Functional Connectivity and Neuronal Variability of Resting State Activity in Bipolar Disorder—Reduction and Decoupling in Anterior Cortical Midline Structures

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Abstract: Introduction: The cortical midline structures seem to be involved in the modulation of different resting state networks, such as the default mode network (DMN) and salience network (SN). Alterations in these systems, in particular in the perigenual anterior cingulate cortex (PACC), seem to play a central role in bipolar disorder (BD). However, the exact role of the PACC, and its functional connections to other midline regions (within and outside DMN) still remains unclear in BD. Methods: We investigated functional connectivity (FC), standard deviation (SD, as a measure of neuronal variability) and their correlation in bipolar patients ($n = 40$) versus healthy controls ($n = 40$), in the PACC and in its connections in different frequency bands (standard: 0.01–0.10 Hz; Slow-5: 0.01–0.027 Hz; Slow-4: 0.027–0.073 Hz). Finally, we studied the correlations between FC alterations and clinical-

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neuropsychological parameters and we explored whether subgroups of patients in different phases of the illness present different patterns of FC abnormalities. Results: We found in BD decreased FC (especially in Slow-5) from the PACC to other regions located predominantly in the posterior DMN (such as the posterior cingulate cortex (PCC) and inferior temporal gyrus) and in the SN (such as the supragenual anterior cingulate cortex and ventrolateral prefrontal cortex). Second, we found in BD a decoupling between PACC-based FC and variability in the various target regions (without alteration in variability itself). Finally, in our subgroups explorative analysis, we found a decrease in FC between the PACC and supragenual ACC (in depressive phase) and between the PACC and PCC (in manic phase). Conclusions: These findings suggest that in BD the communication, that is, information transfer, between the different cortical midline regions within the cingulate gyrus does not seem to work properly. This may result in dysbalance between different resting state networks like the DMN and SN. A deficit in the anterior DMN-SN connectivity could lead to an abnormal shifting toward the DMN, while a deficit in the anterior DMN-posterior DMN connectivity could lead to an abnormal shifting toward the SN, resulting in excessive focusing on internal contents and reduced transition from idea to action or in excessive focusing on external contents and increased transition from idea to action, respectively, which could represent central dimensions of depression and mania. If confirmed, they could represent diagnostic markers in BD. *Hum Brain Mapp 00:000–000, 2014.*

Key words: bipolar disorder; resting state fMRI; functional connectivity; neuronal variability; perigenual anterior cingulate cortex; default mode network

INTRODUCTION

Background

In recent years, a growing number of functional magnetic resonance imaging (fMRI) studies have contributed to elucidating neurofunctional alterations in bipolar disorder (BD) [Vargas et al., 2013]. Though the results obtained from the functional connectivity (FC) studies vary, they suggest differences between patients in any phase of illness (i.e., manic, depressive, and mixed or euthymic phase) and healthy controls in the connectivity patterns of the prefrontal cortex (PFC) and anterior cingulate cortex (ACC) with other cortical and subcortical areas [Vargas et al., 2013]. This suggests a prefrontal dysregulation of affective networks [Strakowski et al., 2005].

Among the resting state networks, the default mode network (DMN) and the salience network (SN), and their relationship and balance with the central executive network (CEN), could be mainly involved in the psychopathology of BD. The DMN mainly concerns cortical midline regions, such as the ACC and posterior cingulate cortex (PCC) and parietotemporal multimodal association cortices [Buckner et al., 2008]. Although the DMN is considered as a single system, in this network the anterior and posterior midline regions seem to show differences in their interactions with other networks [Uddin et al., 2009]. In particular, the anterior regions are involved in affective regulation and favor the representation of emotions in the absence of immediately present positive or negative incentives; they thus play a crucial role in the anticipation of the future affective consequences of sensory-motor patterns and in the persistence of emotion following the cessation of an elicitor [Davidson, 2000]. By contrast, the posterior regions seem to be involved in ideation, internal thoughts and mind wandering [Christoff et al., 2009; Mason et al., 2007]. The SN, which includes regions like the supragenual ACC (SACC) and ventrolateral PFC (VLPFC)—anterior insula [Seeley et al., 2007], responds to the degree of subjective salience, whether homeostatic, intero/exteroceptive or cognitive, and it is also involved in the reward system [Goulden et al., 2014; Menon, 2011]. The CEN, which comprises the dorsolateral PFC (DLPFC) and the posterior parietal cortex, is involved in higher-order cognitive and executive functions [Goulden et al., 2014]. In this context, the FC between different parts of midline regions within DMN could affect the balance between the different resting state networks. In particular, the DMN, which is associated with internal contents, and the CEN, which is associated with external contents, are observed to be anticorrelated [Uddin et al., 2009]. The SN, which is dissociable from CEN and is involved in the detection of salient external stimuli, seems to drive the switching between DMN and CEN, favoring the reduction of the DMN activity and the increase of the CEN activity when attention is externally focused [Goulden et al., 2014; Jilka et al., 2014; Seeley et al., 2007]. Therefore, the cortical midline regions could mediate different functions of DMN and SN (and their relationship with CEN), which are affected in BD.

Especially the peri/subgenual ACC (Brodmann area (BA) 25 and part of 24) seems to be involved in the affective network regulation and in the pathophysiology of BD, since this region has shown anatomical, metabolic and functional alterations during both early stages and different phases of the disease [Fountoulakis et al., 2008]. In particular, volume changes and deficient GABAergic activity were found in the peri/subgenual ACC in BD; moreover,

the functional activity in the left BA 25 showed significant increase during mania and decrease during depressive phases [Fountoulakis et al., 2008]. Furthermore, the perigenual anterior cingulate cortex (PACC) represents a core region of the DMN [Qin and Northoff, 2011], which also shows several alterations in BD [Vargas et al., 2013]. In particular, reduced DMN connectivity was observed in bipolar patients, especially in the anterior regions of this network [Meda et al., 2014; Ongur et al., 2010]. Specific alterations in BD in other networks like the SN and CEN are lacking, but some studies reported FC alterations between PACC/medial PFC and some areas of SN—such as hyperconnectivity between medial PFC and VLPFC (in psychotic manic phase) and hypoconnectivity between PACC and dorsomedial thalamus (like in major depression)—while other studies showed a loss of anticorrelation between medial PFC and DLPFC and a FC reduction within the CEN (in psychotic BD) [Anand et al., 2009; Baker et al., 2014; Chai et al., 2011]. However, the exact role of the PACC, and its functional connections to other midline regions (within and outside the DMN) still remain unclear in BD. Moreover, the exact frequency range of PACC FC as in slower (e.g., Slow-5: 0.01–0.027 Hz) or less slow (e.g., Slow-4: 0.027–0.073 Hz) bands, as recently distinguished in the healthy brain [Buzsaki and Draguhn, 2004; Han et al., 2011; Hoptman et al., 2010; Xue et al., 2014], remain unclear in BD.

In addition to FC, the variability of neural activity has recently been investigated to characterize the resting state [Garrett et al., 2010, 2011, 2013; Zang et al., 2007]. This variability, operationalized as standard deviation (SD), has been shown to be central to both resting state activity and task-evoked activity in the healthy brain [Garrett et al., 2010; Garrett et al., 2011, 2013]. In contrast, variability remains to be investigated in psychiatric disorders, including BD. Moreover, while one recent study in healthy subjects has shown correlation between variability and FC [Di et al., 2013], their relationship has not yet been investigated in BD.

Aims of the Study

The general aim of our study was to investigate different measures of resting state activity—FC and variability—especially in the PACC and its connections in bipolar patients ($n = 40$) and healthy controls ($n = 40$).

The specific aims were the following.

First, based on the above described findings in BD, we aimed to investigate resting state FC in different frequency bands—standard frequency band (SFB: 0.01-0.10 Hz [Fox and Raichle, 2007; Zhang and Raichle, 2010]), Slow-5 and Slow-4 [Buzsaki and Draguhn, 2004; Han et al., 2011; Hoptman et al., 2010; Xue et al., 2014; Zuo et al., 2010]—specifically in the anterior midline structures by taking the PACC as the seed region and the SACC and PCC as control regions. The PACC was used as the seed region because of its documented involvement in the regulation of affective network and pathophysiology of BD and its central role in DMN (see above). To test regional specificity of the PACC, we chose the PCC as the first control region so as to exclude global involvement of the DMN (since the PACC can be regarded as a main part of the anterior DMN, and the PCC as a main part of the posterior DMN [Qin and Northoff, 2011]). The SACC was chosen as the second control region to exclude global involvement of the ACC (since the SACC is close to the PACC but it is a main part of another resting state network, the SN [Seeley et al., 2007]). On the basis of previous findings, we hypothesized that bipolar patients would display decreased FC, specifically in the PACC connections.

Second, we aimed to investigate variability [i.e., SD and fractional SD (fSD)] in the PACC and in those regions that show significant differences in their FC with the PACC in bipolar patients. On the basis of standard measure of SD [Garrett et al., 2010, 2011, 2013] and previous results on variability in other disorders, such as brain injury [Raja Beharelle et al., 2012] and vegetative state [Huang et al., 2013], we hypothesized reduced variability in bipolar patients in those regions showing decreased FC with the PACC.

