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Association between affective temperaments and mood features in bipolar disorder II: the role of insomnia and chronobiological rhythms desynchronization

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Highlights

- Affective temperaments are sub-threshold affective traits precursor in *bipolar disorder*
- Also *insomnia and chronobiological rhythm alterations may play a role in bipolar disorder*
- *They* may mediate the association between affective temperaments and mood symptoms in bipolar disorder type II
- A complex interaction networks among affective temperaments, sleep and chronobiological rhythms may contribute to mood dysregulation
- P*reventive strategies for bipolars should also act on the dysregulation of sleep and circadian rhythms.*

Association between affective temperaments and mood features in bipolar disorder II: the role of insomnia and chronobiological rhythms desynchronization

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Abstract

Background. Bipolar disorders are complex disorders involving the interaction of multiple factors. Affective temperaments, insomnia, and chronobiological rhythms desynchronization may all contribute to bipolar disorder. Since there is a paucity of research examining this topic we aimed to study how they are interrelated and collectively associated with clinical features of bipolar disorder.

Method. One-hundred patients with Bipolar Disorder type II depressive episode with and without mixed features were recruited and compared. Subjects were evaluated with SCID -5, the Biological Rhythms Interview of Assessment in Neuropsychiatry (BRIAN), the Insomnia Severity Index (ISI), and the Temperament Evaluation of Memphis, Pisa, Paris, and San Diego Auto-questionnaire (TEMPS-A) while evaluating depressive (Beck Depression Inventory-BDI-II) and manic (Young Mania Rating Scale-YMRS) symptoms. Logistic regression and mediation analyses were conducted. **Results.** Subjects with mixed features showed a higher scores in both insomnia and chronobiological rhythms scores. When considering affective temperaments not only depressive, cyclothymic and irritable temperaments predicted mood symptoms but also insomnia (depressive symptoms O.R. 4.17, p=0.043) and chronobiological sleep de-synchronization (manic symptoms O.R. 8.69, p=0.001). Insomnia symptoms and chronobiological alterations mediated the association between affective temperaments and mood symptoms. **Limitation**s: the cross-sectional design limited any causal interpretation.

Conclusion. Subjects with mixed features showed a greater severity of insomnia and chronobiological rhythm de-synchronization compared to subjects without. Insomnia and chronobiological alterations may contribute to mood disorders together with affective temperaments in a complex interplay also mediating their effect on mood. Preventive strategies for bipolars should also act on the dysregulation of sleep and circadian rhythms.

Key words: bipolar disorder**,** affective temperaments, chronobiological rhythms, insomnia

1.Introduction

Mood disorders are complex disorders involving the interaction of genetic, physiological, psychological, and environmental factors (American Psychiatric Association, 2013). These factors may result in different clinical manifestations that may include a spectrum of conditions that can encompass elevated mood such as mania/hypomania and depressed mood (American Psychiatric Association, 2013). Major depressive unipolar and bipolar disorders, are amongst the most prevalent and the most likely to be recurrent, chronic and disabling (Wittchen, 2012; American Psychiatric Association, 2013; Schaffer et al., 2015). Therefore the impact on public health represents a major concern leading to global burdens of disease in terms of disability, morbidity, premature mortality (Wittchen, 2012; Whiteford et al. 2013; Ferrari et al., 2014) and to a significant risk for suicidality (Pigeon et al., 2012; Schaffer et al., 2015). The understanding of the mechanism involved in the development and maintenance of bipolar disorders should thus be considered as a priority to identify potential early markers that could help in improving treatment strategies.

Insomnia is a clinically significant feature of bipolar disorder and it is highly prevalent across its entire course, as many as 80-100% of people during the depressive episode, 30-35% during manic and mixed episodes and 45-55% during the inter-episodic phase experience insomnia (Ng et al., 2015; Geoffroy et al., 2015; Rumble et al., 2015; Kanady et al., 2015; Cretu et al., 2016). Hypersomnia is also present in bipolar depression, in at least 10-15% of cases, and it represents a reliable marker of bipolarity (Grigolon et al 2019).

In particular, insomnia has been related to bipolar disorder severity, to emotional hyper-reactivity, emotional impulsivity and to increased suicidality in subjects with bipolar disorder (Pigeon et al., 2012; Pompili et al., 2013; Ng et al., 2015; Geoffroy et al., 2015; Rumble et al., 2015; Etain et al., 2017; Palagini et al., 2019a). Insomnia has been shown to increase the risk of bipolar disorder relapse and recurrence as it is one of the most frequent residual symptoms, it is also an independent risk factor for bipolar disorder and a frequent early sign occurring prior to both depressive and manic episodes (Ritter et al., 2011; Pigeon et al., 2017; Palagini et al., 2019a; Hertenstein et al., 2019; Palagini et al., 2019b). Recently, it has been shown that targeting insomnia may favorably impact on the trajectory of mood and bipolar disorders (Harvey, 2011; Bellivier et al., 2015; Asarnow and Mamber, 2019; Bei et al., 2019). Insomnia might be such a potentially modifiable early marker in bipolar disorder and the understanding of the mechanism involved in

insomnia in bipolar disorders should thus be useful and could help in improving treatment strategies for this mood disorder.

Compelling evidence has also suggested that mood disorders are also frequently associated with a malfunction of the circadian system that may play a pathogenetic role (for an overview see Harvey, 2011; McClung, 2013,; Vadnie and McClung, 2017). According to the "circadian hypothesis of mood disorders" the de-synchronization of the master biological clock of the hypothalamus, the suprachiasmatic nuclei, constitutes a hallmark and a key feature of mood disorders (Harvey, 2011; McClung, 2013; Dellaspezia and Benedetti 2015; Vadnie and McClung, 2017; Wirz-Justice and Benedetti, 2019). It has been shown that the majority of individuals with bipolar disorder presents alterations in the circadian rhythmicity, with abnormalities in physiological and behavioral timekeeping processes across the 24-hours including social life, activities, eating and sleep/wake patterns, prior and during the depressive or manic episodes and euthymia as well (Harvey, 2008; Giglio, et al., 2010; Harvey, 2011; Dellaspezia and Benedetti, 2015; Jones and Benca, 2015; Geoffroy et al., 2015; Slyepchenko et al., 2019). Biological rhythm dysregulation, have been associated with the severity of mood and insomnia symptoms, emotional dysregulation and suicidality in bipolar disorder (Harvey, 2008; Dellaspezia and Bendetti 2015; Bellevier et al., 2015; Geoffroy et al., 2015;Pinho et al., 2016; Charrier et al., 2017, Etain et al., 2017; Palagini et al., 2018; Benard et al., 2019). Similarly to insomnia it has been shown that chronotherapeutic interventions, including total and partial sleep deprivation, may favorably impact on the trajectory of bipolar disorders (Dellaspezia and Bendetti 2015; Bellivier et al., 2015; Wirz-Justice and Benedetti, 2019). Therefore also biological rhythms dysregulation might be such a potentially modifiable early marker in bipolar disorder and the understanding of the mechanism involved could improve treatment strategies for bipolar disorder.

