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Research Paper

Lower morning levels of cortisol and neuropeptides in blood samples from patients with bipolar disorder

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ABSTRACT

Background: Several lines of evidence indicate that circadian rhythm disruption is associated with bipolar disorder (BPD). This strong association, along with evidence from genome wide association studies (GWAS) implicating clock and clock controlled genes with BDP and efficacy of lithium treatment, suggests that BPD circadian rhythm disruption may represent a core etiology feature. Lower morning expression of the neuropeptide somatostatin (SST) has been previously reported in the brain and cerebral spinal fluid of subjects with BPD, coinciding with increased morning severity of anxiety and depression. We aimed to test the hypothesis that levels of neuropeptides involved in circadian rhythm regulation, including somatostatin (SST), neuropeptide-Y (NPY), arginine vasopressin (AVP), vasoactive intestinal peptide (VIP) and cortisol levels, are altered in blood samples collected in the morning from patients BPD.

Method: Thirty nine patients diagnosed as BPD according to DSM-5, and 38 healthy controls were enrolled in the study. Blood were collected at 9 AM from all subjects. Serum levels of SST, NPY, AVP, VIP and cortisol were measured.

Results: We observed significantly lower levels of SST ($p = 0.001$), NPY ($p = 0.001$), VIP ($p = 0.001$) and cortisol levels ($p = 0.001$) in the morning in subjects with BPD compared to control subjects. Significant positive effects of Young Mania Rating Scale and lithium treatment with cortisol, SST, and VIP levels were observed.

Conclusion: Our study suggests that lower morning levels of SST, NPY, VIP and cortisol may represent biomarkers underlying disrupted biological rhythms and behavioral and sleep disturbances observed in patients with BPD.

1. Introduction

Bipolar disorder (BPD) is a chronic disorder affecting approximately 2.8% of adults per year in the United States, and aproximately 4.4% of adults suffer from BPD during their life (Kessler et al., 2005; Merikangas et al., 2010). This life-long disoder, characterized by recurrent episodes of mania, depression, or mixed states, along with periods of healthy mood (euthymic) state, can contribute to impaired functioning and disability due to recurrences of depression and manic episodes together with increased risk of suicide (Kessler et al., 2005; Merikangas et al., 2010). A wide range of factors have been reported to contribute to the etiology of BPD, ranging from genetic, neurobiological, neurodevelopmental, immunological, and environmental factors including psychosocial stressors (Altinoz and Ince, 2017; Brietzke et al., 2012;

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Kato, 2007, 2015; Kloiber et al., 2020; Nakatani et al., 2006; Sibille et al., 2011; Young et al., 2018; Young and Dulcis, 2015).

Growing evidence converges on disrupted circadian rhythms as a potential core component involved in regulating several of these factors. Circadian rhythms are involved in regulating metabolism, nutrition, immune responses, detoxification, hormone secretion and neurobiological processes (Chang and Guarente, 2013; Chen et al., 2016; Fournier et al., 2012; Gachon et al., 2004; Gale et al., 2011; Hofman and Swaab, 1994; Kurose et al., 2011; Libert et al., 2012; Liu et al., 2000; Marcheva et al., 2010; Masri and Sassone-Corsi, 2018; Muller et al., 1985; Perelis et al., 2016; Rana et al., 2003; Richards and Gumz, 2013; Vetter et al., 2015; Wang et al., 2015). Increasing evidence implicates circadian rhythm disruption in BPD (Aydin et al., 2013a; Benedetti et al., 2008a; Bersani et al., 2012b; Etain et al., 2011a; Harvey, 2011a; Jagannath et al., 2013a; Kripke et al., 2009b; McClung, 2011b; Nievergelt et al., 2006a; Pagani et al., 2016; Plante and Winkelman, 2008a; Severino et al., 2009a; Soria et al., 2010b). Genetic studies, including GWAS, have identified gene variants for core clock molecules associated with BPD and responsiveness to lithium treatment (Bollettini et al., 2017; Mansour et al., 2009; McCarthy et al., 2012; McCarthy and Welsh, 2012; Nievergelt et al., 2006a; Pandey et al., 2012; Rybakowski, 2014; Sjoholm et al., 2010; Soria et al., 2010b; Suzuki et al., 2017). In turn, lithium and valproic acid, two of the most effective treatments for BPD, alter expression of core clock molecules and circadian period (Abe et al., 2000; Johansson et al., 2011; Klemfuss, 1992; Li et al., 2012; Noguchi et al., 2016; Yin et al., 2006). Furthermore, sleep disorders are common features of mood disorders, and excessive insomnia or decreased need for sleep are diagnostic criteria for depression and mania respectively (Frank et al., 2015). Consistent with these findings, disruption of circadian rhythms has been reported in BPD (Castro et al., 2015; Geoffroy et al., 2015; Gonzalez, 2014; Harvey et al., 2005; Levenson et al., 2015; McKenna et al., 2014a; Pagani et al., 2016; Rock et al., 2014b; Salvatore et al., 2008; Zanini et al., 2015). Furthermore, anxiety and depression in subjects with mood disorders commonly peak in the morning, consistent with circadian dysregulation of molecular factors that may impact these symptoms (Murray, 2007b, 2008b; Murray et al., 2002a; Wirz-Justice, 2008a). A recent postmortem study demonstrated that protein expression of somatostatin (SST) varies in a diurnal manner in the healthy human amygdala, and is altered in subjects with BPD, with a decrease selectively in the morning (Pantazopoulos et al., 2016). SST and neuropeptide-Y (NPY) in the amygdala have potent anxiolytic and antidepressant effects (Albrecht et al., 2013b; Engin et al., 2008a; Engin and Treit, 2009a; Lin and Sibille, 2015a; Yeung et al., 2011a; Yeung and Treit, 2012a). The decrease of SST in the morning in the amygdala of subjects with BPD therefore may contribute to increased morning severity of anxiety and depression (Murray, 2007b, 2008b; Murray et al., 2002a; Wirz-Justice, 2008a).

