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Total Sleep Time and Kynurenine Metabolism Associated With Mood Symptom Severity in Bipolar Disorder

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Abstract

Objective: Chronic, low-level inflammation is associated with symptomatic bipolar disorder (BD) and with chronic insomnia. Disrupted sleep is a feature of episodes of both mania and depression. We examined the effect of neopterin, a marker of cellular immune activation, and kynurenine (KYN), an inflammatory byproduct of the serotonin pathway, on the association between total sleep time and depression severity in BD.

Method: Twenty-one symptomatic BD participants and 28 healthy controls (HC) were recruited and followed during usual clinical care. At baseline and after symptomatic recovery, total sleep time was objectively measured with actigraphy for one week and blood plasma was collected to measure the serotonin-precursor tryptophan (TRP), KYN, KYN/TRP ratio and neopterin levels. Statistical analyses were conducted using chi square, independent t-tests and hierarchical linear multiple regression models.

Results: Total sleep time was positively correlated with depressive severity and negatively correlated with manic severity. TRP was significantly reduced in BD participants compared to HC. KYN, TRP, and the KYN/TRP were associated with depressive severity when total sleep time and BMI were included in the model. The KYN/TRP ratio trended towards a negative association with mania symptoms, controlling for BMI and total sleep time, in acutely symptomatic BD participants. Neopterin was not associated with sleep or mood severity. After usual clinical care, BD participants showed significantly decreased clinical symptoms but no significant differences in sleep phenotype or biomarkers.

Conclusion: Inflammation, sleep and mood are closely intertwined. Future research into the effect of inflammation on sleep in BD may lead to clinical markers of outcome.

Introduction

Bipolar disorder (BD) is a severe psychiatric disorder, with a complex and sometimes heterogeneous range of symptoms.[1] BD is one of the leading causes of disability demonstrating on average a less favorable prognosis than individuals with major depressive disorder (MDD).[2] As a clinically heterogeneous syndromic disorder, the biological changes in BD have been reported in depressive and manic episodes, and any unifying biological underpinnings of this disorder have remained elusive. [3-5] Research shows that BD presents with numerous immune-inflammatory and sleep abnormalities. [6-8] Activation of the kynurenine (KYN) pathway has been supported in a number of psychiatric disorders, including BD [9, 10].While many studies have independently explored sleep,[11-13] inflammation,[7, 14] and the KYN [9, 15] pathway in BD, there is increasing evidence that inflammation is an important factor in mood, stress, and sleep disturbance [16, 17] and needs to be examined simultaneously for a better understanding of the underlying biological mechanisms. Additionally, there is a dearth of research exploring inflammation, metabolites of the KYN pathway or sleep in acutely ill BD patients. [4]

Mood spectrum disorders (BD, recurrent depressive disorder and seasonal affective disorder) are accompanied by sleep dysregulations that are particularly common among bipolar participants.[8, 12, 13, 18] Sleep dysregulations in mood disorders commonly occur in acute manic or depressive states but are also prevalent during remission. [8, 19-21] A meta-analysis focusing on sleep in remitted BD participants highlights disturbances in several sleep parameters: longer sleep latency, longer sleep duration, and poorer sleep efficiency.[21] Sleep is a remarkably sensitive, but not very specific, indicator of distress and dysregulated sleep can occur for a variety of reasons. Inflammation has been shown to be associated with sleep disturbance and objective short sleep in primary insomnia and obstructive sleep apnea, and may mediate the association between sleep apnea and adeposity.[22-24] By examining the association of sleep dysregulation to inflammatory biomarkers in BD, we may gain an understanding of the sleep, mood and inflammation connection.

The essential amino acid tryptophan (TRP) is a substrate for two important biosynthetic pathways: the serotonin (5-HT) pathway and the KYN pathway (Figure1). During stress, proinflammatory cytokines activate the KYN pathway; deprive the 5-HT pathway of TRP, thereby reducing 5-HT synthesis. The dynamics of this process are characterized best by the kynurenine-to-tryptophan (KYN/TRP) ratio. One of the inflammatory drivers of this diversion of

5-HT to KYN is neopterin, a catabolic product of guanosine triphosphate that is a marker of cellular immune system activation produced by macrophages upon stimulation by interferon-gamma. [25-28] Increased peripheral KYN results in increased central KYN. KYN is further broken down into quinolinic acid, which potently agonizes the N-methyl-D-aspartate (NMDA)-receptor causing excitotoxic effects through receptor over-activation [29, 30] and inhibits glutamate uptake that causes elevated extracellular glutamate concentrations. [31]

In the current study, we investigated the relationship between markers of the KYN pathway, neopterin, sleep and clinical symptoms in acutely ill BD participants. We were also interested in exploring biomarkers and sleep patterns after symptom remission to determine whether these biomarkers were potentially state vs. trait characteristics independent of mood state. We hypothesized:

1) Individuals with acute BD would have significantly lower total TRP and higher KYN/TRP ratio and neopterin levels than healthy controls (HC).