Affective temperaments, described by Akiskal (Akiskal, 1995; Akiskal and Akiskal, 2005), have been hypothesized to play a pathogenetic role in mood disorders. Affective temperaments are

assumed to be long-term subclinical manifestations or phenotypes of mood disorders determining and modeling the emergence, the clinical evolution and several core features of affective disorders including predominant polarity, symptomatic expression, long-term course and outcome as well (Akiskal, 1995; Akiskal and Akiskal, 2005; Perugi et al., 2012; Zeschel et al., 2015; Perugi et al., 2018). Akiskal described five affective temperament types such as depressive (having a sensitivity to suffering), cyclothymic (having rapid shifts in mood and energy), irritable (being moody, and impulsive), hyperthymic (having over-energetic and over-confident traits), and anxious (having exaggerated tendency to be worry) (Akiskal, 1995; Akiskal and Akiskal, 2005) which are considered sub-threshold affective traits precursors and risk factors for mood disorders and are often correlated between them (Rihmer et al., 2010; Zeschel et al., 2015; Perugi et al., 2012; Perugi et al., 2018).

Although both insomnia and chronobiological rhythms dysregulation, being suggested as potential and modifiable early markers for bipolar disorder (Harvey, 2008; Dellaspezia and Bendetti 2015; Bellivier et al., 2015; Pinho et al., 2016; Palagini et al., 2018; Palagini et al., 2019a, 2019b), their association with affective temperaments in bipolar subjects during acute phases is poorly investigated. In one study anxious, cyclothymic and hyperthymic temperaments were related to a polymorphism in the clock genes regulating chronobiological rhythms in a group of subjects with bipolar disorder (Rybakowski et al., 2014). In another study cyclothymic temperament was associated with chonobiological dysregulations in a group of bipolar subjects during remitted phases (Dopierala et al., 2016). Other studies have limited their investigation to circadian preferences, such as chronotype, in association with affective temperaments. Some of these studies have been conducted in non-clinical population and some others in subjects with bipolar disorder during remitted phases (Giglio et al., 2010; Ottoni et al., 2012; Mokros et al., 2017, Jankowski et al., 2017; Chrobak et al., 2018) and have shown that particularly eveningness was associate with depressive and anxious temperaments in some studies and in others to dysthymic, cyclothymic and irritable temperaments . About insomnia, in one study anxious, cyclothymic, depressive and

irritable temperaments have been related to insomnia symptoms in non-clinical population (Oniszczenko et al., 2017).

On these basis we may hypothesized that several factors, such as affective temperaments, insomnia, and chronobiological rhythms desynchronization may all contribute in a relevant degree to determine bipolar disorder signs and symptoms. Since there is a paucity of research examining how insomnia, chronobiological dis-rhythmicity and affective temperaments are interrelated and how they are collectively associated with clinical features of bipolar disorder we aimed to study their association in a sample of subjects with bipolar disorder during acute phases of major depressive episodes. In this study we decided to focus on subjects with bipolar disorder type II because, among bipolar spectrum disorders, its lifetime prevalence has been shown to be higher (Clemente et al 2015), it has a more chronic course of illness leading to an increased risk in suicide and higher rate of depressive episodes with shorter periods among them, and higher rate of comorbidities (Karanti et al 2019). The investigation of insomnia symptoms, chronobiological dysregulation and affective temperaments in subjects with bipolar disorder type II with major depressive episodes may contribute to the understanding of the mechanism involved in the development and maintenance of this type of bipolar disorder that is often misdiagnosed or under recognized in the clinical practice. We aimed to study insomnia symptoms, chronobiological dysregulation and affective temperaments in different subtypes of bipolar disorder type II with major depressive episodes by comparing different subjects with and without mixed features. Secondly we aimed to explore their potential association and their relation with the mood features of bipolar disorder type II. We also explored the potential processes underling the relationship between these variables by conducting mediation analyses.

2.Methods

2.1. Selection of Subjects and Psychometric Questionnaires

From January 2017 to May 2019 the current study included a consecutive series of subjects who were hospitalized at the psychiatric ward of the Azienda Ospedaliero-Univeritaria Pisana AUOP, University of Pisa Italy, with a diagnosis of (Bipolar Disorder type II Diagnostic and Statistical Manual of Mental Disorders, fifth edition-DSM-5criteria, American Psychiatric Association, 2013) for a major depressive episode with and without mixed features. The current study was a cross-sectional observational study approved by the local ethical committee (ID n° 14446, 2017) as a part of an ongoing main research plan aimed at characterizing insomnia and chrono-biological rhythms in several types of mood disorders.

Inclusion criteria for the present study were subjects with 1) a current diagnosis of major depressive episode in Bipolar Disorder type II according to Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) criteria (American Psychiatric Association, 2013), 2) a current diagnosis of major depressive episode with or without mixed features in Bipolar Disorder type II, 2) age between 18 and 65 years and, 3) the willingness to sign an informed consent to the study and 4) were hospitalized at the Psychiatry Unit of the of the Azienda Ospedaliero-Univeritaria Pisana AUOP, University of Pisa Italy.

The exclusion criteria were: 1) a current and lifetime diagnosis of substance abuse, 2) a current depressive episode with psychotic features, 3) other types of bipolar disorders, 4) a cognitive impairment (Mini Mental State Evaluation, cut-off score <24, for the Italian version Measso et al., 1993).

All subjects were evaluated with a set of questionnaires that included the structured interview for DSM-5 (Structured Clinical Interview for Axis I Disorders- SCID-5) (First et al., 2017) to assess the presence of current or lifetime psychiatric diagnosis, the Italian version of the Insomnia Severity Index (ISI) (Morin, 1993; Castronovo et al., 2016) to evaluate insomnia symptoms, the Italian version of the Biological Rhythms Interview of Assessment in Neuropsychiatry (BRIAN) (Giglio et al., 2009; Moro et al., 2014) to evaluate circadian rhythms, the Temperament Evaluation of Memphis, Pisa, Paris, and San Diego Auto-questionnaire (TEMPS-A)

(Akiskal et al., 2005; Preti et al., 2010) to evaluate affective temperaments. The Beck Depression Inventory- II (BDI-II) (Beck et al., 1996) and the Young Mania Rating Scale (YMRS) (Young et al., 1978) were used to evaluate respectively depressive and manic symptoms. At the baseline all the subjects also completed clinical report forms which included current pharmacological therapy.

