

# Reduced plasma orexin-A levels in patients with bipolar disorder

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**Purpose:** Orexins are hypothalamic neuropeptides involved in the regulation of sleep, appetite and arousal. An altered orexin system has been implicated in the pathophysiology of psychiatric disorders. This study aimed to examine whether plasma orexin-A levels differ in patients with schizophrenia, major depressive disorder (MDD), or bipolar disorder (BD) compared to in healthy controls. We also examined the possible correlations between plasma orexin-A levels and clinical variables.

**Patients and methods:** All participants were Japanese. The sample consisted of 80 patients with schizophrenia (42 women, 52.5%; mean age 36.8 years), 80 patients with MDD (43 women, 53.8%; 43.7 years), and 40 patients with BD (24 women, 60%; 41.1 years), as well as 80 healthy controls (48 women, 60%; 47.0 years). Plasma orexin-A levels were quantified by an enzyme-linked immunosorbent assay.

**Results:** Mean orexin-A levels were significantly different across the four diagnostic groups ( $F=4.09$ ;  $df=3$ ;  $p=0.007$ ,  $\eta^2=0.06$ ). In particular, the patients with BD showed significantly lower orexin-A levels than did the controls. When the median value of the control group (109.8 pg/ml) was set as a cut-off value, subjects whose orexin-A levels were below the cut-off were more common in all psychiatric groups (schizophrenia: 73.8%,  $\chi^2=9.56$ ,  $df=1$ ,  $p=0.003$ ,  $OR=2.81$ , 95% CI: 1.45 to 5.45,  $d=0.57$ ; MDD: 78.5%,  $\chi^2=14.02$ ,  $df=1$ ,  $p<0.001$ ,  $OR=3.65$ , 95% CI: 1.82 to 7.29,  $d=0.72$ ; BD: 87.5%,  $\chi^2=16.0$ ,  $df=1$ ,  $p<0.001$ ,  $OR=7.00$ , 95% CI: 2.49 to 19.70,  $d=1.07$ ). We found no association between plasma orexin-A levels and any clinical symptoms, depression severity, or medication doses.

**Conclusion:** Our results suggest that plasma orexin-A levels are reduced in patients with BD.

**Keywords:** orexin-A, plasma, schizophrenia, major depressive disorder, bipolar disorder

## Introduction

Orexin A (hypocretin-1) and B (hypocretin-2) are 33- and 28-amino acid peptides, respectively, and are synthesized in a cluster of neurons in the lateral hypothalamus. Orexins selectively act on two G-protein-coupled receptors: the orexin-1 receptor (OX1R), which has higher affinity to orexin-A, and the orexin-2 receptor (OX2R), which has similar affinities to orexin-A and orexin-B.<sup>1,2</sup> These orexin receptors are expressed throughout the brain, although they are extensively localized within the hypothalamus, hippocampus, raphe nuclei, basal ganglia, locus coeruleus and cortex.<sup>3-5</sup> Orexin neurons are located only in the lateral hypothalamus and perifornical area.<sup>6,7</sup> However, their projection fibers are widely distributed in the central nervous system (CNS), including the basal forebrain, thalamus, and prefrontal cortex.<sup>8</sup>

