

## Original Article

## Functional disconnection in the prefrontal–amygdala circuitry in unaffected siblings of patients with bipolar I disorder

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**Objectives:** Bipolar I disorder (BD) is a highly heritable disorder characterized by mood swings between high-energy and low-energy states. Amygdala hyperactivity and cortical inhibitory hypoactivity [e.g., of the dorsolateral prefrontal cortex (dlPFC)] have been found in patients with BD, as evidenced by their abnormal resting-state functional connectivity (FC) and glucose utilization (GU). However, it has not been determined whether functional abnormalities of the dlPFC–amygdala circuit exist in unaffected, healthy siblings of the patients with BD (BDSib).

**Methods:** Twenty euthymic patients with BD, 20 unaffected matching BDSib of the patient group, and 20 well-matched healthy control subjects were recruited. We investigated seed-based FC (seeds: dlPFC) with resting-state functional magnetic resonance imaging and GU in the regions of interest (e.g., dlPFC and amygdala) using <sup>18</sup>F-fluorodeoxyglucose positron emission tomography.

**Results:** The FC in the dlPFC (right)–amygdala circuit was statistically abnormal in patients with BD and BDSib, but only the patients with BD demonstrated hypoactive GU bilaterally in the dlPFC and hyperactive GU bilaterally in the amygdala. Facilitating differentiation between the BD groups, the altered FC between dlPFC (right) and amygdala (left) was even more prominent in the patients with BD ( $p < 0.05$ ).

**Conclusions:** There was a dysfunctional connection with intact GU in the dlPFC–amygdala circuit of the BDSib, which highlights the vulnerability in families with BD. Diminished top-down control from the bilateral dlPFC, which prevents adequate inhibition of limbic hyperactivity, might mediate the development of BD.

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Bipolar I disorder (BD) is a highly heritable disorder characterized by mood swings between extremely high-energy (mania) and low-energy (depression) states. Such mood fluctuations could be an expression of the emotional dysregulation at the root of BD.

A dorsal system, including bilateral dorsal and lateral regions of the prefrontal cortex (PFC), is predominantly responsible for voluntary emotion regulation, and a ventral system, including the amygdala and ventral regions of the PFC, is involved in the identification of emotionally salient

stimuli and mediation of autonomic responses to stimuli (1). An imbalance between these two neural systems has been proposed to be an underlying mechanism that predisposes people to develop BD (2).

Reduction in the inhibitory function of the PFC [e.g., dorsolateral PFC (dlPFC)] and overactivity in the subcortical limbic regions (e.g., amygdala, parahippocampus, and hippocampus) have been repeatedly reported in functional neuroimaging studies of BD (3–9). Even in resting states, activities in these brain regions are still abnormal in patients with BD. For example, we recently reported that euthymic patients with BD had significantly lower glucose utilization (GU) in the PFC, and higher GU in the limbic structures, as measured by  $^{18}\text{F}$ -fluorodeoxyglucose positron emission tomography ( $^{18}\text{F}$ -FDG PET) (10). The study also identified that, compared to bipolar II disorder (BD-II), bipolar I disorder (BD-I) was associated with more impaired fronto-limbic circuitry (10).

Similarly, the resting-state functional abnormality in the fronto-limbic circuit was revealed by research using task-independent resting-state functional magnetic resonance imaging (fMRI) (rsfMRI). rsfMRI investigates intrinsic low-frequency fluctuations between connected brain structures that may represent a functional connectivity (FC) network of the brain in the resting state (11). The results from rsfMRI have demonstrated functional disconnectivity in the fronto-amygdala circuitry of euthymic patients with BD (5, 12), in spite of some variations in the findings. A recent study examined the connectivity between the amygdala and all PFC voxels in a large sample, and found that patients with BD-I exhibited disconnectivity between the amygdala and right-side dlPFC (5). The dlPFC region is also involved in an intrinsic connectivity network that is reliably identified by rsfMRI, namely an executive control network (ECN). The ECN has been shown to correlate well with cognitive functions measured outside the scanner (13).

Taken together, all of the relevant evidence supports that, during the resting state, functional abnormalities in the dlPFC–amygdala circuit could be an endophenotype of BD, and also the idea that neuroimaging tools should be used to elucidate biomarkers for diagnosing BD (14). We hypothesized that patients with BD and their unaffected, healthy siblings (BDsib) would exhibit similar functional abnormalities in the dlPFC–amygdala circuit as an endophenotype is heritable and might be found both in affected and unaffected family members.

