

Influences of Suvorexant and Mirtazapine on Chronic Pain-related Sleep Disorder in Neuropathic Pain Model Mice

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Abstract

Chronic pain and sleep disorders are independently associated with a reduction in the quality of life. They can be both a cause and consequence of each other; therefore, they should be treated simultaneously. However, optimal treatments for chronic pain-related sleep disorders are not well established. Here, we aimed to investigate the effects of suvorexant, a novel sleep drug, and mirtazapine, a noradrenergic and specific serotonergic antidepressant, on pain-related sleep disorders in a preclinical neuropathic pain mouse model, which was produced by partial sciatic nerve ligation. We calculated the quantity, duration, and depth of sleep by analyzing the electroencephalogram. Voluntary activity was also evaluated by counting the number of wheel rotations with special cages. Daily administration of suvorexant and mirtazapine normalized the reduced rapid eye movement (REM) and non-REM sleep and improved the fragmented sleep, further regaining the depth of sleep at sleep onset in the chronic pain state. Suvorexant decreased voluntary activity, which was prolonged after the end of administration; however, mirtazapine did not decrease it. Both suvorexant and mirtazapine could be potential therapeutic agents for chronic pain-related sleep disorders.

Introduction

Sleep disorder is the most common complication of chronic pain [1-4], and sleep disorders exacerbate pain [5-8]. Sleep disorder and pain make a vicious spiral and should be treated simultaneously in clinical situations. Although benzodiazepines and Z-drugs are frequently used to treat sleep disorders during pain management, these drugs have a narrow role in the management of co-occurring sleep disorders [9]. Furthermore, the use of these drugs should be limited due to their harmful side effects, such as falling [10], cognitive impairment [11], dependence [12], and increased risk of death [13].

It has previously been reported that the hyperactivity of ascending neurons from the brain stem is one of the causes of pain-related sleep disturbance [14,15]. Orexin is a hypothalamic neuropeptide that activates monoaminergic and cholinergic neurons in the hypothalamus/brain stem arousal system to maintain a long, consolidated awake period [16,17]. Suvorexant, a novel sleep drug, promotes normal sleep state by blocking dual orexin receptors [18,19]. Mirtazapine belongs to the class of “noradrenergic and specific serotonergic antidepressants,” which increases the release of serotonin and norepinephrine because of the antagonism of auto-receptors, resulting in the restoration of the balance of noradrenergic and serotonergic neuronal activity in the brain [20]. Mirtazapine is likely to cause sleepiness and is often used for the off-label treatment of insomnia [21] because it acts as an antagonist of the H1 histamine receptor and 5HT₂ serotonin receptor, which produces sedating and calming effects [22]. Because of their pharmacological mechanisms, these drugs could be potential therapeutic agents for pain-related sleep disorders.

Sleep disorders have several aspects and do not simply mean a reduction in sleep time [23]. Sleep disorders include various symptoms, such as difficulty falling asleep, fragmentation, low quality sleep, and abnormal sleep rhythms. Excessive daytime sleepiness is also a symptom of sleep disorders. In the

present study, we administered suvorexant or mirtazapine to a chronic pain mouse model and evaluated the various perspectives of sleep, including not only sleep time but also sleep duration, sleep depth, and effect on voluntary activity of the animals.

Results

Effects of suvorexant on the amount of sleep

Mice underwent an electroencephalogram/electromyogram (EEG/EMG) recording head-mount implantation and partial sciatic nerve ligation (PSNL) surgery under general anesthesia with isoflurane. One week after surgery, we repeated oral administration of vehicle (0.5% methyl cellulose) or suvorexant (30 mg/kg) 15 min before Zeitgeber time (ZT) 0 for 7 days (Figure 1a). We calculated the time change every 2 h of the percentage of sleep and the amount of non-REM and REM sleep (Figure 1b), and compared the values that were added up in the light and dark phases (Figure 1c). The PSNL-vehicle group showed a significant decrease in the percentage of sleep (PSNL-vehicle vs. sham-vehicle: 51.8% vs. 72.1%; $P = 0.0051$) during the light phase, which is the rest period for mice. Both non-REM (PSNL-vehicle vs. sham-vehicle: 5.92 h vs. 7.93 h; $P = 0.040$) and REM sleep (PSNL-vehicle vs. sham-vehicle: 0.29 h vs. 0.72 h, $P = 0.0015$) decreased in the PSNL-vehicle group during the light phase. Daily suvorexant administration significantly normalized the percentage of sleep (76.3%, $P = 0.0007$; vs. PSNL-vehicle), non-REM sleep (8.46 h, $P = 0.0070$; vs. PSNL-vehicle), and REM sleep (0.69 h, $P = 0.0028$; vs. PSNL-vehicle) in nerve-ligated mice during the light phase.

Effects of mirtazapine on the amount of sleep

We repeated intraperitoneal injection of the vehicle (5% dimethyl sulfoxide) or mirtazapine (1 mg/kg) 1 week after head-mount implantation and nerve ligation surgery on the same schedule as that of suvorexant (Figure 1a). We calculated the time change every 2 h of the percentage of sleep and the amount of non-REM and REM sleep (Figure 1d), and compared the values that were added up in the light and dark phases (Figure 1e). The PSNL group showed a significant decrease in the percentage of sleep (47.0% vs. 72.7%, $P = 0.0079$), and the amount of non-REM (5.12 h vs. 8.25 h, $P = 0.0017$) and REM sleep (0.20 h vs. 0.70 h, $P = 0.039$) compared to that in the sham group during the light phase, similarly to the previous suvorexant experiment. Daily mirtazapine injection also significantly normalized the percentage of sleep (74.1%, $P = 0.0050$; vs. PSNL-vehicle), non-REM sleep (7.71 h, $P = 0.0094$; vs. PSNL-vehicle), and REM sleep (1.17 h, $P < 0.0001$; vs. PSNL-vehicle) in nerve-ligated mice during the light phase.

