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Dual orexin receptor antagonist drug suvorexant can help in amelioration of predictable chronic mild stress-induced hyperalgesia

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ABSTRACT

Aims: This study aimed to evaluate the involvement of the orexin system in predictable chronic mild stress (PCMS) and the effects of suvorexant, a dual orexin receptor antagonist, on nociceptive behavior in PCMS.
Materials and methods: Male C57BL/6 J mice were separated into various PCMS groups: a control group with sawdust on the floor of the rearing cage (C), a group with mesh wire on the floor (M), and a group with water just below the mesh wire (W). Activation of lateral hypothalamic orexin neurons was assessed using immunofluorescence. In another experiment, half of the mice in each group were administered an intraperitoneal injection of suvorexant (10 mg/kg), and the remaining mice were injected with the same amount of vehicle (normal saline). Thermal hyperalgesia was examined using tail immersion and hot plate tests, while mechanical hyperalgesia was investigated using the tail pinch test after 21 days of PCMS.
Key findings: Animals subjected to PCMS showed an increased percentage of activated orexin neurons in the lateral hypothalamic region after 21 days. Mice raised in the PCMS environment showed increased pain sensitivity in several pain tests; however, the symptoms were significantly reduced by suvorexant administration.
Significance: The findings revealed that PCMS activates hypothalamic orexin neuronal activity, and the use of suvorexant can help attenuate PCMS-induced thermal and mechanical hyperalgesia.

1. Introduction

Both physical and mental stress have several unexplained effects on the human body. Stress can bring about changes in various physiological processes, including in the immune and metabolic systems, emotional behaviors, sleep cycle, pain sensitivity, and so on (Grafe and Bhatnagar, 2018; McEwen, 2006). Stress can broadly be categorized into two types: acute and chronic. Acute stress response is the body's natural reaction to threats and unpredictable occurrences in the environment (Sargin, 2019). Contrarily, natural physiological coping mechanisms may be hampered by chronic stress and may lead to enduring consequences on the cardiovascular, central nervous, digestive, and immune systems (Sargin, 2019; Yaribeygi et al., 2017). Even though chronic stress is more life-threatening than acute stress, its effects and mechanisms on the body and mind remain unclear due to the wide variety of stress types and their responses (Sargin, 2019).

We have recently found that predictable chronic mild stress (PCMS) induces hyperalgesia and sleep loss in mice (Dalanon et al., 2021). Exposure to PCMS decreased the amount of non-rapid eye movement (NREM) sleep and decreased slow-wave activity (SWA) during NREM sleep, a parameter of sleep depth, in both dark and light phases (Dalanon et al., 2021). In addition, mechanical and aversively hot thermal hyperalgesia were more intensified in the PCMS groups than in the control, and the degree of hyperalgesia was correlated with worse sleep quality (Dalanon et al., 2021). However, the mechanisms underlying how PCMS deprives sleep quantity and quality and enhances hyperalgesia remain unclear.

When discussing stress, sleep, and pain, orexin, a substance involved in the regulation of all of these, is a candidate that is likely to play a part in the mechanism (Sargin, 2019; Ida et al., 2000). Orexins, also known as hypocretins, are neuropeptides produced in the lateral hypothalamic area (Baimel et al., 2015; Yaribeygi et al., 2017). Orexin A and orexin B

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are the two hypothalamic neuropeptides (Carrive, 2017) that act through G-protein coupled receptors (GPCRs) OX1R and OX2R, respectively (Kukkonen, 2017). Orexins have been widely studied since their discovery owing to their role in various physiological functions such as stress responses (Berridge et al., 2010), feeding behavior (Sakurai et al., 1998; Yokobori et al., 2011), sleep and wakefulness (Chemelli et al., 1999), pain sensitivity (Ahmadi-Soleimani et al., 2020), energy homeostasis (Shiuchi et al., 2009; Shiuchi et al., 2019), and emotional regulation (Han et al., 2020). In particular, the role of orexins in behavioral and physiological responses to stress is crucial. Several studies have revealed that the orexin system is activated under high-arousal conditions such as anxiety and stress (Johnson et al., 2012; Berridge et al., 2010).

As for their relationship with pain, orexin receptors are expressed in both pain and sleep modulating centers, such as the locus coeruleus and ventral tegmental area, and are involved in pain processing at both the brain and spinal cord levels (Ahmadi-Soleimani et al., 2020). Activation of orexin type-1 receptors in the locus coeruleus can induce analgesia, and the blockade of these receptors is associated with increased pain sensitivity, that is, hyperalgesia during the formalin test (Mohammad--Pour Kargar et al., 2015). It has also been proven that the analgesic effect of met-enkephalin locus coeruleus neurons is mediated by the orexinergic system (Mohammad Ahmadi Soleimani et al., 2015).