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Until recently, orexin has been studied extensively in patients with narcolepsy whose cerebrospinal fluid (CSF) orexin levels were found to be very low.<sup>9,10</sup> The orexin system plays a role not only in sleep awake status<sup>11</sup> but also in motor control,<sup>12</sup> feeding behavior,<sup>6</sup> stress response<sup>14</sup> and reward,<sup>14</sup> and the regulation of autonomic functions and energy homeostasis.<sup>13</sup> Preclinical and cellular studies suggest that orexin-A and/or orexin afferents modulate noradrenaline, serotonin, dopamine and gamma-aminobutyric acid (GABA) systems<sup>15–17</sup> and that CSF orexin-A levels are correlated with corticotropin releasing hormone (CRH) levels in humans.<sup>18</sup> These neurochemical and neuroendocrine systems have been implicated in the pathophysiology of psychiatric disorders, including mood disorders and schizophrenia.<sup>18–21</sup> For example, orexin receptor antagonists reduced stress-induced anxiety-like behaviors in rodents.<sup>22–25</sup> Intracerebral injection of orexin-A reduced depressive-like behaviors in mice.<sup>26</sup> Lower orexin levels and a reduced number of orexin neurons in the hypothalamus, medial prefrontal cortex (mPFC) and ventral tegmental area (VTA) have been reported in several animal models of depression.<sup>27–29</sup> In rodents, activation of hypothalamic orexin neurons can release orexins that in turn activate dopamine neurons in the VTA, increasing dopamine levels in the prefrontal cortex and/or the striatum.<sup>30,31</sup> These animal studies suggest a possible role of orexin-A in the pathophysiology of psychiatric disorders.

Human studies have also shown that depressed patients had lower orexin-A levels in the CSF and that their depression severity was negatively correlated with orexin-A levels in the CSF.<sup>32–34</sup> Additionally, reduced orexin-A mRNA in peripheral blood cells was reported to be correlated with higher scores on the Hamilton Depression Rating Scale.<sup>35</sup> Salomon et al<sup>36</sup> reported that CSF orexin-A levels in depressive subjects tended to be higher than in control subjects, while the diurnal cycle amplitude was significantly smaller than that in controls. In contrast, Schmidt et al<sup>37,38</sup> found no significant difference in CSF orexin-A levels in patients with either mania or unipolar depression compared to healthy controls. Regarding schizophrenia, Nishino et al<sup>39</sup> reported a positive correlation between CSF orexin-A levels and sleep latency in patients with schizophrenia. Dalal et al<sup>40</sup> found that mean CSF orexin levels were significantly lower in patients with schizophrenia treated with haloperidol than in unmedicated patients.

A previous report showed that orexin-A (but not orexin-B) is highly lipophilic and rapidly crosses from the brain to the periphery through the blood-brain barrier (BBB) by means of a nonsaturable mechanism.<sup>41</sup> Indeed, plasma and CSF orexin-A levels were reported to be lower in patients with narcolepsy.<sup>45,46</sup> Strawn et al<sup>42</sup> reported that CSF and plasma orexin-A concentrations showed a strong correlation and that peripheral orexin-A levels were correlated with CNS serotonergic tone (5-hydroxyindolacetic acid in CSF) in patients with posttraumatic stress disorder (PTSD) and healthy subjects. One study reported that patients with schizophrenia with fewer negative and disorganized symptoms had a significantly higher mean plasma orexin-A level than healthy controls.<sup>43</sup> To our knowledge, no study has examined plasma orexin-A levels in patients with MDD or BD, and only one study compared the plasma orexin-A levels of patients with schizophrenia with those of healthy controls.

As described above, there is a dearth of studies on orexin-A levels in psychiatric disorders. The aim of the present study was to examine whether plasma orexin-A levels are altered in patients with schizophrenia, MDD, and BD compared to in healthy controls. We further examined the possible correlation between plasma orexin-A levels and clinical variables, such as disease severity and medication doses.

## Materials and methods

### Subjects

The subjects included 80 patients with schizophrenia, 80 patients with MDD, 40 patients with BD, and 80 healthy controls. All subjects were biologically unrelated Japanese individuals recruited from the outpatient clinic of the National Center of Neurology and Psychiatry Hospital, Tokyo, Japan, or through advertisements in free local information magazines and on our website announcement. Consensus diagnosis by at least 2 psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria,<sup>46</sup> on the basis of information obtained by the Japanese version of the MINI (Mini International Neuropsychiatric Interview),<sup>47,48</sup> additional unstructured interviews and information from medical records if available. The controls were healthy volunteers with no current or past history of psychiatric disorder based on the information obtained by the M.I.N.I. Participants were excluded if they had prior medical histories of CNS

diseases or severe head injury or if they met the criteria for substance abuse/dependence or mental retardation. The study protocol was approved by the ethics committee at the NCNP. The study was performed according to the Declaration of Helsinki.<sup>49</sup> After the study was described to potential subjects, written informed consent was obtained from every subject.