Our third aim was to investigate the relationship between FC and variability in the resting state in both healthy and bipolar subjects. We hypothesized that variability and FC might be correlated with each other. However, if variability turned out to be in normal ranges in BD, we tentatively hypothesized that variability and FC might be correlated with each other in healthy subjects only, while they might be decoupled from each other in bipolar patients (as this decoupling might be related to the hypothesized decrease in FC in BD).

Finally, we investigated the relationship between FC alterations (in the standard frequency band) and clinical and neuropsychological parameters, to investigate possible clinical correlates of dysconnectivity. Moreover, as preliminary analysis, we explored whether subgroups of patients in different phase of illness present different patterns of FC abnormalities.

METHODS AND MATERIALS

Subjects and Clinical Assessment

Subjects were admitted to the in- and out-patient service of the Psychiatric Clinic at the University of Genoa (IRCCS AOU San Martino—IST, Dipartimento di Neuroscienze, Riabilitazione, Oftalmologia, Genetica e Scienze Materno-Infantili: DINOGMI), from September 2013 to January 2014. The study was conducted on 40 bipolar patients (27 females, 18–60 years old, 11 in manic phase, 11 in depressive phase, 7 in mixed phase, and 11 in euthymic phase) and 40 healthy controls matched for age and sex (Table I).

TABLE I. Demographic and clinical information

	Bipolar disorder patients	Healthy controls
Sample Size, *n*	40	40
Age, mean (SD)	44.6 (11.8)	43.9 (12.8)
Female, *n* (%)	27 (67.5)	26 (65)
Age at Onset, mean (SD)	24.7 (11.1)	
Duration of Illness, mean (SD)	20.0 (11.4)	
Manic patients, *n* (%)	11 (27.5)	
HAM-D, mean (SD)	5.3 (5.3)	
YMRS, mean (SD)	17.2 (5.8)	
Depressed patients, *n* (%)	11 (27.5)	
HAM-D, mean (SD)	24.5 (4.0)	
YMRS, mean (SD)	3.7 (3.0)	
Mixed patients, *n* (%)	7.0 (17.5)	
HAM-D, mean (SD)	18.3 (0.7)	
YMRS, mean (SD)	16.0 (5.5)	
Euthymic patients, *n* (%)	11 (27.5)	
HAM-D, mean (SD)	4.6 (2.3)	
YMRS, mean (SD)	4.1 (2.7)	
Mood Stabilizers, *n* (%)	35 (87.5)	
Antidepressants, *n* (%)	11 (27.5)	
Antipsychotics, *n* (%)	24 (60)	
Benzodiazepines, *n* (%)	12 (30)	
Unmedicated, *n* (%)	1 (2.5)	

The Ethics Committee of San Martino Hospital approved the study, and written informed consent was obtained from all subjects.

Each subject was evaluated by means of the following standardized structured and/or semistructured clinical instruments to obtain information on clinical and diagnostic features, course of illness, family history, and present and past pharmacotherapy: Mini International Neuropsychiatric Interview [Sheehan et al., 1998]; Structured Clinical Interview for DSM-IV Axis II Personality Disorders (SCID-II) [First et al., 1994]; Structured Interview for Mood Disorder—Revised [Cassano et al., 1989]. General, physiologic, pathologic, and psychopathologic history was also investigated.

Furthermore, to evaluate the presence of cognitive impairment, all subjects were administered a brief neuropsychological battery, that included the fluency test, a verbal test (prompted by letter and by category) used to evaluate the integrity of executive functions (frontotemporal functions) [Strauss, 2006]; and the continuous performance test (CPT), a computerized test used to evaluate distractibility and impulsivity in the study of attention functions [Conners, 2003].

Inclusion criteria were as follows: diagnosis of BD type I according to the Diagnostic and Statistical Manual for Mental Disorders-Fourth Edition (DSM-IV) criteria [American Psychatrich Association, 1994], as assessed by the Structured Clinical Interview for Axis-I Disorders/Patient edition (SCID-I/P) [Ventura et al., 1998] for manic, depressed, mixed and euthymic patients; 17-item Hamilton Depression Scale (HAM-D) score ≥ 18 [Hamilton, 1960] and/or Young Mania Rating Scale (YMRS) score ≥ 13 [Young et al., 1978] for manic, mixed and depressed patients; HAM-D score < 8 [Hamilton, 1960] and YMRS score < 8 [Young et al., 1978] for euthymic patients; age between 18 and 60 years; ability to provide written informed consent. Exclusion criteria were as follows: diagnoses of Schizophrenia, Mental Retardation, Dementia, other Cognitive Disorders; history of severe or decompensated somatic diseases, neurological diseases (e.g., former stroke, cerebral vascular malformations, or epilepsy), previous head injury with loss of consciousness (for 5 or more minutes); current alcohol and substance abuse (during the previous 3 months); history of alcohol or substance dependence; history of abuse of synthetic and/or new drugs abuse; pregnancy and lactation; left-handedness; the inability to undergo an MRI examination (claustrophobia, metal implants, etc.); previous treatment with electroconvulsive therapy, chemotherapy, or brain radiotherapy. Healthy controls did not meet the DSM-IV criteria for psychiatric disorders, either currently or in the past; they had a HAM-D score < 8 and a YMRS score < 8; they also met the same exclusion criteria indicated for patients.

In our sample, all the bipolar subjects except one were taking medications, including mood stabilizers ($n = 35$, 87.5%), antidepressants ($n = 11$, 27.5%), antipsychotics ($n = 24$, 60%), and benzodiazepines ($n = 12$, 30%) (Table I).

fMRI Data Acquisition

A 1.5-T GE scanner with a standard head coil was used to acquire all the images. Foam pads were used to reduce head motion and scanner noise. fMRI scanning was carried out in darkness, and the participants were explicitly instructed to keep their eyes closed, to relax and to move as little as possible. Functional images were collected using a gradient echo Echo Planar Imaging sequence sensitive to blood oxygenation level-dependent (BOLD) contrast (TR/TE = 2000/30 ms, flip angle = 90 degree, FOV = 24 cm). Whole-brain volumes were acquired in 33 contiguous 4-mm thick transverse slices, with a 1 mm gap and 3.75×3.75 mm^2 in-plane resolution. For each participant, fMRI scanning lasted 6 min and acquired a total of 150 scans.

In addition, three-dimensional T1-weighted anatomical images were acquired for all subjects in a sagittal orientation by means of a 3D-SPGR sequence (TR/TE=11.5/5 ms, IR=500 ms, flip angle = 8 degree, and FOV = 25.6 cm) with an in-plane resolution of 256×256 and slice thickness of 1 mm.

Data Analysis

Preprocessing steps were implemented in Analysis of Functional NeuroImages (AFNI) (http://afni.nimh.nih.gov/afni [Cox, 1996]). The first 2 volumes of each

functional time series were discarded. The remaining functional images were slice-timing corrected and aligned (head motion correction). Each participant's motion was assessed by means of translation/rotation, and an exclusion criterion (translation > 3 mm, rotation $> 3°$; in each direction) was set. The T1 anatomical images of all subjects were normalized to the Talairach space. Resting state data, masked with the T1 images, were then spatially transformed into the Talaraich space [Talairach and Tournoux, 1988], resampled to $3 \times 3 \times 3$ mm^3 and spatially smoothed (6 mm). The estimated head motion and the mean time series from the white matter and the cerebrospinal fluid were used as covariates in the correlation computation [Fox et al., 2005; Saad et al., 2012]. The data were then filtered with a standard band-pass filter; signals between 0.01 and 0.1 Hz, which are thought to reflect mainly neuronal fluctuations [Biswal et al., 1995; Fox and Raichle, 2007; Zhang and Raichle, 2010], were conserved. On the basis of recent findings in healthy subjects [Buzsaki and Draguhn, 2004; Zuo et al., 2010], we also focused on two separate bands within the standard range of 0.01–0.1 Hz: Slow-5 (0.01–0.027 Hz) and Slow-4 (0.027–0.073 Hz).

Resting-State Analysis

All the resting state analysis was performed using AFNI (http://afni.nimh.nih.gov/afni [Cox, 1996]). A voxel-wise FC analysis of ROI was used. The seed reference time-series of each ROI was obtained by averaging the fMRI time-series of all voxels within the ROI. Correlation analysis was carried out between the seed reference region and the rest of the whole brain in a voxel-wise manner for each frequency band (i.e., 0.01–0.1 Hz, Slow-5 and Slow-4). The correlation coefficients were then transformed to z-values by means of the Fisher r-to-z transformation, to improve normality for group-level t-tests. This produced spatial maps in which the values of voxels represented the strength of the correlation with the ROIs. The PACC was used as the seed ROI for FC computation. Spherical ROI with a radius of 6 mm were placed in the Talairach coordinates of $x = 0$, $y = -45$, $z = 0$ [Qin and Northoff, 2011]. The PCC ($x = 4$, $y = 49$, $z = 25$) [Qin and Northoff, 2011] and SACC ($x = 6$, $y = -19$, $z = 27$) [Seeley et al., 2007] were also used as seed regions of control. To detect the FC group-level differences between bipolar patients and healthy controls, the z-values were entered into a two-sample t-test in a voxel-wise manner, so as to determine which brain regions showed significant differences in positive or negative connectivity to the above three seed regions in each frequency bands (age was used as a covariate to control potential aging effects). All resulting t-maps were thresholded at a corrected P value of 0.05 (multiple-comparison error was corrected using the Monte Carlo simulation).