Effects of suvorexant and mirtazapine on the duration of arousal and sleep

We analyzed the EEG data to calculate the duration of arousal, non-REM sleep, and REM sleep every 6 h. The sham group showed a long duration of arousal during the dark phase, which is an active period for mice. A significant reduction in the duration of wakefulness was detected in nerve-ligated mice (PSNL-vehicle vs. sham-vehicle: 4.94 m vs. 24.1 m, $P < 0.0001$ at ZT 12–18; 3.53 m vs. 15.9 m, $P = 0.0053$ at ZT 18–24; Figure 2a, left; and PSNL-vehicle vs. sham-vehicle: 4.83 m vs. 29.0 m, $P = 0.0098$ at ZT 12–18;

4.27 m vs. 27.8 m, $P = 0.012$ at ZT 18–24; Figure 2b, left), which was improved with suvorexant (15.8 m, $P = 0.012$ at ZT 12–18 and 15.2 m, $P = 0.0063$ at ZT 18–24; vs. PSNL-vehicle; Figure 2a, left) and mirtazapine (33.4 m, $P = 0.0030$ at ZT 12–18 and 30.6 m, $P = 0.0067$ at ZT 18–24; vs. PSNL-vehicle; Figure 2b, left). Analysis of the duration of non-REM sleep revealed fragmented sleep in the PSNL group at almost all periods, which was improved by suvorexant (PSNL-vehicle vs. PSNL-suvorexant: 2.63 m vs. 8.75 m, $P = 0.0001$ at ZT 0–6; 3.05 m vs. 9.36 m, $P < 0.0001$ at ZT 6–12; 2.96 m vs. 7.17 m, $P = 0.0088$ at ZT 12–18; 2.96 m vs. 6.88 m, $P = 0.0162$ at ZT 18–24; Figure 2a, middle) and mirtazapine (PSNL-vehicle vs. PSNL-mirtazapine: 2.28 m vs. 6.06 m, $P < 0.0001$ at ZT 0–6; 2.35 m vs. 5.19 m, $P = 0.0021$ at ZT 6–12; 1.94 m vs. 5.11 m, $P = 0.0005$ at ZT 12–18; 2.04 m vs. 4.92 m, $P = 0.0018$ at ZT 18–24; Figure 2b, middle). There was a downward trend or a significant decrease in the duration of REM sleep with nerve ligation only during the light phase. Suvorexant (PSNL-vehicle vs. PSNL-suvorexant: 0.60 m vs. 1.24 m, $P = 0.022$ at ZT 0–6; 0.65 m vs. 1.23 m, $P = 0.042$ at ZT 6–12; Figure 2a, right) and mirtazapine (PSNL-vehicle vs. PSNL-mirtazapine: 0.52 m vs. 1.53 m, $P < 0.0001$ at ZT 0–6; 0.54 m vs. 1.10 m, $P = 0.023$ at ZT 6–12; Figure 2b, right) significantly increased REM sleep duration in the PSNL group during the light phase.

Effects of suvorexant and mirtazapine on the normalized power density of delta (δ) waves

We analyzed the power density of δ waves, which is one of the factors that define the depth of non-REM sleep. The power density of the δ wave during non-REM sleep was calculated every 3 h, and the ratio with the average value during the entire day was calculated for each individual. In the sham group, the power density of the δ wave in non-REM sleep was particularly strong during the early light phase. The δ power tended to weaken from the late light phase to the early dark phase and became stronger again toward the latter half of the dark phase. However, in the PSNL-vehicle group, diurnal fluctuation of delta power became ambiguous; in particular, the power density of the δ wave in the early light phase was significantly reduced (PSNL-vehicle vs. sham-vehicle: 0.99 vs. 1.13, $P = 0.020$ at ZT 0; Figure 3a; and PSNL-vehicle vs. sham-vehicle: 0.98 vs. 1.09, $P = 0.0042$ at ZT 0; Figure 3b). Both suvorexant (1.15, $P = 0.0074$; vs. PSNL-vehicle; Figure 3a) and mirtazapine (1.12, $P = 0.0004$; vs. PSNL-vehicle; Figure 3b) improved the reduction in δ power density.

Effects of suvorexant and mirtazapine on the voluntary activity

We placed the mice in cages with a running wheel and recorded the number of wheel rotations. PSNL surgery significantly reduced the wheel rotation compared to sham surgery from day 7 to 14 after surgery (sham vs. PSNL: 18,650/day vs. 11,801/day, $P = 0.0040$ at day 7; 19,010/day vs. 11,500/day, $P = 0.0015$ at day 17; Figure 4a). The drug was administered daily for 1 week after PSNL surgery, and the wheel rotation was evaluated at certain test points, as shown in Figure 4b. Daily oral suvorexant administration reduced the number of wheel rotations (8,463/day, $P = 0.0073$ at test point D; 9,759/day, $P = 0.045$ at test point E; vs. test point A, Figure 4c), and the reduction was sustained for 1 week after the end of administration compared to that at the baseline (9,799/day, $P = 0.048$ at test point F; vs. test point A,

Figure 4c). However, daily intraperitoneal injection of mirtazapine did not influence the number of wheel rotations (Figure 4d).

Effects of suvorexant and mirtazapine on pain behavior

We administered vehicle or drug daily for 1 week from postoperative day 7 and evaluated mechanical allodynia and thermal hyperalgesia using the von Frey test and Hargreaves test, respectively, at certain test points shown in Figure 5a. Weekly suvorexant administration improved the paw withdrawal latency for mechanical stimuli with von Frey filament (PSNL-vehicle vs. PSNL-suvorexant: 4.98 s vs. 10.1 s, $P < 0.0001$ at test point C; Figure 5b); however, the effect did not last until the next day. There was no significant difference in the response to thermal stimuli (Figure 5c). However, weekly injection of mirtazapine improved both mechanical allodynia (PSNL-vehicle vs. PSNL-mirtazapine: 4.20 s vs. 9.74 s, $P < 0.0001$ at test point C; Figure 5d) and thermal hyperalgesia (PSNL-vehicle vs. PSNL-mirtazapine: 4.87 s vs. 9.70 s, $P < 0.0001$ at test point C; Figure 5e), and these effects persisted until the next day (PSNL-vehicle vs. PSNL-mirtazapine: 5.06 s vs. 7.23 s, $P = 0.014$ [Figure 5d]; 5.80 s vs. 7.33 s, $P = 0.0440$ [Figure 5e] at test point D).