2) TRP, KYN/TRP and neopterin would be associated with severity of depressive and manic symptoms in acutely symptomatic participants with BD.

3) Sleep duration and biomarkers including kynurenine pathway metabolites (TRP, KYN/TRP), and the pro-inflammatory cytokine neopterin would be associated with depressive and manic symptom severity.

Finally, we were interested in whether these markers changed with symptom remission in asymptomatic patients and explored differences in clinical symptoms, sleep and biomarkers between baseline and follow up.

Participants and Methods

Participants

Thirty BD participants with current mood symptoms participated in the study, and twenty-one completed all measures for this analysis. Participants were recruited when presenting for inpatient or partial hospital care at the Pennsylvania Psychiatric Institute, under Institutional Review Board Protocol No. 39164EP and NIH Office of Human Subject Research Exemption #11509 (7/16/2012). Participants were screened and consented for the study and assessments including sleep and biomarkers were administered while they were receiving inpatient or partial

hospital care. Inclusion criteria for BD participants included adults with a primary diagnosis of BD I or II. Exclusion criteria included use of non-steroidal anti-inflammatory drugs (NSAIDS) on a daily basis, active substance intoxication or withdrawal, major endocrinological or rheumatological illness or pregnancy. Twenty eight healthy control (HC) participants were recruited via advertisement based on the inclusion criteria that they had with no personal or family history of mental illness, rheumatologic or endocrine illness, pregnancy, daily NSAIDS or aspirin use. HCs provided actigraph data in their home environment while clinical and blood measures were collected at the research site.

Measures

Structured clinical interview based on the Mini Neuropsychiatric Interview [32] were conducted and current mood state was assessed with the Hamilton Depression Rating Scale-21 plus atypical items (HDRS-21 + AT) [33], and the Clinician-Administered Rating Scale for Mania (CARS-M). [34] A combination of clinically significant manic and depressed symptoms defined a mixed-manic phenotype. HDRS-21 + AT scores between 0 and 6 indicated no depression, scores between 7 and 17 indicated mild depression, scores between 18 and 24 indicated moderate depression, and scores over 24 indicated severe depression. CARS-M scores below 7 were considered not manic. Scores between 7 and 12 were mild, and above 12 were severe manic phenotypes. Body Mass Index (BMI) for each participant was calculated by measuring height and weight. Participants received usual clinical care and were discharged from the program. After discharge from the hospital or partial hospital program, participants were followed each week by phone and assessed for clinical improvement. A return visit was scheduled to collect a second blood sample when the subject was asymptomatic, or after 3 months had elapsed. Additional measures were collected but not used for this analysis. For more detailed report on methods and results of analysis of fatty acid concentrations, please see Saunders, et al. [35]

Objective measure of Total Sleep Time

During the period of initial evaluation, participants wore an actigraph (Sleepwatch-O, Ambulatory Monitoring, Inc., Ardsley NY), a watch-like device that measures acceleration, on the non-dominant hand for 7 days. Participants also maintained a sleep log, which combined with the actigraphy recording, provides accurate measure of objective sleep measures. ActiLife V.6.4.5 software (ActiGraph Software Department, Pensacola, FL) was used to compute the actigraphy sleep variables, including total sleep time, bedtime variability, sleep latency, sleep

duration, and sleep efficiency. BD participants completed the actigraph during hospitalization and HCs completed it at home. Due to the small sample size, we limited analysis the analysis of sleep variables to the investigation of total sleep time only, which allowed us to conserve statistical power. However, an additional exploratory analysis of mean lag (variability in bedtime from night to night) was also made.

