Research Article

Wen-Dan Decoction Improves Negative Emotions in Sleep-Deprived Rats by Regulating Orexin-A and Leptin Expression

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Wen-Dan Decoction (WDD), a formula of traditional Chinese medicine, has been clinically used for treating insomnia for approximately 800 years. However, the therapeutic mechanisms of WDD remain unclear. Orexin-A plays a key role in the sleep-wake cycle, while leptin function is opposite to orexin-A. Thus, orexin-A and leptin may be important factors in sleep disorders. In this study, 48 rats were divided into control, model, WDD-treated, and diazepam-treated groups. The model of insomnia was produced by sleep deprivation (SD) for 14 days. The expressions of orexin-A, leptin, and their receptors in blood serum, prefrontal cortex, and hypothalamus were detected by enzyme-linked immunosorbent assay, immunohistochemistry, and real time PCR. Open field tests showed that SD increased both crossing movement (Cm) and rearing-movement (Rm) times. Orexin-A and leptin levels in blood serum increased after SD but decreased in brain compared to the control group. mRNA expressions of orexin receptor 1 and leptin receptor after SD were decreased in the prefrontal cortex but were increased in hypothalamus. WDD treatment normalized the behavior and upregulated orexin-A, leptin, orexin receptor 1 and leptin receptor in brain. The findings suggest that WDD treatment may regulate SD-induced negative emotions by regulating orexin-A and leptin expression.

1. Introduction

Insomnia is a common sleep disorder characterized by difficulty falling asleep, staying awake, or both [1]. Worldwide, over 30% of insomnia cases are attributed to high stress or a busy life style [2]. In the USA, about 30–40% of adults have symptoms of insomnia within a given year, and about 10–15% of adults have chronic insomnia [3]. Depression or anxiety, chronic stress, and chronic pain may cause chronic insomnia [4, 5]. Conversely, chronic insomnia may aggravate depression and anxiety due to the dysfunctions of sleep-wake regulating neural circuitries, which have the capacity to reinforce emotional disturbances [6]. The interaction between sleep and emotional changes has been observed both in clinical and subclinical samples, but the molecular mechanisms of the vicious circle are still unclear.

Among many molecular factors, orexin-A and leptin have been associated with insomnia. Orexin-A is a peptide composed of 33 amino acids that have been found in cerebrospinal fluid, lateral and posterior hypothalamus, and medial thalamus [7, 8]. Orexin-A has been shown to play an important role in the sleep-wake cycle and in maintaining the stability of sleep [9–11]. Furthermore, orexin-A administra-
tion may strengthen the awake condition and lengthen awake time [12, 13]. The expression of orexin-A increases slowly in dark periods and decreases slowly in periods of illumination. Orexin receptor 1 (OX1R) is activated by orexin-A and is strongly expressed in cerebral cortex and tissue [14, 15].

Leptin is a polypeptide hormone encoded by the obese gene [16]. In contrast to orexin-A, sleep duration increases leptin secretion and sleep deprivation (SD) reduces leptin secretion [17]. The binding of leptin to its receptor (Ob-R) guarantees the function of orexin-A. Furthermore, SD can affect the expression of Ob-R [18]. However, the relationship between orexin-A and leptin in insomnia and in insomnia-induced emotion changes is still unknown. Therefore, exploring the changes in orexin-A, leptin, and the receptors OX1R and Ob-R in sleep-deprived rats is of great importance.

Currently prescribed sleep-aid medications are often associated with many side effects, including excessive drowsiness, impaired thinking, night wandering, agitation, and balance problems. However, Chinese herbal medicines exert more balanced and nourishing effects in the brain, which improve the symptoms of SD in a different way. Wen-Dan Decoction (WDD), a famous extract, has been used clinically by practitioners of traditional Chinese medicine (TCM) with an effective rate of 93.3% [19–22]. Clinical observations indicate that WDD can improve negative emotions to defend against insomnia [23]. However, the molecular mechanism by which WDD improves emotions and sleep is unknown. In the present study, we demonstrate that WDD may improve insomnia-induced negative emotions by regulating orexin-A and leptin expression.

