Multimodal Brain Changes in First-Episode Mania: A Voxel-Based Morphometry

Functional Magnetic Resonance Imaging, and Connectivity Study

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ABSTRACT

Background: Brain structural and functional changes in bipolar disorder are well established findings but it is uncertain whether these changes are already present in first episode mania (FEM).

Methods: We compared 31 FEM subjects with 31 healthy individuals matched for age, sex and premorbid IQ. Whole-brain voxel-wise morphometry, functional magnetic resonance imaging during the n-back task, and a functional connectivity analysis were performed.

Results: There were no volumetric differences between the two groups. During the 2- back task, FEM patients did not perform differently from controls and activated similar regions, but they showed less deactivation in the ventromedial prefrontal cortex (vmPFC), the anterior hub of the default mode network (DMN). They showed preserved functional connectivity between the vmPFC and other regions of the DMN, but increased connectivity with the superior frontal gyrus."

Conclusions: The absence of volumetric changes in FEM patients suggests that these changes could be related to progression of the illness. On the other hand, the failure of deactivation of the anterior hub of the DMN is present from the onset of the illness and may represent a core pathophysiological feature of bipolar disorder.

INTRODUCTION

Research in neuroimaging has demonstrated that bipolar disorder (BD) is associated with abnormalities in brain regions and systems that modulate emotional behavior (1). However, neurocognitive and neuroimaging studies have also detected abnormalities in executive function and in emotionally neutral memory tasks (2-3) attributed to interference in cognitive functioning through inappropriate activation of brain regions involved in emotional processing (4-5). A further functional abnormality that has been demonstrated in bipolar disorder in recent years is failure of deactivation in parts of the so-called default mode network (DMN), a set of brain regions which are active at rest but deactivate when subjects perform a wide range of cognitive tasks (6). DMN dysfunction is implicated in several psychiatric disorders, including schizophrenia, autism and major depression (7-8). BD patients have been found to show reduced DMN deactivation during cognitive tasks (9-11) as well as reduced DMN connectivity in resting-state functional magnetic resonance imaging (fMRI) (12). The DMN includes two midline regions, an anterior hub at the medial prefrontal cortex (mPFC) and a posterior hub in the posterior cingulate cortex (PCC)/precuneus (7). The DMN is 'anticorrelated' with a broad external attention system (EAS), also known as the taskpositive network, that mediates attention to exogenous stimuli (13-14), and shows increased activation during performance of cognitive tasks (15).

Working memory (WM) is a process that temporarily holds and manipulates information "online" with the goal of performing higher cognitive tasks, such as problem solving, reasoning and language (16). WM is among the cognitive domains that have been shown to be persistently impaired in BD (2). As it shows a large effect size and shared impairment with unaffected relatives, WM might represent a candidate endophenotype for BD (17). Most studies report a loss of connectivity in the prefrontal networks of patients with BD, which are traditionally involved in WM, as well as disturbances in activation of the dorsolateral prefrontal cortex (dlPFC) (18). On the other hand, a previous study on WM in manic patients showed poor deactivation of the DMN (12).

In addition, structural abnormalities have been described in BD. Two meta-analyses found volumetric reductions in the left anterior cingulate cortex (ACC) (19) and the right fronto-insular cortex (19-20). However, progression of the illness makes it difficult to differentiate primary abnormalities from those that may arise on the recurrence of the episodes (21). Moreover, pharmacological treatment can act as a confounder since lithium increases the volume of several grey matter structures (22-23), and lithium, antiepileptic and antipsychotic drugs are associated with cortical thickness in BD patients (24).

The study of individuals presenting a first episode of mania (FEM) can overcome some of these limitations (25). Although the onset of the illness is depressive in around 60% of individuals (26), the first manic episode is the time at which BD is typically diagnosed and at which patients have usually not received lithium. The study of FEM patients is essential from the perspective of the proposed 'staging' models of BD, in which each stage is described with associated neuroimaging findings (27). Total gray matter volume appears to be unaffected at this stage (28), although several studies have described subtle volumetric abnormalities (29-34).

In FEM patients fMRI studies may also help to determine whether disturbances previously reported in multiple-episode patients are present at the onset of the illness or if they are more related to its course. However, the only fMRI study published so far in FEM adults used a response inhibition paradigm (35).

