Differences in Resting-State Functional Magnetic Resonance Imaging Functional Network Connectivity Between Schizophrenia and Psychotic Bipolar Probands and Their Unaffected First-Degree Relatives

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Background: Schizophrenia and bipolar disorder share overlapping symptoms and genetic etiology. Functional brain dysconnectivity is seen in both disorders.

Methods: We compared 70 schizophrenia and 64 psychotic bipolar probands, their respective unaffected first-degree relatives ($n = 70$, and $n = 52$), and 118 healthy subjects, all group age-, gender-, and ethnicity-matched. We used functional network connectivity analysis to measure differential connectivity among 16 functional magnetic resonance imaging resting state networks. First, we examined connectivity differences between probands and control subjects. Next, we probed these dysfunctional connections in relatives for potential endophenotypes. Network connectivity was then correlated with Positive and Negative Syndrome Scale (PANSS) scores to reveal clinical relationships.

Results: Three different network pairs were differentially connected in probands (false-discovery rate corrected $q < .05$) involving five individual resting-state networks: (A) fronto/occipital, (B) anterior default mode/prefrontal, (C) meso/paralimbic, (D) fronto-temporal/paralimbic, and (E) sensory-motor. One abnormal pair was unique to schizophrenia, (C-E), one unique to bipolar, (C-D), and one (A-B) was shared. Two of these three combinations (A-B, C-E) were also abnormal in bipolar relatives but none was normal in schizophrenia relatives (nonsignificant trend for C-E). The paralimbic circuit (C-D), which uniquely distinguished bipolar probands, contained multiple mood-relevant regions. Network relationship C-D correlated significantly with PANSS negative scores in bipolar probands, and A-B with PANSS positive and general scores in schizophrenia.

Conclusions: Schizophrenia and psychotic bipolar probands share several abnormal resting state network connections, but there are also unique neural network underpinnings between disorders. We identified specific connections that might also be candidate psychosis endophenotypes.

Key Words: Bipolar, default mode, functional connectivity, gene, relatives, resting state, schizophrenia

Schizophrenia (SZ) and bipolar disorder (BP) are ostensibly separate clinical entities with distinct clinical courses and outcomes but substantial overlap in phenomenology (1), cognition (2–4), brain structure (5–8), brain function (9,10), and disease risk genes (11–14), especially for SZ and the psychotic subtype of BP (5–8). Approximately 60% of Bipolar I patients display psychotic symptoms (15,16), suggesting that the psychosis domain represents a useful starting point to compare commonalities and differences between these disorders. Similarities might originate in similarly disturbed neurophysiology (17,18).

Both disorders are heritable. Large meta-analytic linkage studies on the basis of clinical SZ and BP phenotypes report several overlapping genetic risk loci (12,13,19). Schizophrenia and affective psychoses co-occur within kindreds, suggesting shared familial risk, consistent with shared genes conferring risk for both disorders (11,20,21), again especially for SZ and psychotic BP (15,22). Finally, psychotic symptoms aggregate familially in BP (23). Thus, some disease risk genes and associated physiologic processes appear common across disorders; others may be unique.

If genetic factors are associated with neurophysiological dysfunction in both illnesses, one would anticipate both common aberrant brain function and illness-specific impairment. Schizophrenia endophenotype research has identified several putative functional and anatomical neural risk markers. Endophenotypes (24) are operationalized as measurable, trait-related, heritable, illness-associated biological features, co-segregating with disease in families and over-represented in unaffected relatives of probands, compared with the general population (25–27). Because illness risk genes are necessarily present in unaffected relatives, one expects them to exhibit some of these neurophysiological dysfunctions. This is true for particular brain imaging and cognitive deficits in both disorders (2,28,29).

Functional dysconnectivity models of SZ suggest that several brain regions subserving different cognitive functions interact ab-

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normally to generate the SZ phenotype (30,31). Empirical support for dysconnection can be found by examining strength of structural or functional connections between different brain regions, which informs understanding organized behavior of cortical functions, either during task-driven cognition or when the brain is at "rest." Functional connectivity (FC) identifies distinct sets of brain regions that are functionally coupled over time, measured as synchronous co-activation of distal neuronal assemblies (30,31). Particular attention has focused on SZ and BP brain activity and FC during "resting" or "baseline" states. At least one dozen distinct, functionally connected resting state networks (RSNs) have been identified, generally reproducible across different study populations and methodologies. These networks comprise regions mediating motor, visual, executive, auditory, and memory functioning and the so-called default-mode network (DMN); these RSN patterns are genetically influenced (32–35).

