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Differential levels of inflammatory and neuroendocrine markers in the hippocampus and anterior cingulate cortex of bipolar disorder subjects: a post-mortem study

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1. Introduction

Bipolar disorder (BD) is a severe and recurrent neuropsychiatric illness that causes high rates of disability, with onset frequently during adolescence and early adulthood¹. Devastating health and social consequences of BD are associated with the fact that the illness is often misdiagnosed, which leads to inappropriate or delayed treatments^{2,3}. Misdiagnosis is also a consequence of the fact that the etiology of BD still poorly understood.

Disturbances in multiple systems have been proposed to play a role in BD pathophysiology, including metabolic, neuroendocrine and immune-inflammatory^{4–6}. Metabolic comorbidities are associated with BD burden, such as diabetes, obesity and cardiovascular diseases, supporting the role of systemic inflammation in BD^{7–9}. In two different meta-analysis, elevated peripheral levels (serum or plasma) of pro-inflammatory cytokines IL-4, IL-6, IL-10, and TNF- α were found in BD patients, when compared to controls^{10,11}. In a meta-analytic study comparing mood episodes, increased levels of peripheral IL-6 were found in mania. In addition, increased levels of TNF- α were found in mania and depression, and increased levels of IL-6 and C-reactive protein (CRP) were

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found in euthymia¹². CRP is an inflammatory factor related to acute immune response¹³ that has been investigated previously in the blood of BD patients¹⁴. Higher levels of CRP were also associated with cognitive dysfunction in BD¹⁵. Cortisol is a glucocorticoid closely involved in inflammation and cytokine regulation¹⁶. Increased cortisol levels in serum/plasma or urine have been found in BD, especially during mania and euthymia¹⁷, supporting a contribution of the hypothalamic–pituitary–adrenal (HPA) axis in this disorder.

Although several shreds of evidence from peripheral measures strongly support the role of inflammation and neuroendocrine changes in BD, the consequences of these systemic alterations to the brain and the potential presence of neuroinflammation still poorly understood. A recent meta-analysis conducted in BD post-mortem brains revealed important evidence of neuroinflammation regarding cell types and molecules involved¹⁸. Among the addressed molecular parameters, the cytokines TNF- α , IL-1 β , and IL-6 were found to be increased in the pre-frontal cortex of BD patients¹⁹. For the purpose of this study, that meta-analysis revealed that the majority of the post-mortem studies in BD brains addressed frontal cortex. There has not been any study focusing on the same inflammatory and neuroendocrine factors in different brain regions. Furthermore, IL-17, CRP and cortisol have not been evaluated in post-mortem brain samples of BD patients. Here, we investigated different neuroinflammatory markers, including a panel of cytokines (IL-1 β , IL-6, IL-10, IL-17A and TNF- α) and CRP, as well as cortisol levels in the hippocampus and anterior cingulate cortex (ACC) in post-mortem brains of BD subjects. These two brain regions have been consistently investigated and implicated in the pathophysiology via neuroimaging studies^{20,21} and are morphologically and biochemically altered in BD showing volume reduction and glutamatergic abnormalities^{22,23}

Methods and materials:

2.1 Participants

This study was conducted in deceased subjects who underwent autopsy at the Sao Paulo Autopsy Service between 2009 and 2016, whose family voluntarily donated the brain to the Biobank for Aging Studies (BAS). Sao Paulo Autopsy Service is a community-based autopsy service responsible for issuing death certificates for subjects who died from natural causes within Sao Paulo city. Death certificates are mandatory in Brazil when the cause of natural death was not determined. After agreeing to participate in the study, the next of kin signed the informed consent, reported the clinical history and donated the brain of the deceased subject. Detailed BAS methodological procedures have been described elsewhere²⁴. Our inclusion criteria were subjects above 18 years old with a non-traumatic cause of death and post-mortem interval (PMI) less than 24 hours with a knowledge informant. A knowledgeable informant was a close family member or caregiver that had at least one weekly contact with the deceased in the last six months before death and was able to recount and provide details of the deceased's health information^{24,25}. The local ethics committee approved the research protocol. Cases with no reliable informant, pH of the cerebral spinal fluid (CSF) ≤ 6.5 , significant cerebral lesions, prolonged agonal state, auto-immune diseases, and chronic inflammatory systemic diseases, such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, scleroderma, ulcerative colitis and Crohn's disease,

sepsis as cause of death, or subjects who were taken corticoids or immunosuppresses were excluded. We used a convenience sample²⁶ to include all subjects with a clinical history of bipolar disorder and selected controls with similar sex, age, cognitive impairment status, and educational background.

