Default mode network shows alterations for low-frequency fMRI fluctuations in euthymic bipolar disorder

Marco Marino[1,2], Zaira Romeo[3], Alessandro Angrilli[3,4], Ilaria Semenzato[3], Angela Favaro[4,5], Gianna Magnolfi[5], Giordano Bruno Padovan[5,6], Dante Mantini[1,2] and Chiara Spironelli[3,4*]*

[1]Department of Movement Sciences, Research Center for Motor Control and Neuroplasticity, KU Leuven, Belgium
[2]IRCCS San Camillo Hospital, Venice, Italy
[3]Department of General Psychology, University of Padova, Italy
[4]Padova Neuroscience Center, University of Padova, Italy
[5]Psychiatric Clinic, Neuroscience Department, University of Padova, Italy
[6]Unit of Penitentiary Medicine, ULSS6 Padova, Italy

Running title: DMN fluctuations in fMRI for euthymic bipolar disorder

***Corresponding authors:**

Marco Marino
Department of Movement Sciences, Research Center for Motor Control and Neuroplasticity, Tervuursevest 101, box 1501, 3001 Leuven, Belgium
Email: marco.marino@kuleuven.be

Chiara Spironelli
Department of General Psychology, Via Venezia 8, 35131 Padova, Italy
Tel. +39 049 8276619
Fax +39 049 8276600
Email: chiara.spironelli@unipd.it

Abstract

Bipolar disorder (BD) is a psychiatric condition causing acute dysfunctional mood states and emotion regulation. Specific neuropsychological features are often present also among patients in euthymic phase, who do not show clear psychotic symptoms, and for whom the characterization from functional magnetic resonance imaging (fMRI) is very limited. This study aims at identifying the neural and behavioral correlates of the default mode network (DMN) using the fractional amplitude of low frequency fluctuations (fALFF). Eighteen euthymic BD patients (10 females; age = 54.50 ± 11.38 years) and sixteen healthy controls (HC) (8 females; age = 51.16 ± 11.44 years) underwent a 1.5T fMRI scan at rest. The DMN was extracted through independent component analysis. Then, DMN time series was used to compute the fALFF, which was correlated with clinical scales. From the between-group comparison, no significant differences emerged in correspondence to regions belonging to the DMN. For fALFF analysis, we reported significant increase of low-frequency fluctuations for lower frequencies, and decreases for higher frequencies compared to HC. Correlations with clinical scales showed that an increase in higher frequency spectral content was associated with lower levels of mania and higher levels of anxious symptoms, while an increase in lower frequencies was linked to lower depressive symptoms. Starting from our findings on the DMN in euthymic BD patients, we suggest that the fALFF derived from network time series represents a viable approach to investigate the behavioral correlates of resting state networks, and the pathophysiological mechanisms of different psychiatric conditions.

Keywords: Default Mode Network (DMN); euthymic; Bipolar Disorder (BD); functional Magnetic Resonance Imaging (fMRI); Independent Component Analysis (ICA); fractional Amplitude of Low-Frequency Fluctuations (fALFF).

Introduction

Functional magnetic resonance imaging (fMRI) data acquired at rest is unaffected by explicit task-related confounds and allows researchers to investigate spontaneous neuronal activity. In this condition, neuronal dynamics appear to be spatially organized in a finite set of coherent patterns commonly called resting-state networks (RSNs) (Fox and Raichle, 2007). The study of RSNs is a viable approach to characterize the functional architecture of both the pathological and the healthy human brain. In particular, in the absence of any explicit cognitive demand, the measure of RSNs can unveil the pathophysiological characteristics of psychiatric disorders (Woodward and Cascio, 2015), especially in those patients who show low compliance at performing a specific task.

Among others, bipolar disorder (BD) is a heterogeneous disease characterized by acute dysfunctional mood states, alternating between mania (in BD-I) or hypomania (in BD-I and BD-II) and depression, and related to dysfunctional emotion regulation (Grande et al., 2016; Phillips and Kupfer, 2013). BD patients are commonly clustered according to their current clinical mood state. Indeed, by alternating periods of depression and elevated mood, at the first onset, according to the main symptoms, they are very likely to be associated either to maniac or to depressed disorder (Grande et al., 2016). Thus, BD patients are frequently misdiagnosed, leading to early inadequate treatment (Dunner, 2003). The picture becomes even more complex when patients show minimum residual mood symptoms and no current psychotic symptoms – a (sub)clinical condition referred to as euthymic state.

Great effort has been dedicated to identify brain functional abnormalities in BD patients and to define how these alterations relate to specific clinical states (Chen et al., 2018). In particular, these functional abnormalities were consistent across emotional and cognitive tasks and particularly in relation to the state of mania, showing enhanced limbic activation during emotional (rather than cognitive) tasks, but still not clearly related to mood states (Chen et al., 2018). Resting state functional connectivity has also been largely used to examine its potential relevance for both affective and cognitive dysfunctions in BD (Lois et al., 2014), especially for investigating the interactions between RSNs. Among these RSNs, the default mode network (DMN), especially, received greater attention. The DMN is composed by a set of brain regions, including left and right angular gyrus (lAG and rAG), posterior cingulate cortex (PCC), and medial prefrontal cortex (mPFC), which are preferentially activated, with fMRI, during internally-generated thoughts and self-reflection, and deactivated during cognitively demanding tasks (Greicius et al., 2003; Raichle et al., 2001). Several studies highlighted abnormalities in the interactions between the regions belonging to the DMN and the regions involved in emotional process regulation, which reflects bipolar mood fluctuations. In particular, these investigations observed selective abnormalities in the connections between the amygdala and DMN regions, including the PCC (reduced) and the mPFC (enhanced) (Rey et al., 2016). Also, DMN has been mainly studied in relation to task-positive networks, such the salience network (Magioncalda et al., 2015), and to brain regions linked to higher cognitive functions (Wang et al., 2015). However, while these studies provided interesting insights into the intrinsic functional connectivity changes associated with the BD condition, still they did not provide any links of brain activity with clinical scales. This lack of correlation is particularly evident for euthymic BD patients as very limited research has been carried out so far (Lois et al., 2014).