Discussion

Recent clinical and basic research suggests that sleep disorder is a heterogenic disorder and requires multifaceted evaluation to correctly determine the state of sleep and the effects of therapeutic agents [23-25]. We evaluated the sleep status of a preclinical pain model so that it would be close to clinical situations by examining the sleep quantity, sleep duration, sleep depth, and the effect on daytime activity using EEG or wheel rotation.

In the present study, PSNL caused a decrease in REM and non-REM sleep based on EEG analysis in mice, which is almost consistent with the results of past studies [15,26-28]. Some studies failed to detect considerable differences in REM sleep, probably because REM sleep in mice is very low and statistically variable. In addition, we found that the duration of non-REM sleep was shortened and interrupted, resulting in fragmented sleep in the neuropathic pain model. Non-REM sleep is classified into stages based on the depth, which correlates with the strength of the δ power density. We calculated the δ power density using a fast Fourier transform and evaluated the time changes. As a result, the sham group showed a sleep rhythm of deep sleep in the early stages of the light phase (i.e., a rest period for mice), which gradually became shallower towards the late stages of the light phase. By contrast, in the neuropathic pain model mice, such sleep rhythms were lost and early deep sleep was not observed. In addition, as a result of analysis focusing on the activity of animals, the sham group showed a long duration of arousal, especially in the first half of the dark phase, which is the active period for mice. However, the duration of arousal was substantially reduced in the neuropathic pain model mice. Furthermore, when the amount of voluntary exercise was evaluated by counting the rotation of the wheel attached to the cages, the amount of exercise decreased in the mice with nerve injury for 1–2 weeks after surgery.

Both experimental and clinical studies have shown that pain is associated with reduced sleep quality and quantity [29-32]. However, the mechanisms underlying the link between chronic pain and sleep disorders remain unclear. In preclinical studies, peripheral nerve damage has been associated with persistent regional cerebral blood flow abnormalities in the somatosensory cortex and thalamus [33]. These findings suggest that persistent pain may promote changes in the ascending arousal system. Previous optogenetic research indicated that ascending noradrenergic excitation in the locus coeruleus induces arousal [34]. Similar reactions in ascending neurons may be a cause of sleep disorders due to chronic pain [14,15].

The orexin receptors (orexin-1 and orexin-2 receptor) are involved in stabilizing arousal and suppressing sleep by maintaining the activity of ascending neurons from the brainstem reticular formation. Because suvorexant causes pharmacological inhibition of dual orexin receptors, it could be a potential therapeutic agent to improve pain-related sleep disorders. In the present study, continuous administration of suvorexant improved chronic pain-related sleep disorders at several points. Suvorexant normalized the reduction of non-REM and REM sleep, improved fragmented sleep, and further regained deep sleep during the early light phase. However, suvorexant resulted in a prolonged decrease in voluntary activity, even after administration. Orexin can potentiate the excitatory synaptic transmission of dopaminergic neurons in the mesolimbic system, causing motivation [35]. Furthermore, the mesopontine tegmentum, including structures relevant to locomotion and muscle tone, is also a major target of orexin [35]. These findings suggest that blocking orexin signaling may lead to lower locomotive behavior.

Recent studies have shown that reduced sleep time and sleep fragmentation exacerbate pain [4-8,36]. Therefore, we evaluated the effects of suvorexant on pain-related behavior. One hour after the last administration of suvorexant, the pain response to mechanical stimuli was delayed with the von Frey filament, whereas it had a slight effect on thermal hyperalgesia. Moreover, the effects of continuously administered suvorexant on allodynia returned to the same levels as those with vehicle control within 24 h after cessation of administration. These results suggest that these changes may be due to a sedative effect rather than a secondary analgesic effect.

Previous studies have shown that mirtazapine, which is commonly used clinically, may improve sleep disorders [21,22,37,38]. Mirtazapine shows a high affinity in the binding assay for the H1 histamine receptor and 5HT2 serotonin receptor and exerts strong antagonistic effects [22]. Therefore, sedative effects are thought to be mediated through the suppression of hyperactivity of histaminergic and serotonergic ascending neurons. The effects of mirtazapine on the endocrine system by normalizing the hypothalamo-pituitary-adrenocortical system overactivation and blunting the melatonin system could be potential mechanisms of sleep improvement [38].

Similar to this study, previous studies [39] have shown that 1 mg/kg of mirtazapine improves sleep quantity in a PSNL mouse model. We injected the same dose daily for a week and evaluated its effects on sleep and pain. As a result, mirtazapine improved the reduction of non-REM and REM sleep, sleep fragmentation, and depth of sleep onset. Furthermore, mirtazapine did not decrease voluntary activity,

contrary to suvorexant. Mirtazapine was reported to promote active motion and have anti-immobility effects in the rat forced swimming test [40]. The enhancing effects of mirtazapine on motivation may be related to an increase in dopamine release in the frontal cortex [41,42]. However, up to 54% of patients reported daytime sleepiness as an adverse effect of mirtazapine in the clinical trial [37]. Mirtazapine predominantly produces anti-histaminergic effects at lower doses, whereas noradrenergic effects become more predominant at higher doses [43]. Because of this unique pharmacological profile, mirtazapine is thought to produce relatively more sedation at lower doses.

Thermal hyperalgesia was also reported to be improved by 1 mg/kg of mirtazapine in the same PSNL model [39]. The suppression of pain behaviors, both mechanical allodynia and thermal hyperalgesia, was prolonged until 24 h after the end of daily administration of mirtazapine. Further investigation is needed to determine whether these effects are due to the secondary effects of sleep improvement or the protracted analgesic effects of mirtazapine.

In summary, the present study demonstrated that both suvorexant and mirtazapine improved pain-related sleep disorders in mice. However, caution should be exercised when administering suvorexant to patients with low motivation for activity. Mirtazapine showed different pharmacological profiles; it suppressed pain behavior during and even after administration, without affecting physical activity. In clinical practice, it is necessary to understand the pharmacological properties of sleep agents to treat pain-related sleep disorders.