Cortisol serves as a broad systemic circadian entrainment factor in addition to its established role as a stress signaling factor (So et al., 2009; Yamamoto et al., 2005). Altered amplitude and phase of cortisol rhythms have been reported in subjects with BPD (Cervantes et al., 2001; Moon et al., 2016), dependent on euthymic, depressed or manic state (Kennedy et al., 1989; Moon et al., 2016), indicating that cortisol expression is a prime candidate as biomarker for circadian rhythm and mood dysfunction. In this study, we aim to test the hypothesis that morning serum levels of cortisol and neuropetides involved in circadian rhythm and mood regulation, are altered in subjects with BPD, coinciding with reported increased severity of anxiety and depression (Murray, 2007b, 2008b; Murray et al., 2002a; Wirz-Justice, 2008a), as a first step in examining if these factors can serve as potential blood biomarkers for circadian rhythm disturbances in people with BPD.

In addition to the key roles that SST and NPY in regulating anxiety and depression, these neuropeptides, along with arginine vasopressin (AVP) and vasoactive intestinal peptide (VIP), are highy expressed in the suprachiasmatic nucleus (SCN) (Dardente et al., 2004), the master circadian clock of mammalian organisms (Moore and Eichler, 1972; Stephan and Zucker, 1972). VIP signaling in the SCN is involved in mediating responses of SCN cells to light, and coordinating molecular clock rhythms between SCN neurons (Aton et al., 2005; Jones et al., 2018). Alteration of VIP signaling strongly impacts circadian period (Aton et al., 2005; Colwell et al., 2003; Pantazopoulos et al., 2010). Furthermore, AVP has also been implicated as a neuropteptide essential for maintenance of normal SCN rhythms both within the SCN and in brain regions outside of the SCN (Li et al., 2009; Mieda et al., 2015). NPY has been reported to mediate the effects of light on the SCN (Weber and Rea, 1997; Yannielli et al., 2004) and injection of NPY into the rodent SCN phase shifts circadian rhythms of wheel-running activity (Huhman and Albers, 1994).

SST is also strongly involved in regulating SCN rhythms. For example, acute in vivo SST depletion in the SCN leads to phase shifts in locomotor activity (Fukuhara et al., 1994). Similar depletion in SCN slices leads to phase advances of neuronal firing rhythms (Fukuhara et al., 1994). Moreover, application of SST to SCN in vitro reflects phase advance and phase delay effects of light pulses, resulting in phase advances or phase delays of neuronal firing rhythms depending on the circadian time of application (Hamada et al., 1993). A genetic polymorphism for SST receptor 5 (SSTR5), expressed abundantly in SCN, has been associated with BPD (Nyegaard et al., 2002), thus genetic polymorphisms regulating SST expression in BPD may contribute to circadian rhythm abnormalities (Aydin et al., 2013a; Benedetti et al., 2008a; Bersani et al., 2012b; Etain et al., 2011a; Harvey, 2011a; Jagannath et al., 2013a; Kripke et al., 2009b; McClung, 2011b; Nievergelt et al., 2006a; Pagani et al., 2016; Plante and Winkelman, 2008a; Severino et al., 2009a; Soria et al., 2010b).

Despite the evidence for involvement of these neuropeptides in the regulation of circadian rhythm and mood, serum levels of these neuropeptides have not been examined together in the same subjects with BPD. In order to test the hypothesis that alterations of these molecules can be detected through minimaly invasive procedures via blood samples in subjects with BPD in a euthemic state, we evaluated the levels of neuropeptide markers (SST, VIP, NPY, AVP and Cortisol) in blood samples collected in the morning (9 am), coinciding with the reported increased severity of anxiety and depression, and decreased levels of SST in the amygdala of subjects with BPD (Murray, 2007b, 2008b; Murray et al., 2002a; Pantazopoulos et al., 2016; Wirz-Justice, 2008a).