Altered functional connectivity during resting state in euthymic bipolar patients revealed differences in the interaction of resting DMN with task-positive networks (Das et al., 2014) and with emotion-related regions (Favre et al., 2014), including the amygdala. However, the spatial pattern of the network itself did not differ between clinical BD subgroups (Das et al., 2014). Another study found differences in bipolar mood state as well as in functional connectivity between bipolar mania (BD-I) and bipolar euthymia phases (Brady Jr et al., 2017), which in turn differed from healthy controls. Also, previous studies have shown that specific clinical variables collected from BD patients showed significant correlations between behavioral variables and peculiar brain activity and connectivity. However, in euthymic BD patients with negligible residual mood symptoms, no significant correlations were found (Linke et al., 2011; Wright et al., 2008).

Independent Components Analysis (ICA) is a data-driven approach that identifies temporally coherent spatial patterns of BOLD signal that are maximally independent from each other. ICA takes into account the BOLD signal from the whole brain to generate functional maps of different brain networks. ICA was first applied to identify an early marker for BD in unmedicated BD-II patients and to investigate the differences in multiple RSNs (Yip et al., 2014), but also to study DMN spatial pattern, which appeared not significantly different among subgroups of BD patients (Thomas et al., 2019). In particular, functional features of BD euthymic patients include preserved whole-brain functional connectivity compared to healthy subjects (Nabulsi et al., 2020), specifically for the DMN (Syan et al., 2018), which suggests that the stability of the DMN may be a neural correlate of an euthymic state. However, it has also been reported that functional changes within the DMN might reflect the history of psychosis (Nabulsi et al., 2020; Syan et al., 2018), so that for example in case of positive history connectivity patterns were found to be similar to the ones identified during bipolar mania or schizophrenia (Brady Jr et al., 2017; Khadka et al., 2013; Syan et al., 2018). This poses limitations in the interpretation of the underlying physiological basis of connectivity which remains rather unclear in euthymic BD patients. In line with this, ICA was not able to show significant alterations in DMN connectivity in BD, suggesting that within-DMN connectivity might be state-dependent, or that any abnormalities during euthymia is not detected by conventional analysis of its spatial pattern (Yoon et al., 2018).

To this end, we explored an alternative approach that could complement conventional connectivity analysis, by looking at the spectral features associated with the DMN. In this context, the fractional amplitude of low-frequency fluctuations (fALFF) (Zou et al., 2008), which measures oscillatory synchronization of regional blood oxygenation level-dependent (BOLD) signal, offers a promising alternative and complementary index (Raichle and Mintun, 2006; Yang et al., 2007). This approach was first used to complement conventional ICA connectivity analysis to investigate the levodopa-induced modulation of resting state sensorimotor network, which notably is affected by Parkison's disease progression (Esposito et al., 2013). Starting from this work, we applied this methodology to better characterize the DMN in BD euthymic patients in terms of both spatial map and spectral content, and improve the understanding of the neural correlate underlying this clinical state.

In the present study, we aimed to test (a) whether euthymic BD patients show DMN alterations, which might reflect the underlying pathophysiology of the disorder, and (b) whether such alterations are a trait marker for the diagnosis of BD, by examining the clinical correlates of the DMN-specific fALFF measurements with behavioral variables. To this end, we first compared the spatial pattern of DMN, as extracted from ICA decomposition analysis, of healthy controls and euthymic BD patients without current psychotic symptoms. In agreement with past literature (Syan et al., 2018; Thomas et al., 2019; Yoon et al., 2018) we expected to find no spatial differences between patients' and healthy controls' DMN distribution. Afterwards, following the extraction of the individual DMN from each participant, we derived the fALFF from the time-course associated with the DMN. We adopted the fALFF to further examine the differences in resting state condition between euthymic BD patients and healthy controls and to test the feasibility of the fALFF as a research and clinical tool to monitor persistent cerebral dysfunction in BD by providing evidences on how it reflects specific behavioral assessment from clinical scales.

Material and methods

Participants

This study has been approved by the Ethics Committee of Padua University Hospital and adheres to the principles of the Declaration of Helsinki. All participants provided informed written consent before study entry.

Patients were recruited at the Mood Disorders Outpatient Unit of the Padova

University Hospital according with the following inclusion criteria: they received a diagnosis of Bipolar Disorder (type I or II) for at least one year, they were non-remitting outpatient, and they were in an euthymic state at the moment of the experimental data collection (Young Mania Rating Scale, YMRS; Young et al., 1978) scores being lower than 8). Therefore, 18 euthymic BD patients (10 females, average age = 54.50 years, Standard Deviation [SD] = ± 11.38 years) took part in the experiment. Demographic, anamnestic and clinical parameters of patients' are summarized in Table 1 and Table 2.