The study conformed to the Declaration of Helsinki and all participants provided written informed consent prior to being enrolled in the study.

Clinical Assessment

Psychiatric diagnosis

The assessment of previous and current psychiatric diagnosis according to the criteria of DSM-5 was performed using the Structured Clinical Interview for Axis I Disorders (SCID-5) (First et al., 2017). Interviews were conducted under the clinical judgment of trained interviewers.

Insomnia symptoms

Insomnia symptoms were evaluated with the Insomnia Severity Index (ISI) (Morin, 1993). The Index is a 7-item self-report questionnaire with a two weeks recall period. The total score ranges from 0 to 28. According to the ISI authors' recommendations, an ISI score of ≥ 8 indicated insomnia symptoms. For the Italian version see (Catronovo et al., 2016).

Chronobiological rhythms

Chronobiological rhythms were assessed with the Biological Rhythms Interview of Assessment in Neuropsychiatry (BRIAN) (Giglio et al., 2009). The BRIAN contains 21 items designed to assess five domains related to biological rhythms: 1) Sleep, 2) Activities, 3) Social aspects, 4) Alimentation, based on the last 15 days, and 5) predominant Rhythm (chronotype) based on the last year (for example: "Are you more active or productive during the evening or the morning?"). The total score may range from 16 to 84 with higher scores denoting greater disturbance in biological

rhythms. It has shown promising validity compared to objective parameters of circadian rhythmicity (Allega et al., 2018). For the Italian version see (Moro et al., 2014).

Affective temperaments

To evaluate the affective temperaments, the Italian version of Temperament Evaluation of Memphis, Pisa, Paris, and San Diego Auto-questionnaire -TEMPS-A. Its 99 constituent items inquire about the subject's life-long traits along depressive, cyclothymic, hyperthymic, irritable, and anxious lines. Individuals answer 'yes' or 'no' when considering their life experience Cut-off scores to determine the dominant temperament were 13 for depressive temperament (18 items), 18 for cyclothymic (19 items), 20 for hyperthymic (20 items), 13 for irritable (18 items), and 18 for anxious (24 items) (Akiskal et al., 2005). For the Italian version see Preti et al. (2010).

Psychiatric scales

Depressive symptoms were assessed using the Beck Depression Inventory-II (BDI-II): the BDI-II is a self-report 21-question inventory, and it is one of the most widely used instruments for measuring the severity of depression. According to the authors' recommendations, a BDI-II score> 13 is indicative of depressive symptoms (Beck et al., 1996; Ghisi et al., 2006). In this study we used an adjusted total BDI-II score which did not included the item 16 (i.e., changes in the amount of sleep) to avoid collinearity with the ISI score.

Manic symptoms were assessed with the Young Mania Rating Scale (YMRS). It is an 11-item scale. The clinician rates the severity of the symptoms from 0 (no symptoms/normal behavior) to 4 (extreme deviation) based on the subjective information provided by the patient about the last 48 hours and the clinical observation of behavior during the interview. A YMRS score> 7 is indicative of manic symptoms (Young et al., 1978, Palma et. al., 1999).

2.2 Statistical analysis

The statistical analysis was performed using SPSS 20.0 for Windows. Results were expressed as Mean + Standard deviation and/or percent values (SD). The Shapiro Wilk Test was used to check the normality of the variables. Differences in means between subjects with bipolar disorder type II depressive episode with and without mixed features were assessed using t-tests for normally distributed variables, or the Mann-Whitney U/Wilcoxon Test for non-normally distributed variables. Categorical variables were analyzed via the Pearson's Chi-Squared Test. An a priori power estimation analysis provided a sample size of n=40 with a power of 0.8. Linear and multiple logistic regression models were then built with depressive/manic symptoms (respectively BDI-II >13 yes/no, YMRS>7 yes/no) insomnia symptoms (ISI>8 yes/no) and chronobiological desynchronization (>median value yes/no), as dependent variables, while taking into account current psychiatric comorbidity, current pharmacological treatments, family history for psychiatric disorders and illness duration. A mediation analysis was performed using the Sobel test (Sobel, 1982) with the aim to investigate the potential processes that may underling the relationship between these variables. All pathways of the mediation were tested.

3.Results

3.1 Descriptive statistics and comparative analyses

Of the 150 potential participants evaluated, 100 subjects [Females =N° 60 (62 %), mean age 48.1±12 years] met the inclusion/exclusion criteria for Bipolar Disorder type II depressive episode with and without mixed features.

Of the 150 subjects 25 who also suffered for a current substance abuse disorder and 25 subjects who did not complete the evaluations were lastly excluded from the final sample.

Forty-five subjects met the inclusion/exclusion criteria for Bipolar Disorder type II depressive episode without mixed features [Females= N° 24 (53,3%) mean age 47.6±12 years], and 55 subjects for Bipolar Disorder type II depressive episode with mixed features [Females: N° 28 (50,9%), mean age 48.6 ± 12.8 years].

Please insert table 1 here

The comparison between subjects with and without mixed features showed that subjects were comparable in terms of age, gender, illness duration, severity of depressive symptoms and current pharmacological therapy with the exception of antidepressants (Table 1).

All subjects have shown a cut off >20 at the BRIAN scale thus all presenting alterations in chronobiological rhythms, but subjects with mixed features showed significantly higher scores indicating a greater severity of chronobiological rhythms dyregulation. In particular subjects with mixed features showed higher scores in the items measuring the dis-rythmicity of sleep and activities. They also showed higher score in the scale measuring the severity of insomnia symptoms. The frequency of affective temperaments was comparable for depressive, cyclothymic, irritable, and anxious temperaments while the hyperthymic temperament was more frequent in subjects with mixed features (Table 1).

3.2 Correlations between variables

Predictors of depressive and manic symptoms

The linear logistic regression analyses in subjects with bipolar disorder showed that depressive temperament (O.R.: 7.18 p<0.001), insomnia symptoms (O.R.: 6.27 p<0.001), and chronobiological rhythm dysregulation (O.R.: 4.88 p=0.004) positively predicted depressive symptoms, while the use of antidepressants (O.R.: 0.27 p=0.011) negatively predicted them (table 2). No other logistic regression resulted significant. In the multiple-logistic regression model including depressive symptoms as the dependent variable, depressive temperament, insomnia symptoms and the use of antidepressants remained significant (Table 2).

