**Effects of inflammation, childhood adversity, and psychiatric symptoms on brain morphometrical phenotypes in bipolar II depression**

**Supplementary materials**

1. **Supplementary Methods**
2. **Supplementary statistical analysis**
3. **Supplementary Results**
4. **Supplementary Discussion**
5. **Supplementary Table 1**
6. **Supplementary Table 2**
7. **Supplementary Table 3**
8. **Supplementary Table 4**
9. **Supplementary Figure 1**
10. **Supplementary Figure 2**
11. **Supplementary Figure 3**

**Supplementary Methods**

1. ***The inclusion criteria of BDII-D patients and HCs***

The inclusion criteria of BDII-D patients were as follows: (1) aged from 15 to 65; (2) right-handed; (3) met the DSM-5 criteria for BD type II during a depressive episode; (4) no MRI contraindication; (5) no anti-inflammatories in the past 4 weeks; (6) no history of electroconvulsive therapy within the last 12 months; (7) no combination of obsessive-compulsive disorder, panic disorder, axis-II psychiatric disorders, neurological disorder, alcohol/substance abuse, history of brain trauma, or pregnancy. HCs were recruited via posters and advertisements in the community and were screened by the Structured Clinical Interview for DSM-5 Nonpatient Edition to ensure that they were free of any psychiatric disorders. The inclusion criteria for HCs were as follows: (1) aged from 15 to 65; (2) right-handed; (3) screened by the Structured Clinical Interview for DSM-V Nonpatient Edition; (4) no MRI contraindication; (5) no history of psychiatric illness in first-degree relatives; (6) no current or past significant medical, neurological illness or chronic inflammatory disease; (7) not catching a cold or acute infection in the past 4 weeks.

1. ***Medicine load calculation***

We used a strategy developed by Hassel et al (Hassel et al., 2008). for measuring total medication load in bipolar individuals by coding the dose of each antidepressant, mood stabilizer, antipsychotic and anxiolytic medication as absent = 0, low = 1, or high = 2. For antidepressants and mood stabilizers, we converted each medication into low- or high-dose groupings using a previously employed approach (Almeida et al., 2009; Phillips, Travis, Fagiolini, & Kupfer, 2008; Sackeim, 2001). Individuals on Levels 1 and 2 of these criteria were coded as low dose, and those with Levels 3 and 4 as high dose. We added a no-dose subtype for those not taking these medications. The antipsychotic doses were converted into chlorpromazine dose equivalents and were coded as 0, 1, or 2, for no medication, chlorpromazine equivalents dose equal or below, or above the mean effective daily dose (ED50) of chlorpromazine as defined by Davis and Chen (Davis & Chen, 2004). The chlorpromazine dose equivalents were calculated by R package “chlorpromazineR”. Alprazolam and lorazepam doses were similarly coded as 0, 1, or 2, concerning the midpoint of the Physician's Desk Reference recommended daily dose range. A composite measure of the total medication load was generated, reflecting the dose and variety of different medications taken, by summing all individual medication codes for each medication category for each bipolar participant.

1. ***VBM and SBM analysis***

First, the T1 images were normalized using the Montreal Neurological Institute's MNI152 template and then segmented into several tissue types including gray matter, white matter, cerebrospinal fluid, etc. During the segmentation process, the signal bias, noise, and global intensities were also corrected. To ensure the quality of the images, and scan with excessive movement (indicated by a blurry scan) or artifacts present were removed. For quality assurance, all segmented anatomical scan files were checked and only scans equal to or over 80% of the acceptable quality assurance threshold will be included in the final analyzed sample. The modulated images were then smoothed with an 8-mm full-width at a half-maximum Gaussian kernel for VBM. Finally, the total intracranial volume (TIV) was extracted from the data as a covariate in statistical steps.

For SBM analysis, default parameters in standard-protocol accordance (http://www.neuro.uni-jena.de/cat12/CAT12-Manual.pdf) were used in segmentation, surface estimation, data resampling (freesurfer 164k), and smooth. Extracted surface parameters included the thickness, gyrification measuring surface complexity in 3D, sulcus depth, and cortical complexity. As recommended, the cortical thickness maps were smoothed with a 15-mm full-width at half-maximum Gaussian kernel while maps of surface complexity and cortical gyrification were smoothed with a 20-mm full-width at half-maximum Gaussian kernel (Luders et al., 2006; Yotter, Nenadic, Ziegler, Thompson, & Gaser, 2011). The surface data were visually inspected for artifacts and homogeneity and the overall image quality was checked in statistical quality control. Desikan-Killiany DK40 Atlas was used to present the results of the SBM analysis.

