the dearth of research examining these affective states concurrently.

MATERIALS AND METHODS

Participants

Participants (aged = 18–60 years) were recruited via the Indiana University Hospital outpatient psychiatry clinic and community advertisement. Patients were included if they satisfied the DSM-IV-TR criteria for BP and a current (hypo) manic or depressed episode (both BP I and II included, see Supplementary Table 1). Diagnoses were determined via Mini International Neuropsychiatric Interview (Sheehan et al., 1998) and a clinical interview by a psychiatrist (AA). Patients were rated on the Hamilton Depression Rating Scale (HAMD) (Hamilton, 1960) and Young Mania Rating Scale (YMRS) (Young et al., 1978) during both screening and scanning day. Depressed participants were required to have HAMD ≥15 and YMRS ≤10 and (hypo)manic participants to have HAMD ≤12 and YMRS ≥12 on scanning day to ensure they continued to be in the mood episode in which they were enrolled.

All participants were medication-free (including ‘rescue’ medications, eg, benzodiazepines) for ≥2 weeks before study inclusion and many for much longer (see Supplementary Material for details regarding medication status). Patients were largely moderately depressed or hypomanic. Although participants were currently medication-free, they reported having had multiple hypomanic/manic and/or depressive episodes for many years, consistent with epidemiological studies that note high rates of BP in community samples, with a large proportion not receiving treatment (Merikangas et al., 2011). See Supplementary Material for exclusion criteria.

MRI Acquisition and Preprocessing

For details see Supplementary Material.

Identification of Disturbed Network Connections

Time series were extracted from a 195-ROI resting-state-based atlas. Fourteen cerebellum ROIs were removed, because of inconsistent spatial coverage, leaving 181 ROIs. Connectivity matrices were entered as dependent variables into the Network Based Statistic (NBS) toolbox (Zalesky et al., 2010). In NBS, the regression model is first tested for each link, following which a t-threshold (t > 3.35) is applied across the network to remove unassociated links. Next, clusters of suprathreshold links (links sharing a node with ≥1 other cluster links) are identified and the corrected significance of each cluster computed. Specifically, a corrected p-value is calculated by comparing observed cluster size (no. of links) against a null distribution of maximal suprathreshold sizes created via permutation (5000 randomizations), resulting in an overall corrected α < 0.05. For all analyses, linear and quadratic trends in mean DVARS were covaried, along with bipolar subtype.

To identify disturbances related to BP overall, the first analysis compared all BP participants with healthy controls (with a covariate modeling the difference between (hypo) manic and depressed). Several strategies exist to fractionate BP network disturbances into those related to (hypo)manic and depressive symptoms. One strategy is to compare BP patients in (hypo)manic and depressed episodes separately to controls. However, this is associated with reduced power/accuracy, because most patients with (hypo)mania also report some depressive symptoms and vice versa (ie, comparisons do not cleanly separate symptoms). Thus, we used continuous YMRS/HAMD predictors. Specifically,
a second analysis was carried out with YMRS and HAMD entered simultaneously as continuous predictors (similar to Spielberg et al., 2015a), thus isolating unique variance associated with each set of symptoms. We use the term ‘continuous’ to mean that actual YMRS/HAMD scores were used as predictors, as opposed to categorical predictors. This does not imply that the YMRS/HAMD distributions were continuous (they were not because of selection criteria).

All participants were included to effectively separate variance unique to YMRS and HAMD and ensure an appropriate multivariate distribution. Although one purpose of these analyses was to fractionate networks observed for BP vs controls, it remained possible that disturbed connections associated specifically with (hypo)manic or depressive symptoms would not emerge in groupwise comparisons. Thus, YMRS/HAMD analyses included all connections. To identify whether BP subtype moderated the relationship between connectivity and YMRS/HAMD, a third analysis included the two-way interactions between subtype and YMRS/HAMD.