Please insert table 2 here

The linear logistic regression analyses in subjects with bipolar disorder showed that the dis-rythmicity of sleep (O.R.: 9.52, p<0.001), cyclothymic (O.R.:6.60, p<0.001) and irritable temperaments (O.R.: 5.31, p=0.001), insomnia symptoms (O.R.: 3.02, p=0.011), and the use of antidepressants (O.R.: 3.11, p=0.010) positively predicted manic symptoms (table 2). In the multiple-logistic regression model including manic symptoms as the dependent variable, the dis-rythmicity of sleep and the use of antidepressants remained significant (table 2).

Predictors of insomnia symptoms

The linear logistic regression analyses in subjects with bipolar disorder showed that depressive (O.R.: 6.27 p<0.001) and manic symptoms (O.R.: 3.02 p<0.011), cyclothymic (O.R.: 4.92 p=0.004), depressive (O.R.: 4.65 p=0.003) and irritable (O.R.: 2.83, p=0.003) temperaments, and chronobiological disruption (O.R.: 4.92 p=0.040), positively predicted insomnia symptoms. No other logistic regression resulted significant. In the multiple-logistic regression model including insomnia symptoms as the dependent variable, depressive symptoms remained significant (table 3).

Please insert table 3 here

Predictors of chronobiological rhythms desynchronization

The linear logistic regression analyses in subjects with bipolar disorder showed that depressive symptoms (O.R.: 4.88 p=0.004), insomnia symptoms (O.R.: 4.75 p=0.001), depressive (O.R.: 3.12, p=0.019),irritable (O.R.: 2.88, p=0.024) and cyclothymic (O.R.: 2.88, p=0.024) temperaments

positively predicted chronobiological rhythms desynchronization**.** No other logistic regression resulted significant. In the multiple-logistic regression model including chronobiological rhythms desynchronization as the dependent variable no variables remained significant (table 4).

Please insert table 4 here

Mediation analyses

The aim of the study was also to investigate the potential processes that may underling the association between variables: we hypothesized that both insomnia symptoms and chronobiological rhythms disruption may mediate the association between affective temperaments and manic/depressive symptoms. A mediation analysis was conducted with chronobiological desynchronization (BRIAN total score) as the mediator between depressive temperament (Temps depressive temperament total score) and depressive symptoms (BDI-II total score). It revealed a mediation effect of chronobiological desynchronization in the relationship between depressive temperaments and depressive symptoms (Fig 1, Z= 2.46, p=0.017). Similarly insomnia symptoms have shown a mediation role between depressive temperament and depressive symptoms (Fig 1, Z= 3.14, p=0.001).

Please insert figure 1 here

Other mediation analyses have shown that both the chronobiological dis-rhythmicity of sleep and insomnia symptoms may mediate the association between cyclothymic temperament and manic symptoms (Figure 2 respectively Z= 2.56, p=0.001 and Z= 3.32, p=0.0008) and that both chronobiological desynchronization of sleep and insomnia symptoms may mediate the association between irritable temperament and manic symptoms (Figure 2, Z= 2.05, p=0.0039, Figure 2 Z= 2.02, p=0.0033)

Please insert figure 2 here

All pathways of mediation have been explored and no other mediation analyses resulted significant.

4.Discussion

We assessed a sample of subjects with bipolar disorder II depressive episode with and without mixed features by evaluating circadian rhythms alterations assessed with The Biological Rhythms Interview of Assessment in Neuropsychiatry (BRIAN) (Giglio et al., 2009), insomnia symptoms and affective temperaments while assessing mood symptoms and taking into account current pharmacological therapy and other clinical/demographic factors that may contribute to bipolar disorders.

Our results firstly showed that subjects with major depressive episode and mixed features may present a greater severity of insomnia and chronobiological rhythms dysregulation compared to subjects without mixed features. In particular, both circadian dis-rhythmicity and insomnia symptoms showed playing a mediation role in the association among depressive, cyclothymic and irritable temperaments and mood disruption in subjects with bipolar disorder type II. Bipolar disorders are complex disorders involving the interaction of multiple factors, that may include not only affective temperaments, but also insomnia and chronobiologial rhythms desynchronization which may contribute to influencing the clinical pictures of bipolar disorder type II. In this framework these data would be useful in order to inform preventive strategies for bipolar disorders by acting not only on affective temperament but also on the dysregulation of sleep and circadian rhythms.

Interestingly, both insomnia symptoms and chronobiological rhythms desynchronization have shown complex interaction networks with depressive, cyclothymic and irritable temperaments resulting in bidirectional pathways. According with these findings we may hypothesized that

together with affective temperaments, chronobiological desynchronization and the alteration of the sleep machinery related to insomnia may play a role in the pathways toward mood dysregulation, thus supporting previous hypotheses (Vadnie and McClung, 2017; Palagini et al., 2019b; Palagini et al., 2019d).

Data of the literature have already shown that bipolar subjects type II may present a greater severity of insomnia symptoms and of chronobiological rhythms dysregulation when compared to healthy controls (Giglio et al., 2009; Duarte Faria et al., 2015; Pinho et al., 2016; Palagini et al., 2018; Palagini et al., 2019a; Slyepchenko et al., 2019). In the present study we aimed to evaluate differences between subtypes by comparing subjects with and without mixed features in subjects with bipolar disorder II. We found that subjects with mixed features may show a greater severity not only of manic symptoms but also of both insomnia symptoms and chronobiological alterations when compared to subjects without mixed features. In particular subjects with mixed features showed an high degree of desynchronization in the rhythms of sleep and of the daily activities. These data are of importance in the clinical practice because both insomnia and chronobiological disruption have been related to bipolar disorder severity, to emotional hyper-reactivity, emotional impulsivity and to increased suicidality in subjects with bipolar disorder (Dellaspezia and Bendetti 2015; Pinho et al., 2016; Etain et al., 2017; Palagini et al., 2018; Palagini et al., 2019a). Subjects with mixed features also presented higher percent in the use of antidepressants, confirming previous observations in bipolar and unipolar depression (Sani et al 2014, Stahl et al 2017). In the present study, in both subjects with mixed and non mixed features, depressive, anxious, cyclothymic and irritable temperaments were largely represented. In particular cyclothymic temperament was the most frequent temperament in subjects with mixed features according with previous reports (Perugi et al 2017) and they also presented an higher frequency of hyperthymic temperament compared to subjects without mixed features. This last observation is not new: starting from Perugi et al (1997) it has emerged that mixed states seemed to arise from both depressive or hyperthymic

temperaments. In particular the use of antidepressants in presence of an hyperthymic temperament have been hypothesized to often trigger a mixed state (Rihmer et al 2010).