Sample collection and biochemical analysis

Participants fasted for at least 6 hours, and blood was drawn in vacutainers containing EDTA in the morning. BD participants had blood drawn between 7:15 and 10:30 AM (average time was 8:24 AM) and HC participants had blood drawn between 8:00 AM and 2:00 PM (average time was 9:19 AM). After centrifugation for 10 min at 654.03 *g*, the plasma supernatant was transferred to plastic tubes and maintained at −80°C until processed. Total TRP (micromoles per liter) and KYN (micromoles per liter) were measured by reversephase high-performance liquid chromatography, as previously described [36, 37], using 3-nitro-L-tyrosine as an internal standard. The KYN/TRP ratio was calculated (expressed as micromoles of KYN per millimoles of TRP). Neopterin was measured by enzyme-linked immunosorbent assay (BRAHMS, Hennigsdorf, Germany) according to the manufacturer's instructions, with a detection limit of 2 nmol/L.

Data and statistical analysis

SPSS version 22, IBM SPSS Statistics (IBM Corporation, Armonk, NY, USA) was used for all statistical calculations. Values of continuous variables were compared between the BD and HC groups using independent samples *t*-tests, and categorical variables were compared using the Pearson chi-square test. Paired *t*-tests were used for baseline and follow-up measurements. In an exploratory analysis, Pearson's *r* was used for separate bivariate correlations on demographics and clinical symptoms, sleep and inflammation variables in the BD sample and the HC sample. Baseline depression was predicted using linear regression models based on a priori variables, controlling for BMI due to the known association between inflammation and obesity.[38] Model one included total sleep time and KYN/TRY, model two, total sleep time and KYN, model three included total sleep time and TRP and model four had total sleep time and neopterin as predictor variables. The same models were implemented to predict baseline manic symptoms controlling for BMI. Paired t-tests were conducted to compare symptoms, total sleep time and inflammatory variables from baseline to follow-up. However, considerably fewer participants were present in

follow-up data. This was due to inability to maintain regular contact with some participants due to a number of issues (e.g., such as unstable housing conditions) (the maximum number of days to follow up was 187; the median was 22 days and average was 52 days). An analysis of dropouts vs. those who continued to be a part of the study, however, did not show any significant differences based on baseline clinical symptoms and demographics including age and sex (p values >.16). Dependent variables were compared between BD smokers and non-smokers with independent t-tests.

Results

Demographic and clinical description of the sample

A cohort of 30 participants with BD was recruited while in a symptomatic mood episode. Six participants did not have sleep assessment data, and 3 did not have biomarker data, thus 21 BD participants were included in the final analysis. A parallel HC group of 31 participants was recruited for the study, of which three participants did not have sleep data (n=28). Participants with BD did not differ in age, gender, race, from the HC group (Table I). A significantly higher proportion of participants with BD were unemployed, not married and smokers than the HC group (Table I). Independent t tests were run between the smokers and non smokers within the BD group to detect any differences based on age, BMI, HRSD, CARSM, TRP, KYN, KYN/TRP and sleep variables. No differences were detected with all p values above .10.

Clinically, BD individuals had a significantly higher BMI and when evaluated for the study, exhibited, on average, severe depressive symptoms and moderate manic symptoms (Table I).

Sleep and Inflammation Variables

The HC group slept an average of 6.9 hours, with a standard deviation of about an hour; and the mean difference in mean lag (variability in bedtime from night-to-night) was 4 minutes with a standard deviation of one hour. The BD group slept an average of 6.5 hours, with a standard deviation of almost two hours; and the mean lag was 23 minutes, with a standard deviation of 60 minutes. Total sleep time was positively correlated with depressive symptom severity and negatively correlated with the severity of manic symptoms (Table II).

1) Group comparisons of TRP, KYN/TRP, and neopterin: As hypothesized, the BD group had significantly lower levels of TRP than healthy controls (p <.03) (Table I). The BD group did not significantly differ from the HC group in mean levels of KYN, KYN/TRP and neopterin (Table I).

2) TRP, KYN/TRP and neopterin and severity of depressive and manic symptoms: Based on Pearson's correlation test, KYN, KYN/TRP, and neopterin did not correlate significantly with depressive and manic symptoms as measured by the HDRS and CARSM clinical measures or sleep measures including total sleep time and bedtime variability. None of the kynurenine pathway markers were associated with total sleep time in either the BD or HC control group. Neopterin levels positively correlated with KYN/TRP (r = .53, p <.01), but were not correlated with clinical severity, TRP and KYN.

3) Association of biomarkers and sleep with depressive and manic severity: The association of depressive severity was tested with four hierarchical regression models using depressive severity as the dependent variable and total sleep time, KYN, TRP and KYN/TRP as the predictor variables controlling for BMI (Table IV). Depressive severity was significantly associated with KYN (model 3) and TRP (model 4), when total sleep time and BMI were accounted for. Models 3 and 4 (Table IV) accounted for 71% and 60% of the variance respectively. Model 1 consisting of KYN/TRP and total sleep time trended toward significance accounting for 60% of the variance (Table IV). Although total sleep time was significantly associated with manic severity in all four models, only the KYN/TRP biomarker trended towards significance (Table V). This model explained 35% of the variance (Table V).