Please insert Table 1 about here

We also enrolled 16 healthy, age-matched, control participants (Table 1), according to the following exclusion criteria: genetic kinship with some members of Patient group, major lifetime psychiatric diagnosis, use of psychotropic drugs.

In addition, all of participants were suitable for MRI scan (e.g., they had no metal bodies in the skull), and none of them suffered from epilepsy or other major brain comorbidities nor the healthy control participants suffered from psychiatric disorders

Clinical Assessment

Before the MRI session, participants completed a psychiatric interview (Structured Clinical Interview for DSM-IV) with a board-certified psychiatrist to determine the presence or absence of current and past psychiatric illness. At the MRI visit, a psychiatrist completed the YMRS as eligibility criterion, as well as the Hamilton Depression Rating Scale (Hamilton, 1967), Altman Self-Rating Mania Scale (ASRM; (Altman et al., 1997) and STAI-Y1 and Y2 (Pedrabissi and Santinello, 1989) with BD participants. Higher scores indicate a greater extent of the measured clinical variable (e.g., depression, mania or anxiety). In the same visit, a clinical psychologist administered the Positive And Negative Affective State (PANAS) questionnaire (Watson et al., 1988). Patients' psychopharmacologic therapy has been recorded as well as other features, such as history of psychotic symptoms, age of onset of BD, duration, mood temporal pattern, number of manic, hypomanic or depressive episodes (details on Table 2).

Please insert Table 2 about here

MR data acquisition

Magnetic resonance imaging was carried out at the Radiology Department of Padua University Hospital with a Siemens MAGNETOM® 1.5 T MRI system (Siemens Healthcare, Erlangen, Germany); specific head coil was mounted to increase image quality of brain tissues. Participants had first a resting state functional MRI (fMRI) scan during which they were instructed to stay relaxed with their eyes open while remaining motionless (201 continuous functional volumes, repetition time=2390 ms, echo time=50ms, flip angle=90°, field of matrix=64x64x36, acquisition voxel size=1.8x1.8x6 mm; acquisition time 8:00 min). Soon after, they were submitted to a high-resolution 3D T1-weighted structural MRI (sMRI) in a gradient-echo sequence (160 sagittal slices, repetition time=2000 ms, echo time=3.13 ms, flip angle=20°, field of matrix=320×320×160, acquisition voxel size=0.656×0.656×1 mm; acquisition time 5:33 min). During the functional scan, subjects were asked to simply stay motionless, awake and relaxed with their eyes closed; no visual or auditory stimuli were presented at any time during functional scanning. None of participants in the study moved, fallen asleep, or reported anxiety or other particular emotion during scanning. All scans were visually inspected by a trained neuroradiologist to exclude gross pathology alterations, excessive motion or major scanner artefacts. A quantitative check was also performed by calculating the framewise displacement (FD) (Power et al., 2012), computed as the sum of the absolute values of the derivatives of the translational and rotational realignment estimates at every timepoint, for which we reported values below 0.5 for both groups (FD_{HC}=0.17 ±0.11, FD_{BD}=0.26±0.14) (Power et al., 2012).

MR data preprocessing

fMRI data were preprocessed by means of an automated pipeline developed using

SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12), including motion correction, spatial alignment to sMRI, bias field correction, co-registration to standard space, and spatial smoothing at 6 mm full width half maximum (Mantini et al., 2013; Marino et al., 2019).

DMN reconstruction using Independent Component Analysis

Connectivity analysis was performed, separately for each subject, using spatial independent component analysis (ICA). ICA, which takes into account of any physiological confounds by extracting specific patterns from independent sources, was used for decomposing the fMRI data into brain activity patterns starting from the spatial covariance of the measured signals (McKeown et al., 1998). We estimated the number of ICs by using the minimum description length criterion (Calhoun et al., 2001). Accordingly, 45–98 ICs were extracted, depending on the specific fMRI dataset. ICs were calculated using the FastICA algorithm, with a deflation approach and hyperbolic tangent non-linearity (Esposito et al., 2005). For each IC, a spatial map and an associated time series are extracted. The spatial map expresses the intensity of the activity across the voxels of that pattern, whereas the time series corresponds to its course over time (Mantini et al., 2009; Mantini et al., 2007). These values express the extent to which a given voxel is modulated by the activation of a specific component (McKeown et al., 1998) and hence reflects the amplitude of the correlated fluctuations within the corresponding functional connectivity network. The spatial map was converted to z-scores by subtracting the average intensity across voxels, and dividing the resulting map by the standard deviation across voxels. To select a possible independent component of interest (i.e., an independent component associated with a given resting-state network, such as the DMN) in each subject and each scan, we used resting-state network spatial templates from a previous study (Mantini et al., 2013). The IC corresponding to DMN was identified using an automated template-matching procedure, in which the considered DMN-template was derived from our previous fMRI study (Mantini et al., 2013). Specifically, the DMN was identified as the IC showing the highest spatial correlation with the DMN template map in Montreal Neurological Institute (MNI) space.