2. Materials and Methods

2.1. Experimental Animals. Adult male Sprague Dawley rats were purchased from Beijing Vital River Laboratory Animal Technology Limited Company (Beijing, China). Animals were held in a room with a constant temperature of 23 ± 1°C; a relative humidity from 30% to 40%; light for 12 h, from 06:00 to 18:00; darkness for 12 h, from 18:00 to 06:00; and purified water ad libitum. Forty-eight rats were randomly divided into four groups as follows: control group (n = 12), model group (n = 12), diazepam-treated group (n = 12), and WDD-treated group (n = 12). The modified multiple platform method was used to generate the SD model rats [24]. Fifteen platforms in a deprivation case (110 cm × 60 cm × 40 cm) were surrounded with water at a temperature between 20°C and 22°C to a depth of 1.0 cm below the surface of the platforms. The rats in the control group were fed routinely for 15 days. The rats in the model, diazepam-treated, and WDD-treated groups were subjected to continuous SD (20 h/day) for 14 days. All animals in the study were maintained in accordance with the guidelines of Chinese legislation on the ethical use and care of laboratory animals. All efforts were made to minimize animal suffering and the number of animals needed to produce reliable data.

2.2. Open Field Test. The open field test (OFT) provides a novel environment in which animal locomotion, exploration, and anxiety are measured. The open field arena (100 cm × 100 cm × 40 cm) is constructed of acrylic, with gray walls and a black floor, which is divided into 25 squares of equal areas as previously described [25, 26]. Instances of crossing-movement (Cm) and instances of rearing-movement (Rm) are usually used as measures of exploration and anxiety [27, 28]. A high frequency of these behaviors indicates increased excitability and exploration. Each of the rats was tested for 3 min on experimental day 7 and 14.

2.3. WDD Preparation. WDD is comprised of eight different Chinese medicinal herbs (Table I). The components were purchased from the Pharmaceutical Department of Dongzhimen Hospital, which is affiliated with the Beijing University of Chinese Medicine (BUCM). Director Shihua Gao identified the components, and the voucher specimens were deposited. All of the components were soaked for 1 h at room temperature and decocted with distilled water for 2 h. The filtrates were condensed and dried by a vacuum desiccator at 60°C and then packaged and stored at room temperature for future use.

2.4. Drugs and Reagents. Diazepam was purchased from Beijing Yimin Pharmaceutical Co. Ltd. (Beijing, China). Rat orexin-A and leptin enzyme-linked immunosorbent assay (ELISA) kits were obtained from Vector Laboratories, Inc. (Burlingame, CA, USA). The rabbit avidin-biotin-peroxidase complex (ABC) detection kit was obtained from Vector Labs, Inc. (USA) and the rabbit leptin antibody (1:200) was obtained from Bioss (Beijing, China). Rabbit orexin-A antibody (1:100) was obtained from Millipore Co. (Billerica, MA, USA). Trizol reagent was purchased from Molecular Research Center (Cincinnati, OH, USA). High-Capacity cDNA Reverse Transcription kit was purchased from Applied Biosystems (Foster City, CA, USA). KAPA SYBR FAST qPCR kit was purchased from Kapa Biosystems (Woburn, MA, USA).

2.5. ELISA. After the 14-day trial, five rats from each group (control, model, diazepam-treated, and WDD-treated) were anesthetized with an intraperitoneal injection of 10% chloral hydrate (0.35 to 0.40 mL/100 g body weight). Rats were sacrificed and blood was collected and centrifuged at 3000 rpm for 15 min. The supernatant was collected and stored at −20°C. If precipitate was found during preservation, centrifugation was repeated. Experimental procedures were conducted according to the kit instructions and the concentrations of orexin-A and leptin in serum were detected.