The Positive and Negative Syndrome Scale (PANSS) was used to evaluate the symptoms in patients with schizophrenia.<sup>50</sup> The 17-item version of the Hamilton Depression Rating Scale (HAM-D-17) was used to assess depressive symptoms in patients with MDD or BD,<sup>51</sup> and the cut-off score for remission was set at  $\leq 7$ .<sup>52</sup> There were no patients with BD who showed a manic state at the time of blood sampling. Daily doses of antipsychotics and antidepressants were converted to chlorpromazine and imipramine equivalent doses according to the published guidelines.<sup>53</sup>

## Measurement of orexin-A

At approximately noon (before lunch), blood samples were collected into EDTA blood tubes and centrifuged to separate the plasma fraction. Plasma samples were then stored at  $-80^{\circ}\text{C}$  until they were used for the assay. Plasma orexin-A levels were measured using a fluorescent enzyme immunosorbent assay (ELISA) kit (FKE-003-30; Phoenix Pharmaceuticals, Burlingame, CA, USA). Using the results from two separate runs of standard concentrations, the interassay coefficient of validation (CV) was less than 10%.

## Statistical analysis

Statistical differences between groups were calculated using Student's unpaired *t*-test, one-way analysis of variance (ANOVA), analysis of covariance (ANCOVA) adjusted for age, sex, and smoking status, and the chi-square test. Correlations were assessed using Pearson's correlation coefficient. Differences with a two-tailed *p*-value of less than 0.05 were deemed statistically significant, and the Bonferroni correction was used for multiple comparisons. Statistical analyses were performed using SPSS Statistics version 23.0 (IBM Japan, Tokyo, Japan).

## Results

The demographic and clinical characteristics of the patients and controls are presented in Table 1. There were no significant differences in the male-to-female ratio ( $\chi^2=1.34$ ;  $df=3$ ;  $p=0.719$ ) or body mass index (BMI)

( $F=1.98$ ;  $df=3$ ;  $p=0.117$ ) across the four diagnostic groups. However, the schizophrenia group was significantly younger than the control and MDD groups ( $F=10.21$ ;  $df=3$ ;  $p<0.001$ ) and had higher smoking status compared with the control ( $\chi^2=7.864$ ,  $df=3$ ,  $p=0.049$ ). There was no correlation between plasma orexin-A levels and age ( $r=0.168$ ,  $p=0.136$ ), and there was no significant sex difference in the healthy control group ( $F=0.793$ ,  $df=78$ ,  $p=0.156$ ) (Figures S1 and S2).

After adjusting for age, sex, and smoking status, mean orexin-A levels were significantly different across the four diagnostic groups ( $F=4.09$ ;  $df=3$ ;  $p=0.007$ ,  $\eta^2=0.06$ ) (Table 1). When each diagnostic group was compared with the control group, there was no significant difference in plasma orexin-A levels between patients with schizophrenia and controls. The plasma orexin-A levels in patients with MDD tended to be lower than those in the control group, although the difference failed to reach statistical significance after the Bonferroni correction was applied ( $p=0.089$ ). The orexin-A levels of the patients with BD were significantly lower than those of controls ( $p=0.010$ ) (Figure 1). As shown in Figure 1, the number of patients with relatively lower orexin-A levels seemed to be higher for all psychiatric groups. When a cut-off point of plasma orexin-A levels was set at 109.8 pg/ml due to the median value of the control group (ie, median split), the rate of subjects below the cut-off was 73.8% in patients with schizophrenia ( $\chi^2=9.56$ ,  $df=1$ ,  $p=0.003$ ; OR =2.81, 95% CI: 1.45 to 5.45;  $d=0.57$ ), 78.5% in patients MDD ( $\chi^2=14.02$ ,  $df=1$ ,  $p<0.001$ ; OR=3.65, 95% CI: 1.82 to 7.29;  $d=0.72$ ), and 87.5% in patients with BD ( $\chi^2=16.0$ ,  $df=1$ ,  $p<0.001$ ; OR=7.00, 95% CI: 2.49 to 19.70;  $d=1.07$ ) (Table 2).