2. Methods

2.1. Participants

Our subject cohort consisted of 39 patients diagnosed as BPD who were in a euthymic state during the study, according to DSM-5 criteria, and 38 healthy control subjects recruited in the Gaziantep University Medical Faculty Hospital Mental Health and Diseases Polyclinic. Clinical data consisted of disease history, mental status examination and psychiatric diagnosis according to DSM-5 criteria. Sociodemographic data included age, sex, marital status, educational level, previous hospitalization history, duration of illness, previous drug use, presence or absence of additional medical and psychiatric diseases, which was obtained through semi-structured interviews and questionaires (refer to Clinical Global Impression Scale (CGI), Young Mania Rating Scale (YMRS), Hamilton Depression Rating Scale (HDRS), Pittsburgh Sleep Quality Index (PSQI)

Subjects with other mental health disorders, mental retardation, and organic brain damage were excluded from the study. For the control group, subjects with any psychiatric or medical condition were excluded from the study. Control subjects were not matched with subjects with BPD for demographic variables such as age sex or education level completed. However, these variables were tested for potential differences between groups as well as effects on our outcome measures in our statistical analysis. Our study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was

obtained from all participants. The study protocol was approved by the Institutional Ethics Committee.

2.2. Blood collection and clinical laboratory measurements

Clinical Global Impression Scale (CGI), Young Mania Rating Scale (YMRS), Hamilton Depression Rating Scale (HDRS), Pittsburgh Sleep Quality Index (PSQI) were administered to the subjects. Patient and control group blood samples were obtained following a 12-hour fasting period, from the antecubital region of the forearm in the sitting position after sterilization with alcohol. Blood samples were taken as 6 mL in anticoagulant tubes, and were used for quantification of serum somatostatin (SST), neuropeptide-Y (NPY), arginine vasopressin (AVP), vasoactive intestinal peptide (VIP) and Cortisol. Samples were centrifuged at 4000 rpm for 10 min, separated into serum, and stored at −80 °C. SST, VIP, NPY, AVP and Cortisol levels were measured by immunochemical methods (ELISA). Serum SST (ElabScience, E-EL-H1923, China, RRID: AB_2893102), VIP (ElabScience, E-EL-H2155, China, RRID: AB_2893103), NPY (ElabScience, E-EL-H1893, China, RRID: AB_2893104), AVP (Anti-Diuretic Hormone) (ElabScience, E-EL-H0272, China, RRID: AB_2893105) and cortisol (ElabScience, E-EL-0157, China, RRID: AB_2893106) levels were measured with an ELISA reader (Biotek, ELx800, USA) using commercial ELISA kit.

2.3. Statistical analysis

Student's *t*-test was used to test for differences of demographic variables between control and BPD subjects. The Mann-Whitney U test was used to compare continuous variables between control and BPD subjects. Correlations between the variables were tested with correlation coefficient analysis.

Differences between groups relative to the main outcome measures in were assessed for statistical significance using stepwise linear regression analysis of covariance (ANCOVA). A logarithmic transformation was uniformly applied to all outcome measure values because the data were not normally distributed. Statistical analyses were performed using JMP Pro v15 (SAS Institute Inc., Cary, NC). Potential effects of covariates were systematically tested in the model for their potential effects on the main outcome measures, and included in the model if they significantly improved the model goodness of-fit. Covariates that were statistically significant, indicating that they accoount for a significant percent of the variance in the data, were included in the model and the resulting p value for the diagnosis group effect represents the p-value after adjusting for all significant covariates that impact the variance in the data, thus the diagnosis group p-value in the ANCOVA model represents the difference accounted for by diagnosis of BPD after accounting for any siginficant effects of other factors. The covariates tested include age, sex, marital status, education level, duration of illness, hospitalization, number of manic or depressive episodes, HDRS, YMRS, PSQI, and medication treatment (valproic acid, lithium, SSRIs, lamotrigen, quetiapine, aripiprazol, risperidon, olanzapine, paliperidone palmitate, clozapine, amisulpride, haloperidol, chlorpromazine, and ziprasidone). All factors that were found to have significantt effects are reported in the results section and corresponding figures.

3. Results

3.1. Sociodemographic characteristics

Euthymic BPD and control subjects were compared across sociodemographic variables. There were no differences between the two groups for age and sex. Control subjects had a significantly higher level of education completed (Table 1).

Table 1
Demographic characteristics.