Therefore, we compared resting-state functional abnormalities in the dlPFC–amygdala circuit among a group of euthymic patients with BD, a group of matched BDsib of the patient group with BD, and a group of well-matched healthy control subjects (HC). Brain imaging scans, including rsfMRI and  $^{18}\text{F}$ -FDG PET, were obtained at rest, while participants were medication free. The combination of the neuroimaging scans allowed us to disentangle the functional abnormalities of BD from different angles. The  $^{18}\text{F}$ -FDG PET scans explored brain regional functional abnormalities at rest, whereas the rsfMRI further examined intrinsic FC patterns between brain regions during the resting state. We also investigated whether impaired executive functioning could be an endophenotype for BD as it was observed in the unaffected relatives of patients with BD (15–17). The correlations of the neuroimaging abnormalities and the cognitive deficits were explored. The reasons why we selected only patients with BD-I were because sometimes it is hard to differentiate other subtypes of BD (e.g., BD-II) from major depression and because BD-I has a high heritability, with a heritability estimate of 0.93 (18).

## Methods and materials

### Participants

Sixty subjects were recruited for the present study, including 20 clinically stable patients with BD, 20 unaffected BDsib from the same families of the patients with BD, and 20 age-, gender-, and ethnicity-matched HC (Table 1). The diagnoses were established by a structured history taking and administration of the Mini-International Neuropsychiatric Interview (MINI) based on the Fourth Edition of the Diagnostic and Statistical Manual system (DSM-IV) criteria (American Psychiatric Association, 1994). Patients were recruited only if they had no history of major medical or neurological illness (e.g., epilepsy, head trauma, or stroke) and had no alcohol or substance abuse history in the previous year. As medication could be a confounding factor in the interpretation of behavioral and neuroimaging data, we recruited only those who had been medication free for at least one week. The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Review Committee of Taipei Veterans General Hospital (for additional details, please refer to the online *supplementary material*).

## Functional abnormalities in BD and siblings

Table 1. Demographic data and clinical variables of the three groups

Variables	BD	BDsib	HC	$F/\chi^2$	p-value	Post hoc (LSD)
Age, years, mean (SD)	41.6 (11.3)	40.6 (10.5)	41.8 (10.6)	0.265	0.768	–
Male/female, n	14/6	11/9	13/7	1.000	0.665	–
Educational levels, n (%)				10.281	0.113	–
7–9 years (junior high)	1 (5)	0 (0)	0 (0)			–
10–12 years (senior high)	10 (50)	5 (25)	3 (15)			–
13–16 years (college)	9 (45)	13 (65)	16 (80)			–
>16 years (graduate)	0 (0)	2 (10)	1 (5)			–
HDRS-17 score	4.7 (3.4)	0.7 (1.1)	1.0 (1.4)	19.020 <sup>a</sup>	<0.001	BD > BDsib BD > HC
YMRS score	3.6 (3.1)	0.2 (0.7)	0.2 (0.7)	30.746 <sup>a</sup>	<0.001	BD > BDsib BD > HC
Age at onset, years, mean (SD)	26.0 (12.1)	–	–	–	–	–
Illness duration, years, mean (SD)	16.1 (10.3)	–	–	–	–	–
Psychotic features, n (%)	12 (60)	–	–	–	–	–
No. of MDE, mean (SD)	4.4 (3.3)	–	–	–	–	–
No. of hypomanic/manic episodes, mean (SD)	6.1 (6.2)	–	–	–	–	–
No. of mixed episodes, mean (SD)	1.8 (3.6)	–	–	–	–	–
No. of psychiatric admissions, mean (SD)	2.8 (3.2)	–	–	–	–	–

BD = bipolar disorder; BDsib = unaffected, healthy siblings of patients with BD; HC = healthy control subjects; HDRS-17 = 17-item Hamilton Depression Rating Scale; LSD = least significant difference; MDE = major depressive episodes; SD = standard deviation; YMRS = Young Mania Rating Scale.

<sup>a</sup>Non-parametric Kruskal–Wallis chi-square test was used because the variable showed a lack of homogeneity of variances among groups.

### Imaging studies

*rsfMRI. Imaging acquisition parameters.* MR images were acquired using a 3.0 GE Discovery 750 whole-body high-speed imaging device. In brief, the resting-state functional images were collected using a gradient echo T2\*-weighted sequence [repetition time (TR)/echo time (TE)/flip = 2,500 msec/30 msec/90°], and high-resolution structural T1-weighted images were acquired in the sagittal plane using a high-resolution sequence (TR = 2,530 msec, echo spacing = 7.25 msec, TE = 3 msec, flip angle = 7°) with isotropic 1-mm voxels and field-of-view (FOV) = 256 × 256 mm (for additional details, please refer to the online *Supplementary material*).