To gain insight into the facets of (hypo)mania/depression driving observed findings, we examined the relationship between significant networks and YMRS/HAMD symptom factor scores. For YMRS, we used weights reported in Hanwella and de Silva (2011) to create factor scores for elevated mood, irritability, and thought disorder. For HAMD, we used weights reported in Pancheri et al. (2002) to create scores for core depressive symptoms, somatic anxiety, psychic anxiety, and anorexia. For each significant finding, a regression was carried out with the relevant factor scores entered simultaneously as predictors to isolate unique variance associated with each.

Identification of Disturbed Graph Properties

Connectivity matrices were entered into the Graph Theoretic GLM (GTG) toolbox v.4.4 (Spielberg et al., 2015a,b), which computes graph properties for each participant (via Brain Connectivity Toolbox; Rubinov and Sporns, 2010). Each matrix was first thresholded to include only positive weights and normalized via division by median weight (excluding zeros) per matrix. Unlike some studies that use higher (and often multiple) density thresholds and binarize networks, we used only one threshold (zero) and retained link weights. This was done because other strategies remove important information (Rubinov and Sporns, 2011) and were used historically because methods were not available for weighted networks. The zero threshold was used because positive and negative weights are typically analyzed separately (Rubinov and Sporns, 2011), and weight normalization was performed, in part, to account for potential bias introduced by thresholding.

Node-specific and mean Clustering Coefficient (indexing Functional Segregation), Global Efficiency (stable variant of Characteristic Path Length; Functional Integration), and Assortativity (Resilience) were examined. Clustering Coefficient reflects the amount of clustering around a node, and mean Clustering Coefficient is the mean across all nodes (indexing global Segregation). Global Efficiency/Assortativity are global network properties. Global Efficiency is the extent to which nodes are connected via the fewest possible paths, reflecting the efficiency of overall network communication.

Assortativity is the extent to which highly connected nodes are linked to other highly connected nodes, reflecting the level of redundancy in communication hubs. Properties were entered as dependent variables in robust regressions in GTG (5000 permutations, same predictors as above).

To limit the number of comparisons, Clustering Coefficient was examined for only the ROI in the equivalent NBS analysis with the highest number of differential connections. An exception was for YMRS, where we also examined right amygdala, given that this region was central to the BP vs HC network. Relationships were tested only for predictors significant in the equivalent NBS analysis.

RESULTS

Participants

Seventy-six patients were enrolled, and data from 15 were excluded because of: not meeting scan day HAMD/YMRS criteria (n = 3), unreliable/unclear information (n = 3), motion (n = 5), sleeping during MRI (n = 1), not completing scan (n = 1), serious susceptibility artifact (n = 2). Close gender/age match was achieved across 30 bipolar (hypo) manic, 30 bipolar depressed, and 30 healthy controls. The remaining (depressed) patient was excluded to maintain gender/age balance. No significant differences were found between BP groups on mean duration off medication (see Supplementary Material for other comparisons).

BP vs Controls

Disturbances in network connections. BP (vs controls) exhibited hyperconnectivity in a 48-node, 61-link network (corrected p = 0.022; Figure 1), and the largest percentage of differential links (42%) were with right amygdala.

Disturbances in Graph Properties. BP exhibited worse Global Efficiency and larger Clustering Coefficient for right amygdala (see Table 1).