2.6. Immunohistochemistry. Expression of orexin-A and leptin in prefrontal cortex and hypothalamus were detected by immunohistochemistry on experimental day 14. Five rats in each group were anesthetized with 10% chloral hydrate (0.35 to 0.40 mL/100 g body weight) and sacrificed. The entire brains were excised and flash-frozen in liquid nitrogen and then moved to −80°C for storage. The brains were dehydrated with 30% sucrose for 24 h and embedded in optimal cutting temperature (OCT) medium after cryoprotection. Frozen brains were cut into slices of 30 μm thickness and fixed with 4% paraformaldehyde for 10 min, then stored at −20°C. A
constant-temperature freezing microtome (Leica CM 1900) was used for tissue sectioning. Tissue sections were immersed in 3% H2O2 to inactivate the endogenous peroxidase and then washed with 0.05 M tris-buffered saline (TBS) three times for 5 min each, blocked by 10% goat serum at room temperature, and incubated with primary antibody at 4°C for over 18 h. After this incubation period, sections were washed with 0.05 M TBS three times for 5 min each, incubated with the secondary antibody at 37°C for 1 h, washed with TBS again, and finally incubated with ABC reagent at 37°C for 1 h. Sections were visualized by DAB for 5 min, dehydrated by graded ethanol solutions, vitrified by dimethylbenzene, and mounted with neutral balsam. Six visual fields were chosen randomly under a microscope at 100× magnification. Quantifications were performed by Image-Pro Plus version 6.0 analysis software to calculate the mean integrated optical density (MOD).

2.7. Real-Time PCR. Five rats in each group were randomly selected for analysis of gene expression by quantitative real-time PCR (qPCR). Total RNA from brain tissue was isolated with Trizol reagent according to the manufacturer’s protocol. Specifically, total brain tissues in Trizol reagent were selected for analysis of gene expression by quantitative real-time PCR (qPCR). To total RNA from brain tissue was isolated with Trizol reagent, according to the manufacturer’s protocol, specifically, total brain tissues in Trizol reagent were selected for analysis of gene expression by quantitative real-time PCR (qPCR). Total RNA from brain tissue was isolated with Trizol reagent according to the manufacturer’s protocol.

| Table 2: Primer sequences, length of PCR products, and optimal annealing temperature for each gene used in real-time quantitative PCR. | Primer | Sequence (5'-3') | Tm (°C) | bp |
|---|---|---|---|---|
| GAPDH | F: 5’GGAAAGCTGTGGCGTGA3’ | 60 | 308 |
|  | R: 5’AAGGTGGAAATGGGAGTTG3’ | 60 | 308 |
| Orexin-A | F: 5’CCCGAGAAGGCTTTCTC3’ | 60 | 88 |
|  | R: 5’GGCGGTTTCCCAAGATTGAG3’ | 60 | 88 |
| Orexin receptor 1 | F: 5’TTCGGGAGGCTTTCAAGG3’ | 60 | 203 |
|  | R: 5’CCCCAGGAAAGATCAA3’ | 60 | 203 |
| OB-R | F: 5’GCAGTCCAGCCTACACTTGG3’ | 60 | 171 |
|  | R: 5’GCTTCACCACATACTCTC3’ | 60 | 171 |

Table 1: Composition and active compounds of WDD.

| Components | Voucher specimens number | Part used | Active Compounds | Amount used (g) |
|---|---|---|---|---|
| Pinelliaternata | 3002305058 | Tuber | Total alkaloids | 6 |
| Immature bitter orange | 10038601 | Young fruit | Flavones | 6 |
| Citrus reticulate Blanco | 100580191 | Mature pericarp | Hesperidin, citrus flavonoids | 9 |
| Bamboo shavings | 100382441 | Interlayer of stem | Phosphodiesterase inhibitor | 6 |
| Liquorice | 100480341 | Rhizome | Triterpenoid saponins | 3 |
| Ginger | 100186533 | Rhizome | Gingerol | 5 |
| Poriacocos | 100382861 | Sclerotium | Polysaccharides | 4.5 |
| Fructusziziphijujubae | 10018527 | Fruits | Alkaloid and glycoside | 5 |