Testing for between-group differences in DMN spatial map

Starting from the individual DMN spatial map, we derived DMN group-level correlation map by performing a one-sample t-test, using a mass-univariate analysis. According to this approach, each voxel showing a significant group effect indicates that there was significant correlation at the group level. We corrected the significance level for multiple comparisons (for multiple voxels involved in the analysis) between single-subject z-scores correlation maps using the Benjamini-Hochberg false discovery rate (BH-FDR) procedure (Benjamini and Hochberg, 1995), which does not make any assumptions about sample dependency. The significance threshold for the DMN group-level correlation map derived from the fMRI data was set to $p < 0.05$, BH-FDR corrected. This was computed separately for each group to visualize the average DMN functional connectivity pattern for both HC and BD groups. We then performed the DMN comparison between the HC and the BD groups by using a two-sample t-test on the individual DMN maps belonging to each group to detect regional differences in the DMN map between the two groups.

Fractional Amplitude of Low Frequency-Fluctuations

Starting from the individual time series associated with the DMN, the fALFF was estimated from the frequency spectrum computed using the Fast Fourier Transform (FFT) function. The fALFF was computed for the whole detectable frequency range, which was subdivided into four separate bands: slow-5 (0.01–0.027 Hz), slow-4 (0.027–0.073 Hz), slow-3 (0.073–0.198 Hz) and slow-2 (0.198– 0.25 Hz). This separation was first suggested by Zou et al. (Zou et al., 2008) to provide a better discrimination compared to the canonical fALFF, which is computed for the frequency range 0.01-0.1 Hz. To limit the effect of individual confound, the fALFF values in the four frequency bands were normalized with respect to the canonical fALFF (Zou et al., 2008; Zuo et al., 2010).

Correlation of fALFF with psychiatric and psychological scales

To identify relevant relationships between neuroimaging measurements and the clinical scores collected from the euthymic BD patients, we carried out a correlation analysis between the normalized fALFF frequency band values and the psychiatric and psychological scales administered during the interview.

Results

As shown in Table 1, no significant differences between HC and BD groups were found. Considering the BD group only, all patients were pharmacologically treated with up to four different psychopharmacologic classes of drugs (mood stabilizers, atypical antipsychotics, antidepressants and anxiolytics – further details on Table S1 in Supplementary Materials). Depressive and manic symptoms assessed with HAM-D and ASRM scales evidenced a negative correlation between these two variables (r_{16}=-0.62, p<0.01). In addition, STAI-Y1 (state) and STAI-Y2 (trait) anxiety levels correlated positively with depressive symptoms (r_{16}=0.72 and r_{16}=0.70, all p<0.001 respectively), but negatively with manic symptoms (r_{16}=-0.63, p<0.01, and r_{16}=-0.54, p<0.05, respectively). Interestingly, positive correlations were found between PANAS-PA (Positive Affect) and ASRM (r_{16}=0.73, p<0.001) and PANAS-NA (Negative Affect) and HAM-D (r_{16}=0.77, p<0.001). Details are fully available in Table S2 and Figure S1 in Supplementary Materials.

Please insert Figure 1 about here

Figure 1 shows the random-effects group-level t-maps of the DMN for HC and BD patients and the random-effects group-level t-map for the difference between HC and BD patients. Consistent with previous studies (Esposito et al., 2005; Mantini et al., 2007) the DMN recruited the PCC and ACC and bilaterally the rANG and lANG. In the between-group comparison, we did not find any significant difference in correspondence to the regions belonging to the DMN[1].

---

[1] The between-group analysis revealed a bilateral activation of Primary Motor cortices (BA 4, MNI coordinates: -47 -10 14 and 43 -4 14 for left and right voxels) in healthy participants (blue

When moving to the normalized fALFF analysis, the DMN in BD patients showed a statistically significant increase in the amplitude of the correlated fluctuations between 0.015 and 0.020 Hz and a decrease between 0.035 and 0.040 Hz and 0.080 and 0.085Hz compared to the HC (Figure 2).

Please insert Figure 2 about here

Notwithstanding no significant group differences were found on the average spectral power level for the full frequency bands (slow-5 = 0.22 ± 0.05 and 0.21 ± 0.05, slow-4 = 0.44 ± 0.07 and 0.44 ± 0.07, slow-3 = 0.35 ± 0.09 and 0.36 ± 0.08, slow-2 = 0.01 ± 0.01 and 0.01 ± 0.01 for HC and BD, respectively), we put forward our investigation to identify relevant relationships between fALFF-derived frequency bands and psychiatric and psychological scales. In particular, as shown in Figure 3, we found that Slow-3 fALFF band showed a significant negative correlation with the ASMR (r_{16}=-0.49, p<0.05) and positive with STAY-Y2 (r_{16}=0.49, p<0.05), whereas the Slow-5 fALFF band was negatively correlated with the PANAS-NA only (r_{16}=-0.49, p<0.05).

Please insert Figure 3 about here

Discussion

In this study, we proposed a data-driven approach that has the potential of unveiling the behavioral correlates of functional connectivity, by extracting fALFF values from the time series associated with RSNs. Here, we focused on the assessment of the DMN in bipolar euthymic patients. In particular, we demonstrated that in contrast to traditional analysis of functional connectivity between HC and BD patients in euthymic states that did not evidence differences, the complementary index provided by the fALFF showed remarkable correlations with psychiatric and

---

color) and a the right primary auditory cortex activation (BA 41, MNI coordinates: 42,-28,14) in BD patients (red color). Note that all these brain regions are not active part of the DMN.

psychological scores. Despite the absence of substantial clinical signs of depression and mania, nevertheless these patients showed an interesting correlation between DMN fALFF and residual symptoms of mania, trait anxiety and positive affect that might unmask a constitutional psychophysiological pattern of vulnerability.