Follow-up data

Exploratory analysis of the follow-up data showed that although the BD participants reported significantly decreased clinical symptoms of depression and mania, [35] no significant differences were detected in total sleep time, TRP, KYN/TRP. Total sleep time appears to have increased at follow-up but was not significantly different from baseline scores. This maybe due to the high variability in total sleep time score for BD participants (Table VI).

Discussion

The current study examined the relationship between objective markers of sleep and biomarkers of TRP metabolism and inflammation in a group of acutely symptomatic BD individuals. We found significantly lower levels of TRP in BD patients compared to HC. We

also found that biomarkers, in conjunction with total sleep time, were positively associated with depressive severity and negatively associated with manic severity in BD patients.

Our first question addressed whether there were group differences between BD and HC in total sleep time, TRP and KYN/TRP. Our finding of lower TRP levels in acutely symptomatic BD participants is concordant with several recent reports examining TRP levels in BD and schizophrenia [10, 39, 40] and the theory that decreased plasma TRP levels in BD participants may potentially lead to dysfunctional brain serotonin synthesis and release. Tryptophan depletion studies [41, 42] provide a useful tool to examine the role and association of serotonin in different psychiatric disorders. Most of the tryptophan depletion studies have focused on unipolar depression due to decreased serotonin as an underlying cause. For example, tryptophan depletion has been associated either with no effect [43] or with a slight but insignificant relapse of manic symptoms in fully recovered BD patients. No tryptophan depletion studies have been carried out in acutely manic BD patients. [39] The present study provides evidence for decreased tryptophan levels in naturalistic occurrence of acute mood episodes. While experimental studies support the notion that decreased levels maybe a precursor to a depressed mood state, however, in our study it is unclear whether the decreased tryptophan levels preceded or resulted from an acute mood state.

Second, we asked if the TRP, KYN/TRP, and neopterin were associated with severity of depressive and manic symptoms. We did not find neopterin, an inflammatory marker, to be significantly different from controls nor was it associated with depression or mania severity. Neopterin levels, however, were positively associated with KYN/TRP, which replicates earlier studies investigating depression and chronic inflammation.[44 45] Further mechanism studies examining the role of neopterin in mood disorders are warranted. For example, one study [25] found significantly higher levels of neopterin predicted post stroke depression in patients who had previously experienced a major depressive episode. Another cross-sectional study found a positive association between MDD with a history of suicide attempt and KYN and but no association with neopterin .[44] However, similar to our findings, Sublette et al. [44] found a positive correlation of the cytokine activation marker neopterin with KYN/TRP, suggesting that KYN production may be influenced by inflammatory processes. The present study supports previous studies finding an association between neopterin and KYN/TRP ratio but does not support a direct association between neopterin and mood disorders.

We hypothesized a significant association between sleep duration and biomarkers (TRP, KYN/TRP, neopterin) with depressive and manic symptom severity. Tryptophan degradation follows one of two main metabolic pathways, which ultimately lead to the production of either serotonin and melatonin, or kynurenine and its metabolites (Fig. 1). Enhanced activation of the kynurenine pathway due to stress or an acute mood state would theoretically hinder melatonin production, thereby potentially affecting sleep patterns of patients. [6, 46, 47] Thus activation of the kynurenine pathway potentially suggests the association of sleep and the kynurenine metabolites with mood states. While we did not find a correlation between these biomarkers and severity, we found an association with severity of clinical mood symptoms when considering KYN/TRP in conjunction with objective total sleep time. Neopterin and tryptophan degradation, as measured by the KYN/TRP is highly associated with immune activation which in turn leads to production of KYN instead of 5-HT from TRP,[48] but in our data neopterin, although correlated with KYN/TRP, was not associated with mood severity, with or without accounting for sleep. The association of KYN and TRP and total sleep time with severity of depression and manic symptoms implies that these pathways are related but further research is necessary to delineate exactly how they influence each other.