To address these issues we undertook a multimodal neuroimaging study in a sample of FEM patients. First, a structural whole-brain analysis was performed to study possible volumetric changes at this initial stage. This was followed by a fMRI study with the nback task, a well-known WM task used to determine whether FEM patients show differential brain activation or deactivation patterns. Finally, a functional connectivity analysis was performed to assess differences in functional connectivity.

METHODS

Subjects

Thirty-one right-handed patients, aged 18 to 45 with a first manic or mixed episode according to DSM-IV-TR criteria were recruited at the Hospital Clínic and Hospital Benito Menni CASM which are two psychiatric hospitals in the Barcelona region. Patients were excluded if they: a) had a history of head trauma or neurological disease, b) had shown alcohol/substance abuse or dependence in the preceding 12 months, and c) had undergone electroconvulsive therapy. Diagnostic status was assessed with the Structured Clinical Interview for DSM-IV for Axis I disorders (36). Information about family history, previous medical and psychiatric history, and current medication was collected. All the subjects were assessed with the Young Mania Rating Scale (YMRS) (37), the Montgomery–Åsberg Depression Rating Scale (MADRS) (38), and the Positive and Negative Syndrome Scale (PANSS) (39).

The healthy control group was composed of 31 right-handed healthy participants recruited from non-medical staff working in the hospitals and via advertisements. They were matched to the patient group for age, sex and IQ and met the same exclusion criteria. Additional exclusion criteria were: a) a history of mental illness and/or treatment with psychotropic medication, b) a positive first-degree family history of a major psychiatric disorder, and c) previous in- or outpatient psychiatric care. The Word Accentuation Test was used to obtain an estimate of the general pre-morbid intellectual ability of all the participants (40). The study was approved by the local research ethics committee, and all participants provided written informed consent.

Neuroimaging

N-back task

The n-back task was administered as a block design, incorporating alternating experimental and baseline blocks. In the experimental blocks (1-back and 2-back) the target letter was defined as any letter that was identical to the one presented 1 or 2 trials back. Each experimental block consisted of 24 letters that were shown every 2 s and contained five repetitions. Participants were instructed to respond to target letters by button press. In the baseline block, an asterisk was flashed with the same frequency as the letters. Blocks were presented pseudo-randomised to

avoid any systematic order effects. Before scanning, all participants received training in a training set.

Task performance

To analyze performance during the n-back task, the sensitivity index d' was calculated. This index derives from signal detection theory and allows the distinction of signal and noise, where a higher d' indicates better signal detection (41). The individual probabilities of hits and false alarms were transformed into z-scores, and measures of sensitivity (d') were computed as follows: d' = z (probability (hits)) - z (probability (false alarms)) (42). Higher d'values indicate more accurate performance. Participants with negative d' values in either or both the 1-back and 2-back task, were excluded from the study.

Image acquisition

fMRI and anatomical data were acquired during the same session in a 1.5 Tesla GE Signa scanner (General Electric Medical Systems, Milwaukee, USA). High-resolution structural T1 MRI data were acquired with the following acquisition parameters: matrix size = 512 x 512; 180 contiguous axial slices; voxel resolution = 0.47 x 0.47 X 1mm3 ; echo time (TE) = 3.93 ms; repetition time (TR) = 2000 ms; inversion time (TI) = 710 ms; flip angle = 150 . A total of 266 T2*-weighted images were acquired using a gradient echo-planar imaging (EPI) sequence depicting blood oxygenation level-dependent (BOLD) contrast. Each volume contained 16 axial planes acquired with the following parameters: TR = 2000 ms, TE = 20 ms, flip angle = 70°, section thickness = 7 mm, section skip = 0.7 mm, in-plane resolution = 3 x 3 mm. The first 10 volumes were discarded to avoid T1 saturation effects.