ICA studies focusing on BD patients at rest reported altered connectivity within the DMN in manic (Lois et al., 2014) or psychotic (Meda et al., 2012) conditions compared to healthy subjects, suggesting that these alterations might be state-dependent (Wang et al., 2020). These abnormalities could be expected in symptomatic rather than in euthymic patients in whom clinical manifestation is not present, as confirmed by the lack of differences found in DMN spatial pattern between BD patients during clinical remission and HC (Du et al., 2015; Lois et al., 2014). Consistently, we also did not find any significant differences in the spatial pattern of the DMN. We then further expanded our work by performing correlation analysis of DMN features with BD clinical characteristics. In particular, we explored the ability of a complementary measure represented by fALFF to identify the alterations in functional connectivity associated with BD conditions.

With respect to the whole-brain fALFF, the fALFF derived from the network time series does not include any other functional sources of variance that might impact on the spectral profile of the considered region or voxel. In particular, the fALFF might represent not only low-frequency correlated neuronal fluctuations but also low-frequency physiological-driven fluctuations, such as cardiac and breathing pattern changes, which contribution cannot be fully excluded as confounding effect on the whole-brain fALFF results (Bu et al., 2019; Cordes et al., 2001), but this risk can be prevented by the network-based fALFF, which only contains the variance associated with the network itself (Esposito et al., 2013). Furthermore, the network-based fALFF makes it possible to disentangle the functional features of spatially overlapping networks that might share brain regions, but potentially present different mechanisms of modulation (Esposito et al., 2013). In this sense, the application of network-based fALFF achieved through ICA, that identifies maximally independent coherent patterns, represents a powerful tool to investigate abnormal RSN connectivity patterns, which may underlie affective and cognitive symptoms of a psychiatric disorder. Accordingly, we proposed the application of a fALFF implementation based on the temporal information of the ICA-derived DMN, as an alternative to the whole-brain approach, which failed to identify clinical correlates in euthymic patients (Martino et al., 2016). In particular, whole-brain fALFF alterations have been reported in regions belonging to the DMN, including decreased power within the rAG and increased power within the ACC for BP patients in depressive state (Yu et al., 2017). Another study combining functional connectivity and fALFF reported alterations for BD patients, which were dependent on the manic or depressive state, and showed negative correlation of fALFF slow-5 with the severity of the depressive and manic symptoms (Magioncalda et al., 2015).

While we did not report significant group differences using the whole frequency bands, locally, within more specific frequency ranges (Figure 2), we found remarkable correlations both with the psychiatric and psychological scales (Figure3). In particular, the correlations reported for the slow-3 fALFF band with ASMR and STAY-Y2 revealed that an increase in the BD spectral content is associated with lower levels of mania and higher levels of anxious symptoms, respectively. On the other hand, the negative correlation of the slow-5 fALFF band evidenced the association of lower depression symptoms with higher spectral amplitude. Previous studies did not report significant correlations, which was interpreted as the consequence of the little variance of symptomatic variables measured from the euthymic patients' sample under investigation (Lois et al., 2014). Still, our approach was able to depict relevant features despite the limited sample size, which suggests that the network-based fALFF might be more sensitive than the whole-brain fALFF to detect clinical correlates.

In addition to resting-state functional connectivity measurements, the fALFF reflects different aspects of brain functioning. In particular, slower oscillations are known to be associated with activity in both cortical and subcortical gray matter regions (Wang et al., 2016), including the insula (Kalcher et al., 2014), which makes them the most suitable in identifying correlates of functional processing and disorders

(Zuo et al., 2010). In this context, investigating different aspects of resting state network functioning is important, as we showed that differences may lie not only in the pattern of connectivity, but also in the spectral power of the associated time series, for which differences between groups might be more evident.

In this study, we focused on within-network assessment and we did not consider the interactions between RSNs, which should be further explored (Das et al., 2014; Lois et al., 2014) also in future studies. Alterations in fALFF were reported between the mPFC and the limbic system (Chen et al., 2018; Li et al., 2012) showing a similar pattern in BD and schizophrenia patients (Khadka et al., 2013). In particular, these alterations were reported in subgroups of BD patients showing both manic and depressive symptoms (Martino et al., 2016) within the DMN, at the level of the ACC and PCC, and between DMN and other brain regions, including the supplementary motor area (Martino et al., 2016). While the quoted studies were able to depict these alterations in BD patients presenting clear symptoms, either to maniac-like or depressed-like, they failed to detect neurophysiological anomalies in euthymic patients lacking clinical symptoms. Showing the possibility of depicting relevant within-network information, in future studies the fALFF analysis could allow to investigate more in depth also the between-network interactions (Lakatos et al., 2008; Sirota et al., 2008).

A number of limitations of this study should be mentioned. The sample size in each group was relatively small, which might result in some subtle functional changes in the brain not being observed. Another aspect is represented by the possible confounding effects of medication (Martino et al., 2016), as almost all of the bipolar patients in our sample were taking medications, including mood stabilizers, antipsychotics, antidepressants, and anxiolytics, which could interfere with the BOLD signal. This study should be considered as a proof-of-concept for the application of this novel methodology and an exploratory analysis in which the p-value <0.05 (corrected for multiple comparisons) level was used for statistical validity. Next studies could further expand the sample size and perform stricter statistical correction to explore essential changes in the function of the brain in BD patients during euthymic condition in order to detect those individuals more vulnerable to relapse.