2.2 Clinical postmortem evaluation

A validated semi-structured interview was conducted with a knowledgeable informant. The interview contained several instruments to collect information on the clinical and functional status of the deceased subject.

Psychiatric history was assessed through the Structured Clinical Interview for Axis I DSM-IV Disorders (SCID)²⁷. If subjects fulfilled any criteria for a psychiatric disorder, according to SCID, an additional interview was conducted to obtain detailed information regarding the clinical course, including treatments, suicide attempts and other clinical variables. The diagnosis of BD was performed through the SCID, according to the informant, and the medical records, when available. Diagnosis of BD was then confirmed based on the final consent between two additional psychiatrists who were blinded to the initial diagnostic hypothesis (consisting of the best-estimate diagnostic, as previously described²⁶). Participants in the control group did not have any mood episodes during their lifetime or any psychiatric diagnosis as screened through the SCID.

Cognitive function was assessed using the Clinical Dementia Rating (CDR)²⁸ and the Questionnaire on Cognitive Decline in the Elderly (IQCODE)^{29,30}, both based on the informant and validated for post-mortem use²⁵. Cognitive impairment was detected by either CDR >0.5 or IQCODE ≥ 3.4 ³¹. Additionally, demographic, death-related conditions, previous medical history (hypertension, diabetes mellitus, coronary artery disease, current alcohol, and tobacco use), and medical treatments were evaluated. Moreover, reported history of pharmacological treatment included all medications taken for the treatment of BD.

Body mass index (BMI in kg/m²) was calculated after measuring the deceased's weight and height in the supine position and without clothes. Post-mortem interval is defined as the time elapsed between the time of death, identified by the coroner in charge, and the beginning of the autopsy.

1.3 Inflammatory markers measurements

During brain processing, different regions from one hemisphere were isolated and snap-frozen at -80°C for biochemical studies. For this study, after thawing, gray matter samples from the anterior hippocampus and ACC (Brodmann area 24) were mechanically homogenized and total protein was extracted using EpiQuik Whole Cell Extraction Kit (Epigentek, Farmingdale, NY, USA) with one modification. Reagents from the extraction buffer of the EpiQuik Whole Cell Extraction Kit interfered in the Enzyme-Linked Immunosorbent Assay (ELISA) readings and were replaced by a phosphate buffer solution (PBS, Sigma-Aldrich, Sao Paulo, Brazil).

Protein concentration was measured using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA USA). Brain homogenates were loaded at a volume of 25 uL per sample for all ILs, 50 uL for cortisol and 100 uL for PCR. Quantitative levels of cytokines (IL-1 β , IL-6, IL-10, IL-17A, TNF- α) were measured using MULTIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel – Immunology Multiplex Assay (HCYTOMAG-60K – Millipore, Darmstadt, Germany), and cortisol using the MULTIPLEX MAP panel HNCSMAG-35K-02 (Millipore, Darmstadt, Germany). The minimum sensibility of cytokine kit ranges from 0.7 to 4.5 $\pm$ 2SD pg/mL depending on the cytokine, the detection range was 3.2 to 10000 pg/mL and the coefficient variation (CV) was 0.21%. For the cortisol, the minimum sensibility 686 $\pm$ 2SD pg/mL, the detection range was 686 to 500000 pg/mL and CV was 0.28%. Levels of CRP were measured using the Sandwich ELISA (Human CRP ELISA Kit – E-EL-H0043 (Elabscience, Houston, Texas, USA), this kit sensibility is 23.2 pg/mL, its detection range is 0.39–25 ng/mL and its CV was 2.83%. The final results for each analyte were acquired after normalization against total protein levels. ELISA and multiplex assays were conducted at once, to avoid batch effects, and by the same technician who were blind to the clinical status of the subjects.