Within each diagnostic group, there was no correlation between plasma orexin-A levels and any clinical variables, including age, BMI, severity scores, or medication doses (Table S1).

## Discussion

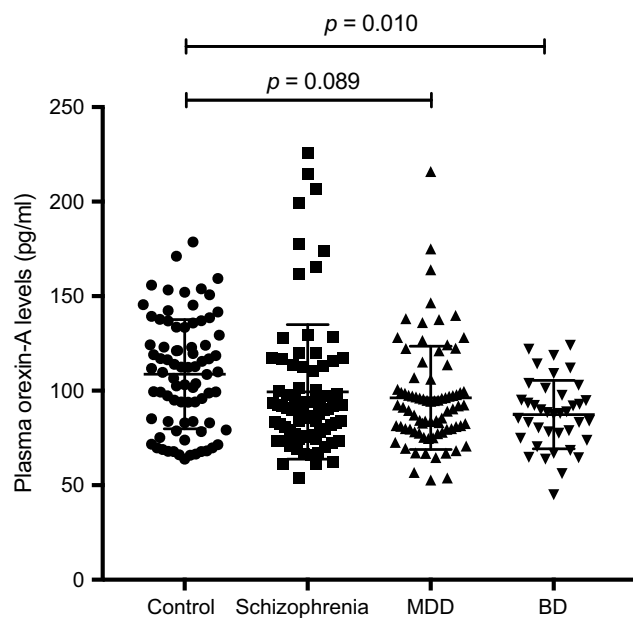
In this study, we examined the plasma orexin-A levels in patients with schizophrenia, MDD, and BD in comparison with those of healthy controls. When each diagnostic group was compared with the control group, mean orexin-A level was significantly lower only in the patients with BD. Individuals who showed plasma orexin-A levels below the cut-off point (109.8 pg/ml; median value of controls group) were significantly more numerous in the schizophrenia, MDD, and BD groups than in the control

Table 1 Demographic and clinical characteristics

	Schizophrenia (n = 80)	MDD (n = 80)	BD (n = 40)	Control (n = 80)	Analysis
Age (y)	36.8 (11.2)	43.7 (10.4)	41.1 (12.1)	47.0 (14.2)	ANOVA: F = 10.21, df = 3, p < 0.001
Sex (M/F)	38/42	37/42	16/24	32/48	$\chi^2$ -test: $\chi^2 = 1.34$ , df = 3, p = 0.719
BMI	23.2 (4.4)	22.7 (3.9)	23.4 (4.4)	21.9 (3.0)	ANOVA: F = 1.98, df = 3, p = 0.117
Smoking status (%)	29.5	16.3	21.1	12.7	$\chi^2$ -test: $\chi^2 = 7.864$ , df = 3, p = 0.049
Plasma orexin-A levels (pg/ml)	99.5 (35.7)	96.2 (27.5)	88.2 (16.8)	108.8 (29.1)	ANCOVA: F = 4.09, df = 3, p = 0.007
Medication status					
on typical antipsychotic medication (%)	65.0	17.9	25.0		
on atypical antipsychotic medication (%)	17.5	5.1	10.0		
on antidepressant medication (%)	15.0	50.0	45.0		
on benzodiazepine medication (%)	21.3	17.9	35.0		
on lithium medication (%)	2.5	7.7	37.5		
on benzodiazepine medication (%)	1.3	0	10.0		
on varproric acid medication (%)	5.0	10.3	17.5		
on lamotrigine medication (%)	2.5	2.6	32.5		
Medications					
Typical antipsychotics (mg/day) <sup>a</sup>	595.5 (1107.4)				
Atypical antipsychotics (mg/day) <sup>a</sup>	247.4 (313.1)				
Antidepressant (mg/day) <sup>b</sup>	172.1 (177.4)	147.6 (103.2)	200.7 (147.4)		
Benzodiazepine (mg/day) <sup>c</sup>	5.2 (2.5)	3.4 (1.7)	6.6 (3.6)		
Lithium (mg/day)			650.0 (290.2)		
Carbamazepine (mg/day)			566.7 (57.7)		
Varproric acid (mg/day)			500.0 (419.5)		
Lamotrigine (mg/day)			185.4 (99.7)		
PANSS					
Positive symptoms	13.8 (5.3)				
Negative symptoms	15.5 (6.2)				
General	31.3 (8.1)				
HAM-D scores					
HAM-D <sub>total</sub>		11.4 (8.3)	10.9 (7.7)		
HAM-D <sub>core</sub>		5.7 (4.3)	5.5 (3.9)		
HAM-D <sub>sleep</sub>		1.6 (1.5)	1.5 (1.7)		
HAM-D <sub>activity</sub>		1.6 (1.7)	1.8 (1.7)		
HAM-D <sub>psychic anxiety</sub>		1.2 (1.2)	1.2 (0.9)		