Finally, we asked whether total sleep time, TRP and KYN/TRP changed with symptom remission in asymptomatic BD patients. Despite subjectively reported decreased clinical symptoms, no differences are detected in the biomarkers or total sleep time. One explanation could be the short follow-up time – perhaps these markers change on a slower time scale, or they may leave a long-term residual mark of the episode, indicating a change in physiology despite remission. [49-51]

The results, however, need to be treated with caution due to the small sample size of the study. Additionally, the patients were all receiving medication for an acute mood episode which makes it difficult it to parse out the effects of medication. Third, in order to maximize statistical power the study investigated only one sleep variable –total sleep time. Sleep, however, is a complex phenomenon and consists of multiple aspects which are easily measured using actigraphic devices. Based on the results of the present study, different sleep variables along with inflammatory markers warrant further investigation with a larger sample size. Another important methodological factor is a structured hospitalized setting compared to a home environment. It may be argued that a hospitalized setting led to patients feeling more stressed than the control

individuals, however, we argue that the patients were already in acute distress unable to cope with their existing resources leading to inpatient hospitalization. The structured setting of the hospital often relieves stress through learning basic skills and teaching patients to generalize those skills to real world naturalistic settings. [52]

Conclusion

In conclusion, the study provides evidence supporting the association of mood severity in acutely symptomatic BD participants with metabolites of the KYN pathway, in association with objective measurements of sleep. Further understanding of the role of in BD is important for development of potential targets for intervention, or markers of treatment response. Targeting sleep and regulation of the TRP breakdown pathway for intervention in BD participants is an area of research warranting further investigation. Targeting these areas for improvement and observing clinically significant changes may lead to more sustained improvement and remission rates.

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References

1. Goodwin FK, Jamison KR. Manic-depressive illness: bipolar disorders and recurrent depression: Oxford University Press; 2007.
2. Cusin C, Serretti A, Lattuada E, Mandelli L, Smeraldi E. Impact of clinical variables on illness time course in mood disorders. Psychiatry research 2000;97(2):217-227.
3. Manji HK, Lenox RH. Signaling: cellular insights into the pathophysiology of bipolar disorder. Biological Psychiatry 2000;48(6):518-530.
4. Manji HK, Quiroz JA, Payne JL, et al. The underlying neurobiology of bipolar disorder. World Psychiatry 2003;2(3):136.
5. Leboyer M, Soreca I, Scott J, et al. Can bipolar disorder be viewed as a multi-system inflammatory disease? Journal of affective disorders 2012;141(1):1-10.
6. Anderson G, Jacob A, Bellivier F, Geoffroy P. Bipolar Disorder: The Role of the Kynurenine and Melatonergic Pathways. Current pharmaceutical design 2015.
7. Berk M, Kapczinski F, Andreazza A, et al. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. Neuroscience & biobehavioral reviews 2011;35(3):804-817.
8. Harvey AG, Schmidt DA, Scarnà A, Semler CN, Goodwin GM. Sleep-related functioning in euthymic patients with bipolar disorder, patients with insomnia, and subjects without sleep problems. American Journal of Psychiatry 2005;162(1):50-57.

9. Miller CL, Llenos IC, Dulay JR, Weis S. Upregulation of the initiating step of the kynurenine pathway in postmortem anterior cingulate cortex from individuals with schizophrenia and bipolar disorder. Brain research 2006;1073:25-37.
10. Clark SM, Pocivavsek A, Nicholson JD, et al. Reduced kynurenine pathway metabolism and cytokine expression in the prefrontal cortex of depressed individuals. J Psychiatry Neurosci 2016;1:8872147.
11. Murray G, Harvey A. Circadian rhythms and sleep in bipolar disorder. Bipolar disorders 2010;12(5):459-472.
12. Harvey AG, Soehner AM, Kaplan KA, et al. Treating insomnia improves mood state, sleep, and functioning in bipolar disorder: A pilot randomized controlled trial. Journal of consulting and clinical psychology 2015;83(3):564.
13. Kanady JC, Soehnera AM, Harvey AG. A Retrospective Examination of Sleep Disturbance across the Course of Bipolar Disorder. Journal of sleep disorders & therapy 2015;4(2).
14. Goldstein BI, Kemp DE, Soczynska JK, McIntyre RS. Inflammation and the phenomenology, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature. The Journal of clinical psychiatry 2009;70(8):1078-1090.
15. Myint AM, Kim Y-K, Verkerk R, et al. Tryptophan breakdown pathway in bipolar mania. Journal of affective disorders 2007;102(1):65-72.
16. Fernandez-Mendoza J, Vgontzas AN. Insomnia and its impact on physical and mental health. Current psychiatry reports 2013;15(12):1-8.
17. Gaines J, Vgontzas AN, Fernandez-Mendoza J, Kritikou I, Basta M, Bixler EO. Gender differences in the association of sleep apnea and inflammation. Brain, behavior, and immunity 2015;47:211-217.
18. Etain B, Milhiet V, Bellivier F, Leboyer M. Genetics of circadian rhythms and mood spectrum disorders. European Neuropsychopharmacology 2011;21:S676-S682.
19. Saunders EF, Novick DM, Fernandez-Mendoza J, et al. Sleep quality during euthymia in bipolar disorder: the role of clinical features, personality traits, and stressful life events. International journal of bipolar disorders 2013;1(1):16.
20. Saunders EF, Fernandez-Mendoza J, Kamali M, Assari S, McInnis MG. The effect of poor sleep quality on mood outcome differs between men and women: a longitudinal study of bipolar disorder. Journal of affective disorders 2015;180:90-96.