2.4 Statistical analysis

Firstly, we conducted the Kolmogorov-Smirnov Test to test which of the continuous variables from our study followed a normal distribution. Between-group differences (BD or control) of continuous variables (age, education, PMI and BMI) were analysed using the Student's t-test or Mann-Whitney U test when did not follow normal distribution. Categorical variables (sex, race, cognitive impairment, current smoking, current alcohol, hypertension, diabetes mellitus, coronary artery disease, dyslipidemia and cause of death), were compared using the chi-squared test or Fisher's exact test. Cause of death was categorized as "cardiovascular" or "other" (cancer, pneumonia, hepatic cirrhosis and chronic obstructive pulmonary disease).

For the BD group, we also conducted a descriptive analysis for the relevant clinical data available in our files, such as disease duration, psychiatric hospitalization (yes or no), suicide attempt and psychotropic in use. Psychotropic were divided into four groups, benzodiazepine, lithium/valproate/carbamazepine, antipsychotics or other.

After descriptive analysis, we tested whether or not clinical, demographic, or biological variables (systemic hypertension, diabetes mellitus, dyslipidemia, current alcohol, current smoking, cause of death, age, sex, PMI, pH, and BMI) could be associated with levels of inflammatory markers and cortisol regardless of the group (BD or control). Continuous variables were assessed using Spearman's correlation test, and the categorical variables were compared using Mann-Whitney U test or Student's t test. In the BD group, we also evaluated potential associations of levels of inflammatory markers and cortisol with hospitalization, and psychotropic in use using Mann-Whitney U test or Student's t test.

Between-group differences (BD or control) of levels of inflammatory factors and cortisol were analysed using the Student's t-test or Mann-Whitney U test if the variable did not follow normal distribution. We also conducted a backward stepwise linear regression

including pH, PMI, BMI, age, cause of death, systemic hypertension and diabetes mellitus as covariates to account for potential confounders.

The level of significance was set at 0.05. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 23.0 (Inc., Chicago, Illinois, EUA).

2. Results

3.1 Descriptive analysis

From the of 266 subjects screened for psychiatric symptoms in the BAS according to SCID and DSM-IV criteria, 89 received full diagnosis of psychiatric disorder through a second interview with a psychiatrist and, finally 17 had a history of BD. The remaining cases had schizophrenia, major depressive disorder, obsessive-compulsive disorder, panic disorder, generalized anxiety disorder, alcohol or drug dependence.

In the BD group, three cases had no ACC tissue available. Because the number of subjects was different depending on the brain region, we will describe separately the demographics for cases with the hippocampus and ACC regions studied. When compared with controls, both BD groups (hippocampus and ACC areas) were similar for demographic characteristics, BMI, pH, PMI, evaluated brain hemisphere, smoking, and alcohol use, as well as for metabolic disease, including diabetes mellitus, coronary artery disease, dyslipidemia or cause of death (Table 1).

We also included the information on clinical characteristics of BD, such as disease duration, psychiatric hospitalization, psychotropic in use, and suicide attempt (Table 1).

3.2 Influence of demographic, clinical and biological characteristics on inflammatory markers and cortisol in the hippocampus and anterior cingulate

In the hippocampus, pH positively correlated with IL-1 β (Correlation Coefficient [ρ]: 0.348, $p=0.044$) and IL-10 (ρ : 0.376 and $p=0.029$). Regarding age, sex, PMI and BMI we did not find any correlations with the inflammatory markers or cortisol. Likewise, we did not find differences regarding levels of inflammatory factors according to cause of death or comorbidities, such as systemic hypertension, diabetes mellitus, dyslipidemia, current alcohol and current smoking.