**Supplementary** **statistical analysis**

***1. VBM differences for*** ***unmedicated BDII-D and HCs***

The set of general linear models (GLMs) with a two-tailed t-test between unmedicated BDII-D and HCs was generated with age, sex, education level, and TIV as covariates by using SPM 12. We used False Discovery Rate (FDR) correction with a p < 0.01 at the voxel level to identify the GMV differences between unmedicated BDII-D patients and HCs. The extent threshold was set at 40 voxels.

***2. SBM differences for unmedicated BDII-D and HCs***

The two-tailed t-test between unmedicated BDII-D and HCs was generated with age, sex, and education level. We initially used False Discovery Rate (FDR) correction with a p < 0.05 at the vertex level to identify the cortical differences between unmedicated BDII-D and HCs. The extent threshold was set at 10 vertices. However, there was no significant difference. So, we used uncorrected p < 0.001 to show the potential different cortical thickness between unmedicated BDII-D and HCs.

**Supplementary Results**

1. ***Group differences in brain phenotypes between unmedicated BDII-D and HCs***

We also performed the VBM analysis (**Supplementary Table 1**) and SBM analysis (**Supplementary Table 2**) between BDII-D patients without medication and HCs. For GMVs, although some minor differences were found between unmedicated BDII-D patients and all BDII-D patients when compared with HCs, the different brain regions in both comparisons were concentrated in the cerebellum, temporal and frontal gyrus, caudate, rectus gyrus, and insula. For cortical thickness, the different brain regions between unmedicated BDII-D patients and HCs were consistence with the main analysis, although this result did not survive the FDR correction threshold.

1. ***Preliminary exploration of brain phenotypes correlations with childhood adversity in BD***

Preliminarily, we observed correlations between higher emotional abuse score (r = -0.190, p = 0.022, q = 0.066) and total childhood adversity score (r = -0.168, p = 0.044, q = 0.088) and smaller mean volume of right MFG. We also found associations between higher sexual abuse score and smaller GMV of right medial SFG (r = -0.179, p = 0.031, q = 0.456) and as well as between higher emotional abuse score and smaller mean GMV of the right insula (r = -0.176, p = 0.034, q = 0.510). Besides, we found correlations between larger mean volumes of right orbital MFG and higher emotional neglect score (r = 0.207, p = 0.013, q = 0.097) and total childhood adversity score (r = 0.191, p = 0.021, q = 0.105) **(Figure 2A)**. However, no correlations survived multiple comparison corrections.

In addition, we also found that the thinner left inferior temporal thickness was correlated with higher CTQ total scores (r = -0.181, p = 0.029, q = 0.1465) and greater emotional neglect (r = -0.173, p = 0.039, q = 0.146), greater sexual abuse (r = -0.177, p = 0.033, q = 0.146), and greater emotional abuse (r = -0.172, p = 0.038, q =0.146), however, no correlations survived multiple comparison corrections **(Figure 2B)**.

1. ***Preliminary exploration of brain phenotypes correlations with inflammatory cytokines in BD***

We also identified several associations between inflammation and brain phenotype, but these results did not survive multiple comparison correction. The associations were preliminarily found between higher CRP and smaller GMVs in left MTG (r = -0.174, p = 0.037, q = 0.108), left orbital IFG (r = -0.169, p = 0.042, q = 0.108), right inferior cerebellum (r = -0.183, p = 0.028, q = 0.108), right insula (r = -0.179, p = 0.031, q = 0.108), right triangular IFG (r = -0.164, p = 0.049, q = 0.108), and left MFG (r = -0.164, p = 0.048, q = 0.108) (**Supplementary Figure 1A**). We also observed associations between the higher counts of WBC and smaller GMVs in the left middle temporal pole (r = -0.179, p = 0.031, q = 0.138), left MFG (r = -0.173, p = 0.037, q = 0.138), right orbital IFG (r = -0.164, p = 0.049, q = 0.147), and right caudate (r = -0.195, p = 0.019, q = 0.138) (**Supplementary Figure 1B**). Higher IL-1β (r = -0.167, p = 0.045, q = 0.42) was also preliminarily found associated with smaller GMV in left MFG (**Supplementary Figure 1C**). Besides, higher NTL was found to be associated with smaller GMV in the right orbital MFG preliminarily (r = -0.179, p = 0.031, q = 0.465; **Supplementary Figure 1D**).