Methods

Animals

This study was carried out in accordance with the ARRIVE guidelines and the Act on Welfare and Management of Animals and the recommendations in the Guidelines for Proper Conduct of Animal Experiments of the Science Council of Japan. The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals at the University of Toyama, as adopted by the Committee on Animal Research of the University of Toyama (Toyama, Japan). Male C57BL/6J mice were purchased from Japan SLC. The mice were maintained at 22–26°C with 12-h light-dark cycle. ZT 0 and ZT 12 represent the light onset (07:00 a.m.) and offset times (19:00 p.m.), respectively. Food and water were provided *ad libitum*. Every effort was made to minimize the number and suffering of the animals used in the following experiments.

Neuropathic pain model

The mice were anesthetized with 3% isoflurane. We produced a partial sciatic nerve ligation model, as described previously [44]. The right sciatic nerve was ligated by a tight ligature with an 8–0 silk suture, approximately half of the diameter. In sham-operated mice, nerves were exposed without ligation.

Compounds and administrations

Suvorexant ([[(7R)-4-(5-chloro-1,3-benzoxazol-2-yl)-7-methyl-1,4-diazepan-1-yl][5-methyl-2-(2H-1,2,3-triazol-2-yl)phenyl] methanone) was synthesized according to previous studies [45,46]. It was dissolved in 0.5% methyl cellulose 400 (Wako Pure Chemicals, Japan) immediately before oral administration. Mirtazapine was purchased from Wako Pure Chemicals (Osaka, Japan) and dissolved in 5% dimethyl sulfoxide and 5% Tween80 just before intraperitoneal injection.

Sleep recordings

The compounds were administered daily for 1 week at the beginning of the light phase 1 week after nerve ligation. We recorded EEG/EMG to evaluate the sleep condition for 24 h on the last day of administration, as previously described [15,26]. Mice were mounted in a stereotaxic head holder and implanted with EEG and EMG electrodes for polysomnographic recordings (Pinnacle Technology, USA) under 3% isoflurane anesthesia in advance. Two stainless steel EEG recording screws were positioned 1 mm anterior to the bregma or lambda, both 1.5 mm lateral to the midline. EMG activity was monitored using Teflon-coated steel wires placed bilaterally into both trapezius muscles. The collected data were analyzed using a software (SLEEPSIGN Kissei Comtec, Japan). The vigilance of every 10-s epoch was automatically classified into three stages, that is, arousal, rapid eye movement (REM), and non-REM sleep, according to the standard criteria: 1) arousal was defined by a high EMG amplitude and low EEG amplitude; 2) REM sleep was defined by a low EMG amplitude, low EEG amplitude, and high θ wave activity; and 3) non-REM sleep was defined by low EMG amplitude, high EEG amplitude, and high δ wave activity [26]. Defined sleep-wake stages were visually examined and corrected, if necessary. Furthermore, we evaluated the time change in the power density of the δ wave to estimate the quality of sleep. The normalized power density of the δ wave was calculated as a value every 3 h for the total power density of the δ wave during non-REM sleep per day.

Assessment of voluntary activity

We measured wheel running to assess voluntary physical performance in neuropathic pain model mice [47]. The mice were allowed to run freely on an open plastic wheel placed inside a standard cage (Melquest, Japan). Wheel rotations were electronically counted and captured in a software program for data storage and analysis at various time points. First, we evaluated the influence of neuropathic pain on the voluntary physical performance. The mice were housed individually in cages and habituated for 4 days before baseline recording. We then measured the number of wheel rotations 1, 2, and 3 weeks after sciatic nerve ligation. Second, we evaluated the influence of compounds on physical activity in a mouse model of neuropathic pain. The mice were habituated to the cage for 4 days before baseline recording. The compounds were administered daily for 1 week at the beginning of the light phase, 1 week after nerve ligation. We then compared the number of wheel rotations at five different time points, the day before administration, day 1 of administration, the day of the end of administration, and 1 day and 1 week after the administration, with the baseline levels.

Measurement of mechanical allodynia

The compounds were administered daily for 1 week at the beginning of the light phase, 1 week after nerve ligation. We then assessed mechanical allodynia at four time points: the day before nerve ligation, the day before administration, the day of the end of administration, and 1 day after administration. To assess the sensitivity to mechanical tactile stimulation, the mice were tested individually using automated von Frey equipment (Dynamic Planter Aesthesiometer; Ugo Basile, Italy). The maximum force was set at 5 g to prevent tissue damage, and the ramp speed was 0.25 g/s to achieve an average baseline paw-withdrawal latency of approximately 8–10 s in naive mice. The paw-withdrawal latency was determined as the average of four measurements per paw. Only quick hind paw movements (with or without licking of hind paws) away from the stimulus were considered to be a withdrawal response. Paw movements associated with locomotion or weight shifting were not considered responses. Before the behavioral responses to the thermal stimulus were tested, the mice were habituated for at least 1 h in a plastic cage with a metal grid bottom. Under these conditions, the latency of paw withdrawal in response to mechanical stimuli was tested. The data represent the average withdrawal latency of the right hind paw.

Measurement of thermal hyperalgesia

The compounds were administered daily for 1 week at the beginning of the light phase 1 week after nerve ligation. We then assessed thermal hyperalgesia at four time points: the day before nerve ligation, the day before administration, the day of the end of administration, and 1 day after administration. To assess the sensitivity to thermal stimulation, mice were tested individually by the Hargreaves test using a well-focused, radiant heat light source (model 33 Analgesia Meter; IITC/Life Science Instruments, USA). The intensity of the thermal stimulus was adjusted to achieve an average baseline paw-withdrawal latency of approximately 8-10 s in naive mice. The paw-withdrawal latency was determined as the average of four measurements per paw. Only quick hind paw movements (with or without licking of hind paws) away from the stimulus were considered withdrawal responses. Paw movements associated with locomotion or weight shifting were not counted as responses. Before the behavioral responses to the thermal stimulus were tested, the mice were habituated for at least 1 h in a clear acrylic cylinder (15 cm high and 8 cm in diameter). Under these conditions, the latency of paw withdrawal in response to thermal stimuli was tested. The data represent the average withdrawal latency of the right hind paw.