In the ACC, IL-1 β (ρ : -0.523, $p=0.04$) and TNF- α (ρ : -0.512, $p=0.005$) were negatively correlated with BMI. IL-10 levels were higher in the current alcohol group (0.0066 ± 0.0023 versus 0.0044 ± 0.0014 , $p=0.033$). We did not find any significant differences regarding age, sex, pH and PMI. Levels of inflammatory factors and cortisol did not differ according cause of death (cardiovascular or other) or the comorbidities (systemic hypertension, diabetes mellitus, dyslipidemia, current alcohol or current smoking).

3.3 Levels of neuroinflammatory markers and cortisol in the hippocampus and anterior cingulate

In the hippocampus of BD group, when compared with controls, we found higher levels of cortisol (109.5 ± 177.2 versus 21.5 ± 30.0 ; $p=0.009$), IL-1 β (0.016 ± 0.013 versus 0.006 ± 0.003 ;

p=0.003), IL-6 (0.111 ± 0.221 versus 0.009 ± 0.045 ; p=0.001), IL-10 (0.028 ± 0.018 versus 0.018 ± 0.008 ; p=0.049), IL-17A (0.015 ± 0.008 versus 0.009 ± 0.004 ; p=0.0140), and TNF- α (0.016 ± 0.009 versus 0.011 ± 0.006 ; p=0.022, Table 2 and Figure 1 for the significantly different results).

In the ACC, there was a significant difference between groups in the levels of cortisol and IL-17A; in the BD group, levels of cortisol were increased (24.8 ± 35.6 versus 2.8 ± 2.6 ; p=0.006), while levels of IL-17A (0.002 ± 0.007 versus 0.003 ± 0.006) were decreased when compared to controls.

Regarding CRP levels, we did not find between-group differences in any of the two investigated brain regions (Table 2, hippocampus: 17.9 ± 21.1 versus 26.4 ± 18.7 ; p>0.06 – ACC: 8.5 ± 6.5 versus 8.6 ± 9.6 ; p=0.65). Levels of IL-1 β , IL-6, IL-10 and TNF- α did not differ between BD and controls in the ACC (Table 2, p>0.05 for all).

After multivariate analysis in the hippocampus, including pH, PMI, BMI, age, cause of death, systemic hypertension and diabetes mellitus as covariates, results regarding IL1 β (coefficient interval [CI]: 0.002 to 0.0019, p=0.022), IL-10 (CI: 0.001 to 0.023, p=0.030) and IL-17A (CI: 0.000 to 0.012, p=0.045) remained significant. For TNF- α , only pH influenced the results (CI: -0.001 to 0.012, p=0.102). Regarding cortisol, including BMI and age in the model generated marginally significant results (CI: -1.616 to 179.955, p=0.054 and CI: -1.488 to 180.053, p=0.054, respectively), as for PMI (CI: -12.847 to 162.735, p=0.092), the results did lose significance. In the case of IL-6, all covariates influenced the results which lose their significance (CI: -0.030 to 0.239, p=0.121). In the ACC, controlling for the same covariates did not change the results for cortisol (CI: 3.196 to 46.812, p=0.027) or IL-17A (CI: -0.001 to 0.00, p=0.030).

3. Discussion

We analysed levels of inflammatory markers and cortisol in the hippocampus and ACC of BD and controls matched by demographic variables. We found in the BD group increased levels of cytokines IL-1 β , IL-10, IL-17A, in the hippocampus. In the ACC of BD subjects, we found increased levels of cortisol, while decreased levels of IL-17A [Figure 2]. Those associations were independent of age, pH, PMI, BMI, cause of death, systemic hypertension and diabetes mellitus. CRP levels did not differ between BD and controls in any of the two brain regions.