**Note:** <sup>a</sup>Daily dosage expressed in chlorpromazine equivalents. <sup>b</sup>Daily dosage expressed in imipramine equivalents. <sup>c</sup>Daily dosage expressed in diazepam equivalents.

**Abbreviations:** MDD, major depressive disorder; BD, bipolar disorder; PANSS, positive and negative syndrome scale; HAM-D, hamilton rating scale for depression.



**Figure 1** Dot plots with plasma orexin-A levels in the study groups. The plasma orexin-A levels in healthy controls and patients with schizophrenia, major depressive disorder, and bipolar are shown. Horizontal bars represent mean values of the groups, and error bars indicate standard deviations. There was a significant difference in plasma orexin levels between the diagnostic groups and healthy controls ( $p=0.007$ ). Post hoc tests showed that bipolar patients had decreased plasma orexin-A levels compared with healthy controls ( $p=0.010$ ).

group. However, we found no association between plasma orexin-A levels and any clinical variable, such as disease severity and medication dose.

To our knowledge, this is the first study that examined plasma orexin-A levels in the three psychiatric disorders in comparison with those of healthy controls. Several studies have reported that orexin-A is present not only in the CSF but also in the peripheral blood of healthy individuals and some narcoleptic patients.<sup>9,10,44,45</sup> With regard to psychiatric disorders, there have been only a few reports that have examined the association between plasma orexin-A levels and mood disorder and schizophrenia. Strawn et al<sup>42</sup> reported that the mean plasma and CSF orexin-A concentrations were significantly lower in patients with PTSD

than in healthy subjects. There was a strong correlation between CSF and plasma orexin-A concentrations in both patients with PTSD and healthy subjects, and plasma orexin-A was negatively correlated with CSF 5-HIAA in healthy subjects.<sup>42</sup> Chien et al<sup>43</sup> showed that patients with schizophrenia ( $N=127$ ) had significantly higher mean plasma orexin-A levels than did healthy controls ( $N=34$ ); however, when the patients were divided into two groups consisting of high and low orexin levels, patients with normal orexin-A levels had significantly more negative symptoms than did patients with high-orexin levels.

Although the origin of plasma orexin-A has not yet been determined, orexin-A neurons are restricted to the lateral hypothalamus area,<sup>7,8</sup> and highly lipophilic orexin-A rapidly crosses the BBB through a nonsaturable mechanism.<sup>41</sup> Therefore, circulating orexin-A could originate from the hypothalamus via the BBB, in which case plasma orexin-A levels may at least partially reflect the production of orexin-A in the hypothalamus. Our results suggest that the observed decrease in plasma orexin-A levels reflects decreased central orexin-A in patients with psychiatric disorders.