In conclusion, the present study extends prior work in BD research by employing a data-driven approach to examine euthymic BD patients without current psychotic symptoms. Understanding the neural correlates of the euthymic phase may have important implications for identifying potential biomarkers to guide clinical recovery of patients with BD. Our work might represent a basis for the study of the pathophysiological mechanisms of this psychiatric condition. In this context, our findings suggest that the fALFF represents a viable approach to investigate the behavioral correlates of RSNs. This finds useful applications in the psychiatric field especially for distinguishing those mental disorders that do not show clear symptoms and are prone to misdiagnosis, and for evaluating shared and unique characteristics of fALFF across the psychotic disorder continuum. Our framework could be applied longitudinally to understand the evolution of network functioning and symptomatology across states of depression, mania, and euthymia in BD, and also in other psychiatric conditions, that eventually share psychotic symptoms with BD, including schizophrenia (Du et al., 2015; Meda et al., 2012) or depression (Qiu et al., 2019). By providing a complementary window of exploration, our work could help to identify which functional abnormalities are state-specific, thus providing clinically useful information for the development of alternative treatment strategies for BD patients during acute episodes or remission.

REFERENCES

Altman, E.G., Hedeker, D., Peterson, J.L., Davis, J.M., 1997. The Altman self-rating mania scale. Biological psychiatry 42(10), 948-955.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal statistical society: series B (Methodological) 57(1), 289-300.

Brady Jr, R.O., Tandon, N., Masters, G.A., Margolis, A., Cohen, B.M., Keshavan, M., Öngür, D., 2017. Differential brain network activity across mood states in bipolar disorder. Journal of affective disorders 207, 367-376.

Bu, X., Hu, X., Zhang, L., Li, B., Zhou, M., Lu, L., Hu, X., Li, H., Yang, Y., Tang, W., 2019. Investigating the predictive value of different resting-state functional MRI parameters in obsessive-compulsive disorder. Translational psychiatry 9(1), 1-10.

Calhoun, V.D., Adali, T., Pearlson, G.D., Pekar, J.J., 2001. A method for making group inferences from functional MRI data using independent component analysis. Human brain mapping 14(3), 140-151.

Chen, L., Wang, Y., Niu, C., Zhong, S., Hu, H., Chen, P., Zhang, S., Chen, G., Deng, F., Lai, S., 2018. Common and distinct abnormal frontal-limbic system structural and functional patterns in patients with major depression and bipolar disorder. NeuroImage: Clinical 20, 42-50.

Cordes, D., Haughton, V.M., Arfanakis, K., Carew, J.D., Turski, P.A., Moritz, C.H., Quigley, M.A., Meyerand, M.E., 2001. Frequencies contributing to functional connectivity in the cerebral cortex in "resting-state" data. American journal of neuroradiology 22(7), 1326-1333.

Das, P., Calhoun, V., Malhi, G.S., 2014. Bipolar and borderline patients display differential patterns of functional connectivity among resting state networks. Neuroimage 98, 73-81.

Du, Y., Pearlson, G.D., Liu, J., Sui, J., Yu, Q., He, H., Castro, E., Calhoun, V.D., 2015. A group ICA based framework for evaluating resting fMRI markers when disease categories are unclear: application to schizophrenia, bipolar, and schizoaffective disorders. Neuroimage 122, 272-280.

Dunner, D.L., 2003. Clinical consequences of under-recognized bipolar spectrum disorder. Bipolar disorders 5(6), 456-463.

Esposito, F., Scarabino, T., Hyvarinen, A., Himberg, J., Formisano, E., Comani, S., Tedeschi, G., Goebel, R., Seifritz, E., Di Salle, F., 2005. Independent component analysis of fMRI group studies by self-organizing clustering. Neuroimage 25(1), 193-205.

Esposito, F., Tessitore, A., Giordano, A., De Micco, R., Paccone, A., Conforti, R., Pignataro, G., Annunziato, L., Tedeschi, G., 2013. Rhythm-specific modulation of the sensorimotor network in drug-naive patients with Parkinson's disease by levodopa. Brain 136(3), 710-725.

Favre, P., Baciu, M., Pichat, C., Bougerol, T., Polosan, M., 2014. fMRI evidence for abnormal resting-state functional connectivity in euthymic bipolar patients. Journal of Affective Disorders 165, 182-189.

Fox, M.D., Raichle, M.E., 2007. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nature reviews neuroscience 8(9), 700-711.

Grande, I., Berk, M., Birmaher, B., Vieta, E., 2016. Bipolar disorder. The Lancet 387(10027), 1561-1572.

Greicius, M.D., Krasnow, B., Reiss, A.L., Menon, V., 2003. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. Proceedings of the National Academy of Sciences 100(1), 253-258.

Hamilton, M.A.X., 1967. Development of a rating scale for primary depressive illness. British journal of social and clinical psychology 6(4), 278-296.

Kalcher, K., Boubela, R.N., Huf, W., Bartova, L., Kronnerwetter, C., Derntl, B., Pezawas, L., Filzmoser, P., Nasel, C., Moser, E., 2014. The spectral diversity of resting-state fluctuations in the human brain. PloS one 9(4), e93375.