Statistics

All data are expressed as mean \pm standard error of mean (SEM). Repeated measures two-way analysis of variance (ANOVA) followed by the Bonferroni multiple comparisons test was used to compare the percentage or amount of each sleep time (Figure 1b-e), the duration of wake, non-REM sleep, and REM sleep time (Figure 2a, b), normalized power density of δ wave among the “sham-vehicle,” “PSNL-vehicle,” and “PSNL-drug (suvorexant or mirtazapine)” groups on the each time point. Repeated measures two-way ANOVA followed by the Bonferroni multiple comparisons test was used to compare the number of wheel rotation between “sham” and “PSNL” groups (Figure 4a). Repeated measures one-way ANOVA followed by the Bonferroni multiple comparisons test was used to compare the number of wheel rotations at each

time point (B–F) with time point A as the baseline (Figure 4c, d). Repeated measures two-way ANOVA followed by the Bonferroni multiple comparisons test was also used to compare the paw withdrawal latency in Hargreaves test and von Frey test between “PSNL-vehicle” and “PSNL-drug (suvorexant or mirtazapine)” groups (Figure 5b-e). Statistical significance was set at $P < 0.05$. All statistical analyses were performed using Prism version 6.0. (GraphPad Software, La Jolla, CA, USA).

Declarations

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions: H.I., H.T., T.S., and M.Y. designed the study. H.I. performed all the experiments and analyses. N.T. synthesized the compounds. H.I. and M.Y. wrote the manuscript. H.I., H.T., T.S., N.T., and M.Y. revised the final version of the manuscript.

Competing interests: The authors declare that they have no competing interests.

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Figures

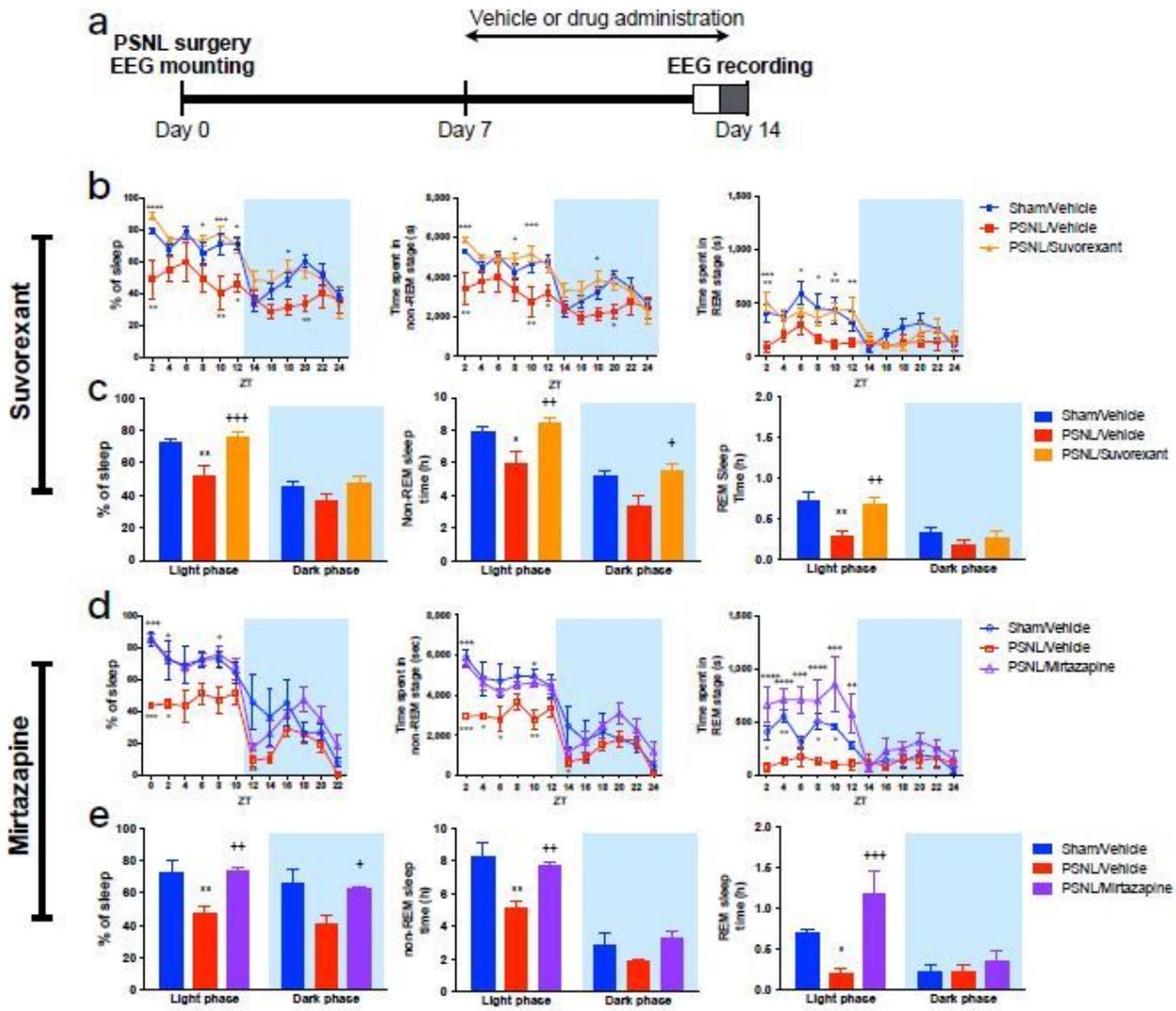


Figure 1

Influence of daily administration of suvorexant and mirtazapine on sleep cycle in neuropathic pain model mice. Nerve-ligated mice were treated with suvorexant (30 mg/kg), mirtazapine (1 mg/kg), or vehicle once daily before ZT0 for 7 days (a). The time changes in the sleep percentage, REM sleep, and non-REM sleep are shown for every 2 h (b, d) and every 12 h (c, e). Data are expressed as mean \pm SEM (sham-vehicle, n = 5-6; PSNL-vehicle, n = 5; PSNL-suvorexant, n = 6; PSNL-mirtazapine, n = 5). *P < 0.05, **P < 0.01, and ***P < 0.001 compared between each sham-vehicle and PSNL-vehicle group, and +P < 0.05, ++P < 0.01, +++P < 0.001, and ++++P < 0.0001 compared between each PSNL-vehicle and PSNL-drug (suvorexant or mirtazapine) group by two-way ANOVA with Bonferroni post-test. Abbreviations: ANOVA, analysis of variance; PSNL, partial sciatic nerve ligation; EEG, electroencephalogram; REM, rapid eye movement; ZT, Zeitgeber time