*Analysis of resting state FC. FC pre-processing:* the motion-corrected functional scans underwent slice timing and motion correction and were registered to the Montreal Neurological Institute (MNI152) atlas using the Functional MRI of the Brain Software Library ([www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl)). The following additional preprocessing steps, which have been described in previous reports (19), were used to prepare the data for FC analysis: (i) spatial smoothing using a Gaussian kernel (6-mm full-width at half-maximum), (ii) temporal filtering (0.009 Hz < f < 0.08 Hz), and (iii) removal of spurious or nonspecific sources of variance by regression of the following variables: (i) the six movement parameters computed by rigid body translation and rotation in preprocessing, (ii) the

mean whole-brain signal, (iii) the mean signal within the lateral ventricles, and (iv) the mean signal within a deep white matter region of interest (ROI). The first temporal derivatives of these regressors were included in the linear model to account for the time-shifted versions of spurious variance. The regression of each of these signals was computed simultaneously, and the residual time course was retained for the correlation analysis.

*FC analysis:* the ECN of each participant was derived from the ROIs in the left and right dlPFC by conducting a seed-based FC analysis (20). In brief, we computed a seed-based dlPFC correlation map by extracting average time series across all voxels in the dlPFC of each subject, which were then correlated with all other brain voxels. Fisher's *r*-to-*z* transformation was used to convert the voxel-wise correlation maps into *z*-maps. The significant difference of the *z*-maps between the patients with BD and HC was evaluated using an independent *t*-test (*Supplementary Fig. 1*). Brain regions that passed a voxel-level uncorrected *p*-value < 0.001 were identified and then submitted for corrections of multiple comparisons. For the bilateral amygdala (i.e., our *a priori* regions), we used AlphaSim, with exact smoothness estimates computed from the general model residuals (*p* < 0.001, Monte Carlo simulations = 10,000 times, *kE* = 17 voxels for amygdala) to correct for type-I errors (5). The method is primarily based on

peak voxels and cluster extent thresholds (21). For other non-*a priori* regions, family-wise errors were adopted to correct for multiple comparisons. The between-group FC values (BD versus HC) in the regions that showed statistical significance were further extracted for all three groups (HC, BD, and BDsib), and one-way analysis of variance (ANOVA) tests were performed on the FC values across the three groups; this analysis was followed by *post hoc* least significant difference (LSD) analyses. The significance level for both was set at a  $p$ -value  $< 0.05$ .

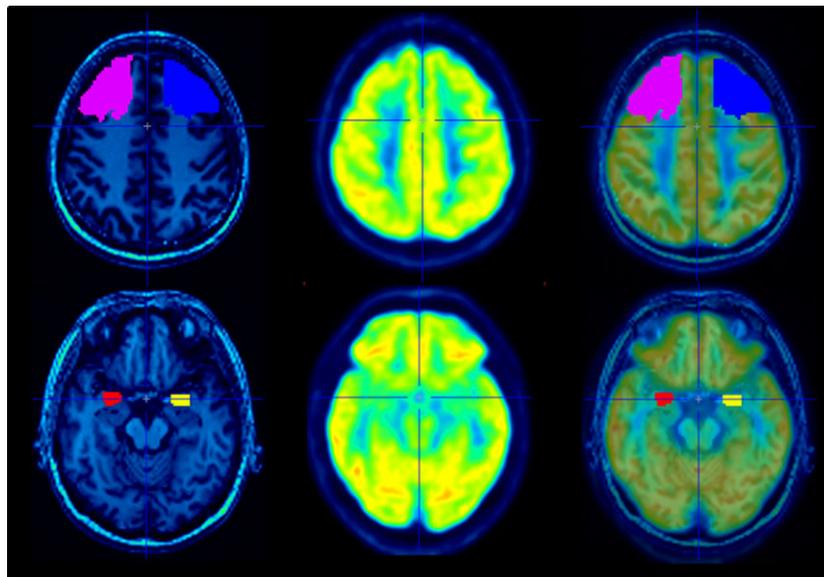
*Positron emission tomography (PET). PET data acquisition.*  $^{18}\text{F}$ -FDG PET scans of at-rest GU were acquired on a PET/computed tomography scanner (Discovery VCT, GE Healthcare, Milwaukee, WI, USA) using the three-dimensional brain mode. The patients fasted for at least eight hours before the PET examination. The PET images were acquired within 45 min after an intravenous injection of approximately 370 MBq of  $^{18}\text{F}$ -FDG. The brain acquisition time was 15 min. Most of the MRI scans and PET scans were done within four days, based on the availability of the machines. The detailed procedures were identical to those in our previously published paper (10).