We found that the patients in psychiatric disorder groups, particularly patients with BD, exhibited low levels of orexin-A than did the healthy controls. Because psychiatric patients frequently present sleep disorders and circadian disruption,<sup>54–56</sup> altered orexin-A signaling may contribute to such arousal and wake-rest disruptions. Specifically, BD patients have a mismatched biological clock and sleep-wake cycle, and the rate of comorbid circadian rhythm sleep-wake disorders in BD subjects was significantly higher than that in MDD subjects.<sup>57,58</sup> During periods of depression in patients with BD, sleep disruptions are commonly exhibited as hypersomnia or excessive sleepiness. Through the use of actigraphy data, research among patients with a current depression diagnosis suggests that patients with bipolar depression are likely to have a delayed sleep phase.<sup>59</sup> In addition, we have

**Table 2** Number of subjects below the cut-off point (109.8 pg/ml) and odds ratios for healthy controls

Group	Frequency, n (%)	OR (95% CI)	$\chi^2$	p-value	d
Schizophrenia	59 (73.8)	2.81 (1.45–5.45)	9.56	0.003	0.57
MDD	62 (78.5)	3.65 (1.82–7.29)	14.02	<0.001	0.72
BD	35 (87.5)	7.00 (2.49–19.70)	16.00	<0.001	1.07
Controls	40 (50.0)	1.00 (reference)	N.A.	N.A.	N.A.

**Note:** Adjusted significance was set at  $p<0.017$  (0.05/3).

**Abbreviations:** MDD, major depressive disorder; BD, bipolar disorder; OR, Odds ratio; CI, confidence interval; N.A., Not applicable.



previously reported that MDD and BD patients showed significant disturbances in sleep and rest-activity rhythms, along with reduced activity levels and increased sleep time each day.<sup>60</sup> Another explanation for the lower orexin-A levels is that the genetic studies addressed the correlation between the orexin system and psychiatric disorders. The carrier of the A allele of the rs2271933 G>A polymorphism (Ile408Val) in the HCRTR1 gene was more common in unipolar depression than in controls.<sup>61</sup> Rotter et al<sup>35</sup> reported that orexin-A mRNA expression in peripheral blood cells was decreased in depressive patients.

We found no significant correlation between orexin-A levels and disease severity or medication dose in any diagnostic group. These findings may have been limited by the fact that patients in the present study are mostly in the chronic phase rather than in the acute phase. Additionally, our measurement of the plasma sample took place in a real-world setting; the majority of patients were taking psychotropic medication, and sampling was not performed until after a whole night of fasting (but before lunch). However, a recent investigation reported that plasma orexin-A levels are not related to circadian rhythms or feeding,<sup>62</sup> it is not yet clear whether plasma orexin-A has any physiological significance in relation to psychiatric disorders. The role of orexin-A in the pathological states of psychiatric disorders such as schizophrenia, MDD and BD needs to be investigated in further studies.

A major limitation is that the patients were under pharmacotherapy and thus the severity of their illness was relatively mild at the time of participation in the study (mean HAM-D scores of 11.4 in MDD and 10.9 in BD; mean PANSS score of 60.6 in schizophrenia), which may have resulted in type II errors, particularly for MDD and schizophrenia groups. To address this issue, further studies in more severe subjects will be required. It is also possible that the medication may have affected the plasma orexin-A level in our patients. However, we found no significant correlation between plasma orexin-A levels and medication dose in any diagnostic group. It is therefore unlikely that medication has a major effect on plasma orexin-A levels.

## Conclusion

Our results suggest that plasma orexin-A levels are reduced in patients with BD than in healthy controls, suggesting that the low plasma levels of orexin-A play a role, at least in a proportion of BD. However, the elucidation of the clinical implications of plasma orexin-A concentrations would

require further clinical studies with larger sample sizes and preclinical studies to examine the mechanisms underlying the altered orexin-A levels in BD.

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## Disclosure

The authors report no conflicts of interest in this work.