Second, we investigated the SD of BOLD signal changes in the resting state (as a measure of variability) and the fSD (as a measure of frequency-specific contributions to variability) within those regions that showed altered FC with the PACC and in each frequency band. SD and fSD values in each frequency bands were entered into a two-sample t-test to compare group-level differences in the variability between bipolar patients and healthy controls. The results were thresholded at corrected $P < 0.05$ (Bonferroni correction was carried out for multiple comparisons).

Finally, Pearson correlation analysis was performed between FC and SD/fSD in the regions showing altered FC with the PACC in each frequency band. This analysis was conducted both in healthy subjects and in bipolar patients. We assessed the potential impact of clinical parameters on the correlation results between FC and SD/fSD in BD using a partial correlation between these factors, with the YMRS and HAMD total scores as covariates. The results were thresholded at corrected $P < 0.05$ (Bonferroni correction was carried out for multiple comparisons and Bootstrap correction was carried out to detect outliers).

Clinical Correlations and Explorative Subgroup Analysis

We examined the potential impact of the psychotropic medication load—that is, the number and dosage of different medications—on FC and SD/fSD in BD [Phillips et al., 2008]. This was done by converting antipsychotics into chlorpromazine dose-equivalents [Baldessarini, 2013], mood stabilizers into lithium dose equivalents [Baldessarini, 2013], antidepressants into imipramine dose-equivalents [Baldessarini, 2013], and benzodiazepines into diazepam dose-equivalents [Arana and Rosenbaum, 2000]. We then used the codes 0, 1, 2, and 3 to indicate: no medication, and dose-equivalents below, equal to or above the mean effective daily dose, respectively [Davis and Chen, 2004]. We generated a composite measure of the medication load by summing all individual medication codes for each category for each individual BD patient [Zanetti et al., 2009]. First, we investigated the potential impact of medications on imaging data by correlating the resulting pharmacological load with FC and SD/fSD. Second, we assessed the potential impact of medications on the loss of correlation between FC and SD/fSD in BD using a partial correlation between these factors, with the pharmacological load as a covariate.

Furthermore, Pearson correlation analysis was performed between altered FC values and clinical variables—that is, YMRS and HAM-D total scores—and neurocognitive variables—that is, fluency prompted by letter and by category, and CPT parameters such as total hits, total omission errors and total commission errors. The results were thresholded at corrected $P < 0.05$ (Bonferroni correction was carried out for multiple comparisons).

Finally, we conduct an explorative analysis of FC in the subgroups, that is, manic, depressed, euthymic, and healthy controls. We applied the same methods that we

used for the main analysis between the whole sample of bipolar patients and controls, using age as coregressor. A P value < 0.05 (corrected for multiple-comparison error using the Monte Carlo simulation), was set for between-subgroup comparisons. However these analysis should be considered preliminary as the subgroups sample size is relatively small.

SD/fSD t-test comparisons and correlation analyses were performed by means of IBM SPSS Statistics® Version 19 for Windows® (Chicago)®.

RESULTS

Functional Connectivity

We compared FC in the standard frequency band between bipolar patients and healthy controls. Taking the PACC as the seed region (with age as a covariate), in bipolar patients we found decreased FC from the PACC to various other regions located predominantly in the posterior DMN, such as the PCC and inferior temporal gyrus (ITG), and in the SN, such as the SACC and VLPFC (Fig. 1a left upper part and Table II first column). In contrast, on taking the PCC and SACC as control regions, we mainly found decreased FC from those ROIs to the PACC only (Fig. 1a left middle and lower part and Table III and Table IV first column, respectively) in bipolar patients. These findings suggest that the main alteration in bipolar patients involves FC in the PACC, while no major alterations occur in the PCC or SACC.

In addition to the standard frequency band, we also focused on Slow-5 and Slow-4, in accordance with recent literature [Buzsaki and Draguhn, 2004; Han et al., 2011; Hoptman et al., 2010; Zuo et al., 2010]. We first performed a two-sample test between groups using the same seed regions as above, but now separately for Slow-5 and Slow-4. This revealed almost the same FC alteration that we found in the standard frequency band (Fig. 1a central and right parts). However, we observed that the FC pattern, especially when the PACC was used as the seed region, was stronger in Slow-5 than in Slow-4 (Fig. 1a upper central and right parts). We, therefore, conducted exclusive masking analyses of the group comparisons between Slow-5 and Slow-4. This showed a greater difference in the pattern of PACC-based FC to various midline regions between bipolar patients and healthy controls in Slow-5 than in Slow-4 (Fig. 1b). These findings suggest that the deficits in FC from the PACC in bipolar patients were greater in Slow-5 than in Slow-4.

In summary, our results show significantly decreased resting state FC from the PACC (the core region of the anterior DMN), mainly to other midline regions such as the SACC and PCC. Moreover, PACC-based FC differences between groups were greater in Slow-5 than in Slow-4, suggesting greater deficits in Slow-5 in bipolar patients. In contrast, no major alterations were observed in FC when the PCC (the core region of the posterior DMN) or SACC (one of the main regions of the SN) was used as the seed region. Taken together, these findings suggest specific anterior DMN deficits in resting state activity in BD, for example, PACC-based FC, mainly occurring in the slowest infraslow frequency band, that is, Slow-5 rather than Slow-4.

Variability

To further understand the deficits in resting state FC in bipolar patients, we investigated variability (SD) and frequency-specific contributions to variability (fSD) within those regions that showed decreased FC with the PACC, in each frequency band. Neither SD nor fSD differed significantly between bipolar patients and healthy controls in any of the regions investigated (Fig. 2, Table V and Table VI). No frequency-band-specific effects were observed in either Slow-5 or Slow-4.

In summary, these findings suggest that there is no deficit in variability of resting state activity in those regions that showed decreased FC with the PACC in BD.

Relationship Between Functional Connectivity and Variability

Finally, we investigated the relationship between resting state FC and variability in the regions showing decreased FC with the PACC in BD, first in healthy controls and second in patients.

We observed significant positive correlations between SD and FC (z-score) in a number of target regions (though not all) in healthy subjects: the higher the variability (SD) in the target regions, the higher their FC (z-score) with the PACC (Fig. 3 left part and Table VII). In contrast, variability in the seed region PACC did not correlate with FC (z-score) between seed and target regions (Table VII). Thus, there is asymmetry in the relationship between variability and FC between seed and target regions, with FC being related to variability in the target region, but not in the seed region.

In contrast to healthy subjects, none of these correlations between SD and FC (z-score) held in bipolar patients (Fig. 3 right part and Table VIII). In BD patients, unlike healthy subjects, no significant correlations emerged between PACC seed-based FC to the target regions (e.g., PCC, SACC, VLPFC, and ITG) and variability in these regions. We also used a partial correlation between FC and SD/fSD, with the YMRS and HAMD total scores as covariates, and no correlations emerged (all $P > 0.05$), suggesting that the loss of correlation between FC and variability in BD did not depend on clinical parameters. These findings suggest that variability in the target regions is decoupled from FC between seed and target regions in BD.

In summary, PACC-based resting state FC correlated with variability in various (but not all) target regions,