Fractionation

Disturbances in network connections. YMRS was associated with hyperconnectivity in a 57-node, 87-link network (corrected p = 0.007; Figure 2), and the largest percentage of differential links (20%) were with left posterior superior frontal gyrus (pSFG; Figure 2f). A midbrain (MB) region (encompassing substantia nigra (SN), red nucleus, and ventral tegmental area (VTA); Figure 2d) and right amygdala (Figure 2e) also accounted for relatively high percentages of differential links (18% and 13%, respectively). This network overlapped that observed across all BP patients by 50%. Overlap consisted largely of connections with amygdala and with lateral occipital cortex. To gain insight into the particular facets of (hypo)mania driving findings, we examined relationships between network connectivity and YMRS symptom factors. The elevated mood factor predicted unique variance in mean connectivity of the entire YMRS network (p = 0.010), along with mean amygdala (p = 0.006) and MB (p = 0.038) links in particular. Irritability predicted unique variance in mean connectivity (p = 0.011), along with pSFG (p = 0.006) links in particular.
HAMD was associated with hyperconnectivity in a 26-node, 26-link network (corrected \( p = 0.038 \); Figure 3), and the largest percentage of differential links (50%) were with right posterior-middle orbitofrontal cortex (pmOFC, located in posterior orbital gyrus). This network overlapped the cross-BP network by 8%. Overlap largely consisted of central operculum connections. The *somatic anxiety* HAMD factor uniquely predicted mean connectivity in the entire HAMD network \( (p < 0.001) \), along with pmOFC \( (p = 0.026) \) links in particular.

YMRS and HAMD networks did not overlap. No interactions with BP subtype were observed.
Disturbances in graph properties. YMRS predicted worse Global Efficiency and larger Clustering Coefficient for right amygdala (see Table 1). The irritability factor uniquely predicted worse Global Efficiency (Table 1). HAMD predicted weaker global Assortativity and larger Clustering Coefficient for right pmOFC (Table 1). The depressed mood factor uniquely predicted weaker Assortativity (Table 1). No findings emerged for mean Clustering Coefficient. See Supplementary Material for additional analyses.

**DISCUSSION**

The aim of the present work was to isolate BP-related disturbances in functional brain networks and fractionate these disturbances into components associated with (hypo)manic and depressive symptoms. Using graph-theory techniques in a sample of medication-free BP patients, we identified a network of connectivity differences present across BP (Figure 1) and fractionated this into segments related to (hypo)manic or depressive symptoms. We also identified symptom-related disturbances in the functional organization of both global and local networks (see Table 1 for a summary of findings).

**Disturbances Present Across (Hypo)mania and Depression**

Across BP participants, greater connectivity was observed in a network in which right amygdala had the largest number of differential links (Figure 1). Examination of graph-theory properties further revealed that BP was associated with greater network clustering (Clustering Coefficient, indexing **Functional Segregation**) around right amygdala, suggesting that amygdala has a greater influence over widespread network processing in BP. These findings are consistent with meta-analyses of activation studies, where amygdala was consistently associated with BP (Chen et al, 2011; Houenou et al, 2011; Delvecchio et al, 2012). In fact, amygdala was one of only two regions consistently observed across extant meta-analyses of BP activation. As evident in Figure 1, the regions showing disturbed connectivity with amygdala are largely in primary, secondary, or higher-order (ie, association) sensory cortices. Given amygdala’s central role in determining
affective salience (Phelps, 2006), hyperconnectivity between amygdala and sensory regions and greater amygdala network clustering may reflect a form of hypersalience, wherein affective significance is inappropriately overlaid on perception.

Present findings of disturbed connectivity with sensory regions may be surprising given that past BP research and theory often focused on regions more typically linked to affect (Phillips and Swartz, 2014). However, ours is the first study to examine ‘whole-brain’ connectivity in BP (ie, examination of connectivity between all nodes), rather than limiting to a small subset (eg, 1–3) of regions. Thus, findings similar to ours could have emerged previously had different analysis been used. Also, we identified disturbed connections, which can be with regions that themselves are not ‘disturbed’, potentially explaining why sensory regions have not emerged in meta-analyses of activation studies.

Consistent with the hypotheses, graph-theory analyses revealed that BP was associated with less efficient global network communication (Global Efficiency, indexing Functional Integration). High integration is crucial for maintaining a ‘small-world’ network organization (Watts and Strogatz, 1998), which is found in diverse networks including the brain (Muldoon et al, 2016). A ‘small-world’ organization is thought to be optimal, because such networks tend to show greater computational power and adaptive reconfiguration (Watts and Strogatz, 1998). Our finding that BP was associated with worse integration suggests that the brain’s ‘small-world’ balance is disturbed in BP.