Image analysis

Image processing and analysis were implemented using Statistical Parametric Mapping (SPM8; www.fil.ion.ucl.ac.uk/spm) software. Structural analysis was performed using voxel-based morphometry (VBM) with unified segmentation (43). Normalized and modulated gray matter segmented images were produced for each participant and smoothed using a Gaussian isotropic kernel of 8 mm full width half maximum (FWHM). The statistical threshold to detect differences between FEM patients and healthy participants was set at $P < 0.05$, using family wise error (FWE) correction.

fMRI preprocessing involved realignment, transformation into standard stereotactic Montreal Neurological Institute (MNI) space, and smoothing with an isotropic Gaussian kernel of 8 mm FWHM. No participant was excluded due to excessive interscan motion (defined as >4-mm translation, >4° rotation). A first-level fixed effect model was computed for each participant. fMRI responses were modeled using a canonical hemodynamic response function (hrf) convolved with the vectors of interest and signal was temporally filtered using a high pass filter (cut-off frequency 130s). Movement parameters were entered as nuisance covariates. Statistical contrast images of the 2-back versus baseline condition were produced for each participant and entered in an independent-sample t test at the second-level random effects analysis. Statistical significance was set at $P < 0.05$ using FWE correction. MarsBaR (www.marsbar.sourceforge.net) was used to extract measures of brain activation (weighted parameter estimates) from 5 mm radius spheres at peak height coordinates within each significant suprathreshold cluster. These

measures were also used in further analyses in SPSS 18.0 (SPSS Inc, Chicago, IL) to examine the effect of IQ, illness duration, antipsychotic medication exposure and clinical scale scores.

Psychophysiological interaction (PPI)

PPI computes functional connectivity between the time series of a seed voxel of interest (VOI) and the time series of all other voxels (44). In our study, the PPI analysis was addressed to examine mPFC (seed region) connectivity during the 2-back task. The mPFC is considered the anterior hub of the DMN. It encompasses a set of areas that lie along the frontal midline comprising the anterior cingulate (ACC) (Brodmann areas 24 and 32ac) and the medial region of the frontopolar cortex (FPC) (BA10) (10). The PPI analysis consists of a design matrix with three regressors: the "psychological variable", representing the experimental task (2-back); the "physiological variable", representing the neural response in the seed region (mPFC) and a third variable representing the "psychophysiological interaction" between the first and the second variables. The coordinates of the mPFC corresponded to the local maximum detected in the between group comparison of the 2-back > baseline contrast. For each participant, the first eigenvariate time was extracted from a sphere of 5 mm radius centered on the peak height coordinates for the region. Subject-specific contrast images were then entered into a random-effects analysis using one-sample t tests (thresholded at $P < 0.05$, FWE). To directly compare group differences in functional connectivity, we examined the interaction between the PPI (interaction between mPFC activity and the 2-back task) and the group (FEM patients and healthy participants) (thresholded at $P < 0.05$, FWE).

For both fMRI and PPI analyses, stereotactic coordinates were converted from MNI spatial array to that of Talairach and Tournoux (www.mrccbu.cam.ac.uk/Imaging/mnispace.html), and corresponding anatomical regions and Broadmann areas (BA) were identified with the Talairach Daemon Client (www.talairach. org).

RESULTS

Participants

Table 1 shows the demographic and clinical information about the sample.

Behavioral data

There were no significant differences between groups in the performance of the 2- back task (Table 1).

Structural results

No suprathreshold clusters were identified (statistical threshold, $P < 0.05$, FWE) in the whole-brain analysis. Even with a more liberal threshold of $P < 0.001$ (uncorrected, cluster size > 50) no significant differences were found between groups.

fMRI results

Within-group activations and deactivations during n-back performance

In the 2-back versus baseline comparison, both the healthy and FEM participants activated similar regions encompassing the dlPFC, the lateral FPC, the inferior and superior parietal lobule (IPL; SPL), the insula, and the cerebellum. Regarding deactivations, in healthy controls the two midline nodes of the DMN were deactivated: an anterior region comprising bilateral medial FPC and ACC, and a posterior region at the PCC and precuneus. They also showed bilateral deactivation of the temporal poles. Instead, FEM subjects only exhibited a limited region of deactivation at the PCC (Table 2).

Between-group comparisons

In the 2-back > baseline contrast, FEM patients showed less deactivation compared to the healthy participants in a large cluster of 665 voxels (Z-value = 3.88) at the left mPFC, including the ACC (x = -10, y = 41, z = 3; BA32) and the left medial FPC (x = -4, y = 50, z = -6; BA10) (Figure 1). The healthy participants did not show greater activation than FEM patients in any brain region.

A significant positive correlation was observed between the mean signal change in the left ACC and the positive PANSS score (r = 0.46, P = 0.024) that did not survive Bonferroni correction.

PPI results

Based on the results of the fMRI analysis, the left medial FPC (BA10) was chosen as the central area for the PPI analysis. The seed voxel was centred on the Talairach coordinates (x = -4, y = 50, z = -6) identified by the effect of diagnosis.