*PET analysis.* The mean brain glucose uptake values in the ROIs (i.e., dlPFC and amygdala) were extracted from MRI-coregistered PET images in the standard stereotactic space (Fig. 1; for

details, please see *Supplementary Material*) using PMOD version 3.0 (PMOD Technologies, Zurich, Switzerland) as we have previously described (22). To prevent bias in the inter- or intra-rater reliability due to manual delineation, an automated anatomical labeling (AAL) template (23) was used to delineate these regions. We report the normalized glucose uptake values in the ROIs; the values were normalized by dividing the glucose uptake values by the global mean uptake values. One-way ANOVA tests were performed on the resting regional cerebral glucose metabolism (rCMglu) values to compare the three groups, and this analysis was followed by *post hoc* LSD analyses. Pearson's correlation tests were performed to study the correlations between the depression and mania scores and between the cognitive measurements and normalized GU in the ROIs. A  $p$ -value  $< 0.05$  was deemed to be statistically significant in the correlation and *post hoc* analyses.

#### Cognitive measurements

The Montreal Cognitive Assessment (MoCA) (24) and the Mini-Mental State Examination (MMSE) (25) were used to detect potential cognitive dysfunction in the patients with BD because cognitive deficits in patients with BD when they are in remission could be relatively mild and less prominent than at other times. Both demonstrated good agreement with executive function in the subjects



*Fig. 1.* Delineations of the regions of interest (ROIs) for positron emission tomography (PET) analysis in an individual. Left panel: individual magnetic resonance imaging (MRI) scans were normalized to the standard MRI-T1 template. Four ROIs were predefined, including the left dorsolateral prefrontal cortex (dlPFC) (blue), right dlPFC (pink), left amygdala (yellow), and right amygdala (red). Middle panel: individual PET images were normalized to the standard stereotactic space via coregistration of the anatomical MRI of the subject. Right panel: the glucose uptake in each ROI was extracted from the MRI-coregistered PET images.

with cognitive impairments (e.g., post-stroke) (26). The MoCA was reportedly more sensitive than the MMSE in detecting mild cognitive impairment (24). In addition, the Wisconsin Card Sorting Test (WCST) (27) was adopted to evaluate executive functions as deficits of such executive functions had been reported in euthymic subjects with BD (10) (see online *Supplementary materials* for additional details regarding the procedures).

Statistical analysis for other variables

For analysis of the demographic data and clinical variables, SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA) was used. One-way ANOVAs (or Student's *t*-test) and chi-square tests were used to compare the continuous and categorical variables among the groups, respectively. A *p*-value < 0.05 was considered statistically significant. Homogeneity of variance was tested using Levene's test. When this test was significant (*p* < 0.05), the non-parametric Kruskal–Wallis test was applied to variables that failed to pass the homogeneity assumption of ANOVA.

Results

Demographic data and clinical variables

There were no differences in the age, gender, or educational levels among the three groups (Table 1). The subjects with BD were mostly in symptomatic remission but had statistically more severe mood symptoms [i.e., Young Mania Rating Scale (YMRS) and 17-item Hamilton Depression Rating Scale (HDRS)-17 scores] (Table 1). The BD groups' cognitive performance was significantly worse than that of the BDsib and HC on the MoCA and MMSE, but there was no statistically significant difference in the WCST performance of the three groups (Table 2).

FC in the dlPFC–amygdala circuit and clinical correlates

We conducted an anatomically defined dlPFC seed-based analysis to examine the FC between the bilateral dlPFC and all other voxels in the brain (i.e., the ECN). The patients with BD had significantly higher connectivity between the right dlPFC and right amygdala (*x* = 18, *y* = 0, *z* = –18; cluster size = 30 voxels) and between the right dlPFC and left amygdala (*x* = –22, *y* = 0, *z* = –16; cluster size = 54 voxels) (Fig. 2A). Both findings were corrected for multiple comparisons. There were also some findings in non-*a priori* regions that passed a voxel-level uncorrected *p*-value < 0.001 (*Supplementary Table 1*). However, none of them survived after correcting for multiple comparisons. We then compared the right dlPFC–amygdala connectivity between the three groups and found that the BDsib FC was similar to that of patients with BD; that is, the right dlPFC–bilateral amygdala connectivity was statistically abnormal in the patients with BD and the BDsib alike (Fig. 2B). As compared to the BDsib, the altered FC between the right dlPFC and left amygdala was even more prominent in the patients with BD (*p* < 0.05) (Fig. 2B). If the seed-based whole-brain analysis was carried out among three groups by using ANOVA, a statistical significance could also be found in the right amygdala (*F* = 18.15, cluster size = 35 voxels, *p* < 0.05 after Monte Carlo correction).