Khadka, S., Meda, S.A., Stevens, M.C., Glahn, D.C., Calhoun, V.D., Sweeney, J.A., Tamminga, C.A., Keshavan, M.S., O'Neil, K., Schretlen, D., 2013. Is aberrant functional connectivity a psychosis endophenotype? A resting state functional magnetic resonance imaging study. Biological psychiatry 74(6), 458-466.

Lakatos, P., Karmos, G., Mehta, A.D., Ulbert, I., Schroeder, C.E., 2008. Entrainment of neuronal oscillations as a mechanism of attentional selection. science 320(5872), 110-113.

Li, C.T., Hsieh, J.C., Wang, S.J., Yang, B.H., Bai, Y.M., Lin, W.C., Lan, C.C., Su, T.P., 2012. Differential relations between fronto-limbic metabolism and executive function in patients with remitted bipolar I and bipolar II disorder. Bipolar Disorders 14(8), 831-842.

Linke, J., Sönnekes, C., Wessa, M., 2011. Sensitivity to positive and negative feedback in euthymic patients with bipolar I disorder: the last episode makes the difference. Bipolar disorders 13(7-8), 638-650.

Lois, G., Linke, J., Wessa, M., 2014. Altered functional connectivity between emotional and cognitive resting state networks in euthymic bipolar I disorder patients. PLoS One 9(10), e107829.

Magioncalda, P., Martino, M., Conio, B., Escelsior, A., Piaggio, N., Presta, A., Marozzi, V., Rocchi, G., Anastasio, L., Vassallo, L., 2015. Functional connectivity and neuronal variability of resting state activity in bipolar disorder—reduction and decoupling in anterior cortical midline structures. Human brain mapping 36(2), 666-682.

Mantini, D., Corbetta, M., Perrucci, M.G., Romani, G.L., Del Gratta, C., 2009. Large-scale brain networks account for sustained and transient activity during target detection. Neuroimage 44(1), 265-274.

Mantini, D., Corbetta, M., Romani, G.L., Orban, G.A., Vanduffel, W., 2013. Evolutionarily novel functional networks in the human brain? Journal of Neuroscience 33(8), 3259-3275.

Mantini, D., Perrucci, M.G., Del Gratta, C., Romani, G.L., Corbetta, M., 2007. Electrophysiological signatures of resting state networks in the human brain. Proceedings of the National Academy of Sciences 104(32), 13170-13175.

Marino, M., Arcara, G., Porcaro, C., Mantini, D., 2019. Hemodynamic correlates of electrophysiological activity in the default mode network. Frontiers in neuroscience 13, 1060.

Martino, M., Magioncalda, P., Huang, Z., Conio, B., Piaggio, N., Duncan, N.W., Rocchi, G., Escelsior, A., Marozzi, V., Wolff, A., 2016. Contrasting variability patterns in the default mode and sensorimotor networks balance in bipolar depression and mania. Proceedings of the National Academy of Sciences 113(17), 4824-4829.

McKeown, M.J., Makeig, S., Brown, G.G., Jung, T.P., Kindermann, S.S., Bell, A.J., Sejnowski, T.J., 1998. Analysis of fMRI data by blind separation into independent spatial components. Human brain mapping 6(3), 160-188.

Meda, S.A., Gill, A., Stevens, M.C., Lorenzoni, R.P., Glahn, D.C., Calhoun, V.D., Sweeney, J.A., Tamminga, C.A., Keshavan, M.S., Thaker, G., 2012. Differences in resting-state functional magnetic resonance imaging functional network connectivity between schizophrenia and psychotic bipolar probands and their unaffected first-degree relatives. Biological psychiatry 71(10), 881-889.

Nabulsi, L., McPhilemy, G., Kilmartin, L., Whittaker, J.R., Martyn, F.M., Hallahan, B., McDonald, C., Murphy, K., Cannon, D.M., 2020. Frontolimbic, frontoparietal, and default mode involvement in functional dysconnectivity in psychotic bipolar disorder. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging 5(2), 140-151.

Pedrabissi, L., Santinello, M., 1989. Verifica della validità dello STAI forma Y di Spielberger. Giunti Organizzazioni Speciali.

Phillips, M.L., Kupfer, D.J., 2013. Bipolar disorder diagnosis: challenges and future directions. The Lancet 381(9878), 1663-1671.

Power, J.D., Barnes, K.A., Snyder, A.Z., Schlaggar, B.L., Petersen, S.E., 2012. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. Neuroimage 59(3), 2142-2154.

Qiu, S., Chen, F., Chen, G., Jia, Y., Gong, J., Luo, X., Zhong, S., Zhao, L., Lai, S., Qi, Z., 2019. Abnormal resting-state regional homogeneity in unmedicated bipolar II disorder. Journal of affective disorders 256, 604-610.

Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., Shulman, G.L., 2001. A default mode of brain function. Proceedings of the National Academy of Sciences 98(2), 676-682.

Raichle, M.E., Mintun, M.A., 2006. Brain work and brain imaging. Annu. Rev. Neurosci. 29, 449-476.

Rey, G., Piguet, C., Benders, A., Favre, S., Eickhoff, S.B., Aubry, J.M., Vuilleumier, P., 2016. Resting-state functional connectivity of emotion regulation networks in euthymic and non-euthymic bipolar disorder patients. European psychiatry 34, 56-63.