An informative variation of FC, "functional network connectivity" (FNC) (36–38), evaluates functional coupling or coherence among large-scale distributed networks. Few studies have examined interactions among different resting networks in SZ or BP (37,39–42). Zhou *et al.* (41) first examined functional connections between different RSNs in SZ, finding significant connectivity differences within and between resting state functional magnetic resonance imaging (fMRI) networks, notably those associated with dorsal prefrontal, lateral parietal, inferior temporal, dorsolateral prefrontal, and dorsal premotor cortices. Jafri *et al.* (37) examined FNC in SZ, employing the same method as the current study, reporting several abnormally higher connections, primarily between DMN, fronto-parietal, and basal ganglia networks. Ongur *et al.* (43) compared DMN (derived from resting state fMRI) in SZ and BP; both had less DMN connectivity in medial prefrontal cortex; abnormal recruitment in BP involved parietal cortex; abnormal recruitment in SZ involved frontopolar cortex/basal ganglia. Patients had significantly higher frequency fluctuation than control subjects, suggesting abnormal functional organization of the core RSN circuit and implicating dysfunction in how broad networks engage/disengage relative to one another over time. Therefore, FNC techniques provide a means to quantify how overall engagement of broad networks is influenced by other large neural systems and permits testing hypotheses about abnormalities in clinical disorders. Evidence for FNC deficits could indicate abnormal mechanisms mediating inter-cellular signaling. Because they affect large-scale network engagement, these mechanisms would likely be found consistently across many brain structures having diverse structure and functional specialization. If FNC abnormalities represent general psychosis intermediate phenotypes (26,44,45) or even unique SZ or BP markers, inquiry could turn toward generalized neuronal signaling mechanisms, ideally related to specific, measureable genetic risk factors and etiological pathways. Therefore it is important to study unmedicated and unaffected relatives of probands to detect psychosis endophenotypes.

We used a dysconnectivity model to investigate how these different RSNs interact in SZ and BP, to better understand such large-scale systems interactions and better delineate their underlying pathology. We expected that such analyses would highlight commonalities and differences in neural systems integration between disorders. Our goals were to: 1) delineate common and unique FNC profiles in SZ and BP; and 2) determine which abnormalities occur in their unaffected relatives, suggesting strong genetic influence. We first used group independent component analysis (ICA) to identify RSNs in all subjects. We then employed a two-stage analytic approach to find connectivity differences among resting state components with our previously published FNC methods (37,46). Our data-driven FNC approach provides a unique means to test brain connectivity, focusing on naturally occurring large-scale networks versus pre-specifying regions or seeds that impose more assumptions and possible bias on the data examined. We hypothesized that both SZ and BP probands would exhibit different FNC between components, including those representing both DMN and other networks (37). We additionally predicted reduced FNC between network pairs supporting cognitive functions impaired in the disorders (e.g., fronto-parietal [SZ] and fronto-temporal [BP] systems). We predicted abnormalities in cerebellar, sensori-motor, and related subcortical structures in SZ (41), consistent with cognitive dysmetria hypotheses. For BP we hypothesized FNC differences in limbic circuits (47), spatial memory/attention, and emotional regulatory areas (42,48,49). Finally, we hypothesized that subsets of aberrant connections in probands would also be observed in their unaffected relatives due to shared genetic risk.

Methods and Materials

Subjects

We assessed 118 normal control subjects, 70 first-degree relatives of SZ, 52 BP first-degree relatives, 64 psychotic BP, and 70 SZ patients (age-, gender-, and ethnicity-matched to control subjects) (Table 1). The DSM-IV (50) consensus diagnoses were established by trained clinical raters and senior diagnosticians with all clinical data and Structured Clinical Interviews for DSM Diagnoses (51) interviews: inter-rater reliability was $>$.90 among raters. Probands were clinically stable with constant medication doses for $\geq$4 weeks as follows: mood stabilizers (19 SZ; 44 BP), typical antipsychotics (7 SZ; 2 BP), atypical antipsychotics (58 SZ; 36 BP), benzodiazepines (13 SZ; 11 BP), anticholinergics (11 SZ; 4 BP), SSRIs (18 SZ; 16 BP), tricyclics or monoamine oxidase inhibitors (9 SZ; 13 BP), and psychostimulants (2 SZ; 4 BP). Relatives of probands were free of Axis 1 psychopathology and not taking psychoactive medications. Participants were recruited via word of mouth and advertisements at the Olin Neuropsychiatry Research Center (ONRC); all provided written informed consent approved by the institutional review boards of Hartford Hospital and Yale. Participants were drawn from the Bipolar Schizophrenia Network on Intermediate Phenotypes and other ongoing ONRC studies, independent from samples in previous publications.

Bipolar subjects all had a lifetime diagnosis of psychosis, on the basis of the presence of hallucinations/delusions during at least one episode (within or distinct from an affective episode) in their illness course, described in Strasser *et al.* (52); each was assessed additionally for current psychosis on day of scanning on the basis of scores $\geq$3, in one or more of the following Positive and Negative Syndrome Scale (PANSS) positive subscales: delusions**,** conceptual disorganization**,** hallucinations, and suspiciousness/persecutory. On the basis of these criteria, 50% of bipolar probands had current psychosis. The BP probands were assessed on scan day with commonly employed cutoff scores for manic and depressive episodes, respectively: Montgomery/Asberg Depression Rating Scale $>$32 and Young Mania Scale $>$20 (53,54). Thus defined, 3 of 64 BP subjects met criteria for major depressive episode, and 8 of 64 met criteria for manic episode. Relatives were further classified for presence or absence of DSM-IV-TR Cluster A personality disorders, on the basis of the Structured Interview for Disorders of Personality (55); there were only 3 SZ and 4 BP relatives, respectively.

Data Pre-Processing

The fMRI images were collected on the ONRC Siemens Allegra 3T system (Siemens, Malvern, Pennsylvania). The echo planar image gradient-echo pulse sequence (repetition time: 1500 msec; echo