Increased levels of inflammatory factors are consistently reported in peripheral blood of BD patients^{10–12} strongly suggesting a contribution of inflammation to the pathophysiology of this disorder. However, the direct relationship between increased circulating inflammatory factors and brain inflammation is still poorly understood, especially in BD. Association between immune deficiency and memory loss³² indicates that the neuro-immune crosstalk deserves further attention in the field of psychiatric disease as well. In fact, treatment of major depressive patients with cyclooxygenase-2 inhibitor, an inhibitor of pro-inflammatory cytokines production, yields significantly greater clinical improvement as a reboxetine adjunctive therapy³³. Likewise, different adjunctive treatments with anti-inflammatory

properties, including aspirin and omega-3 have been used in depressive patients with positive results^{34–36}. To our knowledge, this is the first study investigating several inflammatory markers in two different brain regions in the same BD cohort. Also, to date, cortisol and CRP levels have not been evaluated in post-mortem brain tissue of BD patients. Cytokines can be produced both in blood and brain cells³⁷, while CRP and cortisol are only produced exogenously^{38,39}. Since we measured these molecules in the hippocampus and ACC, our work brings new insights on how brain-regions could differently respond to inflammatory and neuroendocrine factors in BD patients.

We found increased levels of cytokines in both brain regions, suggesting neuroinflammation can take place in both brain regions and may underlie pathophysiology of BD. However, a higher number of cytokine-type inflammatory alterations were found in the hippocampus, when compared to the ACC. It may be the case that the hippocampus is most affected than the ACC, with greater potential to discriminate BD subjects from controls, at least regarding the cytokines IL-1 β , IL-10. A potential explanation is that the hippocampus receives inputs from several other brain regions⁴⁰ and, for this reason, could be more vulnerable to the allostatic load^{41,42} occurring during mood episodes and disease progression. In fact, allostatic load has been related to chronic inflammation and disease progression, as a result of disrupted cellular homeostasis, which can also lead to cell death and tissue loss. Interestingly, reduction of hippocampal volumes in BD patients was previously associated with increased peripheral levels of cytokines IL-1 β s, TNF-R1, and sIL-2R⁴³, as well as with illness duration in BD²¹. Consistent with these neurobiological findings, deficits in memory function, subserved by the hippocampus are the cognitive abnormalities often identified in individuals with BD, even during euthymia^{44,45}. The fact that we found association of increased levels of IL-1 β , IL-6, IL-17A, antipsychotic use and past hospitalization (including TNF- α for the last) in the hippocampus, reinforces the possibility of association between disease severity, often a result of neuroprogression, and neuroinflammation in this brain region. Brain-region vulnerability to specific molecular changes is also a known process of other chronic mental illnesses such as neurodegenerative diseases⁴⁶, and our results suggest that this could be the case for psychiatric disorders as well.

Although the ACC has also been implicated in BD pathophysiology in neuroimaging and molecular studies^{20,22} data on cytokine levels in postmortem brain tissue were still lacking. Microglia is one of the sources of cytokine production⁴⁷ and has been suggested to behave differently across brain regions^{48,49}. Distinct microglial phenotype in the ACC could explain differences in cytokine levels in comparison with the hippocampus. Regarding IL-17A because this molecule is mainly produced by immune blood cells, discrepancies we found between the two brain regions could be explained by region-specific permeability of the BBB, a mechanism that has been explored in animal models^{50–52}.

Unfortunately, due to our cross-sectional study design, the question regarding the source of cytokines remains, especially in the case of IL1- β and TNF- α that can be produced in both blood and brain cells. Increased levels of these cytokines we found in the brain could either, be a result of the infiltrating peripheral immune cells into the brain, or a product of microglia activation. One way to address this question would be evaluating cytokine levels in different brain regions, as well as in the blood in a cell-specific approach. The study could present

with a targeted approach using sorted microglia, and specific T-cells, or could have an exploratory approach using single-cell/nuclei high throughput sequencing. A recent single-cell large methylome study analyzing both brain and blood tissue provided mechanistic insights into how the immune system may interact with the brain to affect major depressive disorder susceptibility⁵³. Astrocytes-yielded results suggest that epigenetic stress-linked changes may activate the innate immune system leading to systemic inflammatory responses that impact cells in both brain and blood.

Although increased levels of CRP have been related to manic episodes and cognitive decline in BD patients^{14,15}, we did not find significant between-group differences. One possibility is that our small sample size masked the difference in the case of the hippocampus ($p=0.06$). This trend in the hippocampus, but not in the ACC also speaks in favor of our hypothesis that the hippocampus could be more vulnerable to sustained/chronic cytokine neuroinflammation along the BD course.