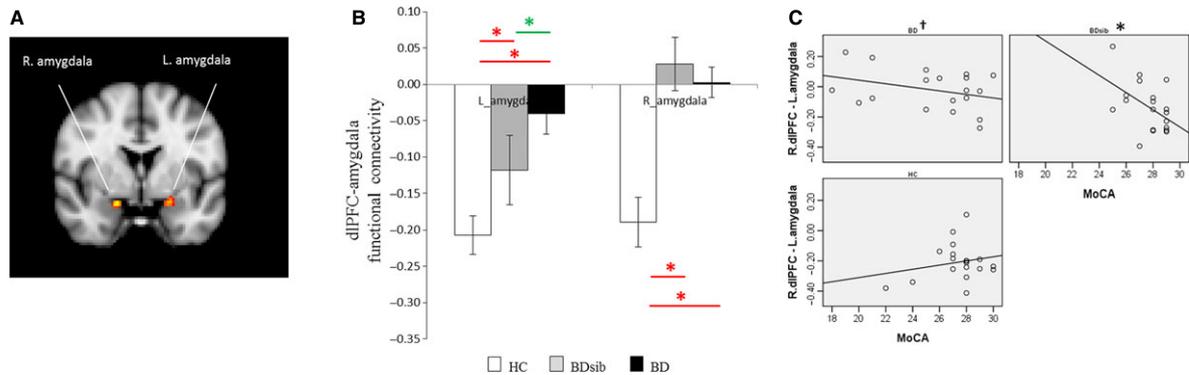
The right dlPFC–left amygdala connectivity was negatively correlated with MoCA in the BDsib group (*r* = –0.499, *p* = 0.035) and, to a lesser degree, in the BD group (*r* = –0.487, *p* = 0.066), whereas a contrasting positive correlation was found in the HC group (*r* = 0.348) (Fig. 2C). The right dlPFC–left amygdala connectivity was also correlated with high manic severity, as measured by the YMRS scores (*r* = 0.274, *p* = 0.045) (*Supplementary Fig. 2*). If the correlation analyses were

Table 2. Cognitive performance of the three groups

	BD	BDsib	HC	<i>F</i> -value	<i>p</i> -value	<i>Post hoc</i> (LSD)
<b>MoCA</b>	25.4 (3.6)	27.7 (1.3)	27.6 (1.9)	5.325	0.008	BD < BDsib, BD < HC
<b>MMSE</b>	27.6 (2.0)	29.2 (1.6)	29.3 (0.9)	6.915	0.002	BD < BDsib, BD < HC
<b>Executive function (WCST)</b>						
Percent errors	27.1 (13.1)	19.8 (8.0)	23.6 (13.7)	1.947	0.152	– <sup>a</sup>
Percent conceptual level responses	66.6 (16.9)	76.5 (11.3)	70.4 (19.0)	1.912	0.157	– <sup>a</sup>
Categories completed	5.3 (1.6)	5.8 (1.1)	5.3 (1.5)	0.824	0.444	– <sup>a</sup>

Values are indicated as mean (standard deviation). BD = bipolar disorder; BDsib = unaffected, healthy siblings of patients with BD; HC = healthy control subjects; LSD = least significant difference; MMSE = Mini-Mental State Examination; MoCA = Montreal Cognitive Assessment; WCST = Wisconsin Card Sorting Test.

<sup>a</sup>Analysis of variance showed no differences among the three groups, but independent *t*-tests demonstrated that subjects with BD performed worse than BDsib on different measures of the WCST.



**Fig. 2.** Altered prefrontal–amygdala connectivity in bipolar disorder (BD) and in unaffected, healthy siblings of patients with BD (BDsib). **(A)** Voxel-wise magnetic resonance imaging analysis of seed-based functional connectivity in the executive control network [i.e., bilateral dorsolateral prefrontal cortex (dIPFC) as seeds] showed significant group differences in the right dIPFC and bilateral amygdala between the patients with BD and the healthy control subjects (HC). **(B)** Direct comparison of right dIPFC–amygdala connectivity among the three groups additionally revealed a similar pattern of altered dIPFC–amygdala functional connectivity in the patients with BD and the BDsib, which was different from the HC (marked in red). The subjects with BD demonstrated significantly more altered functional connectivity in the left amygdala than did the BDsib (marked in green). The asterisks (\*) represent a statistically significant difference in the *post hoc* least significant difference analysis of variance ( $p < 0.05$ ). **(C)** The altered functional connectivity between the right dIPFC and left amygdala was negatively correlated with Montreal Cognitive Assessment (MoCA) scores in the patients with BD ( $r = -0.437$ ) and in the BDsib ( $r = -0.499$ ), but positively correlated in the HC ( $r = 0.348$ ). \*significant correlation ( $p < 0.05$ ); †non-significant trend for correlation ( $p < 0.08$ ); L = left; R = right.