In our bipolar patients during acute depressive phases, the logistic regression analyses revealed that depressive symptoms were predicted by depressive temperament confirming data from the literature (Perugi et al., 2012; Perugi et al., 2018) and by both insomnia (O.R. 6.27), as already known (Hertenstein et al., 2019), and by chronobiological alterations (O.R. 4.88) confirming the data found in remitted bipolar subjects (Pinho et al., 2016; Slyepchenko et al., 2019). Interestingly not only the association between insomnia and depressive symptoms was, as expected, bidirectional (Fang et al., 2019) but also chronobiological alterations could predict depressive symptoms and *viceversa*. This last finding may be in line with the "circadian hypothesis of mood disorders" which suggests that mood disorders are frequently associated with a malfunction of the circadian system that may play a pathogenetic role (for an overview see Harvey, 2011; McClung, 2013,; Vadnie and McClung, 2017, Wirz-Justice and Benedetti, 2019).

Similarly in the present study, the logistic regression analyses revealed that manic symptoms were predicted by the use of antidepressants, by cyclothymic and irritable temperaments confirming previous works showing their association (Perugi et al., 2012; Sani et al 2014, Perugi et al., 2018) and by both insomnia (O.R. 6.27) and the chronobiological alterations of sleep (O.R. 4.88) confirming some data about the association among insomnia, circadian sleep disorders and manic symptoms across the course of bipolar disorder (Kanady et al., 2015). Particularly manic symptoms have shown a bidirectional association with insomnia symptoms and with the dis-rythmicity of sleep, which in the multiple regression model remain the factor mostly implicated in manic symptoms prediction (OR:8.69, p=0.001). Basing on these findings we may hypothesized that alteration of both the homeostatic sleep process related to insomnia and of the circadian pacemaker causing the dis-rythmicity of sleep may play a pivotal role in manic symptoms. These findings are in line with previous hypotheses: the dysfunction of the sleep machinery *in toto,* with the

impairment of both the circadian and homeostatic regulation of sleep, may favor manic/mood symptoms by dysregulating most of the systems involved in mood regulation thus contributing to the insurgence and the chronicization of bipolar disorder (Vadnie and McClung, 2017; Palagini et al., 2019b;Wirz-Justice and Benedetti, 2019).

As shown in table 3 the logistic regression analyses revealed that insomnia symptoms in subjects with bipolar disorder were predicted by cyclothymic, depressive and irritable temperaments confirming previous works in non clinical population (Oniszczenko et al., 2017). Indeed the pathway of prediction resulted bidirectional with affective temperaments predicting insomnia and *viceversa* in a complex interaction networks. We may hypothesized that the alteration of the sleep machinery related to insomnia may contribute to affective temperament and *viceversa* playing a role in the pathways toward mood dysregulation, thus supporting previous hypotheses (McClung, 2013,Vadnie and McClung, 2017; Palagini et al., 2019b; Palagini et al., 2019d). In particular by adopting a life course perspective we may hypothesized sleep playing a key role in early brain developmental processes as well as in personality, temperament, character (for an overview see Palagini et al 2019d).

As expected, within the framework of a bidirectional relationship mutually reinforcing insomnia symptoms were predicted by mood dysregulation such as both depressive (O.R. 6.27) and manic symptoms (O.R. 4.88) confirming previous reports (Talbot et al 2012, Fang et al 2019). Similarly chronodistruption (O.R. 4.75) could predict insomnia symptoms confirming some clinical data about the association between circadian sleep disorders and insomnia across the course of bipolar disorder (Harvey, 2008; Harvey, 2011; Kanady et al., 2015). We may hypothesize that insomnia and chronobiological rhythms desyncronization may predict each other within the framework of a self-reinforcing feedback loop (Harvey, 2011) thus completely disrupting the sleep machinery. At the end, the alteration of the sleep machinery may play a pivotal role by dysregulating most of the systems involved in mood regulation and contributing to bipolar disorders (McClung, 2013;Vadnie

and McClung, 2017; Palagini et al., 2019b; Palagini et al., 2019d; Wirz-Justice and Benedetti, 2019).

As shown in table 4 the logistic regression analyses revealed that not only depressive and insomnia symptoms may predict chronobiological alterations in subjects with bipolar disorder type II but also cyclothymic, depressive and irritable temperaments may have a role. Indeed the pathway of prediction resulted bidirectional with affective temperaments predicting chronobiological alterations and *viceversa* in a complex interaction networks. We may hypothesized that the alteration of the circadian clock machinery may influence affective temperaments and *viceversa* playing a role in the pathways toward affective and mood dysregulation, thus supporting previous hypotheses (McClung 2013; Vadnie and McClung, 2017). In particular by adopting a life course perspective we may hypothesized sleep and circadian rhythms regulation playing a key role in early brain developmental processes as well as in personality, temperament, character (for an overview see Palagini et al 2019d).

Mediation analyses showed a mediation role of both insomnia and chronobiological rhythms desynchronization in the relationship between depressive, cyclothymic and irritable temperaments and mood features (Figure 1,2). In addition insomnia symptoms have shown a mediation role in the relationship between chronobiological rhythms desyncronization and depressive symptoms.

Bipolar disorder are in fact complex disorders involving the interaction of multiple factors, that may include not only affective temperaments but also chronobiologial desynchronization and insomnia, which may contribute to influencing the clinical pictures of the disorder. Thus we could hypothesize a complex interplay among affective temperaments, chonobiological alterations, insomnia and mood dysregulation. According to recent theories (McClung, 2013; Vadnie and McClung, 2017) chronobiological dis-rhythmicity may contribute to mood disorders by dysregulating most of the systems involved in mood and affective regulation, contributing to the insurgence and the chronicization of mood disorders (for an overview see Palagini et al., 2019b). The impairment of

the sleep machinery may represent the "chain" among these different factors that collectively contribute to bipolar disorder (for an overview Palagini et al., 2019b; Palagini et al., 2019d).