These analyses captured variance common to BP, but did not isolate disturbances specific to each state. Thus, we examined the relationship between networks and (hypo)manic depressive symptoms simultaneously, thus isolating the unique variance associated with each state.

**Disturbances Related to (Hypo)manic Symptoms**

We identified a network in which (hypo)manic symptoms (YMRS) were related to hyperconnectivity (Figure 2). This network considerably overlapped (by 50%) that observed across all BP patients and right amygdala again had a large percentage of differential links (Figure 2e). Similar to the findings for all BP patients, YMRS was associated with less efficient global communication and greater amygdala.
network clustering. Thus, although amygdala disturbance and worse overall communication were observed across all BP patients, these disturbances appear to have a more crucial role in (hypo)mania. As mentioned above, increased connectivity between amygdala and sensory regions may reflect a form of hypersalience. If true, individuals with (hypo)manic symptoms may perceive affective connections where none exist, which may contribute to the elevated mood characteristic of (hypo)mania. This hypothesis is supported by our finding that the elevated mood YMRS symptom factor uniquely predicted amygdala hyperconnectivity.

Along with amygdala, a large percentage of differential links in this network were with a MB region (Figure 2d) encompassing SN, red nucleus, and VTA. Because this node includes multiple structures, we cannot determine which region(s) are driving findings. However, SN and VTA are the brain’s primary sources of dopaminergic innervation, and are thus crucial components of reward circuitry (Haber and Knutson, 2010). Thus, it is unsurprising that the elevated mood YMRS factor uniquely predicted MB hyperconnectivity. Thus, (hypo)mania-related increases in the pursuit of pleasurable activities may stem, in part, from hyperactivation of mesocortical/mesolimbic dopaminergic pathways.

The largest percentage of differential links in the YMRS network was with left pSFG. These connections were primarily with association cortex (e.g., superior parietal lobule), and research suggests that such pathways have a crucial role in goal-directed behavior (Banich, 2009). In particular, posterior prefrontal cortex is thought to bias parietal association cortex towards preferential processing of goal-relevant stimuli. Thus, hyperconnectivity between pSFG and association cortex may reflect the excessive goal-striving crucial to (hypo)mania. Combined with research indicating that hindered goal pursuit can lead to irritable mood in both healthy and BP populations (Harmon-Jones, 2003; Urošević et al, 2008), our finding that the YMRS irritability factor uniquely predicted pSFG hyperconnectivity may reflect both increased goal-striving and the frustration of such pursuits.

Disturbances Related to Depressive Symptoms

We identified a network in which depressive symptoms (HAMD) were associated with hyperconnectivity (Figure 3), and the largest percentage of differential links were with right pmOFC. This network had fairly small (8%) overlap with the cross-BP network. The observed pattern of HAMD-related hyperconnectivity differs from a recent meta-analysis of resting-state connectivity in MDD (Kaiser et al, 2015). Thus, it is possible that the neural circuitry supporting BP-related depression is fundamentally different from that in MDD. This is consistent with a recent meta-analysis, which found several differences when comparing BP and MDD activation during facial affect paradigms (Delvecchio et al, 2012). The BP patients in this meta-analysis were not necessarily depressed, and thus these findings may reflect differences between mania and depression. However, several activation studies have found differences when directly comparing MDD with depressed BP (Fournier et al, 2013; Lawrence et al, 2004; Rive et al, 2015), and similar studies are needed to directly compare MDD and depressed BP networks.