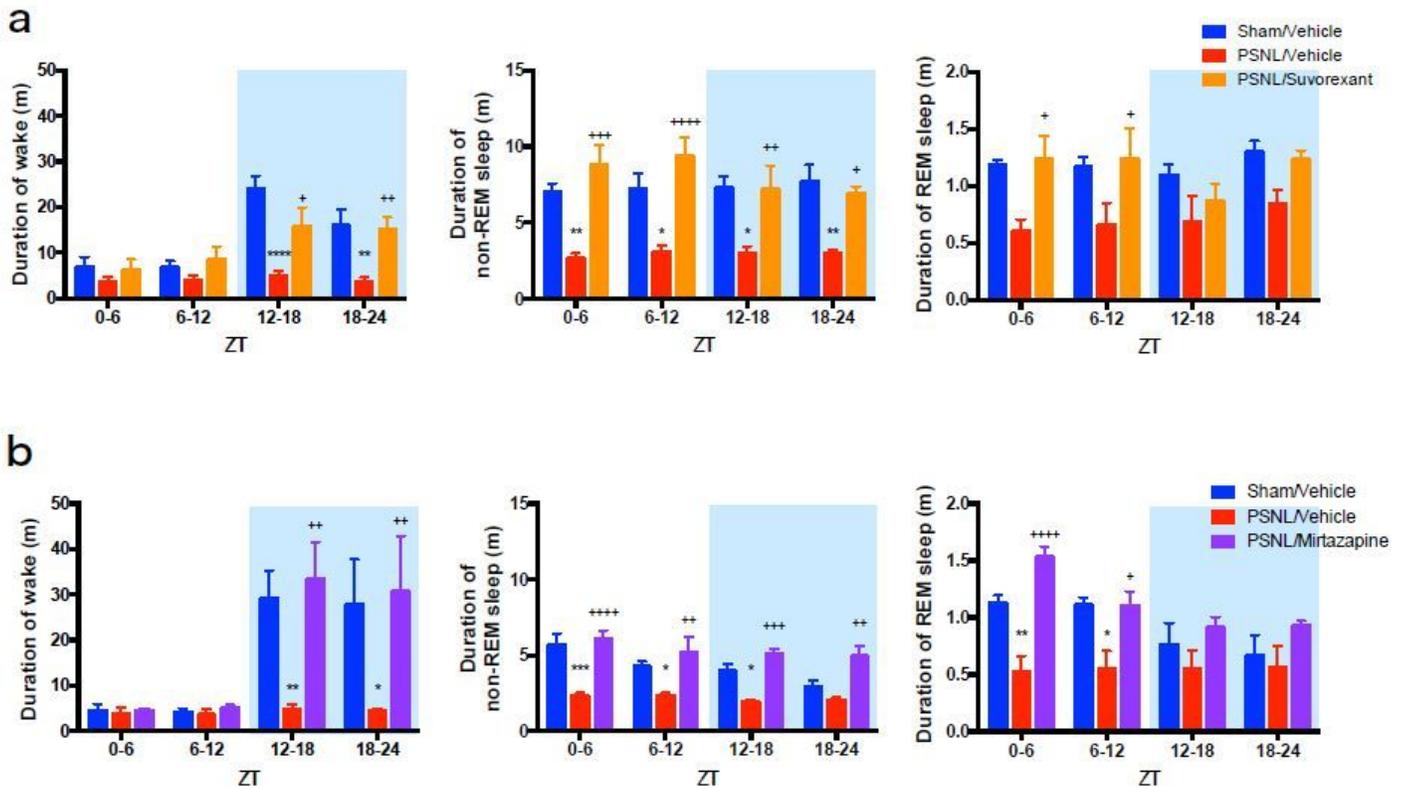


Figure 2

Influence of daily administration of suvorexant or mirtazapine on the duration of arousal and sleep in neuropathic pain model mice. Nerve-ligated mice were treated with suvorexant (30 mg/kg) (a), mirtazapine (1 mg/kg) (b), or vehicle once daily before ZT0 for 7 days. The duration of arousal, non-REM sleep, and REM sleep were recorded every 6 h. Data are expressed as mean \pm SEM (sham-vehicle, n = 5-6; PSNL-vehicle, n = 5; PSNL-suvorexant, n = 6; PSNL-mirtazapine, n = 5). *P < 0.05, **P < 0.01, ****P < 0.0001, and ***P < 0.001 compared between each sham-vehicle and PSNL-vehicle group, and +P < 0.05, ++P < 0.01, +++P < 0.001, and ++++P < 0.0001 compared between each PSNL-vehicle and PSNL-drug (suvorexant or mirtazapine) group by two-way ANOVA with Bonferroni post-test. Abbreviations: ANOVA, analysis of variance; PSNL, partial sciatic nerve ligation; REM, rapid eye movement; ZT, Zeitgeber time