Data are expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) tests were used to analyze the data with statistical package for the social sciences (SPSS) version 17.0 software. In addition, the least significant difference (LSD) method was chosen for multiple comparisons. Table 2: Primer sequences, length of PCR products, and optimal annealing temperature for each gene used in real-time quantitative PCR.
was adopted for comparisons between groups. The repeated measures procedure of the general linear model (GLM) in SPSS version 17.0 was used to conduct one-way ANOVA analysis for repeated measures data (body-weight and food intake), and multivariate analysis process of variance was used to make comparisons between groups on each time point (the LSD method). P values <0.05 were considered statistically significant.

3. Results

3.1. WDD Treatment Ameliorated SD-Induced Increases in Cm and Rm Times. 7 days and 14 days after SD, the Cm and Rm times in the model group were significantly increased compared to the control group (P < 0.01). However, the Cm times and Rm times in the WDD-treated group were decreased when compared to the model group at 14 days after SD (Cm time: P < 0.01; Rm time: P < 0.05). Values of both parameters in the WDD-treated group were lower than the corresponding values in the diazepam-treated group (P < 0.01) (Table 3).

3.2. WDD Inhibited SD-Induced Increase of Orexin-A Content in Blood Serum. Serum levels of orexin-A in the model group were significantly higher than those in the control group (P < 0.01), while serum levels of orexin-A in the WDD group were lower than those of the model group (P < 0.05) (Figure 5).

3.3. The Mean Integrated Optical Density (MOD) of Orexin-A and Leptin in Both Prefrontal Cortex and Hypothalamus. In both prefrontal cortex and hypothalamus, orexin-A and leptin expressions in the model group were significantly lower than those in the control group (P < 0.01) (Figures 1, 2, 3, and 4). Remarkably, the expressions of orexin-A and leptin in the WDD group were significantly higher than those in the model group (P < 0.01). Compared to the diazepam group, the expressions of both orexin-A and leptin in the WDD group were significantly higher (P < 0.05) (Table 4).

3.4. The mRNA Expression of Orexin-A, OXIR, and Ob-R in Prefrontal Cortex and Hypothalamus. Table 5 shows that the mRNA levels of orexin-A in the diazepam- and WDD-treated groups were significantly higher than that in the model group in the prefrontal cortex (P < 0.01). Regarding the mRNA level of OXIR in the prefrontal cortex, the diazepam- and WDD-treated groups showed increased expressions when compared to the model group (P < 0.05). Compared to the control group, the mRNA levels of Ob-R in the model group in both prefrontal cortex and hypothalamus were significantly increased (P < 0.01).

**Table 3: WDD treatment ameliorated SD-induced increases in Cm and Rm times.**

| Parameters | Groups       | Before SD  | 7 days after SD | 14 days after SD |
|------------|--------------|------------|-----------------|------------------|
|            | Control group| 40.9 ± 19.7| 31.3 ± 23.1     | 8.8 ± 4.4        |
| Cm times   | Model group  | 50.2 ± 11.4| 89.0 ± 21.3**   | 53.3 ± 15.6**    |
|            | Diazepam group| 39.4 ± 16.5| 105.4 ± 15.0**  | 44.4 ± 22.8**    |
|            | WDD group    | 45.7 ± 23.8| 96.6 ± 35.7**   | 25.0 ± 12.7***   |
| Rm times   | Control group| 7.7 ± 4.4  | 2.1 ± 1.8       | 1.0 ± 1.0        |
|            | Model group  | 6.4 ± 2.2  | 9.8 ± 2.5**     | 4.0 ± 2.1**      |
|            | Diazepam group| 5.3 ± 3.5  | 10.6 ± 4.8**    | 4.3 ± 1.2**      |
|            | WDD group    | 6.8 ± 3.7  | 11.4 ± 2.5**    | 2.0 ± 0.9**      |

*P < 0.05, **P < 0.01: versus the control group; *P < 0.05: versus the model group; **P < 0.01: versus the diazepam group.