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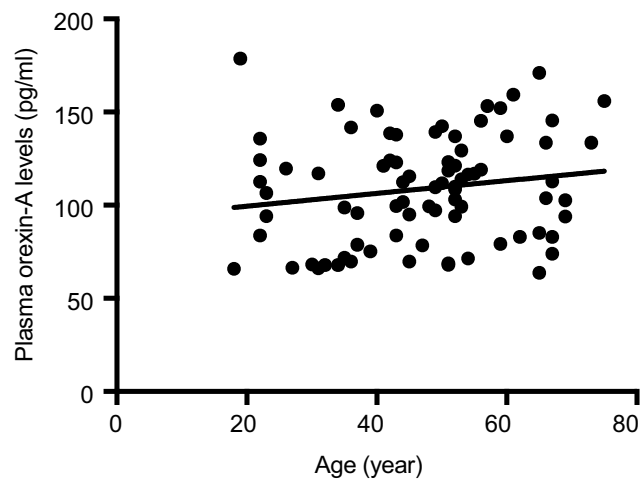


## Supplementary materials

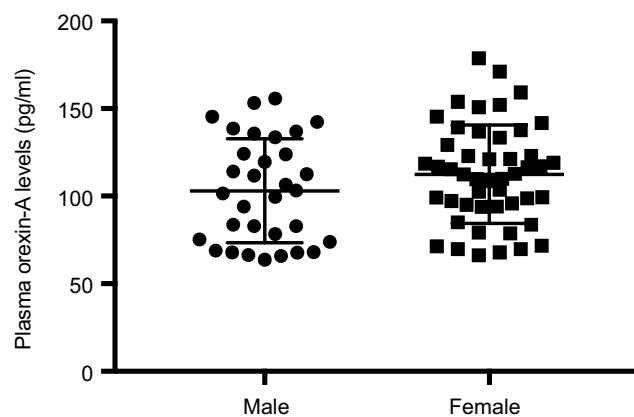
**Table S1** Simple correlations between plasma orexin-A levels and clinical variables

	Schizophrenia (n = 80)		MDD (n = 80)		BD (n = 40)	
	r	p	r	p	r	p
Age (y)	0.182	0.106	-0.098	0.939	-0.134	0.410
BMI	-0.045	0.701	-0.055	0.628	-0.316	0.470
Medications						
Typical antipsychotics (mg/day)	-0.124	0.286				
Atypical antipsychotics (mg/day)	0.035	0.765				
Antidepressant (mg/day)	0.142	0.210	-0.082	0.621	-0.013	0.960
Benzodiazepine (mg/day)	0.041	0.721	0.216	0.642	-0.036	0.904
Lithium (mg/day)					-0.006	0.982
Carbamazepine (mg/day)					0.179	0.821
Valproic acid (mg/day)					-0.145	0.757
Lamotrigine (mg/day)					-0.190	0.535
PANSS						
Positive symptoms	0.024	0.835				
Negative symptoms	-0.102	0.381				
General	-0.171	0.145				
HAM-D scores						
HAM-D <sub>total</sub>			0.102	0.381	-0.100	0.550
HAM-D <sub>core</sub>			0.059	0.604	-0.038	0.818
HAM-D <sub>sleep</sub>			0.110	0.337	0.087	0.594
HAM-D <sub>activity</sub>			-0.023	0.841	-0.029	0.861
HAM-D <sub>psychic anxiety</sub>			0.117	0.306	-0.016	0.921
HAM-D <sub>somatic anxiety</sub>			0.038	0.742	-0.04	0.805

**Abbreviations:** MDD, Major depressive disorder; BD, Bipolar disorder; BMI, Body mass index; PANSS, Positive and Negative Symptoms Scale; HAM-D, Hamilton Rating Scale for Depression.



**Figure S1** Correlation between plasma orexin-A levels and age. There was no significant correlation between plasma orexin-A levels and age in healthy controls (N=80;  $r=0.168$ ,  $p=0.136$ ).



**Figure S2** Comparison of plasma orexin-A levels in healthy male and female controls. There was no significant difference in plasma orexin-A levels between males (N=32) and females (N=48) ( $p=0.156$ ). Horizontal bars represent mean values of the groups, and error bars indicate standard deviations.

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