Group	BPD group		Control group		*P*
	M/n	*SD/%*	*M/n*	*SD/%*	
Mean Age	33.85±	9.56	32.24	±7.96	0.42
Sex	***n***	***%***	***n***	***%***	0.45
Female	19	48.7	20	52.6	
Male	20	51.3	18	47.4	
Marital Status	***n***	***%***	***N***	**%**	0.11
Single	13	33.3	16	42.1	
Married	22	56.4	22	57.9	
Divorced/Widowed	4	10.3	0	0	
Educational Status	***N***	***%***	***n***	**%**	**0.01***
None	1	2.6	1	2.6	
Primary School	9	23.1	1	2.6	
Secondary School	5	12.8	1	18.4	
High School	9	23.1	7	73.7	
University/College	15	38.5	28	2.6	

3.2. Clinical characteristics of the BPD group

The clinical characteristics of the BPD group are displayed in Table 2. All patients maintained their current treatments throughout the study. The medications used were typical antipsychotics, atypical antipsychotics, mood stabilizers, and combinations of these medications. The most commonly used mood stabilizator was valproic acid (41%) and the most commonly used antipsychotic was aripiprazole. Medication status of the subjects with BPD is reported in Table 3. Subjects with BPD displayed significantly higher YMRS, HDRS, and PSQI scores compared to control subjects (Fig. 1).

3.3. Neuropeptide and cortisol levels

Cortisol, NPY, SST and VIP levels were significantly lower in BPD subjects in comparison to control subjects (Fig. 2). Significant positve effects of lithium and YMRS were observed for cortisol, SST, and VIP and included in the ANCOVA models (lithium: cortisol $p = 0.01$, SST $p = 0.04$, VIP $p = 0.009$; YMRS: cortisol $p = 0.003$, SST $p = 0.001$, VIP $p = 0.005$. In addition, significant effects of age ($p = 0.002$) and sex ($p = 0.05$) were detected for VIP and included in the ANCOVA model. AVP level was not statistically significant different between groups ($p = 0.772$), (Fig. 2).

Table 2
BPD group features.

Diagnosis group	BPD	
	M/n	*SD/%*
Mean age of BPD onset	25.26	6.07
Duration of BPD	7.84	6.67
Hospitalization	***N***	***%***
Yes	30	76.9
No	9	23.1
Number of manic episodes	***n***	***%***
1–3	35	92.3
4–6	3	7.7
Number of depressive episodes		
1–3	33	84.7
4–6	4	10.2
> 7	2	5.1
HAM-D	3.82	2.23
YMRS	1.46	1.33
Pittsburgh	5.05	2.76
Pittsburgh Group		
Normal	15	38.5
Abnormal	24	61.5

Table 3
Medication Status of BPD Subjects.

Medication type	On medication (yes/no)	Specific medication
VPA	16 Yes / 23 No	N/A
Lithium	4 Yes / 35 No	N/A
Lamotrigine	11 Yes / 28 No	N/A
Quetiapine	18 Yes / 21 No	N/A
Aripiprazole	19 Yes / 20 No	N/A
Risperidone	12 Yes / 27 No	N/A
SSRIs	5 Yes / 34 No	Escitalopram (2) Venlafaxine (2) Fluoxetine (1)
Benzodiazepines	4 Yes / 35 No	Clonazepam (2) Lorazepam (2)
Stimulants	1 Yes / 38 No	Methylphenidate
Olanzapine	2 Yes / 37 No	N/A
Paliperidone Palmitate	2 Yes / 37 No	N/A
Clozapine	0 Yes / 39 No	N/A
Amisulpride	1 Yes / 38 No	N/A
Haloperidol	1 Yes / 38 No	N/A
Chlorpromazine	1 Yes / 38 No	N/A
Ziprasidone	2 Yes / 37 No	N/A
ECT	0 Yes / 39 No	N/A

VPA: Valproic acid.
SSRIs: Selective Serotonin Reuptake Inhibitors.
ECT: Electroconvulsive Therapy.

3.4. Correlations of neuropeptide and cortisol levels with measures of clinical scores, medication treatment, or demographic factors

In control subjects, we observed a positive significant correlation of AVP levels with SST, VIP, NPY and cortisol levels (R square = 0.40, $p < 0.001$ with SST, *R*square = 0.54, $p < 0.001$ with VIP, *R*square = 0.25, $p < 0.02$ with NPY, *R*square = 0.54, $p < 0.001$ with cortisol respectively). Strong positive correlations of cortisol levels with SST, VIP, and AVP were also detected (*R*square = 0.73, $p < 0.001$ with SST, *R*square = 0.48, $p < 0.001$ with VIP, *R*square = 0.54, $p < 0.02$ with AVP respectively). We also observed a positive correlation of SST with VIP levels (*R*square = 0.36, $p < 0.001$). There was no significant correlation of NPY with VIP, SST, or cortisol.