**Table 4: The effect of sleep deprivation on the mean integrated optical density (MOD) of orexin-A and leptin in the prefrontal cortex and hypothalamus and the regulation of WDD.**

| Part             | Groups          | Orexin-A   | Leptin     |
|------------------|-----------------|------------|------------|
| Prefrontal cortex| Control group   | 0.17 ± 0.03| 0.40 ± 0.02|
|                  | Model group     | 0.14 ± 0.02** | 0.19 ± 0.04** |
|                  | Diazepam group  | 0.14 ± 0.01** | 0.22 ± 0.03**   |
|                  | Wen-dan group   | 0.16 ± 0.01** | 0.27 ± 0.03**   |
| Hypothalamus     | Control group   | 0.17 ± 0.01 | 0.27 ± 0.03 |
|                  | Model group     | 0.12 ± 0.02** | 0.15 ± 0.04**   |
|                  | Diazepam group  | 0.13 ± 0.02** | 0.18 ± 0.03**   |
|                  | Wen-dan group   | 0.14 ± 0.01** | 0.21 ± 0.02**   |

**P < 0.01: versus the control group; *P < 0.05: versus the model group; **P < 0.01: versus the model group; *P < 0.05: versus the diazepam group; **P < 0.01: versus the diazepam group. Data is presented as mean ± standard error of the mean (SEM).**
Table 5: The effect of sleep deprivation on mRNA expression of orexin-A, OX1R, and Ob-R in prefrontal cortex and hypothalamus and the regulation of WDD.

| Part           | Group             | Orexin-A       | OX1R       | Ob-R       |
|----------------|-------------------|----------------|------------|------------|
|                | Control group     | 1.62 ± 0.54    | 0.80 ± 0.34| 1.10 ± 0.10|
|                | Model group       | 0.70 ± 0.20    | 0.34 ± 0.14| 0.61 ± 0.13**|
|                | Diazepam group    | 1.65 ± 0.22**  | 0.64 ± 0.11*| 1.91 ± 0.07***|
|                | Wen-dan group     | 2.15 ± 0.04*** | 0.79 ± 0.10*| 0.79 ± 0.07***|
| Prefrontal cortex | Control group     | 2.41 ± 1.41    | 0.82 ± 0.23| 1.28 ± 0.45|
|                | Model group       | 1.91 ± 1.01    | 0.99 ± 0.04| 2.55 ± 0.23**|
|                | Diazepam group    | 1.22 ± 0.34    | 0.78 ± 0.26| 2.71 ± 0.24***|
|                | Wen-dan group     | 2.09 ± 0.17    | 1.65 ± 0.74| 4.30 ± 0.76***|

*P < 0.05: versus the control group; **P < 0.01: versus the control group; *P < 0.05: versus the model group; **P < 0.01: versus the model group; *P < 0.05: versus the diazepam group; and **P < 0.01: versus the diazepam group. Data are presented as mean ± standard error of the mean (SEM).

Figure 1: Protein expression of orexin-A in prefrontal cortex. (Tissue sections were viewed at 40x magnification.) Pale brown cells, which are positive for orexin-A expression, are oval-shaped and spread over the endochylema in the prefrontal cortex. No orexin-A positive cells were observed in the cytomembrane.