Within-group PPI analyses showed significant positive interaction in healthy participants between WM (2-back > baseline) and the left ventromedial prefrontal cortex (vmPFC) in regions belonging to the DMN such as the dorsal mPFC (medial frontal BA9, and anterior cingulate BA24) and the posterior cingulate (BA31). Positive interactions were also found in the premotor cortex (precentral gyrus, BA6), the superior parietal lobule, the fusiform gyrus, and the thalamus (Table 3). In FEM patients, we also observed significant positive connectivity between the left FPC and most of the mentioned regions except for the fusiform and the thalamus (Table 3).

Group comparisons revealed a significant difference in functional connectivity during 2-back between the left FPC seed and the superior frontal gyrus (x = -20, y = 16, z = 53, Z-value = 4.62; BA8), with FEM patients showing increased connectivity compared to healthy participants (Figure 2).

DISCUSSION

The structural analysis did not show any significant volumetric differences in FEM bipolar patients. Even when a more liberal statistical threshold was used, no differences were found between groups. Three previous meta-analyses in BD found lateral ventricle enlargement (22) and volumetric reductions in the left rostral anterior cingulate cortex (ACC) (19) and the right fronto-insular cortex (19-20). A recent study by the ENIGMA consortium found reduced cortical gray matter thickness in the frontal, temporal and parietal regions (24). However, it is not clear if all these findings were present at the onset of the illness as volumetric studies in FEM patients

show conflicting results. On the other hand, a meta-analysis conducted with FEM studies did not find changes in total grey matter volume, but only 4 studies and 66 patients were analyzed (28). In fact, most volumetric studies in FEM patients included relatively small samples, usually ranging from 12 to 24 patients. A considerable risk of both type I (false positives) and type II (false negatives) errors (22) could partially explain the differences among studies. In this context, we acknowledge our sample size as a limitation as it is still far from avoiding these errors, especially type II errors, even if no differences between groups emerged even when the analysis was uncorrected. Nonetheless, this can also be considered a relative strength as this is the largest sample in structural neuroimaging with FEM patients described so far. The study by Koo et al (45) included 38 patients, but the sample was restricted to patients with psychosis, which entails a risk of bias by selecting the most severe subgroup of bipolar patients. Half of our sample had a depressive onset and therefore presented several years of illness. However, this did not have an impact on volumetric measurements and reinforces our finding that volumetric abnormalities are not present at the beginning of BD. Instead, they may appear as a consequence of the course of the illness, reflecting its underlying neurobiological progression (21).

Regarding the functional analysis, to our knowledge, this is the first fMRI study assessing WM in FEM patients. During the 2-back task similar regions were activated in both FEM patients and healthy individuals, including the dlPFC, the lateral FPC, the inferior and superior parietal lobules, the insula, and the cerebellum. However, while a set of regions belonging to the DMN, such as the vmPFC, the PCC, precuneus, and the lateral temporal cortex were deactivated in healthy individuals, only a much smaller region in the PCC was deactivated in FEM patients (Table 2). In the between-group comparison, FEM patients showed a pattern of poor deactivation in a region corresponding to the anterior hub of the DMN, also known as ventral mPFC, comprising the left ventromedial FPC, and the ACC (ventral and dorsal) (Figure 1).

Brain activations in the DMN regions are greater during rest than during engagement in a broad range of goal-directed tasks. The DMN is hypothesized to mediate taskindependent or intrinsic thought rather than task-dependent or extrinsic stimulus processing. In the healthy brain, greater suppression of the default network is associated with better memory formation (46). As a task becomes more difficult, DMN suppression increases as if attentional resources are allocated away from intrinsic thoughts and toward difficult extrinsic tasks (11). Poor deactivation of the DMN during WM tasks has been found in schizophrenia (47-48) and schizoaffective disorder (49). In BD, it has been reported in acute mania (12), depression (13), and euthymia as a persistent trait-like feature (14). In our analysis, only the anterior hub of the DMN was found to be differentially deactivated in FEM patients. No significant differences in deactivation in other regions of the DMN were found.