In subjects with BPD, we observed a positive significant correlation of AVP levels with SST, VIP, and cortisol levels (*R*square = 0.41, $p < 0.001$ with SST, *R* square = 0.41, $p < 0.001$ with VIP, *R* square = 0.25, *R* square = 0.30, $p < 0.003$ with cortisol respectively). In contrast to control subjects, no significant correlation between AVP and NPY levels was detected in subjects with BPD. Similar to control subject, subjects with BPD displayed strong positive correlations of cortisol levels with SST, VIP, and AVP were also detected (*R* square = 0.80, $p < 0.001$ with SST, *R* square = 0.57, $p < 0.001$ with VIP, *R* square = 0.30, $p < 0.003$ with AVP respectively). We also observed a positive correlation of SST with VIP levels (*R* square = 0.65, $p < 0.001$) similar to control subjects. There was no significant correlation of NPY with any of the outcome measures in subjects with BPD.

We detected significant, positive relationships of cortisol, SST and VIP levels with YMRS in subjects with BDP (Fig. 3). There were no statistically significant relationships of VIP, NPY, AVP, and cortisol levels with CGI or HDRS scores. In addition, numbers of manic episodes and depressive episodes were not associated with levels of SST, VIP, NPY, AVP or cortisol.

No significant differences between medication type and any of the outcome measures. Although significant effects of lithium treatment were detected in ANCOVA models, comparisons of lithium treatment with neuropetpide and cortisol levels revealed non-significant positive effects of lithium (Fig. 4). Furthermore, no significant correlations were detected for age, sex, marital status, educational level, duration of illness, number of hospitalizations, or recreational drug use with SST, VIP, NPY, APV and cortisol levels.

4. Discussion

We observed significantly lower levels of SST, VIP, NPY and cortisol in blood samples collected in the morning from subjects with BD. These decreases coincide with previously reported increased severity of anxiety and depression in the morning across mood disorders (Murray, 2007b, 2008b; Murray et al., 2002a; Wirz-Justice, 2008a), as well as a reported decrease of SST expression in the morning in the amygdala of subjecs with BD (Pantazopoulos et al., 2016). Furthermore, significant positive effects of YMRS and lithium were detected for cortisol, SST, and VIP levels, suggesting that decreases of these measures may reflect depression symptoms and may be partially corrected by lithium treatment. Our data suggests that lower morning levels of neuropeptides involved in circadian rhythm regulation may represent a biological signature of circadian disruption in this disorder, detectable through blood analysis.

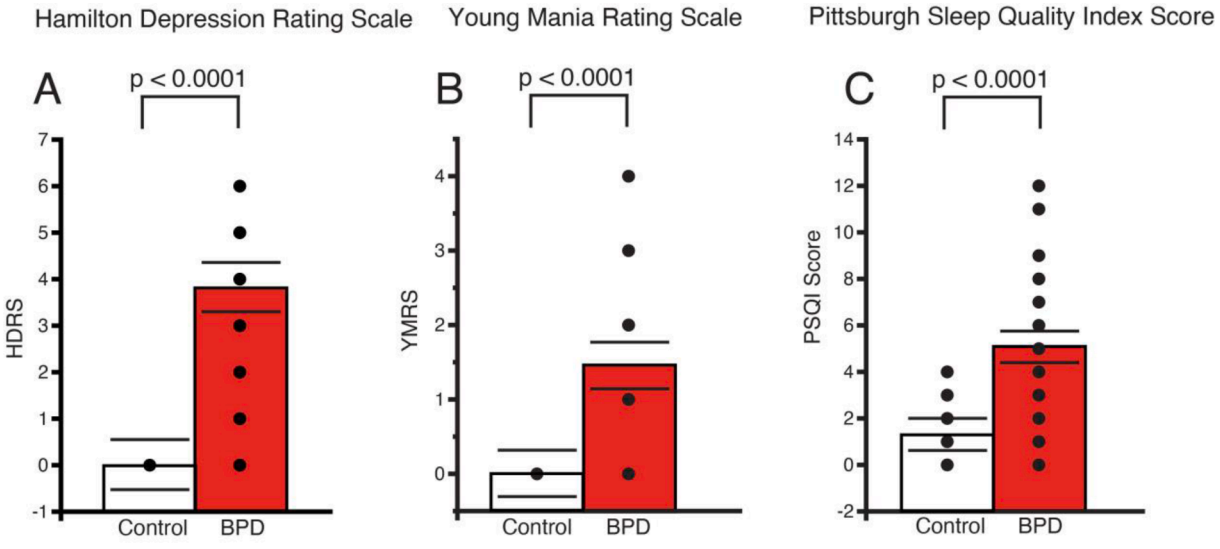

Fig. 1. Increased Depression, Mania, and Sleep Disturbance Measures in Subjects with Bipolar Disorder. Scatterplots depicting Hamilton Depression Rating measures (A), Young Mania Rating Scale measures (B) and Pittsburgh Sleep Quality Index scores (C) in control subjects and subjects with bipolar disorder. Significant increases of MDRS (A) YMRS (B) and PSQI (C) were detected in subjects with Bipolar Disorder. Scatterplots show the mean (histogram) and 95% confidence intervals (black lines).