Cortisol levels were increased in both hippocampus and ACC of BD subjects, when compared to controls. In the hippocampus, after multivariate analysis including age and BMI, results became marginally significant, and in the case of PMI, results did lose significance. Both brains regions are directly connected with the paraventricular nucleus, suggesting they may play a role in bi-directionally inter-regulatory mechanisms of the HPA axis⁵⁴. However, in the case of the ACC, this regulatory activity could be specifically related to BD pathophysiology. On the other hand, the role of the HPA dysfunction in the hippocampus could be influenced by postmortem biochemical changes, and potentially age and BMI. Cortisol levels have been associated with age^{55,56} and for BMI, although literature is conflicting, one study found either underweight and overweight could be associated with increased cortisol levels and may activate HPA axis⁵⁷. Our results suggest further attention should be given for the role of the ACC in the context of HPA axis hyperactivity in BD, as well as for role of hippocampus with attention to this disorder's comorbidities and clinical variables. Future studies comparing the expression of the glucocorticoid receptors between these brain regions may further elucidate the role of increased cortisol in the context of HPA axis hyperactivity in BD.

Considering the mean age at death (65 years old) and the relationship between age and inflammation⁵⁸, our results suggest BD patients, during their life course, could be more susceptible to chronic neuroinflammatory process than their age-matched controls. Although we did not find a correlation between age at death and inflammatory factors in any of the brain regions, this could be due to the strict age range we had in our sample. In the multivariate analysis, the inclusion of age at death in the model did change the results for IL-6, while cortisol became marginally significant. Age itself could partially explain increased levels of IL-6 and cortisol in our BD cases. Increased plasma concentrations of IL-6 were previously associated with age^{59–61} and elevated cortisol levels during late evening and early night were associated with age^{55,56}. Even though our findings cannot be generalized to other age ranges, increased cortisol and IL-6 levels could also be found in the brain of younger BD patients compared to their age-matched controls, due to accelerated epigenetic aging and circadian rhythm alterations⁶².

Other confounding factors influenced differences between groups. In the case of IL-6, besides age, each of the covariates (pH, PMI, BMI, cause of death, systemic hypertension and diabetes mellitus) changed the results. It is possible the IL-6 may be influenced by a wide range of inflammatory processes in the cell, but not specific to BD pathophysiology. TNF- α was influenced by pH in the hippocampus, although these results should be interpreted with caution, since pH was measured in the CSF and a direct association in brain tissue remains to be answered. In the case of cortisol and IL-6, because we found a wide variation in these data, it is not clear whether there is a biological relevance for these findings. The question remains whether these variables would still be able to change the between group differences in the case of a higher sample size.

Limitations of our study include retrospective nature with reliance on informants for the collection of clinical information. Our small sample size and number of observations in covariates (such as cause of death, alcohol use, coronary artery disease) may have provided false-negative results, especially in the multivariate analysis, that should be interpreted in the light of its limitations. Lack of full neuropathological evaluation of neurodegenerative pathological changes is also a limitation given the mean age at death of our subjects. Likewise, because our study involved mostly older adults, generalization to other BD age groups is limited. We attempt to minimize the potential effects of neurodegenerative-neuropathological alterations by selecting controls matched for age and cognitive status. Additionally, it would be relevant to test the specificity of these inflammatory alterations to BD, evaluating other psychiatric conditions, such as MDD and schizophrenia. Unfortunately, lack of sample size for schizophrenia and limited research funding for evaluating other psychiatric disorders did not allow us to conduct these analyses.

In summary, our results suggest that brain regions may be differentially affected by inflammatory and neuroendocrine responses along the BD course. Hippocampus may be more susceptible to cytokine-mediated neuroinflammation, which could be related to increased allostatic load, disease severity and cognitive alterations (including subsyndromal) in BD. Increased cortisol levels in the ACC suggest these brain regions could be especially involved in hyperactivity of the HPA axis in BD and could contribute to stress response and executive alterations in this disorder. Future studies with larger sample sizes including younger BD subjects and other psychiatric disorders to evaluate cross-diagnostic differences may further clarify whether these neuroinflammatory abnormalities are specific to BD.