HAMD was also related to worse global network resilience (Assortativity), suggesting that network processing in depressed BP is more vulnerable to disruption (e.g., by distraction). Furthermore, HAMD was associated with greater clustering in the local network surrounding pmOFC (Clustering Coefficient), suggesting that pmOFC has a greater influence over widespread network processing in BP depression. Given the role of OFC in maintaining the motivational value of stimuli (Kennerley et al, 2011), elevated clustering may relate to the biases in affective value observed in depression (Anderson et al, 2014). Specifically, OFC clustering may reflect more intense/frequent value-related processing, potentially occurring because depressed individuals are less able to discriminate value and attempt to compensate by overengaging OFC. In turn, disturbances in stimulus valuation may be reflected in the difficulty making decisions found in depression.

Fractionation of Network Observed Across BP Patients

Interestingly, only (hypo)manic symptoms were related to amygdala disturbance, which formed the core of the network observed across all BP patients. Thus, the YMRS network appears to be more analogous to the cross-BP network, consistent with the fact that (hypo)mania is the uniquely defining characteristic of BP. Given that no paths were shared between YMRS and HAMD networks, they may reflect at least partially separable pathophysiological processes. Past work indicates that family transmissions of manic and depressed episodes in BP may be independent, which the authors suggest is reflective of partially distinct biological pathways in mania and depression (Merikangas et al, 2014). Interestingly, both YMRS and HAMD were associated with hyperconnectivity, indicating that transitioning between (hypo)mania and depression is not simply a matter of tuning circuitry up or down.

Although not examined here, BP is also associated with mixed episodes, characterized by both manic and depressive symptoms. Thus, although YMRS and HAMD networks did not overlap in the present study, the brain states associated with these symptoms cannot be exclusive. Instead, there are likely neural processes promoting the onset of both (hypo) manic and depressive symptoms, and present findings should not be interpreted as indicative that the brain states associated with these symptoms operate in an all-or-none manner.

Strengths and Limitations

The present study benefited from a number of strengths, including a medication-free sample, examination of (hypo) manic and depressed BP patients simultaneously, and use of graph-theory methods. Several limitations must also be considered. Although participants were medication-free, they were not medication-naïve. As such, present findings may be driven, in part, by long-term medication use. Furthermore, this work was cross-sectional and could not differentiate between factors predisposing toward the development of BP from factors arising as a consequence. An ideal study would longitudinally examine the same individuals through both depressive and (hypo)manic episodes. However, such work is extremely difficult and it is unethical to keep patients...
medication-free. Therefore, present findings should be regarded as complementary to studies with medicated participants. In addition, although we can speculate as to the real-world impact of the observed network disturbances, we did not collect behavioral data to confirm these hypotheses. Thus, these hypotheses should be considered preliminary and their veracity examined in future research. As this study was conducted in medication-free outpatients, only two patients met the criteria for a full manic episode (the rest hypomanic) and the majority of the depressed patients were only moderately depressed. Thus, although we examined symptoms continuously, the extent to which present findings are representative of more severe BP (ie, mania, severe depression) remains unknown. Finally, present findings should be considered preliminary until replicated in both resting-state fMRI and other methodologies. For example, task fMRI can probe the dependence of the network disturbances on the affective context.

Summary and Conclusions
We found network differences present across all BP participants and fractionated these disturbances into components related to (hypo)manic and depressive symptoms. We also found symptom-related differences in the functional organization of the network: (hypo)manic symptoms were related to less efficient communication across the global network, whereas depressive symptoms were associated with a less resilient organization of the global network. Further, both (hypo)manic and depressed symptoms predicted greater local clustering around key nodes in their respective networks.

Present findings provide deeper insight into the pathophysiological processes associated with (hypo)mania and depression. This insight may have key implications for the development and refinement of therapeutic interventions. For example, patients with disturbance primarily in one symptom network may respond differentially to treatment. Thus, quantifying the magnitude of disturbance in each network could prove useful in treatment selection. In summary, we demonstrated that BP-related pathology has important and heterogeneous effects on brain networks and related graph properties. Results move us closer to understanding the precise networks disturbed in BP and highlight the importance of examining the shared and unique network pathology related to (hypo)manic and depressive symptoms.

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