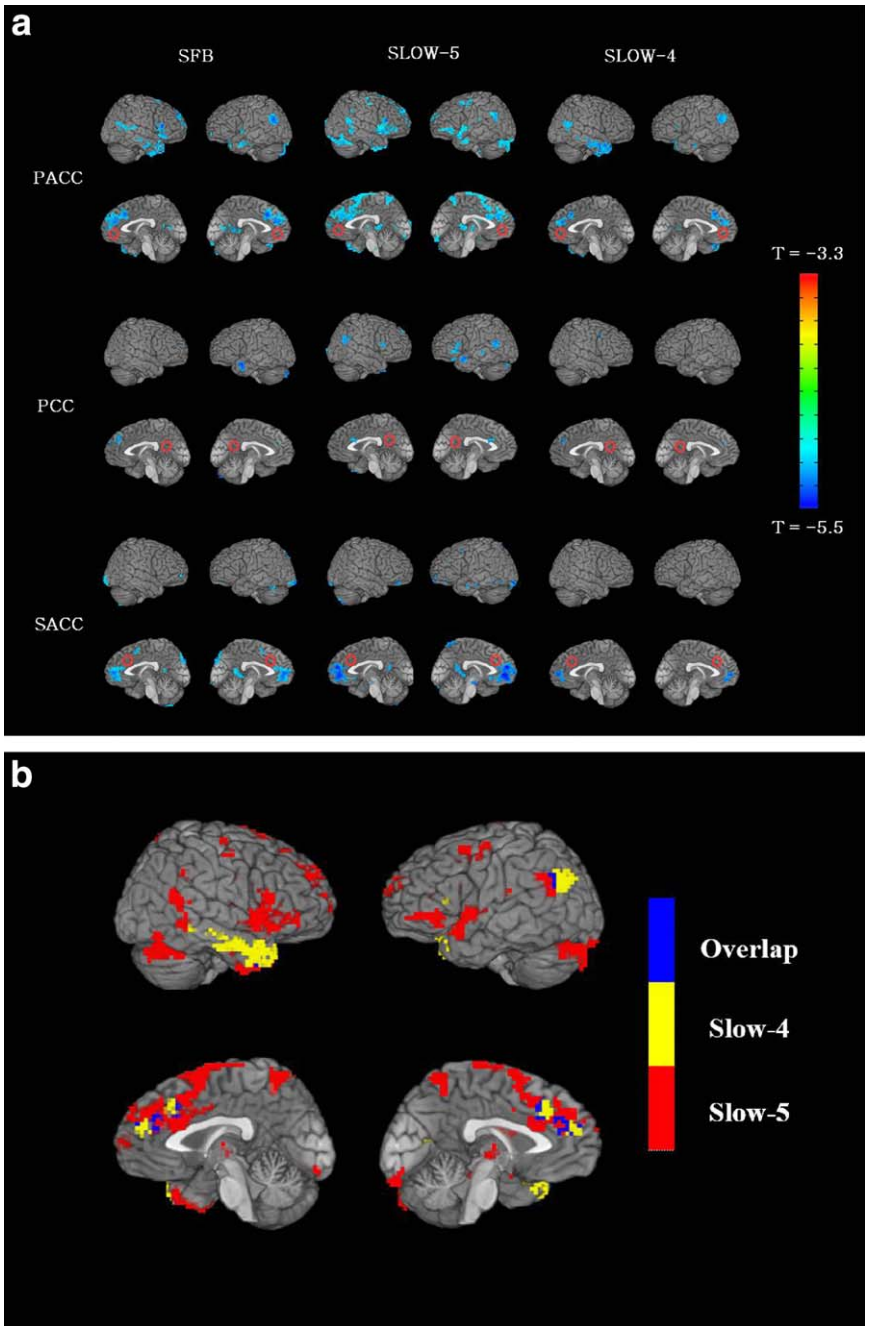

Figure 1.

a. FC maps: BD versus healthy controls. Abbreviations: PACC, perigenual anterior cingulate cortex; PCC, posterior cingulate cortex; SACC, supragenual anterior cingulate cortex are used as seed regions in the different frequency bands, FSB (Standard Frequency Band: 0.01–0.10 Hz), Slow-5 (0.01–0.027 Hz), and Slow-4 (0.027–0.073 Hz). All t-maps were thresholded at corrected $P < 0.05$. The color bar shows voxel-wise t-values. **b.** Differences in FC between Slow-5 (0.01–0.027) and Slow-4 (0.027–0.073) in the PACC (perigenual anterior cingulate cortex) T maps (BD patients versus Healthy Controls). All t-maps were thresholded at corrected $P < 0.05$. The color bar shows the different t-maps (blue: overlap map; yellow: Slow-4 map; red: Slow-5 map).

TABLE II. PACC seed region (x = 0, y = 45, z = 0)

	SFB		Slow-5		Slow-4	
Anatomical region	*x, y, z*	CS (voxel)	*x, y, z*	CS (voxel)	*x, y, z*	CS (voxel)
DLPFC R	59, 26, 14	544	59, 26, 14	1070		
VLPFC L/Ins L	−40, 23, 14	371	−34, 5, 17	342	−43, 23, 14	117
SACC L	−4, 23, 35	848	11, 17, 23	1307	−7, 26, 35	183
OFC L	−25, 23, 13	128	−49, 17, −13	97	−28, 14, −22	108
PG L/SMA L			−40, −7, 53	104		
ITG R	44, − 4, −34	384	44, 8, 37	269	59, −13, −25	353
ITG L	−55, −10, −13	85				
TPJ R			62, −46, 11	118		
TPJ L	−52, −61, 23	163	−52, −55, 20	67	−37, −55, 26	145
Pc R			2, −52, 59	73		
PCC R	11, −58, 17	114				
PCC L/Cu L	−10, −73, 17	248			−10, −73, 11	103
Th L			−16, −10, 14	375		

Notes: Peak coordinates at corrected $P < 0.05$ for standard frequency band (SFB: 0.01–0.10 Hz), Slow-5 (0.01–0.027 Hz), and Slow-4 (0.027–0.073 Hz).

Abbreviations: PACC, Perigenual Anterior Cingulate Cortex; DLPFC R, Dorsolateral Prefrontal Cortex Right; VLPFC L/Ins L, ventrolateral prefrontal cortex left /insula left; SACC L, supragenual anterior cingulate cortex left; OFC L, orbitofrontal cortex left; PG L/SMA L, precentral gyrus left/supplemental motor area left; ITG R, inferior temporal gyrus right; ITG L, inferior temporal gyrus left; TPJ R, temporal parietal junction right; TPJ L, temporal parietal junction left; Pc R, precuneus right; PCC R, posterior cingulate cortex right; PCC L/Cu L, posterior cingulate cortex left/cuneus left; Th L, thalamus left; CS, cluster size.

including the SACC, VLPFC, PCC/Precunues, and ITG (Fig. 3 left part). This correlation seems to be lost in BD, in which PACC-based FC is decoupled from variability in the various target regions (Fig. 3 right part). This is most interesting since the variability itself was not reduced in either seed or target regions in BD (see above).

Clinical Correlations and Explorative Subgroup Analysis

To assess the potential impact and interference of pharmacotherapy with the BOLD signal [Phillips et al., 2008], we correlated the medication load with FC and SD/fSD. The medication load did not correlate with FC and SD/fSD (except for SACC in Slow-4, $P = 0.009$, but there were no differences in SD between patients and controls), suggesting that resting state parameters were not greatly affected by pharmacotherapy. Second, we used a partial correlation between FC and SD/fSD, with the medication load as a covariate. No correlations emerged (all $P > 0.05$), suggesting that the loss of correlation between FC and variability in BD did not depend on pharmacotherapy.

With regard to clinical correlations of altered PACC FC, significant inverse correlation was found between HAM-D total score and PACC-SACC FC ($r = -0.331$; $P = 0.003$); significant inverse correlations were found between YMRS total score and PACC-PCC FC ($r = -0.308$; $P = 0.005$), PACC-OFC L FC ($r = -0.396$; $P < 0.000$), and PACC-TPJ L FC ($r = -0.339$; $P = 0.002$).

With regard to neuropsychological evaluation, bipolar patients showed significant deficits in fluency prompted by letter ($F = 3.969$; df = 81; $P = 0.001$), but not in fluency prompted by category, when compared to HC. In terms of CPT, bipolar subjects showed significant lower number of total hits ($F = 11.203$; df = 81; $P < 0.001$) and higher number of total omission errors ($F = 7.760$; df = 81; $P < 0.001$), but no difference in total commission errors, when compared to HC. Fluency prompted by letter showed a direct correlation with PACC-SACC FC ($r = 0.354$; $P = 0.001$) and with

TABLE III. PCC seed region (x = −4, y = −49, z = 25)

	SFB		Slow-5		Slow-4	
Anatomical Region	*x, y, z*	CS (voxel)	*x, y, z*	CS (voxel)	*x, y, z*	CS (voxel)
PACC R/ MPFC R	11, 62, 20	110				
SACC R			17, 32, 26	97		
VLPFC L			−40, 23, 8	167		
Ins L			−28, −28, 11	325		
TPJ R/ STG R			50, −19, 2	230		

Notes: Peak coordinates at corrected $P < 0.05$ for standard frequency band (SFB: 0.01–0.10 Hz), Slow-5 (0.01–0.027 Hz), and Slow-4 (0.027–0.073 Hz).

Abbreviations: PCC, posterior cingulate cortex; PACC R/MPFC R, perigenual anterior cingulate cortex right/medial prefrontal cortex right; SACC R, supragenual anterior cingulate cortex right; VLPFC L, ventrolateral prefrontal cortex left; Ins L, insula left; TPJ R/STG R, temporal parietal junction right/superior temporal gyrus right; CS, cluster size.

TABLE IV. SACC seed region ($x = -6$, $y = 19$, $z = 27$)

	SFB		Slow-5		Slow-4	
Anatomical region	*x*, *y*, *z*	CS (voxel)	*x*, *y*, *z*	CS (voxel)	*x*, *y*, *z*	CS (voxel)
PACC	−7, 47, −1	812	−7, 44, 2	762	17, 41, 14	317
ITG L			−49, −52, −13	139		
OFC L			−28, 56, −4	75		

Notes: Peak coordinates at corrected $P < 0.05$ for standard frequency band (SFB: 0.01–0.10 Hz), Slow-5 (0.01–0.027 Hz), and Slow-4 (0.027–0.073 Hz).
Abbreviations: SACC, supragenual anterior cingulate cortex; PACC, perigenual anterior cingulate cortex; ITG L, inferior temporal gyrus left; OFC L, orbitofrontal cortex left; CS, cluster size.