The DMN is anticorrelated with an external attention system (EAS) that mediates attention to exogenous stimuli (7-8). The EAS is a broad task-positive and extrinsic network in which subsystems have been described. It comprises regions of lateral prefrontal and parietal cortex, dorsal anterior cingulate, and anterior insula/frontoopercular regions, implicated in attentional and cognitive control functions (9). The DMN and the EAS systems are believed to shift activations in an opposite direction balance depending on the type of mental activity the individual is involved in (7) although they can sometimes interact cooperatively (9). It is noteworthy that in our study only the DMN side of this balance was impaired. FEM patients did

not show differences compared with controls in relation to the pattern of activations, including key regions for WM tasks such as the dlPFC (50), unlike previous studies in mania which have described hypoactivation of the dlPFC during the n-back task (12, 51). The dlPFC is believed to play an essential role in increasing task performance during WM tasks (50). However, our result is consistent with the absence of significant differences in task performance. It is also consistent with a previous study in which psychotic FEM subjects did not show impairment in WM (52). On the other hand, recent studies with the n-back task found an increased activation of the dlPFC in mostly euthymic bipolar patients (18, 53), suggesting inefficient processing. There were no differences in performance in one of the studies (18) whereas BD patients performed worse than healthy controls but better than schizophrenics in the second study (53). Altogether, our findings suggest that dlPFC functioning is not impaired at this early stage of the illness. Thus, the impairment observed in other studies could be attributed to the progression of the illness, although an alternative explanation is that dlPFC dysfunction is subtle in BD and only becomes apparent with high mental loads (54).

It could be hypothesized that DMN dysfunction might be related to the symptoms of mania, such as distractibility. However, failure of deactivation has also been documented in depressed bipolar patients (13) and other conditions, and therefore, it is more likely to represent an interference in concentration secondary to different clinical states. Reassessment of patients after the remission of the manic episode could help to distinguish whether it is a state-dependent phenomenon or an initial disturbance of the DMN activity that might represent a core pathophysiological abnormality in the cascade of bipolar illness. The scanning of chronic BD subjects in mania, depression, and euthymia supports the latter view, as failure of deactivation in the ventromedial frontal cortex has been found in all three states (14).

The functional connectivity analysis focused on the vmFPC. As expected, healthy individuals showed a pattern of increased connectivity in two other regions of the DMN, the nearby dmPFC (10) and the PCC. FEM patients' pattern was quite similar, which involves preservation of the functional connectivity within the DMN. As disturbances in connectivity between vmPFC and regions such as PCC have been observed (55), we could hypothesize that they would appear along the progression of the illness into more advanced stages. However, FEM patients showed additional increased connectivity with the superior frontal gyrus (BA8) that was not observed in healthy individuals. Besides including the frontal eye field, activation of this area has been shown to increase when test subjects experience uncertainty (56). This could be an indirect sign of an increased level of demand and difficulty set by the WM task in FEM patients, even if they achieve a normal performance.

The fact that half of the sample had a depressive onset and, therefore, were not studied right at the beginning of the bipolar illness could be considered a limitation. However, the approach of excluding these patients would involve selecting a subgroup of patients with different clinical features (26) that would become a bias as well. In fact, following the DSM definition, it is not possible to make a diagnosis of BP in patients with no manic or hypomanic episodes in the past. Another limitation is that the patients were not drug-naive. Conducting neuroimaging studies with drug-naive bipolar I patients, especially if manic, is a great challenge for researchers. A recent study included 20 drug-naive BD individuals, but only bipolar II and not otherwise specified patients were included (57). Nevertheless, the individuals in our sample had been

treated for the current manic episode for no longer than 3 weeks, and the impact of the medication, especially in the structural data, was very limited. However, the impact of sedative drugs, mainly antipsychotics and benzodiazepines, on vigilance should be taken into account, as lower levels of vigilance assessed through EEG involve changes in the BOLD signal in fMRI (58). All patients but one were treated with antipsychotics, and about one third received more sedative antipsychotics, mainly olanzapine. And 25% of the patients also received benzodiazepines. Moreover, mania itself is associated with vigilance dysregulation (59). Nevertheless, performance control in the n-back task with the sensitivity index, excluding patients with a poor performance in the task should limit the impact of this phenomenon. The sample size is a previously mentioned limitation, especially regarding the structural analysis, even if it is large compared to other studies in FEM.