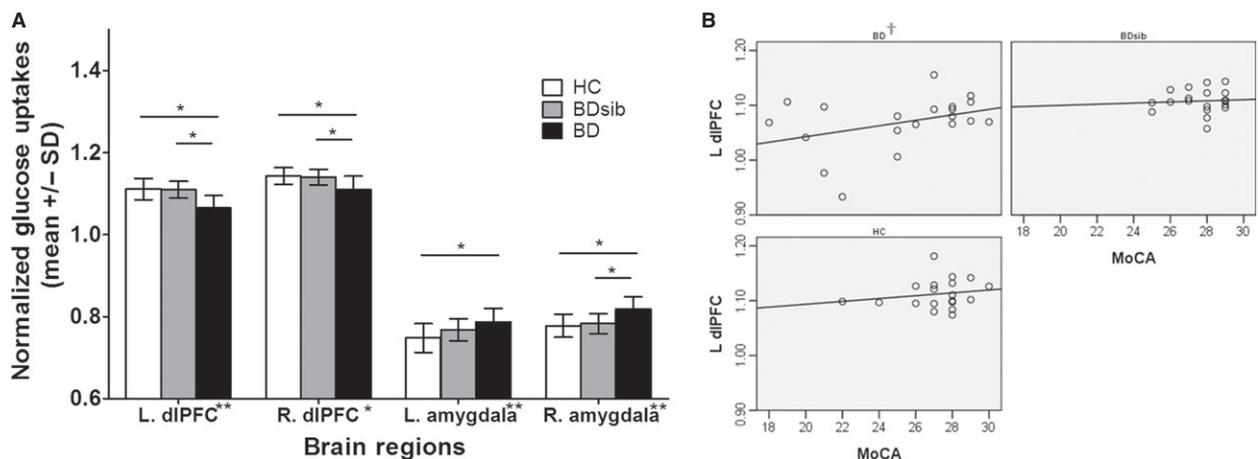
carried out separately in each group, however, there was no correlation between the right dIPFC–bilateral amygdala connectivity and YMRS scores.

#### Regional GU and clinical correlates

The patients with BD, but not the BDsib, had lower GU in the bilateral dIPFC and higher GU in the bilateral amygdala than did the HC (Fig. 3). In all four ROI, the BDsib presentations were similar to those of the HC. There was a reciprocal relationship between the dIPFC and amygdala because

there were strong negative correlations in the GU between the left dIPFC and left amygdala ( $r = -0.477$ ,  $p < 0.001$ ), between the left dIPFC and right amygdala ( $r = -0.583$ ,  $p < 0.001$ ), between the right dIPFC and left amygdala ( $r = -0.512$ ,  $p < 0.001$ ), and between the right dIPFC and right amygdala ( $r = -0.562$ ,  $p < 0.001$ ).

We found that the GU in the bilateral amygdala correlated positively with the YMRS scores in the BD group ( $p < 0.05$ ) (Table 3), if correlation analyses were separately performed in each group. There was also a trend to suggest that GU in the



**Fig. 3.** Glucose utilization in the bilateral dorsolateral prefrontal cortex (dIPFC) and amygdala. **(A)** The patients with bipolar disorder (BD), but not the unaffected, healthy siblings of patients with BD (BDsib), had significantly lower glucose utilization in their bilateral dIPFC and higher glucose utilization in their bilateral amygdala than did the healthy control subjects (HC). The statistical comparison of the groups was conducted by analysis of variance for each region of interest, and this was followed by a *post hoc* least significant difference analysis. **(B)** The correlation analysis revealed a positive correlation in the patients with BD between left-dIPFC glucose utilization and Montreal Cognitive Assessment (MoCA) scores. †non-significant trend for correlation ( $p < 0.08$ ); L = left; R = right; SD = standard deviation. \*significant correlation ( $p < 0.05$ ). \*\*significant correlation ( $p < 0.005$ ).