The role of orexin in the regulation of sleep and wakefulness is well established. Optogenic activation of orexin neurons results in immediate wakefulness and activity (Adamantidis et al., 2007; Carter et al., 2009), while optogenic inactivation of orexin neurons results in NREM sleep, especially during wakefulness (Tsunematsu et al., 2011; Sasaki et al., 2011). Suvorexant, a dual orexin receptor antagonist, has recently been commercialized as a treatment for insomnia. In rodents, suvorexant administration has also been reported to have an effect on PTSD-like symptoms in stress-re-stress (SRS)-exposed rats (Prajapati and Krishnamurthy, 2021) and overall sleep-promoting effects in adult rats (Sanchez-Alavez et al., 2019).

Thus, orexin is involved in all aspects of stress, pain, and sleep, and this fact seems to suggest that the increased pain sensitivity associated with PCMS-induced sleep loss observed in our previous study described above may be mediated by changes in the orexin system. If so, suppression of the orexin system by suvorexant treatment might alter the pain sensitivity induced by PCMS. This study aimed to investigate the role of the orexin system in PCMS and examine whether the continuous administration of orexin receptor antagonists can alleviate the hyperalgesia caused by PCMS.

2. Materials and methods

2.1. Animals

Eight-week-old male C57BL/6 J mice were used in this experiment (Japan SLC, Shizuoka, Japan). Mice were housed in a humidity-(40%) and temperature-controlled (23 ± 1 °C), 12-hour light-dark (L/D) cycle (lights on at 0800) animal containment system. The mice were provided with water and food ad libitum.

The Animal Study Committee of Tokushima University approved this study (Lic. No. T30–30). The Guidelines for the Use of Animals in Research from the International Association for the Study of Pain, as well as the Council of the Physiological Society of Japan's Guidelines for the Care and Use of Animals, were adapted for this study. All efforts were made to decrease the number of mice used and to reduce animal suffering.

2.2. PCMS environment models

All mice were placed in single cages (136 mm × 208 mm × 115 mm). The experimental groups (n = 5/group) included the control cohort (C) as well as the mesh wire (M) and water (W) groups, which were the PCMS condition groups. Mice in group C were reared in bedding filled with normal sawdust; mice in group M were placed on a wire net over normal sawdust; and mice in group W were reared in a cage with water, 2 mm below the wire net, for 21 days (Chikahisa et al., 2017; Dalanon et al., 2021).

2.3. Collection of brain tissue

Mouse brains were fixed by perfusion with paraformaldehyde (PFA). After 48 h, whole-brain tissue was post-fixed in 4% PFA for 24 h, followed by incubation at 4 °C in a solution of 20% (w/v) sucrose and phosphate-buffered saline (PBS). Later, the harvested brain tissue was snap-frozen in OCT (optimal cutting temperature) compound (Sakura Fine Technical, Tokyo, Japan), and 30 μm sections were prepared for use in immunofluorescence staining.

2.4. Immunofluorescence staining

Brain sections were subjected to 3% normal donkey serum at room temperature for 2 h and incubated at 4 °C with rabbit antibodies against c-Fos (1:1000 dilution; Cell Signalling Technology (CST), Danvers, MA, USA) and goat antibodies against orexin (1:2000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 3 days. Following this, the sections were washed with PBS and incubated with an Alexa Fluor-594-labeled donkey anti-goat IgG antibody (Molecular Probes, Eugene, OR, USA) for 2 h at room temperature at a dilution of 1:500 or with an Alexa Fluor-488-labeled donkey anti-rabbit IgG antibody (1:500; Molecular Probes). Finally, using a microscope (DM4000B; Leica), the sections were examined and carefully fitted according to the shape of the brain structures. Manual counting of the co-expression sites of orexin and c-Fos was performed, as well as the calculation of the co-expression rate in orexin neurons.

2.5. Drug administration

Mice were randomly allocated to six groups (n = 7 in each group): control-vehicle, control-suvorexant, mesh wire-vehicle, mesh wire-suvorexant, water-vehicle, and water-suvorexant. Suvorexant (Belsomra 20 mg) tablets were procured from MSD-KK (Tokyo, Japan). Suvorexant suspended in normal saline (10 mg/kg) was administered intraperitoneally every morning. The vehicle group received normal saline intraperitoneally. The PCMS conditions were maintained for 21 days.

2.6. Pain assessment

Mechanical hyperalgesia was determined using the tail-clip test. Tail immersion and hot plate tests were performed in this study to assess thermal hyperalgesia. The tests were performed after 21 days of stress.