Longitudinal studies are encouraged in order to discriminate what changes are secondary to the course of the illness, to the treatment, or are just epiphenomenon (21). From the structural point of view, it would be advisable to reassess the sample in 2 to 5 years time in order to assess the development of possible volumetric abnormalities. Regarding the functional study, reassessment of FEM patients after remission of the manic episode would help to understand if the DMN abnormality observed is state- or trait-related and persistent from this early stage.

In summary, we did not find volumetric differences in a sample of FEM patients suggesting that these changes may be more related to the progression of the illness. However, in the first fMRI study using a WM paradigm, FEM patients showed poor deactivation in the anterior hub of the DMN, implicating this network in the early pathophysiology of mania and BD. No differences were observed in activation of dlPFC or any other region. On the other hand, functional connectivity within the DMN was preserved but FEM patients showed increased connectivity between the vmPFC and the superior frontal gyrus.

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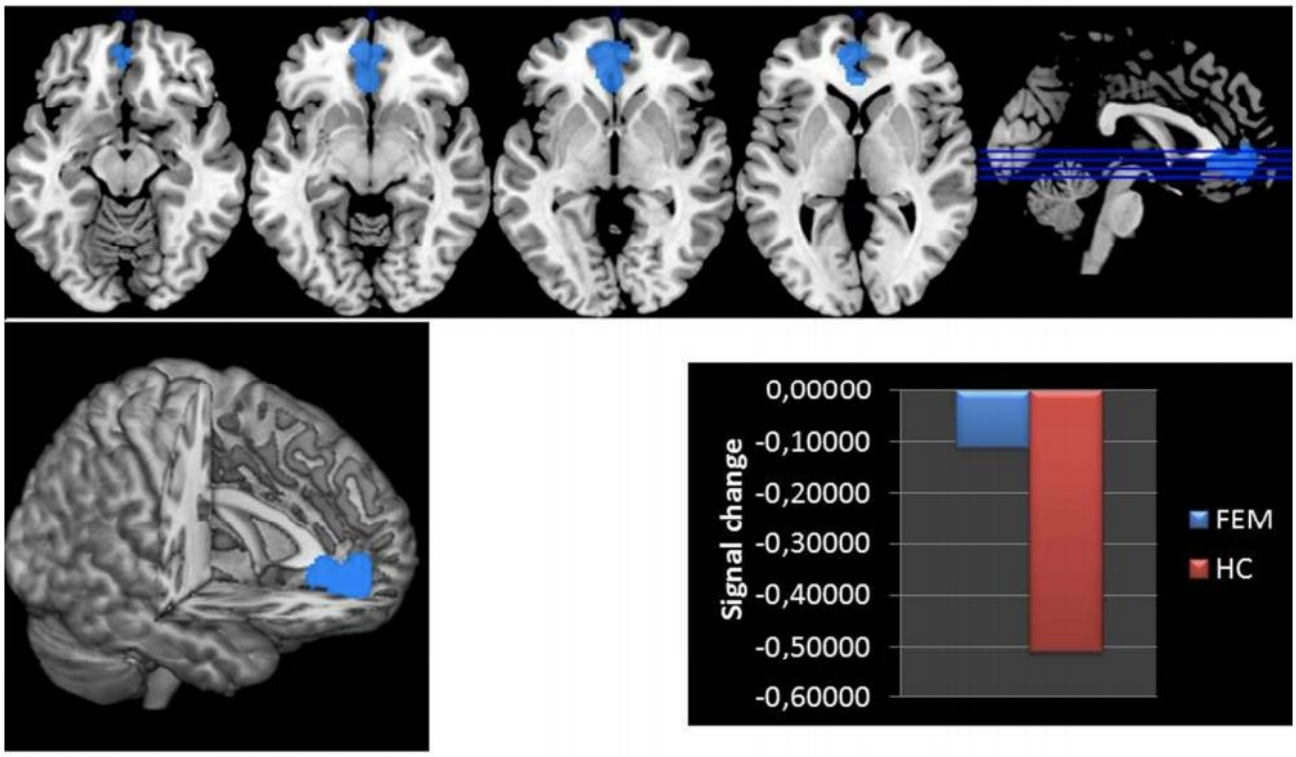

Figure 1a. Brain regions showing significant differences between FEM patients and controls for the 2-back vs baseline contrast. Areas where the patients showed less deactivation are shown in blue.
a) Axial sections
b) Render image

Figure 1b. Signal change for the 2-back vs baseline contrast in FEM vs. HCs (at -4, 50, -6) (BA10)