In this light the evaluation, not only of chronotypes, but also of the different aspects of circadian rhythmicity including the pattern of sleep/wake, of daily activities, of social aspects and alimentation should be included in the routinary clinical evaluation of bipolar patients. It has already been suggested that chronobiology may help provide preventive strategies and/or improve the treatment of mood disorders (Harvey, 2008; Mondin et al., 2015; Dellaspezia and Benedetti 2015; Wirz-Justice and Benedetti, 2019; Gottlieb et al 2019). To prevent and treat the clinical manifestations of the chronobiological rhythms dysregulations may reduce their consequences on affective and mood state and on sleep regulation. We may hypothesized that by re-synchronizing chronobiological rhythms we may improve the regulation of mood, affective aspects and sleep. Similarly the evaluation of sleep disturbances and namely of insomnia in subjects with bipolar disorder should be included in the routinary clinical evaluation of subjects with bipolar disorder for its potential therapeutic implications and preventive treatment strategies with long-term outcome. As already postulated, the treatment of sleep disturbances in bipolar disorder may improve the trajectory of the disorder (Harvey, 2011; Bellivier et al. 2015; Asarnow and Mamber, 2019, Bei et al., 2019) and may improve the regulation of mood, affective aspects and circadian rhythms.

4.1.Limitations

These results should be interpreted in light of several limitations including the lack of physiological measures of chronobiological rhythms. Secondly, the cross-sectional design limits any causal interpretation. Consequently, longitudinal studies are needed with larger samples of subjects with bipolar disorder type II and other types of mood disorders to examine the direction of risk and generalizability of the current findings. We have to consider also that depressive temperaments may predict for depressive symptoms: when acutely depressed, a self-report questionnaire, such as the

one assessing temperamental features, may be biased, as it could be difficult for a patient during a major depressive episode to differentiate between 'acute depressive symptoms' and 'temperamental depressive features'.

In conclusion, this study may suggest that: i) subjects with bipolar disorder type II depressive episode with mixed features may hold a greater severity of insomnia symptoms and chronobiological dis-rhythmicity compared to subjects without mixed features ii) not only affective temperaments but also both insomnia symptoms and chronodistruption may contribute to mood dysregulation in bipolar disorder type II iii) both insomnia symptoms and chronobiological desynchronization may be related to cyclothymic, depressive and irritable temperaments and may mediate the effects of these temperaments on mood features iv) These findings may have clinical implications. In particular the assessment and treatment of sleep and chronobiological rhythms in subjects with bipolar disorder should be a priority in order to identify those who may benefit from prevention and early intervention strategies.

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None

Conflict of Interest

Authors statement

The work described has not been published previously and it is not under consideration for publication elsewhere.

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All authors contributed to the idea and the design of the study, Danila Caruso, Lucia Massa, Martina Novi Francesco Pardini, Gianluca Salarpi collected the data, Mario Miniati, Stefano Pini, Etain Bruno, Riemann Dieter worked to obtain the final version of it. This form of the paper is approved by all authors

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Legend.

Table 1. Demographic and psychometric variables. Description of the total sample and comparison between subjects with bipolar disorder type II, depressive episode with and without mixed features. N=Number, %=percentage, SD: Standard Deviation, **t**= t test**, χ2:**chi square. [a] = cells expected count less than 5. ISI: Insomnia Severity Index, BRIAN: Biological Rhythms Interview of Assessment in Neuropsychiatry, TEMPS-A: Temperament Evaluation of Memphis, Pisa, Paris, and San Diego Auto-questionnaire. Cut-off scores on total scores to determine the dominant temperament: depressive temperament >13, cyclothymic>18, hyperthymic> 20, irritable >13, anxious >18. BDI-II: Beck Depression Inventory-II, YMRS:Young Mania Rating Scale. Significance in bold

Table 2. Linear and multiple logistic regression analyses on depressive and manic symptoms in subjects with bipolar disorder II. Results of the linear and multiple logistic regression analyses among the BDI-II: Beck Depression Inventory-II (upper part of the table) and the YMRS: Young Mania Rating Scale (lower part of the table) and other variables. ISI: Insomnia Severity Index, BRIAN: Biological Rhythms Interview of Assessment in Neuropsychiatry, Temperaments evaluated with the TEMPS-A: Temperament Evaluation of Memphis, Pisa, Paris, and San Diego Auto-questionnaire. B= unstandardized regression coefficient. S.E.: Standard Error; O.R.: Odds Ratio; C.I. 95%: confidence interval at 95%.Significance in bold.

Table 3. Linear and multiple logistic regression analyses on insomnia symptoms in subjects with bipolar disorder II. Results of the linear and multiple logistic regression analyses among the ISI: Insomnia Severity Index and other variables: BRIAN: Biological Rhythms Interview of Assessment in Neuropsychiatry, BDI-II: Beck Depression Inventory-II, YMRS: Young Mania Rating Scale. Temperaments evaluated with the TEMPS-A: Temperament Evaluation of Memphis, Pisa, Paris, and San Diego Auto-questionnaire. B= unstandardized

regression coefficient. S.E.: Standard Error; O.R.: Odds Ratio; C.I. 95%: confidence interval at 95%.Significance in bold.

Table 4. Linear and multiple logistic regression analyses on chronobiological rhythms desynchronization in subjects with bipolar disorder II. Results of the linear and multiple logistic regression analyses among the BRIAN: Biological Rhythms Interview of Assessment in Neuropsychiatry and other variables. ISI: Insomnia Severity Index:, BDI-II: Beck Depression Inventory-II, YMRS: Young Mania Rating Scale. Temperaments evaluated with the TEMPS-A: Temperament Evaluation of Memphis, Pisa, Paris, and San Diego Auto-questionnaire. B= unstandardized regression coefficient. S.E.: Standard Error; O.R.: Odds Ratio; C.I. 95%: confidence interval at 95%.Significance in bold.