21. Geoffroy P, Scott J, Boudebesse C, et al. Sleep in patients with remitted bipolar disorders: a meta-analysis of actigraphy studies. Acta Psychiatrica Scandinavica 2015;131(2):89-99.
22. Gaines J, Vgontzas AN, Fernandez-Mendoza J, et al. Inflammation mediates the association between visceral adiposity and obstructive sleep apnea in adolescents. American Journal of Physiology-Endocrinology and Metabolism 2016;311(5):E851-E858.
23. Li Y, Vgontzas AN, Fernandez-Mendoza J, et al. Objective, but not subjective, sleepiness is associated with inflammation in sleep apnea. Sleep 2017;40(2).
24. Fernandez-Mendoza J, Baker JH, Vgontzas AN, Gaines J, Liao D, Bixler EO. Insomnia symptoms with objective short sleep duration are associated with systemic inflammation in adolescents. Brain, behavior, and immunity 2017;61:110-116.
25. Tang C-Z, Zhang Y-L, Wang W-S, Li W-G, Shi J-P. Elevated Serum Levels of Neopterin at Admission Predicts Depression After Acute Ischemic Stroke: a 6-Month Follow-Up Study. Molecular neurobiology 2015:1-11.
26. Schröcksnadel K, Widner B, Bergant A, et al. Longitudinal study of tryptophan degradation during and after pregnancy. Life sciences 2003;72(7):785-793.
27. Schröcksnadel H, Baier-Bitterlich G, Dapunt O, Wachter H, Fuchs D. Decreased plasma tryptophan in pregnancy. Obstetrics & Gynecology 1996;88(1):47-50.
28. Fuchs D, Hausen A, Reibnegger G, Werner ER, Dierich MP, Wachter H. Neopterin as a marker for activated cell-mediated immunity: application in HIV infection. Immunology today 1988;9(5):150-155.
29. Schwarcz R, Whetsell WO, Mangano RM. Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. Science 1983;219(4582):316-318.
30. Stone T, Perkins M. Quinolinic acid: a potent endogenous excitant at amino acid receptors in CNS. European journal of pharmacology 1981;72(4):411-412.
31. Tavares RG, Tasca CI, Santos CE, et al. Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes. Neurochemistry international 2002;40(7):621-627.
32. Sheehan DV, Lecrubier Y, Sheehan KH, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 1998;59 Suppl 20:22-33;quiz 34-57.
33. Hamilton M. A rating scale for depression. Journal of neurology, neurosurgery, and psychiatry 1960;23(Journal Article):56-62.