4. Discussion

The present study showed that SD significantly increased Cm and Rm time in rats in the open field, while WDD treatment reversed these changes. Especially, at 14 days after SD, the Rm time in the WDD-treated group was significantly lower than that in the diazepam-treated group (P < 0.01). Although the anxiolytic effects of sleep deprivation on OFT results has been demonstrated in a previous study [29], the paradigm used previously (72 h total) differed from ours (20 h/day for 14 days). In an OFT, many factors, such as breeding environment, sex, and stress reaction to water and food deprivation, may affect the results [30]. Diazepam is popularly used to treat insomnia; however, in the present study, the behavioral measurements did not show any beneficial effect after SD in both Cm time and Rm time in the diazepam-treated group. Similar results were reported by others [31], which may indicate that diazepam works through
other behavioral aspects, such as stretch attend and wall-following (thigmotaxis) [31], rather than the locomotor and exploring activities, as observed in the OFT.

After long-term SD, significantly decreased protein expression of orexin-A was observed in prefrontal cortex and hypothalamus, but mRNA expression of orexin-A in prefrontal cortex and hypothalamus of the model group was lower than the control group, though this latter difference did not reach statistical significance. A previous study [32] showed that long-term (48 h) SD downregulated the positive expression of orexin-A, decreased OX1R mRNA expression in the prefrontal cortex, and increased OX1R mRNA expression in the hypothalamus. Because orexin-A can maintain the awake state, our results indicate that long-term SD (≥14 d) can affect the rat’s ability to wake up, as well as vigilance, which may be related to reductions in orexin-A expression induced by high consumption of glucose and restrained protein synthesis. On the other hand, SD decreased the expression of 

leptin in the serum, prefrontal cortex, and hypothalamus and decreased mRNA expression of Ob-R in prefrontal cortex. Previously, leptin expressions have been reported to decline after SD in the serum of young rats [32]. The mechanism of leptin may be mediated by its binding with Ob-R in the central nervous system and peripheral tissues. Acute illumination for 4 h did not change orexin expression in lateral hypothalamus, while SD for 6 h significantly increased orexin expression [33–35]. These results demonstrate that SD may induce the dysfunction of this pair of molecules.

As mentioned above, WDD is a famous Chinese medicine that has been prescribed for hundreds of years and that has a remarkable effect in the treatment of insomnia.

It is read that “Ban xia, Zhu ru, Zhi shi 2 liang each, Chen pi 3 liang, Zhi gancao 1 liang, Fu ling 1.5 liang, Sheng jiang 5 pieces, and Chinese date, 1 piece.” in Treatise on Three Categories of Pathogenic Factors written by Chen Yan [36] in Song dynasty. Phlegm-fire disturbing heart and the heart spirit restless cause insomnia was written in Jing Yue Quan Shu Bu Mei. Chinese clinical literature shows that WDD is effective in the treatment of insomnia. Researchers [19] conducted a clinical trial with 150 insomnia patients, using WDD as a treatment medication and Ambien as a control medicine. The results showed that, compared to the control group, sleep time, sleep efficacy, sleep disorder, and daytime activity function of the treatment group were significantly improved.

Results from the present study show that the excitability behavior of rats is enhanced on day 14 after SD. Improvements were observed after both WDD and diazepam treatments, and WDD effects were superior to those of diazepam. WDD does not function as a sedative-hypnotic; rather, WDD regulates the emotional disorders caused by insomnia, thus improving sleep quality. The observed enhanced excitability
caused by SD in our study is in accordance with other researchers’ findings [37, 38]. WDD can significantly improve rat excitability behavior caused by SD on day 14 after SD. The beneficial effects of WDD are also superior to diazepam. After 14 days of SD, WDD treatment decreases Cm and Rm time, indicating that WDD can improve SD-induced excitability behavior. As well, WDD significantly improves the protein expression of orexin-A in the serum, the prefrontal cortex, and hypothalamus, though no statistically significant improvements in mRNA expression of orexin-A and OXIR in the hypothalamus were observed, which indicates that WDD may regulate orexin-A at the protein, rather than mRNA, level. Even though WDD could not improve Ob-R expression in the prefrontal cortex, Ob-R expression in the hypothalamus was remarkably increased. Considering that Ob-R is located mainly in the hypothalamus, the results indicate that the hypothalamus is the key target of leptin. Furthermore, WDD treatment increased leptin and promoted its binding to Ob-R, inhibiting the synthesis and secretion of orexin-A, a hypothalamic neuropeptide that may enhance sympathetic nervous system activity, decrease appetite, and promote energy consumption. As a result, the emotional changes induced by SD are changed. Protein expressions of orexin-A in the prefrontal cortex and hypothalamus, as well as mRNA expression of orexin-A and OXIR in the prefrontal cortex, increased significantly, which demonstrates that WDD can activate OXIR by inducing the secretion and expression of orexin-A in the brain, thus reducing glucose consumption and promoting protein synthesis. In a similar manner, diazepam also elevated the protein level of leptin in the serum and hypothalamus. However, WDD is superior to diazepam, with the advantage of multitargeted regulation, as well as the improvement of negative emotions.