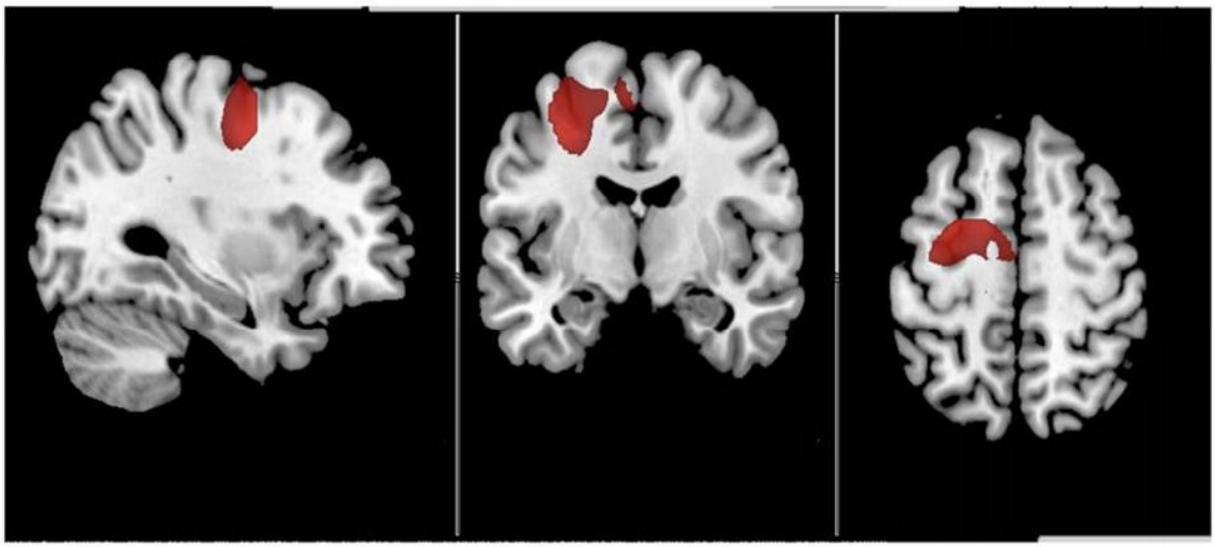

Figure 2. PPI analysis. Between group comparison: FEM patients showed significantly increased connectivity during 2-back between the left mFPC seed and the superior frontal gyrus (x = -20, y = 16, z = 53; BA8) (red coloured) compared to healthy participants.

Table 1. Demographic, behavioural and clinical characteristics of the sample

Characteristics	FEM patients (N=31)	Healthy Controls (N=31)	P - value
Demographic and behavioural			
Age (y)	30.52 (9.05)	31.06 (8.76)	0.809
Sex (male/female)	16/15	16/15	1
Years of education	13 (3.75)	14.13 (4.33)	0.27
Premorbid IQ estimation from TAP	100.79 (11.7)	104.35 (7.01)	0.195
Performance 2-back task	2.72 (1.07)	3.167 (1.19)	0.173
Clinical			
Age of onset of BD (y)	27.28 (6.78)	-	-
Duration of BD (y)	3.33 (4.24)	-	-
First episode polarity, depression, N (%)	16 (51.6)	-	-
Duration of episode (days)	36.83 (23.79)	-	-
Psychotic symptoms in current episode, N (%)	23 (74.19%)	-	-
YMRS score	18.03 (8.06)	-	-
MADRS score	4.3 (4.17)	-	-
General PANSS score	53.4 (13.31)	-	-
Positive PANSS score	16.63 (4.99)	-	-
Negative PANSS score	10.57 (5.08)	-	-
Pharmacological		-	-
Lithium N (%)	14 (45.2%)	-	-
Valproate N (%)	8 (25.8%)	-	-
Antipsychotics N (%)*	30 (96.77%)	-	-
Atypical Antipsychotics N (%)	29 (93.54%)	-	-
Typical Antipsychotics N (%)	4 (12.9%)	-	-
Benzodiazepines, N (%)	8 (25.8%)	-	-
Antipsychotic medication (mg/day; CPZE)	387.681 (205.35)	-	-

Note: Except for sex, and otherwise stated, data are presented as mean (SD).