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Highlights

The brain-immune interplay in psychiatric disorders is still poorly understood

Most neuroimmune post-mortem studies in bipolar disorder have focused on the frontal cortex

	Hippocampus (n=17)			Anterior Cingulate (n=14)		
Characteristic	BD	controls	p	BD	controls	p
Age (years), mean (SD) ^o	68.4 (13.4)	65.9 (13.5)	0.60	66.2 (13.9)	64.9 (12.3)	0.60
Education (years), mean (SD) [†]	6.1 (4.2)	5.8 (3.4)	0.82	6.3 (4.3)	6.0 (3.4)	0.87
Women, n (%) [*]	12 (70.6)	12 (70.6)	1.00	11 (78.6)	11 (78.6)	-
Race white, n (%) [*]	12 (70.6)	12 (70.6)	1.00	9 (64.3)	6 (42.9)	0.39
Cognitive impairment, n (%)	11.8 (2.0)	11.8 (2.0)	1.00	14.3 (2.0)	14.3 (2.0)	1.00
IQCODE, mean (SD) [†]	3.05 (0.13)	3.19 (0.52)	0.26	3.04 (0.14)	3.20 (0.52)	0.73
BMI, mean (SD) [†]	23.9 (5.1)	23.1 (3.6)	0.65	23.7 (5.2)	22.9 (3.6)	0.64
pH, mean (SD)	7.0 (0.59)	6.7 (0.18)	0.21			
PMI (hours), mean (SD) [†]	15.5 (4.9)	14.1 (2.6)	0.50	15.2 (5.0)	14.9 (3.3)	0.65
Right brain hemisphere, n (%) [*]	12 (70.6)	13 (76.5)	1.00	10 (71.4)	7 (63.6)	1.00
Current smoking, n (%) [*]	4 (23.5)	6 (35.3)	0.45	5 (35.7)	4 (28.6)	0.67
Current alcohol use, n (%) [*]	5 (29.4)	3 (17.6)	0.68	4 (28.6)	2 (14.3)	0.36
Hypertension, n (%) [*]	10 (58.8)	9 (52.9)	0.73	7 (50.0)	5 (35.7)	0.45
Diabetes mellitus, n (%) [*]	7 (41.2)	3 (17.6)	0.27	5 (35.7)	4 (28.6)	0.67
Coronary artery disease, n (%) [*]	4 (23.5)	4 (23.5)	1.00	5 (35.7)	3 (21.4%)	0.40
Dyslipidemia, n (%) [*]	3 (17.6)	3 (17.6)	1.00	3 (21.4)	3 (21.4)	1.00
Cause of death, n (%) ^{**}						

	Hippocampus (n=17)			Anterior Cingulate (n=14)		
Characteristic	BD	controls	p	BD	controls	p
Cardiovascular	10 (58.8)	14 (82.4)	0.13	8 (57.1)	11 (78.6)	0.42
Other	7 (41.2)	3 (17.6)		6 (42.9)	3 (21.4)	
Disease duration, mean (SD) [†]	22.47 (18.5)	–	–		–	–
Psychiatric hospitalization, n (%)*	9 (52.9)	–	–	12 (85.7)	–	–
Suicide attempt, n (%)*	1 (5.9)	–	–	2 (14.3)	–	–
Current psychotropic treatment n (%)*						
Benzodiazepines	5 (29.4)	–	–	5 (35.7)	–	–
Lithium/valproate/ carbamazepine	5 (29.4)	–	–	5 (35.7)	–	–
Antipsychotic	8 (47.1)	–	–	7 (50.0)	–	–
Other	3 (17.6)	–	–	3 (21.4)	–	–

We investigated inflammatory related-factors in hippocampus and anterior cingulated

Hippocampus may be more susceptible to cytokine-mediated neuroinflammation

Brain region vulnerability to inflammation may affect bipolar disorder clinical course

Fig 1. Neuroinflammatory markers that significantly differed between bipolar disorder subjects and controls. A) Cortisol, IL-1 β , IL-6 levels, IL-10, IL-17A and TNF- α among BD and controls in the hippocampus. B) Cortisol and IL-17A among BD and controls in the anterior cingulate. For all * indicates $p < 0.05$ and ** $p < 0.01$.