5. Conclusion

In conclusion, the present study demonstrated that SD is a good model to induce insomnia-related negative emotions. WDD treatment effectively improved SD-induced negative emotions by regulating the function of orexin-A and leptin, such as the upregulated orexin-A and leptin in blood serum and brain tissue of SD rats compared to control animals. Furthermore, our study demonstrated that WDD can upregulate orexin-A, OXIR, and Ob-R in the prefrontal cortex of the SD rats, while only upregulating Ob-R in hypothalamus. WDD treatment has been proven effective in improving SD-induced negative emotions by regulating orexin-A and leptin, an effect that may have a great impact on the treatment of patients with insomnia.
**Figure 4:** Protein expression of leptin in hypothalamus. (Tissue sections were viewed at 40x magnification.) The dark brown cells, which are positive for leptin expression, are oval-shaped and spread over the cell membrane and the endochylema. Fewer positive cells are apparent in the model group and the diazepam- and WDD-treated groups.

**Figure 5:** The effect of sleep deprivation on orexin-A concentrations in serum and the regulation of Wen-Dan Decoction. **P < 0.01:** versus the control group; *P < 0.05:** versus the model group. Data are presented as mean ± standard error of the mean (SEM).

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

**Authors’ Contribution**

Fengzhi Wu, Yuehan Song, and Feng Li were equal contributors to this paper.
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References

[1] R. M. Golub, “Insomnia,” The Journal of the American Medical Association, vol. 307, no. 24, pp. 2653–2653, 2012.

[2] T. Roth, “Insomnia: definition, prevalence, etiology, and consequences,” Journal of Clinical Sleep Medicine, vol. 3, supplement 5, pp. 7–10, 2007.

[3] D. N. Neubauer, “Can’t sleep? What to know about insomnia,” [EB/OL], 2014, http://www.sleepfoundation.org/article/sleep-related-problems/insomnia-and-sleep.

[4] S. C. Park, J. M. Kim, T. Y. Jun et al., “Prevalence and clinical correlates of insomnia in depressive disorders: the CRESCEND study,” Psychiatry Investigation, vol. 10, no. 4, pp. 373–381, 2013.

[5] P. Alföldi, T. Wiklund, and B. Gerdle, “Comorbid insomnia in patients with chronic pain: a study based on the Swedish quality registry for pain rehabilitation (SQRP),” Disability and Rehabilitation, 2013.

[6] C. Baglioni, K. Spiegelhalder, C. Lombardo, and D. Riemann, “Sleep and emotions: a focus on insomnia,” Sleep Medicine Reviews, vol. 14, no. 4, pp. 227–238, 2010.

[7] T. Sakurai, A. Amemiya, M. Ishii et al., “Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior,” Cell, vol. 92, no. 4, pp. 573–585, 1998.

[8] G. J. Hervieu, J. E. Cluderay, D. C. Harrison, J. C. Roberts, and R. A. Leslie, “Gene expression and protein distribution of the orexin-1 receptor in the rat brain and spinal cord,” Neuroscience, vol. 103, no. 3, pp. 777–797, 2001.