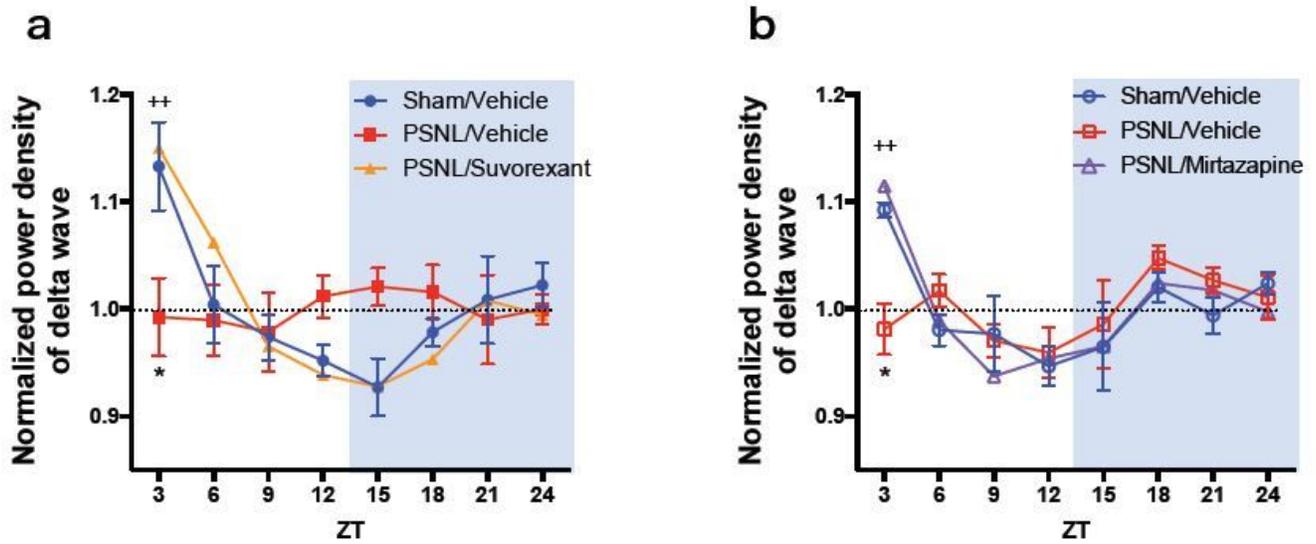


Figure 3

Influence of daily administration of suvorexant or mirtazapine on the changes in power density of δ wave in neuropathic pain model mice. Nerve-ligated mice were treated with suvorexant (30 mg/kg) (a), mirtazapine (1 mg/kg) (b), or vehicle once daily before ZT0 for 7 days. The change in the normalized power density of the δ wave in non-REM sleep was observed every 3 h. Data are expressed as mean \pm SEM (sham-vehicle, n = 5; PSNL-vehicle, n = 4-5; PSNL-suvorexant, n = 5; PSNL-mirtazapine, n = 5). *P < 0.05, compared between each sham-vehicle and PSNL-vehicle group, and ++P < 0.01, compared between each PSNL-vehicle and PSNL-drug (suvorexant or mirtazapine) groups by two-way ANOVA with Bonferroni post-test. Abbreviations: ANOVA, analysis of variance; PSNL, partial sciatic nerve ligation; REM, rapid eye movement; ZT, Zeitgeber time