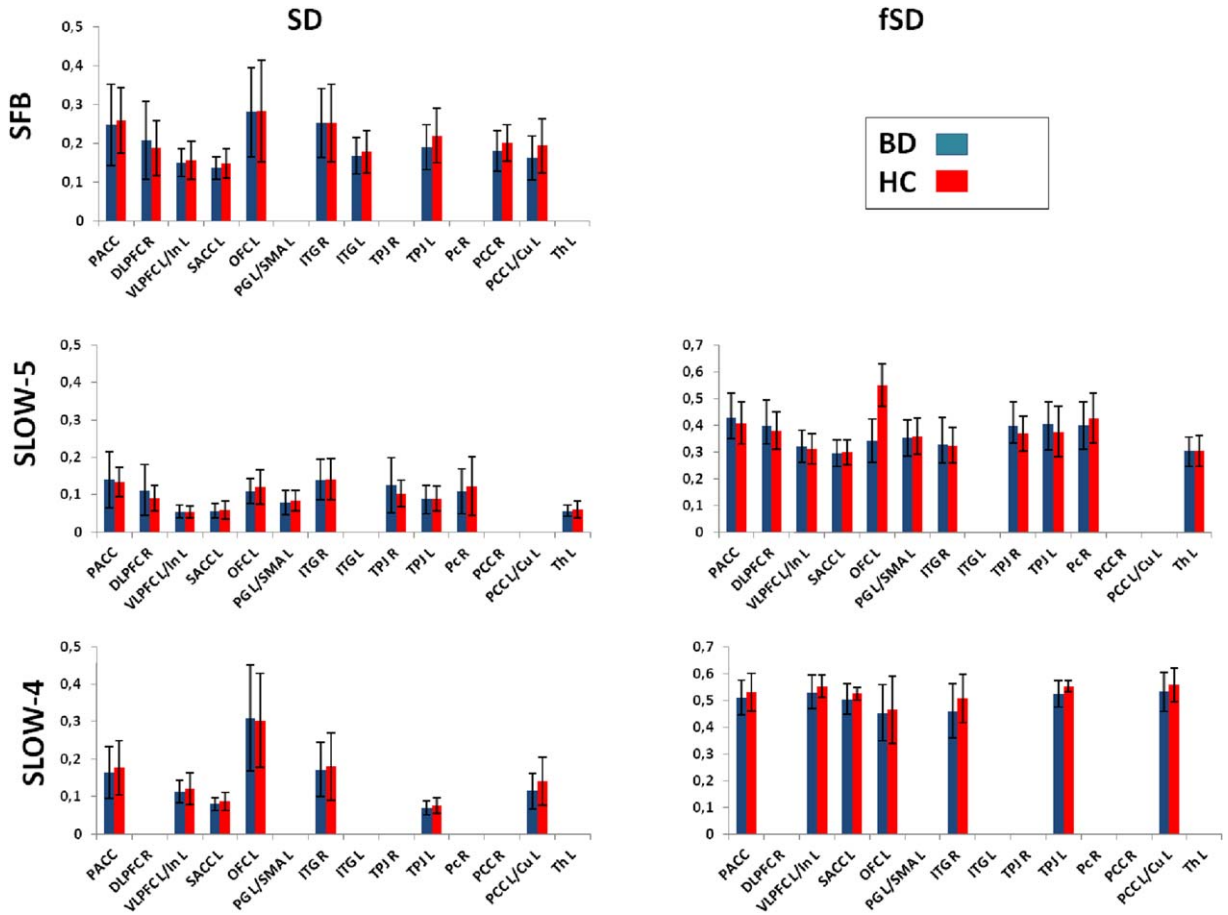

Figure 2.

SD and fSD in Healthy Controls (HC) versus BD patients. Abbreviations: PACC, perigenual anterior cingulate cortex; DLPFC R, dorsolateral prefrontal cortex right; VLPFC L/Ins L; ventrolateral prefrontal cortex left /insula left; SACC L, supragenual anterior cingulate cortex left; OFC L, Orbitofrontal Cortex Left; PG L/SMA L, precentral gyrus left/supplemental motor area left; ITG R, inferior temporal gyrus right; ITG L, inferior temporal gyrus left; TPJ R, temporal parietal junction right; TPJ L, temporal parietal junction left; Pc R, precuneus right; PCC R, posterior cingulate cortex right; PCC L/Cu L, Posterior cingulate cortex left/cuneus left; Th L, thalamus left; FSB, standard frequency band (0.01–0.10 Hz), Slow-5 (0.01–0.027 Hz), and Slow-4 (0.027–0.073 Hz).

TABLE V. FC and SD in HC

	SFB		Slow-5			Slow-4		
Anatomical region	FC (sd)	SD (sd)	FC (sd)	SD (sd)	fSD (sd)	FC (sd)	SD (sd)	fSD (sd)
PACC		0.259 (0.084)		0.134 (0.039)	0.408 (0.079)		0.177 (0.072)	0.529 (0.104)
DLPFC R	0.126 (0.142)	0.187 (0.071)	0.271 (0.282)	0.090 (0.034)	0.379 (0.070)			
VLPFC L/In L	0.152 (0.184)	0.156 (0.049)	0.169 (0.222)	0.054 (0.016)	0.312 (0.058)	0.167 (0.274)	0.121 (0.042)	0.552 (0.084)
SACC L	0.138 (0.143)	0.147 (0.038)	0.227 (0.261)	0.059 (0.024)	0.300 (0.047)	0.164 (0.167)	0.087 (0.024)	0.525 (0.508)
OFC L	0.248 (0.163)	0.283 (0.132)	0.417 (0.485)	0.121 (0.046)	0.355 (0.080)	0.197 (0.191)	0.303 (0.126)	0.465 (0.096)
PG L/SMA L			0.194 (0.296)	0.084 (0.028)	0.360 (0.068)			
ITG R	0.157 (0.159)	0.252 (0.101)	0.378 (0.305)	0.141 (0.056)	0.342 (0.066)	0.181 (0.216)	0.181 (0.090)	0.508 (0.115)
ITG L	0.261 (0.207)	0.178 (0.055)						
TPJ R			0.338 (0.411)	0.103 (0.035)	0.368 (0.064)			
TPJ L	0.208 (0.149)	0.219 (0.070)	0.279 (0.271)	0.089 (0.034)	0.376 (0.095)	0.146 (0.204)	0.076 (0.021)	0.553 (0.059)
Pc R			0.365 (0.414)	0.123 (0.079)	0.426 (0.093)			
PCC R	0.356 (0.184)	0.200 (0.047)						
PCC L/Cu L	0.282 (0.207)	0.194 (0.070)				0.243 (0.249)	0.141 (0.064)	0.557 (0.091)
Th L			0.135 (0.241)	0.060 (0.023)	0.303 (0.058)			

Notes: Mean values of functional connectivity, standard deviation, and fractional standard deviation in standard frequency band (SFB: 0.01–0.10 Hz), Slow-5 (0.01–0.027 Hz) and Slow-4 (0.027–0.073 Hz) in Healthy Controls (HC)
Abbreviations: PACC, Perigenual Anterior Cingulate Cortex; DLPFC R, Dorsolateral Prefrontal Cortex Right; VLPFC L/Ins L; Ventrolateral Prefrontal Cortex Left /Insula Left; SACC L, Supragenual Anterior Cingulate Cortex Left; OFC L, Orbitofrontal Cortex Left; PG L/SMA L, Precentral Gyrus Left/Supplemental Motor Area Left; ITG R, Inferior Temporal Gyrus Right; ITG L, Inferior Temporal Gyrus Left; TPJ R, Temporal Parietal Junction Right; TPJ L, Temporal Parietal Junction Left; Pc R, Precuneus Right; PCC R, Posterior Cingulate Cortex Right; PCC L/Cu L, Posterior Cingulate Cortex Left/Cuneus Left; Th L, Thalamus Left; CS, Cluster Size; sd (standard deviation)

PACC-TPJ L FC ($r = 0.400$; $P < 0.000$). CPT total hits showed a direct correlation with PACC-OFC L FC ($r = 0.381$; $P < 0.000$) and with PACC-TPJ L FC ($r = 0.340$; $P = 0.002$). CPT total omission errors showed an inverse correlation with PACC-OFC L FC ($r = -0.411$; $P < 0.000$) and with PACC-TPJ L FC ($r = -0.350$; $P = 0.001$). No