34. Altman EG, Hedeker DR, Janicak PG, Peterson JL, Davis JM. The Clinician-Administered Rating Scale for Mania (CARS-M): development, reliability, and validity. Biol Psychiatry 1994 Jul 15;36(2):124-134.
35. Saunders EF, Reider A, Singh G, Gelenberg AJ, Rapoport SI. Low unesterified: esterified eicosapentaenoic acid (EPA) plasma concentration ratio is associated with bipolar disorder episodes, and omega-3 plasma concentrations are altered by treatment. Bipolar disorders 2015;17(7):729-742.
36. Laich A, Neurauter G, Widner B, Fuchs D. More rapid method for simultaneous measurement of tryptophan and kynurenine by HPLC. Clinical Chemistry 2002;48(3):579-581.
37. Widner B, Werner ER, Schennach H, Wachter H, Fuchs D. Simultaneous measurement of serum tryptophan and kynurenine by HPLC. Clinical Chemistry 1997;43(12):2424-2426.
38. Jung UJ, Choi M-S. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. International journal of molecular sciences 2014;15(4):6184-6223.
39. Bell C, Abrams J, Nutt D. Tryptophan depletion and its implications for psychiatry. The British Journal of Psychiatry 2001;178(5):399-405.
40. Chiappelli J, Postolache TT, Kochunov P, et al. Tryptophan Metabolism and White Matter Integrity in Schizophrenia. Neuropsychopharmacology 2016.
41. Benkelfat C, Seletti B, Palmour RM, Hillel J, Ellenbogen M, Young SN. Tryptophan depletion in stable lithium-treated patients with bipolar disorder in remission. Archives of general psychiatry 1995;52(2):154-155.
42. Booij L, Van der Does W, Benkelfat C, et al. Predictors of mood response to acute tryptophan depletion: a reanalysis. Neuropsychopharmacology 2002;27(5):852-861.
43. Cassidy F, Murry E, Carroll BJ. Tryptophan depletion in recently manic patients treated with lithium. Biological psychiatry 1998;43(3):230-232.
44. Sublette ME, Galfalvy HC, Fuchs D, et al. Plasma kynurenine levels are elevated in suicide attempters with major depressive disorder. Brain, behavior, and immunity 2011;25(6):1272-1278.
45. Schröcksnadel K, Wirleitner B, Winkler C, Fuchs D. Monitoring tryptophan metabolism in chronic immune activation. Clinica chimica acta 2006;364(1):82-90.
46. Brzezinski A, Vangel MG, Wurtman RJ, et al. Effects of exogenous melatonin on sleep: a meta-analysis. Sleep medicine reviews 2005;9(1):41-50.

47. Dijk D-J, Cajochen C. Melatonin and the circadian regulation of sleep initiation, consolidation, structure, and the sleep EEG. Journal of biological rhythms 1997;12(6):627-635.
48. Widner B, Laich A, Sperner-Unterweger B, Ledochowski M, Fuchs D. Neopterin production, tryptophan degradation, and mental depression—what is the link? Brain, behavior, and immunity 2002;16(5):590-595.
49. Benkelfat C, Ellenbogen MA, Dean P, Palmour RM, Young SN. Mood-lowering effect of tryptophan depletion: enhanced susceptibility in young men at genetic risk for major affective disorders. Archives of general psychiatry 1994;51(9):687-697.
50. Van der Does AW. The effects of tryptophan depletion on mood and psychiatric symptoms. Journal of affective disorders 2001;64(2):107-119.
51. Riedel WJ, Klaassen T, Schmitt JA. Tryptophan, mood, and cognitive function. Brain, behavior, and immunity 2002;16(5):581-589.
52. Björgvinsson T, Kertz SJ, Bigda-Peyton JS, Rosmarin DH, Aderka IM, Neuhaus EC. Effectiveness of cognitive behavior therapy for severe mood disorders in an acute psychiatric naturalistic setting: A benchmarking study. Cognitive behaviour therapy 2014;43(3):209-220.

Table I:

Demographic, Clinical Symptoms and Differences in Sleep and Biomarkers between Healthy Controls and Bipolar Disorder Individuals

	Healthy Control N (%)	Bipolar Disorder N (%)	p
Total sample size	28	21	

Sex (F)	16 (57%)	10 (48%)	0.57
Race			
Asian	3 (11%)	0	0.19
Black	1 (4%)	0	
White	24 (86%)	21 (100%)	
Marital Status			0.02
Married	14 (50%)	4 (18.2%)	
Not married	14 (50%)	17 (81.8%)	
Employment			<0.001
Unemployed	2 (7%)	11 (55%)	
Disabled	0	4 (20%)	
Employed	16 (57%)	4 (20%)	
Student	10 (36%)	1 (5%)	
Smoking Status	1 (4%/)	11 (52%)	<.001
	Mean (SD)	Mean (SD)	
Age (in yrs)	31.57 (10.33)	36.10 (11.33)	0.16
Body Mass Index($kg*m^{-2}$)	25.01 (4.91)	30.32 (5.49)	.001
Mania (CARS-M)	0	16.52 (13.87)	<.001
Depression (H-21)	.32 (.61)	33.62 (15.37)	<.001
Total Sleep Time (mins)	414.68 (70.98)	392.56 (117.23)	.45
Bedtime variability (mins)	-3.67 (58.31)	-22.62 (61.17)	.26