Figures

Figure 1
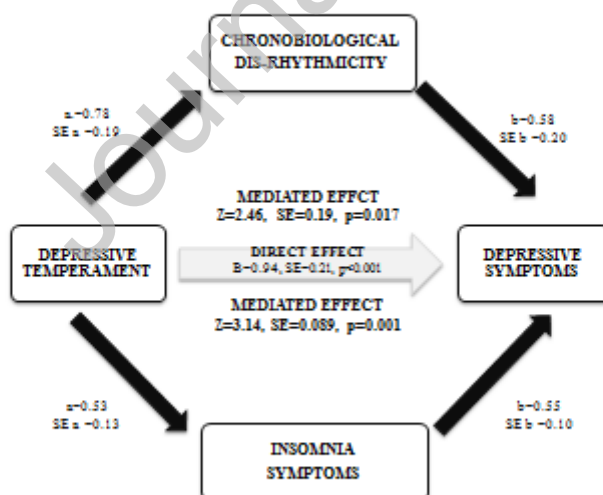
Figure 1. Mediation analyses

Both chronobiological dis-rhythmicity and insomnia symptoms mediated the association between depressive temperament and depressive symptoms. a: unstandardized regression coefficient for the

association between the independent variable and mediator, SEa=standard error of a. b: coefficient for the association between the mediator (in presence of independent variable) and the dependent variable. SEb=standard error of b. Z= Sobel test value. SE= Standard Error. Significance in bold

Figure 2
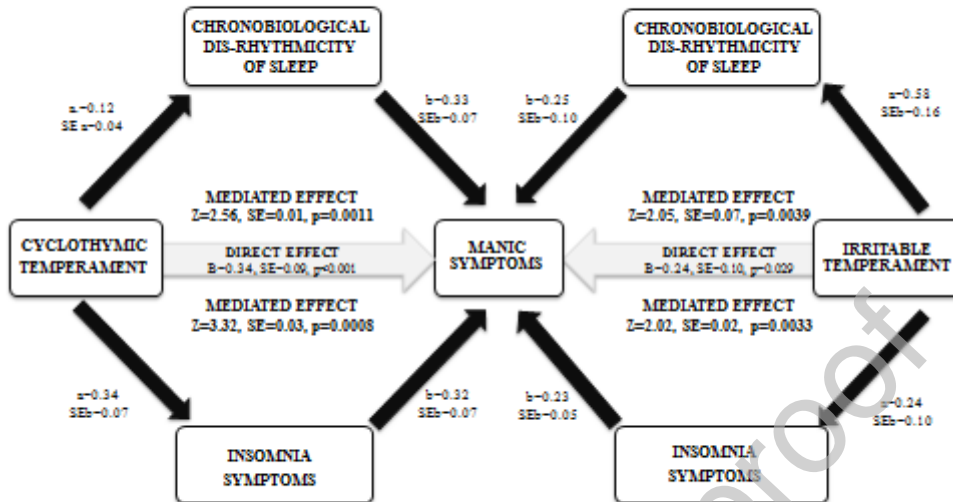
Figure 2. Mediation analyses

Both chronobiological dis-rhythmicity of sleep and insomnia symptoms mediate the association between and cyclothymic temperament and manic symptoms. Chronobiological dis-rhythmicity mediates the association between irritable temperament and manic symptoms. a: unstandardized regression coefficient for the association between the independent variable and mediator, SEa=standard error of a. b: coefficient for the association between the mediator (in presence of independent variable) and the dependent variable, SEb=standard error of b. Z= Sobel test value. SE= Standard Error. Significance in bold

Table 1. Demographic and psychometric variables

|	Subjects with Bipolar Disorder II (N=100)	Bipolar Disorder II Depressive episode non mixed features (N=45)	Bipolar Disorder II Depressive episode with mixed features (N=55)	t or χ2 (df=2)	*p*
Age (years)	48.1±12	47.6±12	48.6 ± 12.8	0.24	0.723
Gender (female) N°(%)	62(62)	24 (53,3)	28 (50.9.)	0.60 [a]	0.080
Illness duration (years)	18.2±11.6	18.2±11.6	17.9 ± 11.6	0.98	0.754
ISI-Insomnia Symptoms	10.7±7.3	9.3±5.8	12.6±6.3	4.07	**0.041**
Chronobiological rhythms- BRIAN					
BRIAN total score	47.7±10.4	47.4 ±9.1	48.3 ±11	5.3	**0.022**
BRIAN Sleep/wake pattern	11.6±3.5	10.3±3.3	12.±3.7	5.4	**0.034**
BRIAN Activities	13.3±4.2	12.35±4.3	14.1±3.9	4.3	**0.039**
BRIAN Social life	8.7±2.8	8.5±2.5	8.9±2.7	0.49	0.489
BRIAN Alimentation	8.4±2.9	8.5±2.1	8.6±3.2	1.04	0.309
BRIAN chronotype	5.8±2.5	5.7±1.4	6.04±1.5	1.16	0.284
Affective temperaments TEMPS-A					
Depressive temperament	46(46)	19(42.2)	27(49.1)	0.57 [a]	0.299
Cyclothymic temperament	60(60)	25(55.5)	35(66.6)	0.24 [a]	0.094
Hyperthymic temperament	30(30)	9(20)	21(38.1)	4.35 [a]	**0.031**
Irritable temperament	37(37)	15(33.3)	22(40)	0.53 [a]	0.509
Anxious temperament	52(52)	24(53.3)	28(50.1)	0.10 [a]	0.469
Psychiatric scales					
BDI-II total score	23.3±10.4	23.3±9.1	23.8±11.5	0.6	0.801
YMRS total score	9.3±6.3	4.3±2.2	13.4±2.6	24.5	**<0.001**
Current drug treatments	N° (%)	N° (%)	N° (%)		
Antidepressants	60(60)	34(75.5.0)	27(91.2)	5.5 [a]	**0.015**
Mood stabilizers	78(78)	36(80.0)	42(76.3)	0.07 [a]	0.493
Lithium	65(65)	28(62.2)	37(67.2)	0.31 [a]	0.361
Benzodiazepines	61(61)	26(57.7)	35(63.6)	0.51 [a]	0.305
Neuroleptics	77(77)	32(71.1)	45(81.8)	2.7 [a]	0.079
Sleeping pills	61 (61)				

Neuroleptics		36(80.0)	45(81.8)	0.02 [a]	0.555
Benzodiazepines		27(60.00)	31(56.3)	2.7 [a]	0.072
Z-Drugs		2(4.44)	6(10.0)	1.2 [a]	0.228
Antihistamines		4(8.88)	9(16.3)	1.0 [a]	0.272
Antidepressants		4(8.88)	9(16.3)	1. 0 [a]	0.234
Anxiety comorbidity	21(21)	8(17.7)	13(23.6)	3.0 [a]	0.066

Legend. Demographic and psychometric variables. Description of the total sample and comparison between subjects with bipolar disorder type II, depressive episode with and without mixed features. N=Number, %=percentage, SD: Standard Deviation, **t**= t test**,** [a] =**χ2:**chi square. ISI: Insomnia Severity Index, BRIAN: Biological Rhythms Interview of Assessment in Neuropsychiatry, TEMPS-A: Temperament Evaluation of Memphis, Pisa, Paris, and San Diego Auto-questionnaire. Cut-off scores on total scores to determine the dominant temperament: depressive temperament >13, cyclothymic>18, hyperthymic> 20, irritable >13, anxious >18. BDI-II: Beck Depression Inventory-II, YMRS:Young Mania Rating Scale. Significance in bold .