Table 3. Correlations between the neuroimaging findings and clinical variables in the three groups

		BD		BDsib		HC	
		YMRS	MoCA	YMRS	MoCA	YMRS	MoCA
<b>rsfMRI</b>	Right dlPFC–left amygdala	0.196 (0.444)	−0.437 (0.066)	−0.050 (0.838)	−0.499* (0.035)	−0.090 (0.722)	0.348 (0.158)
	Functional connectivity						
	Right dlPFC–right amygdala	0.092 (0.754)	−0.288 (0.317)	0.147 (0.547)	−0.180 (0.481)	0.189 (0.454)	−0.196 (0.435)
<b><sup>18</sup>F-FDG PET</b>	Left dlPFC	−0.157 (0.575)	0.473 (0.075)	−0.344 (0.149)	0.065 (0.793)	−0.165 (0.514)	0.256 (0.306)
	Regional glucose uptake						
	Right dlPFC	−0.391 (0.149)	0.168 (0.549)	−0.240 (0.323)	0.144 (0.556)	−0.106 (0.677)	0.294 (0.237)
	Left amygdala	0.536* (0.039)	−0.193 (0.490)	−0.135 (0.888)	−0.289 (0.232)	0.144 (0.529)	−0.340 (0.167)
	Right amygdala	0.666* (0.007)	−0.429 (0.111)	−0.305 (0.668)	0.012 (0.963)	0.062 (0.806)	−0.253 (0.312)

Values are expressed as *r* (p-value). BD = bipolar disorder; BDsib = unaffected, healthy siblings of patients with BD; dlPFC = dorsolateral prefrontal cortex; <sup>18</sup>F-FDG = <sup>18</sup>F-fluorodeoxyglucose; HC = healthy control subjects; MoCA = Montreal Cognitive Assessment; PET = positron emission tomography; rsfMRI = resting-state functional magnetic resonance imaging; YMRS = Young Mania Rating Scale. \*significant correlation (*p* < 0.05).

left dlPFC was correlated negatively with the MoCA scores in the BD group (*r* = 0.473, *p* = 0.075) (Fig. 3B, Table 3).

## Discussion

Strengths and the most important finding

The present study combined PET and rsfMRI to investigate directly the neural substrates of the ECN in patients with BD-I and BDsib. Our findings revealed that functional abnormalities in the dlPFC–amygdala circuit, but not cognitive deficits, could be endophenotypes for BD as the abnormalities were found both in affected and unaffected family members. Moreover, one of the merits of the present functional neuroimaging study is that we examined unmedicated subjects so that the potential effects of medication on the behavioral and neuroimaging results did not bias our interpretation of the group differences.

The most important finding was the altered FC in the dlPFC–amygdala circuit in the BDsib, which was also found in the patients with BD. The results of previous rsfMRI studies have been inconsistent, which might have been because of biases from medication effects (most studies recruited patients taking medications), different mood states (manic, euthymic, or depressed), and methodological differences (ROI or independent component analysis) across studies, which is an issue that was described in a recent systematic review (12). The results of the present study are in line with those of the only previously published rsfMRI study in medication-free patients with BD, in which Anticevic et al. (5) found that

euthymic patients with BD-I had disconnectivity between their amygdala and right dlPFC. The present study extended the findings to BDsib. Moreover, a growing body of evidence supports mitochondrial dysfunction as a principal cause of BD, and certain mitochondrial DNA (mtDNA) polymorphisms, such as mtDNA 10398A, have been identified as BD risk genes (28, 29). The resting brain is never truly resting and most energy utilized by brain cells is generated from plasma glucose in the mitochondria. Mitochondrial dysfunction is associated with increased oxidative stress and apoptosis, both of which have been consistently associated with the pathophysiology of BD and are therapeutic targets (e.g., lithium treatment could increase Bcl-2, which in turn reduces oxidative stress and apoptosis) (30). As mtDNA is single stranded and maternally inherited, and BD and BDsib share identical mtDNA genes, the common findings of the altered FC in the BD and BDsib might be a result of mitochondrial dysfunction in the BD family members. However, this remains to be investigated further.

BDsib: functional disconnectivity with intact GU in the dlPFC–amygdala circuit

In spite of the lack of available fMRI studies on BDsib thus far, our results are consistent with recent evidence that demonstrates that the unaffected offspring of bipolar patients also have alterations in the functioning of their fronto-limbic systems (31). Using an fMRI emotional working memory paradigm, the investigators found that, compared to healthy subjects, unaffected offspring