KYN (μmol/L)	1.98 (.55)	1.79 (.50)	.22
TRP (μmol/L)	68.74 (11.62)	60.25 (14.30)	.03
KYN/TRP	28.93(7.26)	29.88(6.21)	.63
Neopterin (nmol/N)	5.45 (1.39)	5.80 (1.66)	.46

Note.KYN = Kynurenine, TRP = Tryptophan, Kyn/Try= Kynurenine/Tryptophan

Table II:

Bivariate correlations among clinical symptoms of depression, mania, inflammation and sleep variables in bipolar participants only (n=21)

	HRDS21	CARSM	BMI	Total Sleep Time	Mean Lag	TRYP	KYN	Kyn/Trp	Neopterin
HRDS21		-.62**	0.11	.72**	-0.32	0.36	0.44	0.14	-0.11
CARSM			-0.05	-.52*	0.32	-0.04	-0.25	-0.25	0.36
BMI				-0.03	0.21	-.46*	-0.04	0.38	0.24
TotalSleep Time					-0.25	0.21	0.03	-0.26	-0.34
MeanLag						-0.05	0.21	0.33	0.25
TRYP							.67**	-0.22	-0.19
KYN								.56*	0.24
Kyn/Trp									.53*

*Note. N = 21; *p < .05, **p < .01; BMI = Body Mass Index, Kyn/Try = Kynurenine/Tryptophan*

Table III

Bivariate correlations among inflammation and sleep variables in healthy control participants only (n=28)

	BMI	Total Sleep Time	meanLag	TRYP	KYN	Kyn/Trp	Neopterin
BMI		-0.25	0.04	-0.12	-0.08	0.01	-.010
TotalSleepTime			-0.12	-0.07	-0.19	-0.14	.01
meanLag				-0.23	0.12	0.31	.32
TRYP					.479**	-0.12	.18
KYN						.81**	.40*
Kyn/Trp							.60**

*Note. N = 28; *p < .05, **p < .01; BMI = Body Mass Index, Kyn/Try = Kynurenine/Tryptophan*

Table IV

Depressive symptom severity is positively associated with Total Sleep Time and KYN/TRP in Bipolar Disorder (controlling for BMI)

Variable	β	t	R	Adj.R^2
Model 1			.81	.60
BMI	.05	.34		
Total Sleep Time	.85	5.42**		
KYN/TRP	.32	2.0^		
Model 2			.54	.24
BMI	.50	3.85**		
Total Sleep	.27	2.15*		

Time				
Neopterin	.17	.92		
Model 3			.85	.71
BMI	.21	1.53		
Total Sleep Time	.78	5.88**		
KYN	.39	2.93**		
Model 4			.81	.60
BMI	.30	1.83		
Total Sleep Time	.66	4.53**		
TRP	.35	2.10*		

*Note. N = 21; *p < .05, **p < .01; ^ =.06; BMI = Body Mass Index, Kyn/Try = Kynurenine/Tryptophan*

Table V

Manic symptom severity is negatively associated with Total Sleep Time and KYN/TRP in Bipolar Disorder Group (controlling for BMI)

Variable	β	t	R	Adj.R^2
Model 1			.66	.35
BMI	.04	.19		
Total Sleep	-.69	-3.59*		

Time				
KYN/TRP	-.39	-2.00^		
Model 2			.40	.10
BMI	.17	1.21		
Total Sleep Time	-.29	-2.12*		
Neopterin	.12	.90		
Model 3			.60	.25
BMI	-.21	-.57		
Total Sleep Time	-.57	-2.97*		
KYN	-.21	-1.07		
Model 4			.57	.21
BMI	-.06	-.28		
Total Sleep Time	-.56	-2.76*		
TRP	.05	.20		

*Note. N = 21; *p < .05, ^p = .06; BMI = Body Mass Index, Kyn/Try = Kynurenine/Tryptophan*

Table VI

Paired sample t test comparing clinical symptoms of depression, mania, sleep and inflammation variables at baseline and follow up in BD

	Baseline	Follow Up	N	P Value
Mania	19.69 (15.11)	3.75 (5.47)	16	<.001
Depression	32.40 (14.32)	11.13 (10.60)	15	<.001

Total Sleep Time (minutes)	353.34 (124.68)	421.51 (125.70)	8	.24
KYN (μmol/L)	1.69 (.48)	1.70 (.50)	13	.92
TRP (μmol/L)	61.24 (15.91)	62.04 (13.78)	13	.82
KYN/TRP	27.81(5.65)	27.34(5.81)	13	.82

Note. Kry/Try = Kynurenine/Tryptophan

Figure 1. The Kynurenine Pathway