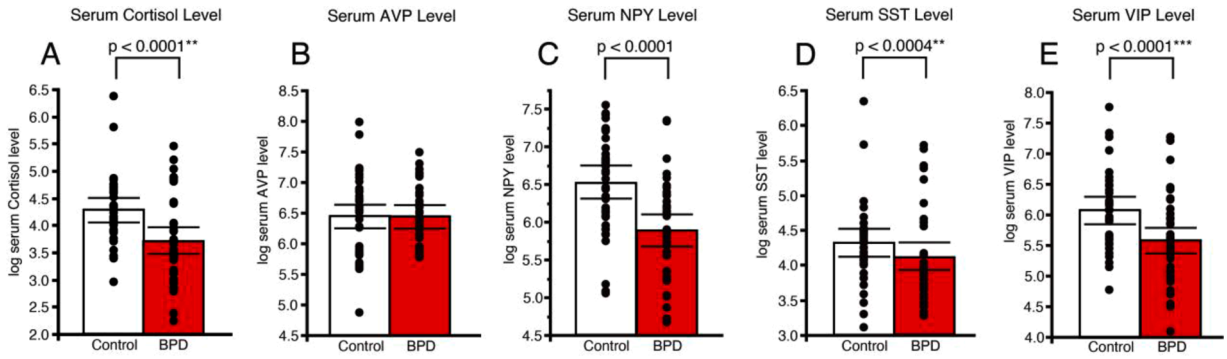

Fig. 2. Decreased Morning Levels of Serum Cortisol and Neuropeptides in Subjects with Bipolar Disorder. Scatterplots depicting serum cortisol (A), AVP (B), NPY (C), SST (D), and VIP (E) in control subjects and subjects with bipolar disorder. Significant decreases of cortisol (A) NPY (C), SST (D) and VIP (E) were detected in subjects with Bipolar Disorder. Scatterplots show the mean (histogram) and 95% confidence intervals (black lines). ** Adjusted for significant effects of YMRS and lithium. ***Adjusted for significant effects of age, sex, YMRS, and lithium.

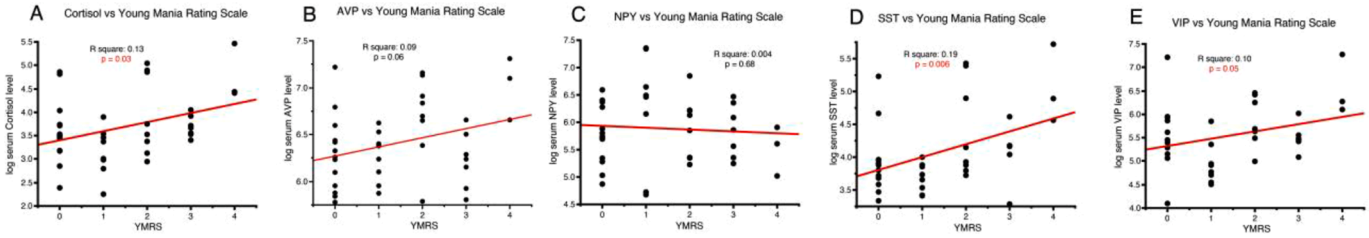

Fig. 3. Association of YMRS with Serum Cortisol and Neuropeptide Levels in Subjects with Bipolar Disorder Scatterplots depicting associations of serum cortisol (A), AVP (B), NPY (C), SST (D), and VIP (E) with Young Mania Rating Scale scores in subjects with bipolar disorder. Significant positive correlations of cortisol (A) SST (E), and VIP (E) were detected with YMRS in subjects with Bipolar Disorder. Scatterplots show the value for each subject plotted by YMRS scores.

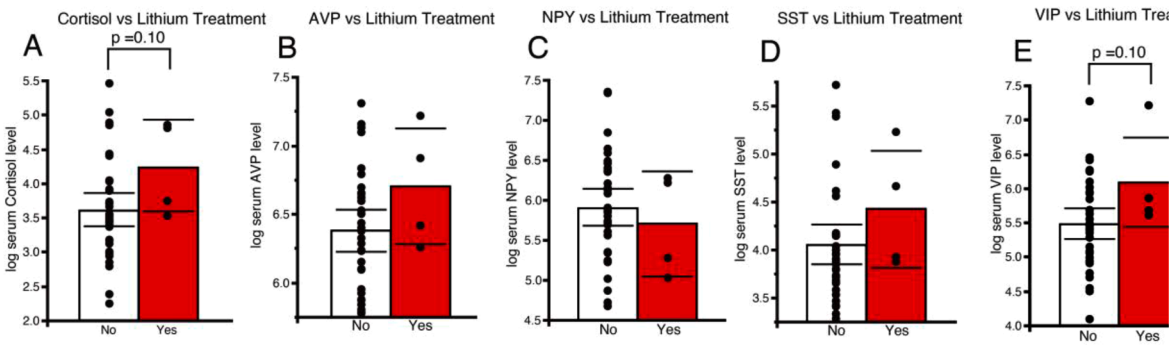

Fig. 4. Relationship of Lithium Treatment With Levels of Serum Cortisol and Neuropeptides in Subjects with Bipolar Disorder. Scatterplots depicting serum cortisol (A), AVP (B), NPY (C), SST (D), and VIP (E) levels in subjects with bipolar disorder with or without lithium treatment. Non-significant increases of cortisol (A) and VIP (E) were detected in subjects with lithium treatment. Scatterplots show the mean (histogram) and 95% confidence intervals (black lines).