As for surface-based brain phenotypes, we preliminarily found correlations between higher WBC and thinner right rostral middle frontal thickness (r = -0.198, p = 0.029, q = 0.270) and left lateral orbitofrontal thickness (r = -0.173, p = 0.017, q = 0.255) (**Supplementary Figure 2A**). We also observed associations between higher IL-6 and thinner left lateral orbitofrontal thickness (r = -0.180, p = 0.038, q = 0.45) (**Supplementary Figure 2B**), and higher CPR and thinner left rostral middle frontal thickness (r = -0.183, p = 0.027, q = 0.405) (**Supplementary Figure 2C**). However, no correlations between surface-based brain phenotypes and inflammatory cytokines survived multiple comparison corrections.

1. ***Preliminary exploration of brain phenotypes correlations with psychiatric symptoms in BD***

We also preliminarily explored associations between significantly different GMV and inflammation and psychiatric symptoms (including depressed mood, anxiety mood, and psychotic symptoms) in the BD II depression group.

Preliminarily, we found higher PANSS positive score was correlated with larger GMVs of the left rectus (r=0.187, p=0.022, q=0.165) (**Supplementary Figure 3A**). Similarly, a higher PANSS negative score was associated with the GMVs of left MTG (r=0.188, p=0.021, q=0.195), right MFG (r=0.182, p=0.026, q=0.195), and right orbital IFG (r=0.163, p=0.046, q=0.198) (**Supplementary Figure 3B**). Besides, relationships were found between higher PANSS total score and larger GMV of the left middle temporal pole (r=0.186, p=0.023, q=0.308) as well as GMV of right orbital IFG (r=0.167, p=0.041, q=0.308) in BD II depression (**Supplementary Figure 3C**).

In addition, a relationship was found between higher HAMD score and smaller mean GMVs of left orbital IFG (r=-0.167, p=0.044, q=0.24) and right caudate (r=-0.184, p=0.027, q=0.24), respectively (**Supplementary Figure 3D**). The higher HAMA score was also associated with smaller mean GMV of the right caudate (r=-0.207, p=0.012, q=0.155), while significant associations were found between a higher HAMA score and larger right middle temporal pole (r=0.179, p=0.031, q=0.155) as well as right orbital IFG (r=0.183, p=0.028, q=0.155), respectively (**Supplementary Figure 3E**). However, no correlations survived multiple comparison corrections.

**Supplementary Discussion**

In the BDII-D group, we observed severer depression was correlated with smaller GMV in the right caudate and orbital IFG. However, a severe anxious mood presented relationships with smaller GMV in the right caudate but larger GMV in the right orbital IFG and temporal pole. It should be noted, because the correlation results did not survive multiple comparison corrections, our study only provided correlation trends. Several studies have reported that orbital IFG is involved in affective processes and cognitive control, and reduced volume or cortical thickness in orbital IFG is closely related to suicide behavior (Kuusinen, Cesnaite, Peräkylä, Ogawa, & Hartikainen, 2018; Wagner et al., 2012). The caudate nucleus is a pair of brain structures that make up part of the basal ganglia involved in movement control, cognitive function, and emotions (Grahn, Parkinson, & Owen, 2008; Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008). We speculated that the correlation between reduced orbital IFG and caudate volumes and severer depressive symptoms may underlie the impaired cognitive regulation of emotion and lay potentially relevant to hopeless feelings implicated in the pathophysiology of BDII-D. However, the exact mechanism of the positive correlation between higher HAMA scores and larger GMV in the right orbital IFG and the temporal pole is not entirely clear yet. It needs to be further verified by other studies.

Interestingly, unexpected results were found in significant relationships between worse positive symptoms and larger GMV in the temporal pole. Although the majority of studies demonstrated severer psychotic symptoms showed smaller GVM in psychotic disorders (Lui et al., 2009). However, a study found a similar trend of relationship to our study between GMV and PANSS core in schizophrenia comorbid with depressive symptoms (Wei, Ge, Chen, Cao, & Zhang, 2021). We speculated here that psychotic symptoms combined with depressive symptoms may increase GMV in some brain regions and whether the nerve remodeling involves still needs further clarification.