Table 2. Linear and multiple logistic regression analyses on depressive and manic symptoms in subjects with bipolar disorder II

Depressive symptoms	Linear				Multiple			
	B (SE)	O.R.	C.I. 95%	p	B (SE)	O.R.	C.I. 95%	p
ISI	**1.83 (0.52)**	**6.27**	**2.271-17.471**	**<0.001**	**1.43 (0.70)**	**4.17**	**1.049-16.645**	**0.043**
BRIAN tot	**1.58 (0.55)**	**4.88**	**1.636-14.602**	**0.004**	0.60 (0.03)	2.91	0.981-1.144	0.109
Depressive temperament	**1.97 (0.63)**	**7.18**	**2.069-24.945**	**<0.001**	**1.44(0.72)**	**5.07**	**1.283-20.113**	**0.021**
Antidepressants	**-1.29 (0.50)**	**0.27**	**0.102-0.740**	**0.011**	**-1.37 (0.66)**	**0.26**	**0.067-0.969**	**0.045**
Manic symptoms	Linear				Multiple			
	B (SE)	O.R.	C.I. 95%	p	B (SE)	O.R.	C.I. 95%	p
ISI	**1.10 (0.43)**	**3.02**	**1.282-7.117**	**0.011**	0.4 (0.71)	1.51	0.373-6.148	0.561
BRIAN tot	0.13 (0.19)	1.01	0.975-1.052	0.519	-	-	-	-
BRIAN SLEEP	**2.25 (0.49)**	**9.52**	**3.636-24.977**	**<0.001**	**2.16 (0.65)**	**8.69**	**2.413-31.356**	**0.001**
Cyclothymic temperament	**1.88(0.53)**	**6.60**	**2.327-18.768**	**<0.001**	1.06(0.72)	2.89	0.895-1296	0.144
Irritable temperament	**1.67(0.51)**	**5.31**	**1.930-14.624**	**0.001**	1.06(0.60)	2.88	0.879-9.473	0.080
Antidepressants	**1.13 (0.44)**	**3.11**	**1.312-7.376**	**0.010**	**1.45 (0.63)**	**4.27**	**1.225-14.998**	**0.023**

Legend: Results of the linear and multiple logistic regression analyses among the BDI-II: Beck Depression Inventory-II (upper part of the table) and the YMRS: Young Mania Rating Scale (lower part of the table) and other variables. ISI: Insomnia Severity Index, BRIAN: Biological Rhythms Interview of Assessment in Neuropsychiatry, Temperaments evaluated with the TEMPS-A: Temperament Evaluation of Memphis, Pisa, Paris, and San Diego Auto-questionnaire. B= unstandardized regression coefficient. S.E.: Standard Error; O.R.: Odds Ratio; C.I. 95%: confidence interval at 95%.Significance in bold.

Table 3. Linear and multiple logistic regression analyses on insomnia symptoms in subjects with bipolar disorder II

Insomnia symptoms	Linear				Multiple			
	B (SE)	O.R.	C.I. 95%	p	B (SE)	O.R.	C.I. 95%	p
BRIAN tot	**1.55 (0.48)**	**4.75**	**1.857-12.177**	**0.001**	1.07 (0.60)	2.92	0.896-9.550	0.075
BDI-II	**1.83 (0.52)**	**6.27**	**2.271-17.471**	**<0.001**	**1.47 (0.70)**	**4.37**	**1.100-17.409**	**0.036**
YMRS	**1.10 (0.43)**	**3.02**	**1.282-7.117**	**0.011**	0.50 (058)	1.65	0.528-5.171	0.388
Depressive temperament	**1.53 (0.51)**	**4.65**	**1.703-12.746**	**0.003**	0.37 (0.67)	1.45	0.390-5.492	0.273
Cyclothymic temperament	**1.59(0.54)**	**4.92**	**1.680-14.458**	**0.004**	1.11 (0.64)	3.05	0.390-5.492	0.083
Irritable temperament	**1.04(0.50)**	**2.83**	**1.047-7.685**	**0.040**	0.71 (0.65)	2.04	0.570-7.306	0.575

Legend: Results of the linear and multiple logistic regression analyses among the ISI: Insomnia Severity Index and other variables: BRIAN: Biological Rhythms Interview of Assessment in Neuropsychiatry, BDI-II: Beck Depression Inventory-II, YMRS: Young Mania Rating Scale. Temperaments evaluated with the TEMPS-A: Temperament Evaluation of Memphis, Pisa, Paris, and San Diego Auto-questionnaire. B= unstandardized regression coefficient. S.E.: Standard Error; O.R.: Odds Ratio; C.I. 95%: confidence interval at 95%.Significance in bold.

Table 4. . Linear and multiple logistic regression analyses on chronobiological desynchronization in subjects with bipolar disorder II

BRIAN tot	Linear				Multiple			
	B (SE)	O.R.	C.I. 95%	p	B (SE)	O.R.	C.I. 95%	p
ISI	**1.55 (0.48)**	**4.75**	**1.857-12.177**	**0.001**	0.64 (0.64)	1.91	0.921-8.322	0.317
BDI-II	**1.58 (0.55)**	**4.88**	**1.636-14.602**	**0.004**	1.01(0.56)	2.76	0.537-6.807	0.070
Depressive temperament	**1.14 (0.48)**	**3.12**	**1.209-8.087**	**0.019**	0.55 (0.49)	1.74	0.655-4.552	0.259
Irritable temperament	**1.05 (0.47)**	**2.88**	**1.148-7.250**	**0.024**	0.60 (0.54)	1.08	0.655-5.274	0.267
Cyclothymic temperament	**1.09 (0.51)**	**2.97**	**1.093-8.093**	**0.033**	1.04 (0.66)	2.84	0.775-10.471	0.115

Legend: Results of the linear and multiple logistic regression analyses among the BRIAN: Biological Rhythms Interview of Assessment in Neuropsychiatry and other variables. ISI: Insomnia Severity Index:, BDI-II: Beck Depression Inventory-II, YMRS: Young Mania Rating Scale. Temperaments evaluated with the TEMPS-A: Temperament Evaluation of Memphis, Pisa, Paris, and San Diego Auto-questionnaire. B= unstandardized regression coefficient. S.E.: Standard Error; O.R.: Odds Ratio; C.I. 95%: confidence interval at 95%.Significance in bold.