TABLE VI. FC and SD in BD

	SFB		Slow-5			Slow-4		
Anatomical region	FC (sd)	SD (sd)	FC (sd)	SD (sd)	fSD (sd)	FC (sd)	SD (sd)	fSD (sd)
PACC		0.248 (0.105)		0.140 (0.075)	0.427 (0.093)		0.164 (0.068)	0.509 (0.064)
DLPFC R	0.044 (0.119)	0.208 (0.100)	0.105 (0.263)	0.112 (0.068)	0.399 (0.095)			
VLPFC L/In L	0.051 (0.124)	0.150 (0.035)	0.047 (0.181)	0.054 (0.017)	0.320 (0.060)	0.032 (0.129)	0.114 (0.030)	0.530 (0.063)
SACC L	0.054 (0.082)	0.136 (0.029)	0.048 (0.120)	0.057 (0.020)	0.294 (0.053)	0.064 (0.108)	0.080 (0.018)	0.505 (0.055)
OFC L	0.116 (0.147)	0.280 (0.115)	0.211 (0.286)	0.110 (0.033)	0.343 (0.080)	0.061 (0.151)	0.310 (0.141)	0.453 (0.104)
PG L/SMA L			0.065 (0.193)	0.079 (0.033)	0.353 (0.068)			
ITG R	0.091 (0.129)	0.252 (0.089)	0.190 (0.342)	0.139 (0.054)	0.326 (0.104)	0.102 (0.153)	0.172 (0.072)	0.460 (0.100)
ITG L	0.139 (0.152)	0.167 (0.047)						
TPJ R			0.147 (0.216)	0.125 (0.074)	0.398 (0.089)			
TPJ L	0.086 (0.111)	0.190 (0.058)	0.128 (0.193)	0.088 (0.038)	0.403 (0.084)	0.036 (0.109)	0.069 (0.018)	0.523 (0.050)
Pc R			0.179 (0.271)	0.108 (0.060)	0.402 (0.086)			
PCC R	0.214 (0.181)	0.180 (0.053)						
PCC L/Cu L	0.160 (0.123)	0.162 (0.057)				0.112 (0.120)	0.115 (0.047)	0.531 (0.073)
Th L			0.020 (0.158)	0.057 (0.015)	0.304 (0.053)			

Notes: Mean values of functional connectivity, standard deviation, and fractional standard deviation in standard frequency band (SFB: 0.01–0.10 Hz), Slow-5 (0.01–0.027 Hz), and Slow-4 (0.027–0.073 Hz) in bipolar disorder patients.
Abbreviations: PACC, perigenual anterior cingulate cortex; DLPFC R, dorsolateral prefrontal cortex right; VLPFC L/Ins L; ventrolateral prefrontal cortex left /insula left; SACC L, supragenual anterior cingulate cortex left; OFC L, orbitofrontal cortex left; PG L/SMA L, precentral gyrus left/supplemental motor area left; ITG R, inferior temporal gyrus right; ITG L, inferior temporal gyrus left; TPJ R, temporal parietal junction right; TPJ L, temporal parietal junction left; Pc R, precuneus right; PCC R, posterior cingulate cortex right; PCC L/Cu L, posterior cingulate cortex left/cuneus left; Th L, thalamus left; CS, cluster size; SD, standard deviation.

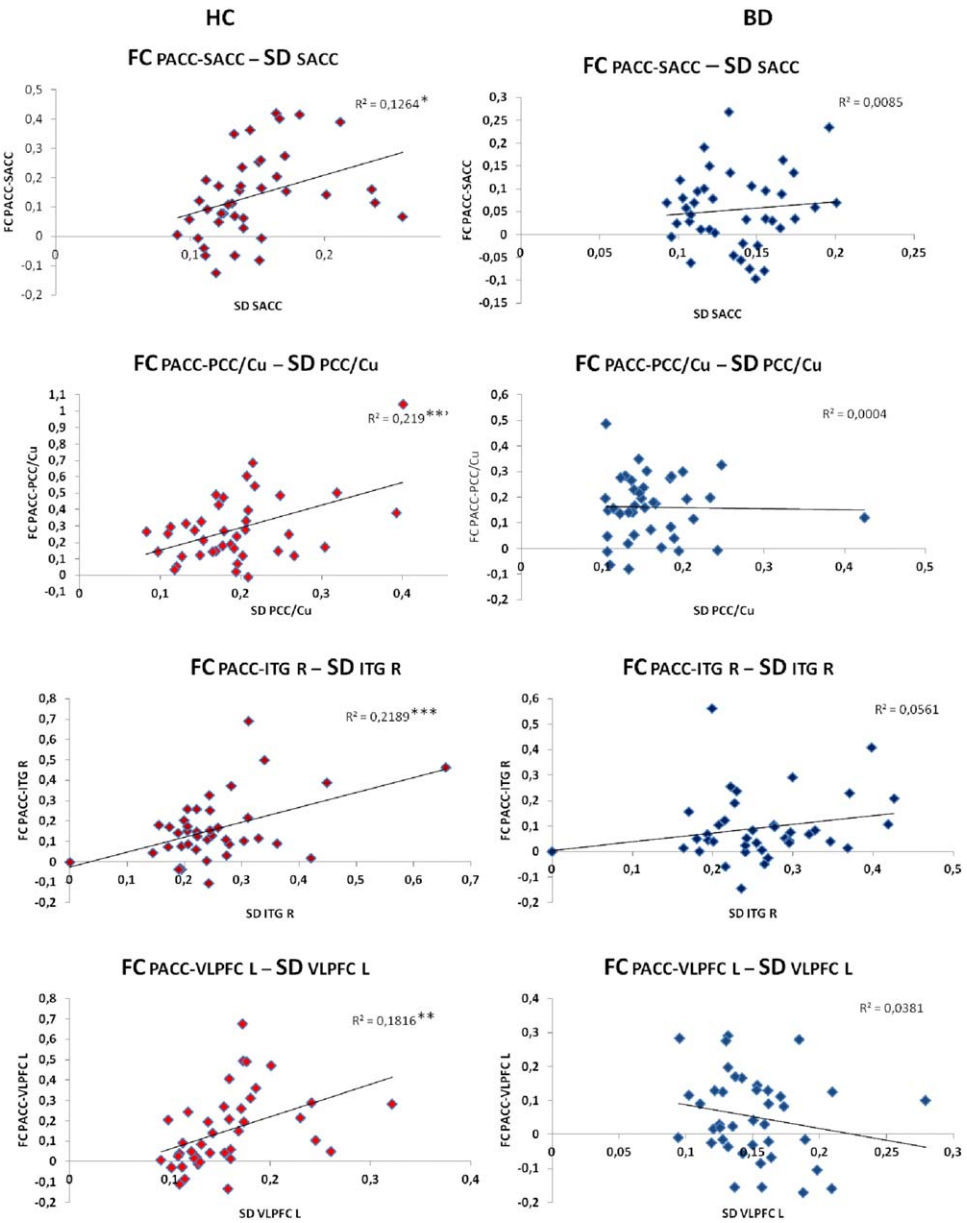

Figure 3.

Pearson correlations between FC and SD in standard frequency band (SFB 0.01–0.10 Hz) in healthy controls (HC) and BD patients. Abbreviations: SACC, supragenual anterior cingulate cortex; ITG R, inferior temporal gyrus right; VLPFC L, ventrolateral prefrontal cortex left; PCC/Cu, posterior cingulate cortex/cuneus.

additional correlations between the altered FC values and clinical parameters were found.

Finally, we carried out explorative subgroup comparisons of FC in the standard frequency band, by taking the PACC as the seed region (with age as a covariate). In manic patients, when compared to controls, we found decreased FC between the PACC and PCC (peak coordinates: $x = -1$, $y = -40$, $z = 17$) and between the PACC and OFC L (peak coordinates: $x = -22$, $y = 20$, $z = 19$). By contrast, in depressed patients, when compared to controls, we found decreased FC between the PACC and SACC (peak coordinates: $x = 11$, $y = 11$, $z = 44$). No additional differences were found in the other subgroup comparisons. Interestingly, these FC findings on mania and depression confirmed clinical correlations with HAMD and YMRS.

TABLE VII. FC and SD/fSD correlations in HC

	SFB		Slow-5				Slow-4			
	SD		SD		fSD		SD		fSD	
Anatomical Region (FC: SR-ROI)	SR (*r*)	ROI (*r*)	SR (*r*)	ROI (*r*)	SR (*r*)	ROI (*r*)	SR (*r*)	ROI (*r*)	SR (*r*)	ROI (*r*)
PACC—DLPFC R	−0.117	−.044	0.045	−0.123	0.119	0.072				
PACC—VLPFC L/In L	0.325	**0.426**[b]	**0.570**[c]	**0.381**[a]	0.335	0.132	0.344	**0.355**[a]	**0.479**[c]	**0.394**[a]
PACC—SACC L	0.246	**0.356**[a]	0.151	0.352	0.169	0.304	0.244	0.333	0.204	**0.358**[a]
PACC—OFC L	0.166	−.172	0.275	**0.454**[c]	0.085	**0.529**[c]	**0.488**[c]	0.275	**0.643**[c]	**0.640**[c]
PACC—PG L/SMA L			0.335	0.292	**0.407**[b]	0.232				
PACC—ITG R	**0.357**[a]	**0.468**[c]	0.348	0.314	0.197	0.206	0.067	0.032	0.349	0.323
PACC—ITG L	0.039	0.048								
PACC—TPJ R			0.277	0.184	0.023	0.188				
PACC—TPJ L	0.082	0.274	0.155	0.134	0.079	0.132	0.036	0.292	0.233	0.318
PACC—Pc R			0.261	0.289	0.093	0.352				
PACC—PCC R	−0.136	0.250								
PACC—PCC L/Cu L	0.050	**0.468**[c]					0.133	0.261	**0.308**[a]	0.221
PACC—Th L			0.213	0.004	0.345	0.052				

Notes: Correlation values (*r*) of functional connectivity and standard deviation or fractional standard deviation in standard frequency band (SFB: 0.01–0.10 Hz), Slow-5 (0.01–0.027 Hz), and Slow-4 (0.027–0.073 Hz) in HC. The bold numbers represent significant correlations.
[a]$P \leq 0.025$
[b]$P \leq 0.01$
[c]$P \leq 0.005$.
Abbreviations: PACC, perigenual anterior cingulate cortex; DLPFC R, dorsolateral prefrontal cortex right; VLPFC L/Ins L; ventrolateral prefrontal cortex left /insula left; SACC L, supragenual anterior cingulate cortex left; OFC L, orbitofrontal cortex left; PG L/SMA L, precentral gyrus left/supplemental motor area left; ITG R, inferior temporal gyrus right; ITG L, inferior temporal gyrus left; TPJ R, temporal parietal junction right; TPJ L, temporal parietal junction left; Pc R, precuneus right; PCC R, posterior cingulate cortex right; PCC L/Cu L, posterior cingulate cortex left/cuneus left; Th L, thalamus left; CS, cluster size; SR, seed region; ROI, region of interest.