**Reference**

Almeida, J. R., Akkal, D., Hassel, S., Travis, M. J., Banihashemi, L., Kerr, N., . . . Phillips, M. L. (2009). Reduced gray matter volume in ventral prefrontal cortex but not amygdala in bipolar disorder: significant effects of gender and trait anxiety. *Psychiatry Research, 171*(1), 54-68. doi:10.1016/j.pscychresns.2008.02.001

Davis, J. M., & Chen, N. (2004). Dose response and dose equivalence of antipsychotics. *Journal of Clinical Psychopharmacology, 24*(2), 192-208. doi:10.1097/01.jcp.0000117422.05703.ae

Grahn, J. A., Parkinson, J. A., & Owen, A. M. (2008). The cognitive functions of the caudate nucleus. *Progress in Neurobiology, 86*(3), 141-155. doi:10.1016/j.pneurobio.2008.09.004

Hassel, S., Almeida, J. R., Kerr, N., Nau, S., Ladouceur, C. D., Fissell, K., . . . Phillips, M. L. (2008). Elevated striatal and decreased dorsolateral prefrontal cortical activity in response to emotional stimuli in euthymic bipolar disorder: no associations with psychotropic medication load. *Bipolar Disorders, 10*(8), 916-927. doi:10.1111/j.1399-5618.2008.00641.x

Kuusinen, V., Cesnaite, E., Peräkylä, J., Ogawa, K. H., & Hartikainen, K. M. (2018). Orbitofrontal Lesion Alters Brain Dynamics of Emotion-Attention and Emotion-Cognitive Control Interaction in Humans. *Frontiers in Human Neuroscience, 12*, 437. doi:10.3389/fnhum.2018.00437

Luders, E., Thompson, P. M., Narr, K. L., Toga, A. W., Jancke, L., & Gaser, C. (2006). A curvature-based approach to estimate local gyrification on the cortical surface. *Neuroimage, 29*(4), 1224-1230. doi:10.1016/j.neuroimage.2005.08.049

Lui, S., Deng, W., Huang, X., Jiang, L., Ma, X., Chen, H., . . . Gong, Q. (2009). Association of cerebral deficits with clinical symptoms in antipsychotic-naive first-episode schizophrenia: an optimized voxel-based morphometry and resting state functional connectivity study. *American Journal of Psychiatry, 166*(2), 196-205. doi:10.1176/appi.ajp.2008.08020183

Phillips, M. L., Travis, M. J., Fagiolini, A., & Kupfer, D. J. (2008). Medication effects in neuroimaging studies of bipolar disorder. *American Journal of Psychiatry, 165*(3), 313-320. doi:10.1176/appi.ajp.2007.07071066

Sackeim, H. A. (2001). The definition and meaning of treatment-resistant depression. *Journal of Clinical Psychiatry, 62 Suppl 16*, 10-17.

Wager, T. D., Davidson, M. L., Hughes, B. L., Lindquist, M. A., & Ochsner, K. N. (2008). Prefrontal-subcortical pathways mediating successful emotion regulation. *Neuron, 59*(6), 1037-1050. doi:10.1016/j.neuron.2008.09.006

Wagner, G., Schultz, C. C., Koch, K., Schachtzabel, C., Sauer, H., & Schlösser, R. G. (2012). Prefrontal cortical thickness in depressed patients with high-risk for suicidal behavior. *J Psychiatric Research, 46*(11), 1449-1455. doi:10.1016/j.jpsychires.2012.07.013

Wei, G. X., Ge, L., Chen, L. Z., Cao, B., & Zhang, X. (2021). Structural abnormalities of cingulate cortex in patients with first-episode drug-naïve schizophrenia comorbid with depressive symptoms. *Human Brain Mapping, 42*(6), 1617-1625. doi:10.1002/hbm.25315

Yotter, R. A., Nenadic, I., Ziegler, G., Thompson, P. M., & Gaser, C. (2011). Local cortical surface complexity maps from spherical harmonic reconstructions. *Neuroimage, 56*(3), 961-973. doi:10.1016/j.neuroimage.2011.02.007