4.1. Technical considerations

Limitations of our study include is a cross-sectional study with a small sample size. Blood samples were collected at only one timepoint from patients in a euthymic phase, limiting the interpretation of our results. It is possible that the molecules we examined are decreased across the 24-hour cycle, or alternatively, they may be increased later in the day or at night due to altered circadian expression of these factors. We focused on a single timepoint in the morning due to the reported moorning increased severity of anxiety and depression symtptoms (Murray, 2007b, 2008b; Murray et al., 2002a; Wirz-Justice, 2008a) and the observed morning decrease of SST expression in the brain and csf of people with BDP (Pantazopoulos et al., 2016) (Rubinow, 1986) for this initial study. However, future studies with sampling intervals across the 24-hour cycle will provide further insight into the interpretation and underlying cause of these changes. Our study consisted of 39 patients diagnosed as BPD and 38 healthy controls. Future studies are needed ideally in larger cohorts of 100 subjects per group and studies using subjects from multiple geographic locations with a wider range of diversity in order to confirm and expand upon our findings. Any negative findings from replication studies should also be reported. Furthermore, for ethical purposes, all patients in our study were taking psychiatric medications, and their treatment continued during the study. Therefore, the effect of drugs on hormones could not be evaluated. Our study consisted only of subjects with BPD and control subjects, no other related psychiatric disorders were analyzed. Therefore, our observed changes may not be specific for BPD and may be present to some extent in related disorders such as major depressive disorder and schizophrenia.

4.2. Lower morning levels of neuropeptides involved in circadian rhythm regulation

We observed lower levels of cortisol, SST, VIP, and NPY in samples taken in the morning from euthymic BPD patients compared to the control subjects. These decreases coincide with reported morning severity of anxiety and depression in mood disorders (Murray, 2007b, 2008b; Murray et al., 2002a; Wirz-Justice, 2008a), as well as with

reported morning decreases of SST in the amygdala (Pantazopoulos et al., 2016) and csf of subjects with mood disorders (Rubinow, 1986). Taken together, our results suggest that lower morning levels of these molecules involved in circadian rhythm and mood regulation may represent a biomarker for BPD, detectable in blood samples. Further studies in larger subject cohorts including subjects in manic states and subjects in depressed states are needed to confirm and expand on these findings.

Lower morning SST, VIP, NPY and cortisol may reflect overall decreased levels or an altered circadian phase in subjects with BPD. The increasing evidence supporting the role of circadian rhythm abnormalities in mood disorders provides support for the latter (Aydin et al., 2013b; Benedetti et al., 2008b; Bersani et al., 2012a; Etain et al., 2011b; Harvey, 2011b; Jagannath et al., 2013b; Kripke et al., 2009a; McClung, 2011a; Nievergelt et al., 2006b; Plante and Winkelman, 2008b; Severino et al., 2009b; Soria et al., 2010a). Studies have shown that circadian rhythm synchronization is reduced in patients with BPD. (Gonzalez, 2014; McKenna et al., 2014b; Rock et al., 2014a). In addition, studies using bright light therapy also support altered circadian phase in BPD. Bright light therapy, effective in the treatment of major depression in the morning, triggers mixed-manic conditions when administered in the morning to subjects with BPD in a depressed state (Kaladchibachi and Fernandez, 2018; Sit et al., 2007, 2018). However, this treatment is effective when administered to patients with bipolar depression in the middle of the day (Kaladchibachi and Fernandez, 2018; Sit et al., 2007, 2018). The actions of pharmacological treatment also support altered circadian phase in BPD. Lithium and valproic acid represent two of the most effective treatments for BPD. These two therapeutic agents both modulate the expression of core clock molecules and the circadian period (Abe et al., 2000; Johansson et al., 2011; Klemfuss, 1992; Li et al., 2012; Yin et al., 2006). In addition genetic polymorphisms of clock molecules, including in findings from GWAS studies, have been associated with BPD and with lithium responsiveness (Bollettini et al., 2017; Mansour et al., 2009; McCarthy et al., 2012; McCarthy and Welsh, 2012; Nievergelt et al., 2006a; Pandey et al., 2012; Rybakowski, 2014; Sjoholm et al., 2010; Soria et al., 2010b; Suzuki et al., 2017)).