BD: Bipolar Disorder; **CPZE:** chlorpromazine equivalents; **MADRS:** Montgomery–Åsberg Depression Rating Scale; **N:** number of sample; **PANSS:** Positive and Negative Syndrome Scale; **y:** years; **TAP:** Test de Acentuación de Palabras (Word Accentuation Test); **YMRS:** Young Mania Rating Scale

* Antipsychotics: Risperidone 14 subjects, Olanzapine 9, Aripiprazole 5, Asenapine 1, Zuclopenthixol 1, Haloperidol 3 (on top of olanzapine).

Table 2. Voxel-Based Whole-Brain Analysis: Clusters of Significant Task-Related Activation and Deactivation During the Two-Back vs. Baseline (FWE, P<0.05)

Region	Gyrus	Laterality	Brodmann Area	Talairach and Tournoux Coordinates			Z-value
				x	y	z	
Healthy Participants							
Activation							
Frontal	Middle frontal	Left	6	-30	-3	50	6.63
		Right	6	36	-2	42	6.05
		Right	46	50	28	24	4.88
		Right	10	36	42	18	4.84
Parietal	Inferior parietal lobule	Right	40	46	-41	44	6.92
	Superior parietal lobule	Left	7	-28	-58	45	6.35
Temporal	Insula	Left	13	-36	18	8	5.91
		Right	13	32	23	3	5.69
	Inferior temporal	Left	19	-38	-60	0	5.77
Cerebellum		Left	N/A	-4	-53	-14	5.52
		Right	N/A	28	-63	-22	5.38
Deactivation							
Frontal	Medial frontal	Left	10	-2	48	-6	5.63
Limbic	Anterior Cingulate	Right	32	2	39	11	5.44
		Left	24	-6	37	4	5.34
	Posterior cingulate	Left	31	-10	-55	29	5.68
		Right	31	2	-45	30	5.27
Parietal	Precuneus	Right	31	14	-57	25	5.18
	Angular	Left	39	-46	-70	35	5.25
Temporal	Superior Temporal	Right	38	30	14	-29	5.34
		Left	38	-30	10	-31	5.23
FEM subjects							
Activation							
Frontal	Middle frontal	Left	6	-42	0	48	6.44
	Inferior frontal	Right	6	46	7	31	5.59
	Middle frontal	Left	46	-48	31	28	5.15
		Left	10	-42	49	3	4.95
Parietal	Inferior parietal lobule	Right	40	50	-37	44	5.72
	Superior parietal lobule	Left	7	-28	-60	49	5.61
Temporal	Insula	Right	13	38	20	3	5.55
	Inferior temporal	Left	19	-46	-62	-2	4.72
Cerebellum		Left	N/A	-30	-61	-24	4.82
Deactivation							
Limbic	Posterior cingulate	Right	31	2	-43	32	4.61

Note: Coordinates refer to the cluster peak voxel in mm in Talairach space; x = sagital plane; y =coronal plane; z = axial plane.

Table 3. Regions expressing functional connectivity between left frontopolar cortical activity and working memory in healthy participants and FEM patients (FWE, $P < 0.05$)

Region	Gyrus	Laterality	Brodmann Area	Talairach and Tournoux Coordinates			Z-value
				x	y	z	
Healthy Participants							
Seed	*Frontopolar*	*Left*	*10*	*-4*	*50*	*-6*	
Frontal	Precentral	Left	6	-22	-24	60	5.62
	Medial	Left	9	-2	46	33	5.15
Parietal	Superior parietal lobule	Right	7	8	-23	45	5.59
Limbic	Cingulate	Right	24	6	0	42	6.05
	Posterior cingulate	Right	31	2	-66	11	5.81
		Left	31	-2	-35	39	5.47
Temporal	Fusiform	Left	37	-51	-55	-16	5.36
Thalamus		Left	N/A	-4	-30	18	6.02
FEM subjects							
Seed	*Frontopolar*	*Left*	*10*	*-4*	*50*	*-6*	
Frontal	Middle frontal	Left	8	-30	21	41	5.38
	Superior frontal	Right	6	4	26	54	5.08
Limbic	Cingulate	Right	24	2	-11	45	5.22
	Posterior cingulate	Left	31	-2	-51	28	5.01
Parietal	Superior parietal lobule	Left	7	-10	-41	43	5.31

Note: Coordinates refer to the cluster peak voxel in mm in Talairach space; x = sagital plane; y =coronal plane; z = axial plane.