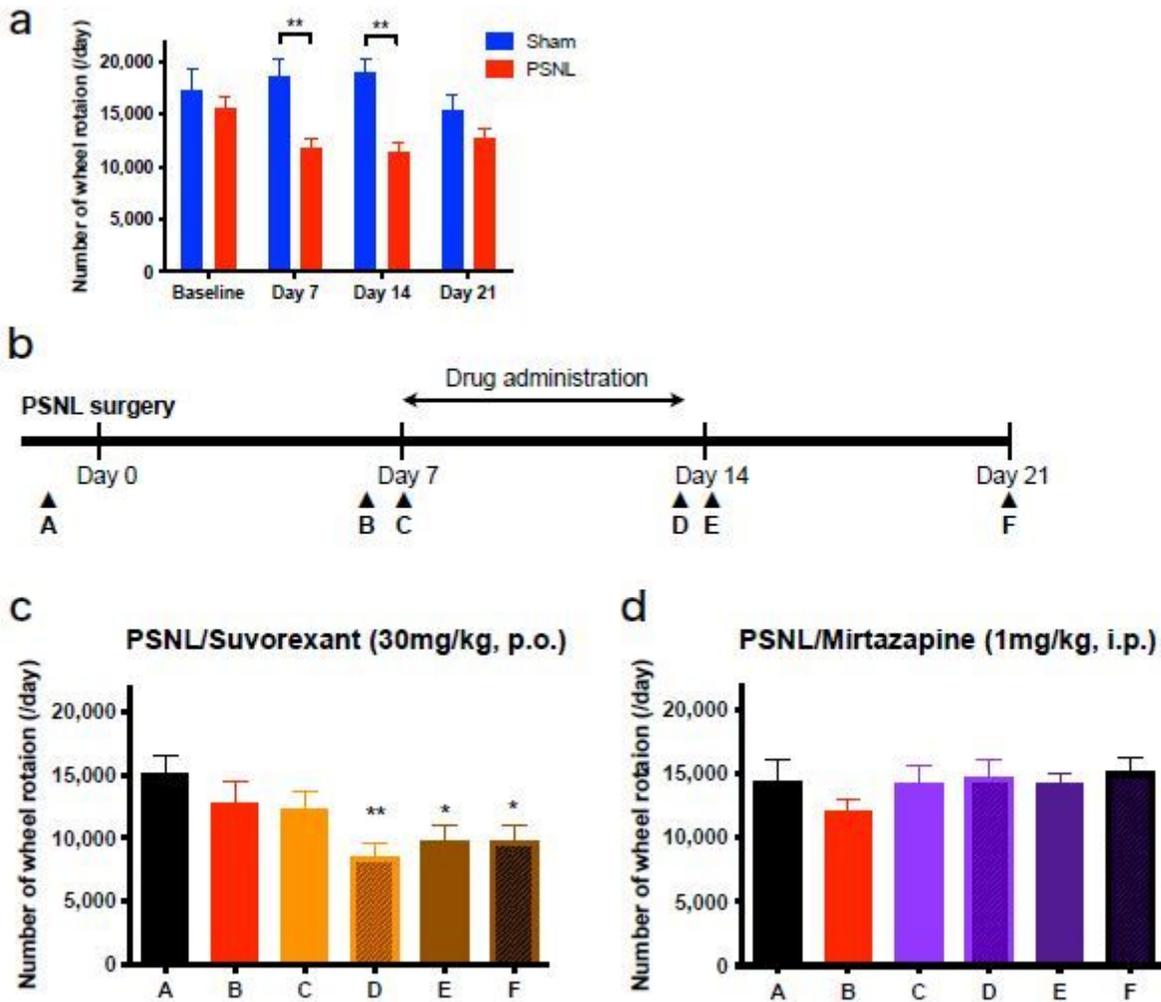


Figure 4

Changes in voluntary physical performance after PSNL and daily administration of suvorexant and mirtazapine. The mice were placed in a cage with a plastic wheel and the total number of wheel rotations were counted to assess voluntary physical performance as per the schedule (a). The number of rotations was evaluated before and 3 weeks after surgery, and the PSNL and sham groups were compared. $**P < 0.01$, two-way ANOVA with Bonferroni post-test ($n = 6$, b). The mice were administered suvorexant (30 mg/kg, c) or mirtazapine (1 mg/kg, d) once daily at ZT0 for 7 days after nerve ligation. We compared the number of wheel rotations at six different time points around the daily suvorexant administration with the baseline. $*P < 0.05$, $**P < 0.01$, one-way ANOVA with Bonferroni post-test ($n = 8$). Each time point is defined as follows: A: baseline, B: 1 day before daily administration, C: day 1 of administration, D: the day of the end of daily administration, E and F: 1 day and 1 week after the end of administration. Data are expressed as mean \pm SEM. Abbreviations: ANOVA, analysis of variance; PSNL, partial sciatic nerve ligation.

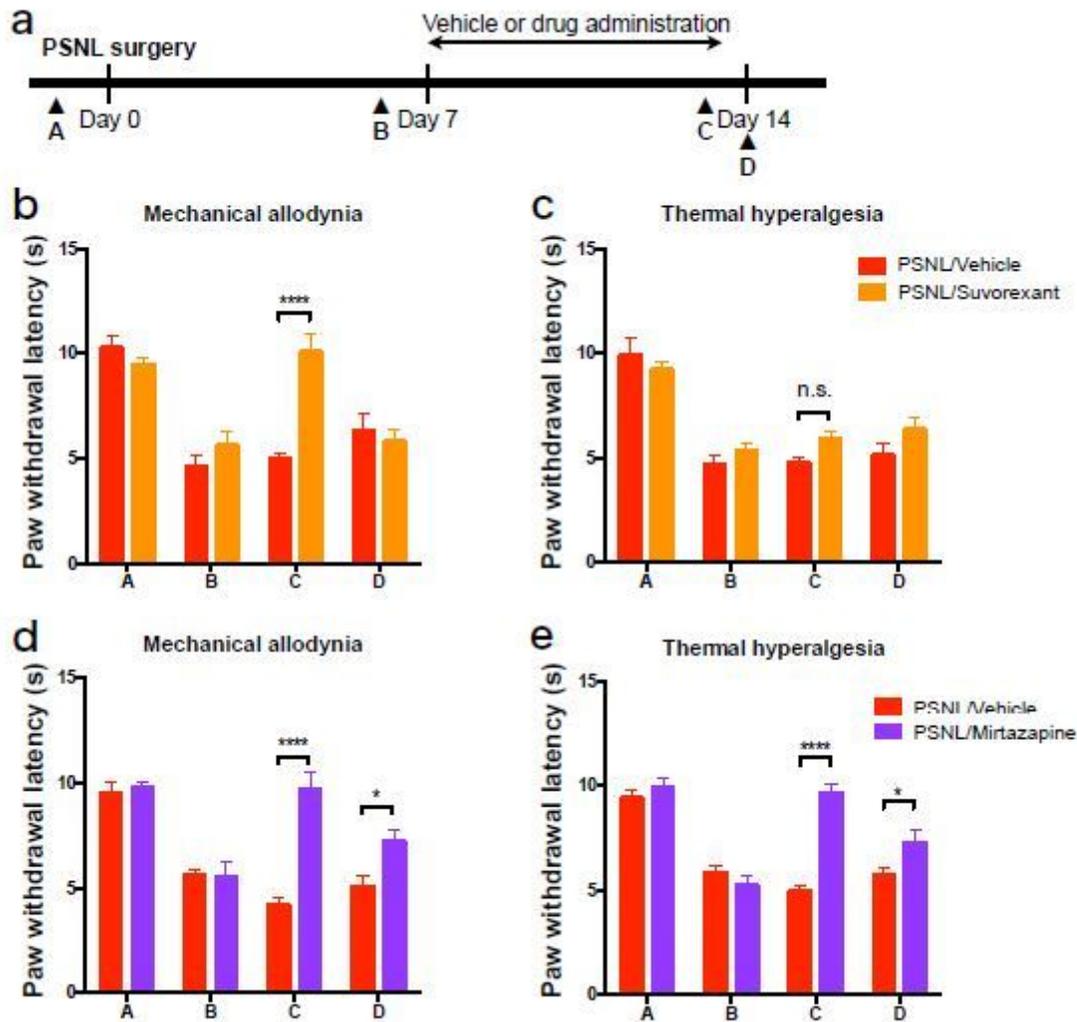


Figure 5

Changes in mechanical allodynia and thermal hyperalgesia after daily administration of suvorexant and mirtazapine. The mice were treated with suvorexant (30 mg/kg), mirtazapine (1 mg/kg), or vehicle once daily at ZT0 for 7 days after nerve ligation (a). They were assessed for mechanical allodynia (b, d) and thermal hyperalgesia (c, e) with automated von Frey test and planter test at four time points. Data are expressed as mean \pm SEM (PSNL-vehicle, n = 8-9; PSNL-suvorexant, n = 8; PSNL-mirtazapine, n = 9). *P < 0.05, ****P < 0.0001, compared between PSNL-vehicle and PSNL-suvorexant or PSNL-vehicle and PSNL-mirtazapine by two-way ANOVA with Bonferroni post-test. Each time point is defined as follows: A: baseline, B: 1 day before daily administration, C: 1 h after the last administration, D: 1 day after the end of administration. Abbreviations: ANOVA, analysis of variance; PSNL, partial sciatic nerve ligation; ZT, Zeitgeber time