Sirota, A., Montgomery, S., Fujisawa, S., Isomura, Y., Zugaro, M., Buzsáki, G., 2008. Entrainment of neocortical neurons and gamma oscillations by the hippocampal theta rhythm. Neuron 60(4), 683-697.

Syan, S.K., Smith, M., Frey, B.N., Remtulla, R., Kapczinski, F., Hall, G.B.C., Minuzzi, L., 2018. Resting-state functional connectivity in individuals with bipolar disorder during clinical remission: a systematic review. Journal of psychiatry & neuroscience: JPN 43(5), 298.

Thomas, S.A., Christensen, R.E., Schettini, E., Saletin, J.M., Ruggieri, A.L., MacPherson, H.A., Kim, K.L., Dickstein, D.P., 2019. Preliminary analysis of resting state functional connectivity in young adults with subtypes of bipolar disorder. Journal of affective disorders 246, 716-726.

Wang, J., Wang, Y., Wu, X., Huang, H., Jia, Y., Zhong, S., Wu, X., Zhao, L., He, Y., Huang, L., 2020. Shared and specific functional connectivity alterations in unmedicated bipolar and major depressive disorders based on the triple-network model. Brain imaging and behavior 14(1), 186-199.

Wang, L., Kong, Q., Li, K., Su, Y., Zeng, Y., Zhang, Q., Dai, W., Xia, M., Wang, G., Jin, Z., 2016. Frequency-dependent changes in amplitude of low-frequency oscillations in depression: a resting-state fMRI study. Neuroscience letters 614, 105-111.

Wang, Z., Meda, S.A., Keshavan, M.S., Tamminga, C.A., Sweeney, J.A., Clementz, B.A., Schretlen, D.J., Calhoun, V.D., Lui, S., Pearlson, G.D., 2015. Large-scale fusion of gray matter and resting-state functional MRI reveals common and distinct biological markers across the psychosis spectrum in the B-SNIP cohort. Frontiers in psychiatry 6, 174.

Watson, D., Clark, L.A., Tellegen, A., 1988. Development and validation of brief measures of positive and negative affect: the PANAS scales. Journal of personality and social psychology 54(6), 1063.

Woodward, N.D., Cascio, C.J., 2015. Resting-state functional connectivity in psychiatric disorders. JAMA psychiatry 72(8), 743-744.

Wright, K.A., Lam, D., Brown, R.G., 2008. Dysregulation of the behavioral activation system in remitted bipolar I disorder. Journal of Abnormal Psychology 117(4), 838.

Yang, H., Long, X.-Y., Yang, Y., Yan, H., Zhu, C.-Z., Zhou, X.-P., Zang, Y.-F., Gong, Q.-Y., 2007. Amplitude of low frequency fluctuation within visual areas revealed by resting-state functional MRI. Neuroimage 36(1), 144-152.

Yip, S.W., Mackay, C.E., Goodwin, G.M., 2014. Increased temporo-insular engagement in unmedicated bipolar II disorder: an exploratory resting state study using independent component analysis. Bipolar disorders 16(7), 748-755.

Yoon, H.-K., Dev, S.I., Sutherland, A.N., Eyler, L.T., 2018. Disruptions in resting state functional connectivity in euthymic bipolar patients with insomnia symptoms. Psychiatry Research: Neuroimaging 275, 1-4.

Young, R.C., Biggs, J.T., Ziegler, V.E., Meyer, D.A., 1978. A rating scale for mania: reliability, validity and sensitivity. The British journal of psychiatry 133(5), 429-435.

Yu, E., Liao, Z., Mao, D., Zhang, Q., Ji, G., Li, Y., Ding, Z., 2017. Directed functional connectivity of posterior cingulate cortex and whole brain in Alzheimer's disease and mild cognitive impairment. Current Alzheimer Research 14(6), 628-635.

Zou, Q.-H., Zhu, C.-Z., Yang, Y., Zuo, X.-N., Long, X.-Y., Cao, Q.-J., Wang, Y.-F., Zang, Y.-F., 2008. An improved approach to detection of amplitude of low-frequency fluctuation (ALFF) for resting-state fMRI: fractional ALFF. Journal of neuroscience methods 172(1), 137-141.

Zuo, X.-N., Di Martino, A., Kelly, C., Shehzad, Z.E., Gee, D.G., Klein, D.F., Castellanos, F.X., Biswal, B.B., Milham, M.P., 2010. The oscillating brain: complex and reliable. Neuroimage 49(2), 1432-1445.

FIGURE CAPTIONS

Figure 1. Random-effects group-level t-maps of the DMN for HC (top row, winter color scale) and BD patients (middle row, hot color scale), and random-effects group-level t-map of the differences between HC and BD patients (bottom row, hot/winter color scales depending on the group contrast). The reported p-values ($p<0.05$) were corrected for the FDR both for the random-effects group-level t-maps of each group and for the random-effects group-level t-map of their comparison.

Figure 2. Normalized fALFF analysis carried out on DMN time series in BD patients and HC (red and blue lines, respectively). The black dots at the bottom of the panel indicate the frequency range where normalized fALFF is significantly different between BD and HC groups (two-sample t-test, $p<0.05$).

Figure 3. Significant Pearson's correlations between normalized fALFF DMN analysis and ASRM mania symptoms severity (left panel), Trait Anxiety levels (middle panel) and PANAS-NA scores (right panel).