(aged 8–17 years) of patients with BD-I exhibit greater right PFC activation in response to positive emotional distracters, and reduced PFC modulation of the amygdala in response to both the positive and negative emotional distracters. Our results extend our knowledge of the abnormal functional connections in unaffected BD family members from task-dependent fMRI to task-free rsfMRI. Furthermore, the identified functional abnormality in the dlPFC–amygdala circuit could be associated with the cognitive functions in the BD families because we found an abnormal pattern of negative correlations between the right dlPFC–left amygdala connectivity and MoCA in the BD and BDsib ( $r = -0.420$ ,  $p = 0.009$ ). The amygdala has been critically linked to transient emotional response and subsequent subjective emotional experience, which usually follows the enhanced perception of emotional stimuli in patients with BD (2). Abnormal left, but not right, amygdala activity in response to mild sad and neutral faces is a characteristic feature of BD (32). On the other hand, the PFC is thought to play a crucial role in the top-down suppression of the activated emotion and the reappraisal of the stimulus meaning in human subjects, including healthy subjects and patients with BD (2). The altered right dlPFC–left amygdala might be speculated to be a result of a reactive engagement of the right dlPFC in the top-down regulation of the subcortical affective circuitry, and specifically of the amygdala. We found that the GU in the bilateral dlPFC of the BDsib was normal, which suggests that the PFC function was intact in the recruited BDsib. Hence, in the presence of altered dlPFC–amygdala FC, we speculated that a successfully compensatory regulation of emotion from the PFC might protect BDsib from developing BD.

BD: impaired FC and GU in the dlPFC–amygdala circuit

Previously published task-dependent fMRI studies have reported functional impairment of the brain areas related to fronto-limbic dysregulation, such as the ventral and dorsal PFC (8, 33–36), cingulate cortex (8, 36), and amygdala (8, 36, 37). However, the findings on the direction and exact location of the PFC abnormalities are not consistent, and this is possibly because of the use of differing experimental paradigms (task dependent or independent), unequal between-group task performance, and medication effects. For example, during high attention-related executive tasks (e.g., the n-back paradigm), patients with BD have reduced activity in the dlPFC and dorsal anterior cingulum (36). On the other hand, during high atten-

tion-related executive tasks that are conducted in the presence of emotional distracters, patients with BD demonstrate abnormally greater activity in the same dlPFC regions in response to greater amygdala activity (36). The PFC is involved in both the control of emotion and cognitive function in patients with BD. Our task-free rsfMRI results demonstrated that functional abnormality in the dlPFC–amygdala circuit was present in patients with BD and BDsib in the resting state. In addition, the abnormal connectivity was more prominent in the BD group than in the BDsib (Fig. 2), which could be because the functional deficits of the dlPFC fail to exert top-down regulation of amygdala activity as we found that patients with BD had abnormal GU in the bilateral dlPFC (decreased) and amygdala (increased). Furthermore, the FC in the right dlPFC–left amygdala circuit was correlated with MoCA, but not YMRS scores, in the BD and BDsib. The observations on the dysfunctional dlPFC–amygdala circuit in euthymic BD subjects echo the findings that executive function remains impaired during the euthymic or relatively stable periods (15, 38). The findings of the dysfunctional dlPFC–amygdala circuit in euthymic BD suggested that cognitive impairment could be more sensitive than YMRS in detecting BD diathesis. Although cognitive impairment was not found in our BDsib, cognitive impairment has been reported in some high-risk but unaffected first-degree relatives of BD (15–17).

Two limitations might be worth considering while interpreting the present results. First, the sample size was not large. However, the fact that the patients with BD and the BDsib were from the same family, with an age difference less than five years, and that we recruited only patients with BD-I who were clinically stable and without medication use for at least one week ensured that BD and BDsib theoretically had identical mitochondrial genes and a comparable developmental background. The recruitment criteria were to ensure that BD and BDsib have theoretically identical mitochondrial genes and comparable developmental background. Second, in spite being medication free at the time of scanning, all the patients had been taking medication. It is arguable that a history of medication exposure could have contributed to the present findings. However, it is almost impossible to recruit a patient with BD with an identified history of mania who is drug naïve. Furthermore, our main finding of altered dlPFC–amygdala connectivity was present not only in the patients with BD, but also in the BDsib, who had not been taking medication.

## Conclusions

There was altered FC with intact GU in the dlPFC–amygdala circuit of the unaffected siblings of BD probands. Such abnormal FC existed both in the BD and the BDsib and could be considered as an endophenotype of BD. Although the identified dlPFC–amygdala functional abnormality in the BDsib was milder compared to that in the patients with BD, this finding highlights the vulnerability of families with BD. Diminished top-down control from the bilateral dlPFC to regulate limbic hyperactivity, as demonstrated by the decreased GU in the bilateral dlPFC in the presence of the dysfunctional dlPFC–amygdala connectivity, might mediate the development of BD.

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## Disclosures

The authors of this paper do not have any commercial associations that might pose a conflict of interest in connection with this manuscript.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Seed-based analysis of functional connectivity with seeds at the bilateral dorsolateral prefrontal cortex (dlPFC).

**Figure S2.** Correlations of YMRS scores and functional connectivity between the right dorsolateral prefrontal cortex (R.dlPFC) and left amygdala (L.AMG).

**Table S1.** Seed-based functional connectivity analysis (seeds: bilateral dorsolateral prefrontal cortex).