Several lines of evidence suggest our observed morning decreases in neuropeptide levels may contribute to symptom severity. The most severe symptoms of anxiety and depression consistently occur in the morning (Murray, 2007b, 2008b; Murray et al., 2002a; Wirz-Justice, 2008a), indicating a severity of a circadian component. Our observed decrease of SST levels in the morning in patients with BPD corresponds with reported morning decrease of SST in the CSF (Rubinow, 1986) and in postmortem amygdala samples with a morning time of death (Pantazopoulos et al., 2016), suggesting that lower SST in the morning is a consistent feature of BPD, reflected in brain regions involved in mood regulation (Pantazopoulos et al., 2016), and detectable in blood samples. Rodent studies have demonstrted a criticial role of SST and NPY amygdala neurons in anxiety regulation (Albrecht et al., 2013a; Engin et al., 2008b; Engin and Treit, 2009b; Lin and Sibille, 2015b; McDonald et al., 1995; Yeung et al., 2011b; Yeung and Treit, 2012b).

Rodent studies have demonstrated a critical role of SST in mood regulation. For example, mice with SST deficiency display neuroendocrine, molecular and behavioral abnormalities similar to those seen in human subjects with depression (Albrecht et al., 2013a; Lin and Sibille, 2015b) . Moreover, circadian expression of SST and NPY in the mouse amygdala is associated with increased periods of anxiety at specific circadian times (Albrecht et al., 2013a). Therefore, changes in amygdala SST levels in the morning may be associated with abnormalities of the circadian rhythm associated with symptom severity (Albrecht et al., 2013b; Murray, 2007a, 2008a; Murray et al., 2002b; Wirz-Justice, 2008b).

In addition to lower levels of SST, our observed decreases of cortisol, VIP and NPY may reflect altered circadian rhythm regulation. VIP and NPY expression have both been shown to regulate circadian rhythms in the SCN in mice (Aton et al., 2005; Colwell et al., 2003; Huhman and Albers, 1994; Pantazopoulos et al., 2010; Weber and Rea, 1997; Yannielli et al., 2004), and cortisol serves as a broad systemic circadian rhythm entraiment factor (So et al., 2009; Yamamoto et al., 2005). Together with lower SST levels, decreases of VIP and NPY may reflect core cicadian rhythm distrubances in subjects with BPD, detectable in minimally invasive blood tests. The significant positive correlation of cortisol with SST and NPY levels supports the hypothesis that these changes may reflect a core circadian rhythm disturbance. Future studies in which serum samples are collected across multiple timepoints from the same subjects will shed light into this hypothesis.

4.3. Lower morning cortisol levels in BPD

Our observed decrease of cortisol levels in subjects with BPD compared to control subjects from samples collected at 9 am is in contrast to reported increases of cortisol in subjects with BDP (Cervantes et al., 2001). This discrepancy may be due to sampling time, as previously published studies report a phase advance of cortisol peak expression in BPD, occuring at approximately 6–7 am (Cervantes et al., 2001). Our sampling time of 9 am may be past the peak of expression, and is in agreement with the reported lower cortisol levels at 9–10 am in subjects in a euthymic state compared to control subjects during this time range (Cervantes et al., 2001). A recent study reporting lower salivary cortisol levels in the morning in subjects with depressive disorder supports our findings in blood samples (Izakova et al., 2020). In addition, our samples were taken from BPD subjects in a euthymic state, which may also affect these results, as differences in cortisol rhythms were reported between mood states in BPD subjects (Cervantes et al., 2001; Kennedy et al., 1989; Moon et al., 2016). Based on reports of altered cortisol levels in BPD dependent on mood state, we expect that our observed findings in euthymic patients would be more pronounced in subjects in a depressed state, and would be differentially affected in subjects in a manic state. Future studies using additional sampling timepoint on subjects across mood states of cortisol together with neuropetide levels will provide insight into this question.

In conclusion, we report evidence for alterations in morning plasma SST, VIP, NPY and cortisol levels together in the same subjects from euthymic BPD patients. Cortisol, NPY, SST, and VIP are involved in regulating mood and circadian rhythms, and thus may represent a biomarker for circadian rhythm abnormalities in BPD detectable in blood tests. The positive effects of YMRS and lithum with cortisol, SST and VIP suggest that decreases in these measures may be associated with depression features, and partially corrected by lithium. Further studies with larger patient groups and sampling times are needed to confirm and expand on our findings, in order to establish a blood test for circadian rhythm dysfunction that we can link to circadian alterations of these peptides in brain regions involved in mood regulation, such as the reported morning decrease of SST in the amygdala (Pantazopoulos et al., 2016).

Data sharing

Data will be shared upon request.

Declaration of Competing Interest

Authors declare that they have no conflict of interest.

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