DISCUSSION

Main Findings

Our main findings on the different measures of resting state activity in BD are the following. First, we found decreased FC (especially in Slow-5) from the PACC to other regions located predominantly in the posterior DMN, such as the PCC and ITG, and in the SN, such as the SACC and VLPFC. Second, we found evidence of decoupling between PACC-based FC and variability in the various target regions (without alteration in variability itself).

Functional Connectivity and Variability in Anterior Cortical Midline Resting State in BD

Our findings confirm a general resting state functional dysconnectivity of the medial PFC, that is, PACC, in BD, which is one of the most frequent findings in the literature on the resting state in affective disorders [Vargas et al., 2013]. Extending the current literature, our study also investigated the specificity of PACC hypoconnectivity using the SACC and PCC as control regions and confirmed a central role of the PACC in BD. Furthermore, our findings extend the focus of study to specific frequency bands, and suggest a greater deficit in a lower frequency band, Slow-5, when compared to the next higher one, Slow-4. If confirmed in future studies, this finding highlights the central role of the lowest infraslow frequency fluctuations, which are especially marked in anterior cortical midline structures and may therefore be crucial to predisposing consciousness [Xue et al., 2014]. Interestingly, some of the altered PACC FC values correlated with HAMD, YMRS and neurocognitive parameters, also suggesting a role of functional dysconnectivity in neurocognitive deficits in BD.

Finally, our group demonstrated the relationship between FC and variability in healthy subjects. Both FC and SD are measured with fMRI and can therefore be traced back to the BOLD effects whose exact neuronal mechanism remains unclear. The physiological mechanisms underlying the relationship between FC and SD remain unclear. FC is supposed to reflect synchronization between the neuronal activities in different regions [Fingelkurts et al., 2004] while the neuronal substrate of SD remains unclear. While FC has been related to especially Glutamate (but also GABA) [Duncan et al., 2014], this remains to be reported for SD. One can only speculate that higher degrees of variability in neuronal activity

TABLE VIII. FC and SD/fSD correlations in BD

	SFB		Slow-5				Slow-4			
	SD		SD		fSD		SD		fSD	
Anatomical region (FC: SR-ROI)	SR (*r*)	ROI (*r*)	SR (*r*)	ROI (*r*)	SR (*r*)	ROI (*r*)	SR (*r*)	ROI (*r*)	SR (*r*)	ROI (*r*)
PACC—DLPFC R	0.018	0.125	0.034	−0.135	0.105	−0.121				
PACC—VLPFC L/In L	0.080	−0.195	−0.154	−0.097	−0.039	−0.094	−0.025	−0.258	−0.085	−0.273
PACC—SACC L	−0.181	0.092	−0.012	−0.092	−0.008	−0.009	0.085	0.312	−0.178	0.266
PACC—OFC L	−0.155	−0.220	0.148	−0.215	0.279	−0.149	−0.034	−0.221	0.268	0.007
PACC—PG L/SMA L			−0.083	−0.008	0.042	0.223				
PACC—ITG R	0.215	0.237	0.123	0.026	0.140	0.076	0.277	−0.063	0.284	0.068
PACC—ITG L	−0.157	−0.017								
PACC—TPJ R			0.107	−0.024	0.276	0.154				
PACC—TPJ L	0.128	0.243	0.126	−0.104	**0.409**[b]	0.016	−0.047	0.117	0.011	−0.038
PACC—Pc R			−0.212	−0.117	0.069	−0.047				
PACC—PCC R	−0.301	0.117								
PACC—PCC L/Cu L	−0.166	−0.020					−0.050	−0.076	**0.372**[a]	0.162
PACC—Th L			−0.228	−0.112	−0.013	0.031				

Notes: Correlation values (*r*) of functional connectivity and standard deviation or fractional standard deviation in standard frequency band (SFB: 0.01–0.10 Hz), Slow-5 (0.01–0.027 Hz), and Slow-4 (0.027–0.073 Hz) in bipolar disorder patients. The bold numbers represent significant correlations.

[a] $P \leq 0.025$

[b] $P \leq 0.01$

$P \leq 0.005$.

Abbreviations: PACC, perigenual anterior cingulate cortex; DLPFC R, dorsolateral prefrontal cortex right; VLPFC L/Ins L; ventrolateral prefrontal cortex left /insula left; SACC L, supragenual anterior cingulate cortex left; OFC L, orbitofrontal cortex left; PG L/SMA L, precentral gyrus left/supplemental motor area left; ITG R, inferior temporal gyrus right; ITG L, inferior temporal gyrus left; TPJ R, temporal parietal junction right; TPJ L, temporal parietal junction left; Pc R, precuneus right; PCC R, posterior cingulate cortex right; PCC L/Cu L, posterior cingulate cortex left/cuneus left; Th L, thalamus left; CS, cluster size; SR, seed region; ROI, region of interest.

make more likely the synchronization between the neuronal activities of the respective regions. That remains tentative at best at this point, however. In our study, the correlation between FC and variability seems to be lost in BD, though without alteration, for example, reduction, in variability itself. The asymmetry in the correlation between seed and target regions (i.e., FC between the seed and target region correlates with variability in the target region but not in the seed region) suggests that FC from seed to target regions impacts variability in the respective target regions in healthy subjects; this has been linked to transfer of information from seed-target FC to SD in the target region [Maki-Marttunen et al., 2013]. We observed reduction in FC in bipolar patients and decoupling of variability in target regions from FC, whereas we did not obtain a reduction in variability itself. In other words, FC from seed to target region seems to be no longer transformed and transmitted into variability of neural activity in the target regions [Maki-Marttunen et al., 2013]. This suggests that the communication between regions no longer works properly in BD: the PACC may affect and reduce information transfer to other regions inside (e.g., SACC and PCC) and outside (e.g., VLPFC and ITG) the midline structures (and their respective networks, such as the DMN and SN). This is a tentative hypothesis and awaits future support from specific investigations on entropy, for example, transmission entropy in BD [Maki-Marttunen et al., 2013].

Resting state abnormalities in BD and other psychiatric disorders—Hypothesis on spatiotemporal alterations of resting state and psychopathology of BD

Alterations in cortical midline structures and in the modulation of the resting state networks balance were found to be affected in several psychiatric disorders.

In schizophrenia, various anterior and posterior midline regions of the DMN, including PACC and PCC, showed hypoactivity [Kuhn and Gallinat, 2013]. In schizophrenia these alterations were associated with the reduction of the anticorrelation between DMN and CEN and its replacement by positive correlation, hypothetically resulting in confusion between internal and external contents that can manifest in psychotic symptoms [Northoff, 2014a, b].

In major depressive disorder (MDD), the anterior midline regions, including PACC, showed hyperactivity, while the posterior midline regions, such as PCC, showed hypoactivity [Kuhn and Gallinat, 2013]. In MDD these alterations were associated with an abnormal shifting of the

anticorrelation between DMN and CEN toward the DMN, hypothetically resulting in excessive focusing on internal contents at the expense of external contents that can manifest in increased mind wandering and depressive ruminations [Northoff, 2014b].

In our sample of BD, we found connectivity alterations within the same midline regions as in schizophrenia and MDD, but they are characterized by a different pattern. Furthermore, in the subgroups analysis we observed different FC abnormalities: PACC showed reduced connectivity with SACC in depressed patients and with PCC in manic patients. Since our subsamples size was relatively small, these results should be considered preliminary and our considerations are thus tentative.

We speculate that hypoconnectivity between PACC and SACC could be associated with a deficit in the anterior DMN-SN connectivity and consequent abnormal shifting toward the DMN at the expense of CEN, hypothetically resulting in excessive focusing on internal contents at the expense of external contents that can manifest in increased mind wandering and depressive ruminations. Moreover, the same alterations could be associated with a reduced salience attribution to external stimuli, resulting in volitive inhibition and consequently in a reduced transition from idea to action, which represent the core dimension of depression in the kraepelinian model of BD [Kraepelin, 1902]. These alterations could be similar to those observed in MDD.