Fig 2.

Summary of our findings with the potential mechanistic interactions between neuroinflammatory markers and cortisol in the hippocampus and anterior cingulate in bipolar disorder. Cortisol and IL-17A are produced by blood cells, cross the blood-brain-barrier and acts over both brain regions. Cortisol levels in our study were increased in the BD group in the anterior cingulate, independently of comorbidities. In the case of IL-17A, opposite levels are observed when comparing both brain regions. Hippocampus show the highest number of cytokine changes, which could suggest higher vulnerability of this brain region to increased allostatic load, stress-response and cognitive alterations

Table 1:

Comparison of clinical and demographic variables in bipolar disorder cases and controls

Inflammatory marker (pg/ug of total protein)	Hippocampus (n=17)			Anterior Cingulate (n=14)		
	BD	controls	p	BD	controls	p
Cortisol	109.5 (177.2)	21.5 (30.0)	0.009 [*]	24.8 (35.6)	2.8 (2.6)	0.006 [*]
CRP	17.9 (21.1)	26.4 (18.7)	0.06 [*]	8.5 (6.5)	8.6 (9.6)	0.65 [*]
IL-1 β	0.016 (0.013)	0.006 (0.003)	0.003 [*]	0.003 (0.004)	0.001 (0.001)	0.64 [*]
IL-6	0.111 (0.221)	0.009 (0.045)	0.001 [*]	0.018 (0.027)	0.005 (0.010)	0.09 [*]
IL-10	0.028 (0.018)	0.018 (0.008)	0.049 [*]	0.004 (0.001)	0.005 (0.022)	0.10 [°]
IL-17A	0.015 (0.008)	0.009 (0.004)	0.014 [*]	0.002 (0.007)	0.003 (0.006)	0.019 [°]
TNF- α	0.016 (0.009)	0.011 (0.006)	0.022 [°]	0.005 (0.006)	0.003 (0.001)	0.77 [*]

Note: BD: bipolar disorder; SD: standard deviation; IQCODE Informant Questionnaire on Cognitive Decline in the Elderly; BMI: body mass index; PMI: postmortem interval

[°] Student's t test;

[†] Mann-Whitney U test;

^{*} Chi-square test,

^{**} Fisher's exact test

Table 2:

Comparison of inflammation markers in bipolar disorder cases and controls in the hippocampus and anterior cingulate.

Inflammatory marker	BD		Control	
	Correlation Coefficient	p [*]	Correlation Coefficient	p [*]
Cortisol	0.766	0.001	0.500	0.082
CRP	0.254	0.254	0.277	0.384
IL-1 β	0.604	0.022	-0.104	0.734
IL-6	0.877	0.001	0.165	0.590
IL-10	0.103	0.725	0.291	0.334
IL-17A	-0.055	0.852	-0.445	0.128
TNF- α	0.701	0.005	0.302	0.316

Values are given as means and (standard deviation)

* Mann-Whitney U test

^o Student's t test

Note: BD: bipolar disorder; CRP: C Reactive Protein; IL: interleukin; TNF: tumor necrosis factor.

Table 3

Correlations of inflammatory factors and cortisol across the hippocampus and anterior cingulate in bipolar disorder subjects and controls.

Inflammatory marker	BD		Control	
	Correlation Coefficient	p*	Correlation Coefficient	p*
Cortisol	0.766	0.001	0.500	0.082
CRP	0.254	0.254	0.277	0.384
IL-1 β	0.604	0.022	-0.104	0.734
IL-6	0.877	0.001	0.165	0.590
IL-10	0.103	0.725	0.291	0.334
IL-17A	-0.055	0.852	-0.445	0.128
TNF- α	0.701	0.005	0.302	0.316

* Spearman's rank correlation

Note: BD: bipolar disorder; CRP: C Reactive Protein; IL: interleukin; TNF: tumor necrosis factor.