[9] T. Mochizuki, A. Crocker, S. McCormack, M. Yanagisawa, T. Sakurai, and T. E. Scammell, “Behavioral state instability in orexin knock-out mice,” Journal of Neuroscience, vol. 24, no. 28, pp. 6291–6300, 2004.

[10] C. Anaclert, R. Parmentier, K. Ouk et al., “Orexin/hypocretin and histamine: distinct roles in the control of wakefulness demonstrated using knock-out mouse models,” Journal of Neuroscience, vol. 29, no. 46, pp. 14423–14438, 2009.

[11] T. Mochizuki, E. Arrigoni, J. N. Marcus et al., “Orexin receptor 2 expression in the posterior hypothalamus rescues sleepiness in narcoleptic mice,” Proceedings of the National Academy of Sciences of the United States of America, vol. 108, no. 11, pp. 4471–4476, 2011.

[12] R. Tose, T. Kushikata, H. Yoshiida et al., “Orexin A decreases ketamine-induced anesthesia time in the rat: the relevance to brain noradrenergic neuronal activity,” Anesthesia and Analgesia, vol. 108, no. 2, pp. 491–495, 2009.

[13] T. Shirasaka, T. Yonaha, S. Onizuka, and I. Tsumeyoshi, “Effects of orexin-A on propofol anesthesia in rats,” Journal of Anesthesia, vol. 25, no. 1, pp. 65–71, 2011.

[14] J. N. Marcus, A. schkenasi CJ, C. E. Lee et al., “Differential expression of orexin receptors 1 and 2 in the rat brain,” Journal of Comparative Neurology, vol. 435, no. 1, pp. 6–25, 2001.
[31] E. Choleris, A. W. Thomas, M. Kavaliers, and F. S. Prato, “A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field,” *Neuroscience and Biobehavioral Reviews*, vol. 25, no. 3, pp. 235–260, 2001.

[32] Q. M. Sun, Y. Wang, D. Y. Li et al., “Effects of sleep deprivation on leptin of adolescent rat,” *Hei Long Jiang Medical Journal*, vol. 9, no. 33, pp. 663–664, 2009.

[33] Y. Yoshida, N. Fujiki, T. Nakajima et al., “Fluctuation of extracellular hypocretin-1 (orexin A) levels in the rat in relation to the light–dark cycle and sleep–wake activities,” *European Journal of Neuroscience*, vol. 14, no. 7, pp. 1075–1081, 2001.

[34] X. F. Hui, H. Jiang, Y. P. Cheng, L. Wang, and C. J. Su, “Expressions of orexin-A neurons in rat’s hypothalamus after rapid eye movement sleep deprivation, revival and modafinil intervention,” *China Journal of Modern Medicine*, vol. 20, no. 23, pp. 3576–3579, 2010.

[35] Z. Wang, X. W. Shan, C. Q. Zhang et al., “Effects of long-term continuous operations on orexin-A andOX1R expression in the rat brain,” *Acta Academiae Medicinae Militaris Tertiae*, vol. 28, pp. 1046–1048, 2006.

[36] C. Yan, *San Yin Ji Yi Bing Zheng Fang Lun*, People’s Medical Publishing House, Beijing, China, 1983.

[37] J. L. Tartar, C. P. Ward, J. W. Cordeira et al., “Experimental sleep fragmentation and sleep deprivation in rats increases exploration in an open field test of anxiety while increasing plasma corticosterone levels,” *Behavioural Brain Research*, vol. 197, no. 2, pp. 450–453, 2009.

[38] L. Zheng, H. Li, H. Wang et al., “The effects of prolonged sleep deprivation on brain serotonin metabolism and behavior in rats,” *Chinese Journal of Behavioral Medical Science*, vol. 7, no. 4, pp. 256–257, 1998.