By contrast, we speculate that hypoconnectivity between PACC and PCC could be associated with a deficit in the posterior DMN and consequent abnormal shifting toward the CEN, hypothetically resulting in excessive focusing on external contents at the expense of internal contents that can manifest in distractibility and flight of ideas. Moreover, these networks unbalances could favor the transition from idea to action, which represent the core dimension of mania in the kraepelinian model of BD [Kraepelin, 1902]. Interestingly, the same decreased connectivity between the anterior and posterior regions of the DMN has been demonstrated in ADHD, which is also characterized by distractibility and hyperactivity, some of the typical symptoms of mania [Uddin et al., 2009]. Finally, we speculate that if the anticorrelation between DMN and CEN shifted toward the CEN (which hypothetically characterized mania) decreases and it is replaced by positive correlation, psychotic symptoms will emerge and psychotic mania will partially overlap with some feature of schizophrenia.

According to the hypothesis that psychiatric disorders represent spatiotemporal disorders of brain's resting state activity, we assume that specific spatial and temporal alterations in the structure of resting state produce basic disturbance which lead to BD psychopathology. The alterations in the temporal structure of resting state activity that we observed in our sample, that is, a greater deficit in the lowest frequency range of PACC-related FC, could indicate alterations specific for midline structures. Conversely, the alterations in the spatial structure of resting state activity that we observed in our sample could be associated to unbalances between the different resting state networks, leading to disturbances of transition from idea to action, as a core psychopathological dimension of mania and depression.

Limitations

The main limitation of the present study concerns medications. Indeed, almost all the bipolar subjects in our sample were taking medications, including mood stabilizers, antipsychotics, antidepressants, and benzodiazepines, which could interfere with the BOLD signal. To assess this issue, we examined the potential impact of the psychotropic medication load—that is, the number and dosage of different medications—on FC and SD/fSD in BD [Phillips et al., 2008]. The medication load did not correlate with FC and SD/fSD. Moreover, no correlations emerged between FC and SD/fSD using a partial correlation between these factors with the pharmacological load as a covariate. These results suggested that resting state parameters were not greatly affected by pharmacotherapy and that the loss of correlation between FC and variability in BD did not depend on pharmacotherapy.

Furthermore, the duration of illness could be a confounding factor. Our sample was made up of patients at different stages of the disease. To assess the potential role of the duration of illness in our findings, we correlated FC and SD/fSD with the duration of illness. No significant correlations were observed (all $P > 0.05$), suggesting the absence of major effects of this clinical factor on these resting state parameters.

Finally, the heterogeneity of the sample could constitute another confounding factor, as the different clinical phases of illness—that is, manic, depressive, mixed, and euthymic—might differently affect FC and variability. This also makes it difficult if not impossible to distinguish between trait and state markers of the disease. This leaves open whether PACC functional hypoconnectivity and decoupling from variability of the anterior midline regions could represent a trait marker or a state marker. The number of subjects within the different subgroups of our sample that included depressed, manic and euthymic BD patients allowed us to perform subgroup comparisons only as explorative analysis. Future investigation involving larger subsamples of BD patients in different phases including manic, depressed and euthymic will be needed to confirm our preliminary findings and to properly distinguish between trait- and state-specific resting state markers.

Conclusions

Taken together, our findings suggest that deficits in the PACC, as a part of anterior midline regions of the DMN, could induce reduced information transfer from this

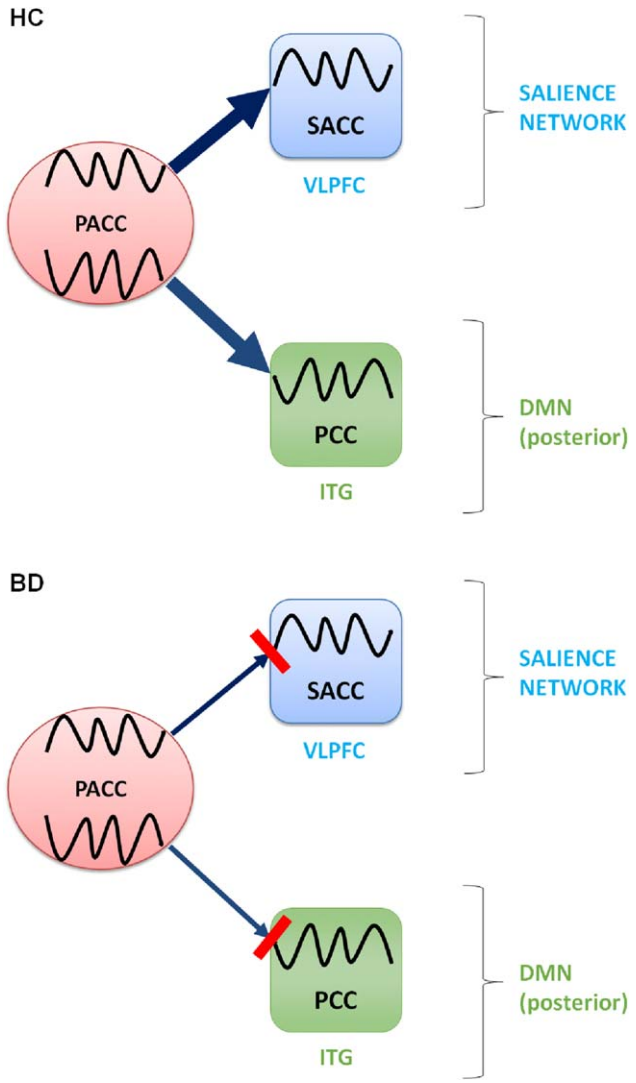

Figure 4.
Scheme of main findings FC deficit in PACC connections and FC—SD decoupling in healthy controls (HC) and BD patients. The arrows represent FC from seed (PACC) to target regions (SACC and VLPFC from SN and PCC and ITG from DMN): FC is reduced in BD (thinner arrows). The waves represent variability: this is normal also in BD. The red bars represent the decoupling between FC and variability in BD. Abbreviations: PACC, perigenual anterior cingulate cortex; VLPFC L, ventrolateral prefrontal cortex left; SACC, supragenual anterior cingulate cortex, ITG, inferior temporal gyrus; PCC, posterior cingulate cortex; DMN, default mode network

region to the other midline regions, such as the PCC and SACC (Fig. 4). This results in dysmodulation, which is sustained by reduced FC and decoupling between SD and FC in those target regions, and which ultimately causes imbalance between the different portions within the midline regions/cingulate gyrus, for example, between the PACC and PCC/SACC.

On shifting from the regional to the network level, this imbalance between the different portions of the cingulate gyrus could alter the balance between the respective networks, for example, DMN and SN that are associated with for instance PACC/PCC (DMN) and SACC (SN). The imbalance between the anterior DMN, posterior DMN and SN could, in turn, induce abnormal changes in those functions associated with these networks, for example, emotions [Holtzheimer and Mayberg, 2011], internal thoughts or mind wandering [Christoff et al., 2009; Mason et al., 2007], and reward-based impulsive behavior [Mendelsohn et al., 2014]. In particular, a deficit in the anterior DMN-SN connectivity could lead to an abnormal shifting toward the DMN, while a deficit in the anterior DMN-posterior DMN connectivity could lead to an abnormal shifting toward the SN, resulting in excessive focusing on internal contents and reduced transition from idea to action or in excessive focusing on external contents and increased transition from idea to action, respectively, which could represent central dimensions of depression and mania. Though as yet speculative, this mechanism may well account for Kraepelin's original description of core alterations in mood, ideation and volition in BD [Akiskal, 1996]. Furthermore, if these results are confirmed in future studies on larger subsample which include bipolar patients in the different phase of illness—that is, in manic, depressive and euthymic phases—they could represent diagnostic markers in BD.

In conclusion, this study is the first to report specific PACC-based deficits in midline cortical FC, especially in the lowest of the slow frequency bands, in BD. Moreover, it also demonstrates that FC is decoupled from neuronal variability in the target regions in BD. Most importantly, as this study used novel measures such as variability, our findings contribute to improving characterization of the resting state in BD, a step which may pave the way to its use as a biomarker in clinical diagnosis and therapy.

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AUTHOR CONTRIBUTIONS

Paola Magioncalda, Matteo Martino, Mario Amore, and Georg Northoff conceived the study; Paola Magioncalda, Matteo Martino, Benedetta Conio, Andrea Escelsior, Andrea Presta, Valentina Marozzi, Rocchi Giulio, Loris Anastasio, Linda Vassallo recruited all the subjects and managed the clinical assessment; Niccolò Piaggio, Luca

Roccatagliata and Matteo Pardini managed the neuroimaging acquisitions; Paola Magioncalda, Matteo Martino, Francesca Ferri, and Zirui Huang performer the data and the statistical analysis; Paola Magioncalda and Matteo Martino wrote the manuscript; Mario Amore and Georg Northoff supervised the study.

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