2.6.1. Tail clip test for mechanical hyperalgesia

The tail clip test (n = 7/group) was adapted from Takagi et al.'s revision of Haffner's tail-pinch test (Takagi et al., 1966). The time of a reaction such as attempting to dislodge the clip or turning around to bite the clip was calculated from when the clip was fastened at the base of the tail until such a reaction occurred. Each session was repeated three times at an interval of five minutes, and the mean latency was calculated. To avoid permanent nerve damage, a cutoff time of ten seconds was used.

2.6.2. Tail immersion test for thermal hyperalgesia

This test (n = 7/group) is a modified version of the test used by Kotlinska et al (Kotlinska et al., 2013). The distal half of the tail was dipped in a hot water bath sustained at 50.0 ± 0.5 °C. The reaction time was measured in seconds for each participant. A 20-s time limit was sustained to prevent tissue damage to the tail skin. The test was repeated three times at an interval of five minutes, and the average reaction time

was calculated.

2.6.3. Hot plate test for thermal hyperalgesia

The hot plate test was performed at 50.0 °C ± 0.5 °C, and continuous monitoring of the surface temperature was performed with a digital thermometer. Each mouse (n = 7/group) was gradually positioned on a hot plate, and the reaction time to a nocifensive behavior, such as paw licking and rearing, was recorded in seconds. In this study, mice that were unable to exhibit these behaviors within 60 s were taken out (to avoid thermal injury), and a value of 60 s was assigned. The frequency of each nocifensive behavior was recorded.

2.7. Plasma corticosterone levels detection and adrenal gland extraction

To determine the amount of plasma corticosterone, trunk blood was extracted from every cohort (n = 4/group). Plasma was separated from whole blood samples mixed with 1 mg/ml EDTA–2Na through centrifuge (4 °C, 4000 rpm, 15 min). Plasma corticosterone levels were measured using an enzyme immunoassay kit (Yanaihara Institute Inc., Fujinomiya, Japan) according to the manufacturer's instructions. After the animals were euthanized, the bilateral adrenal glands were removed and weighed immediately using an electronic scale.

2.8. Food consumption and body weight measurement

The amount of food consumed was calculated by weighing the food pellets. New pellets were added to all groups simultaneously, and the amounts consumed were noted. Body weights were measured by weighing the mice on an electronic scale.

2.9. Statistical analyses

One-way analysis of variance (ANOVA) was used to compare different groups using immunofluorescence staining, followed by Tukey's posthoc test. To compare body weight and food consumption, a two-way repeated ANOVA was performed, followed by Tukey's multiple comparison test. The results of the different adrenal gland weights, plasma corticosterone levels, and tail-pinch, tail immersion, and hot plate tests were compared in each treatment group (vehicle or suvorexant) using one-way ANOVA. Tukey's multiple comparison test was used for post hoc tests, as required. Student's t-test was used for comparison between control-vehicle and control-suvorexant (non-stress). The Shapiro-Wilk test was performed to verify that the data followed a normal distribution. Data are reported as mean ± standard error of the mean (SEM). All statistical analyses were performed using IBM SPSS software.

3. Results

3.1. PCMS increases the activity of orexin neurons

Constant chronic stress for 21 days leads to the stimulation of lateral hypothalamic orexin neurons in the mouse brain. Activated orexin, as indicated by c-Fos neurons, was observed in the lateral hypothalamic region (Fig. 1A). Since c-Fos immunohistochemistry detects the activation of specific neurons in response to experimental conditions, we calculated the number of activated neurons and found a higher percentage of activated orexin neurons co-labeled with c-Fos in both PCMS (M and W) groups compared to in the C group (Fig. 1B) ($F_{(2,12)} = 5.333$, $P = 0.022$). The M ($P < 0.05$) and W ($P < 0.05$) groups showed a significantly higher percentage of c-Fos-active orexin neurons than the C group did.

3.2. PCMS leads to increased food consumption in both the water groups

Since PCMS was observed to activate orexin neurons in the lateral hypothalamus, we injected suvorexant, an orexin receptor antagonist, or vehicle (normal saline) into each PCMS group and observed the changes in body weight, food intake, and pain test results (Fig. 2 A). The body weights of the animals remained fairly constant throughout the PCMS protocol, and no significant differences between the groups were observed (Fig. 2B). In the 21-day PCMS timeline, there was a difference in food intake between the W group with and without orexin receptor inhibition (Fig. 2 C). Food consumption increased in weeks 1, 2, and 3 in the W group injected with vehicle compared to the M group injected with vehicle and the C group injected with vehicle ($F_{(2,18)} = 16.890$,

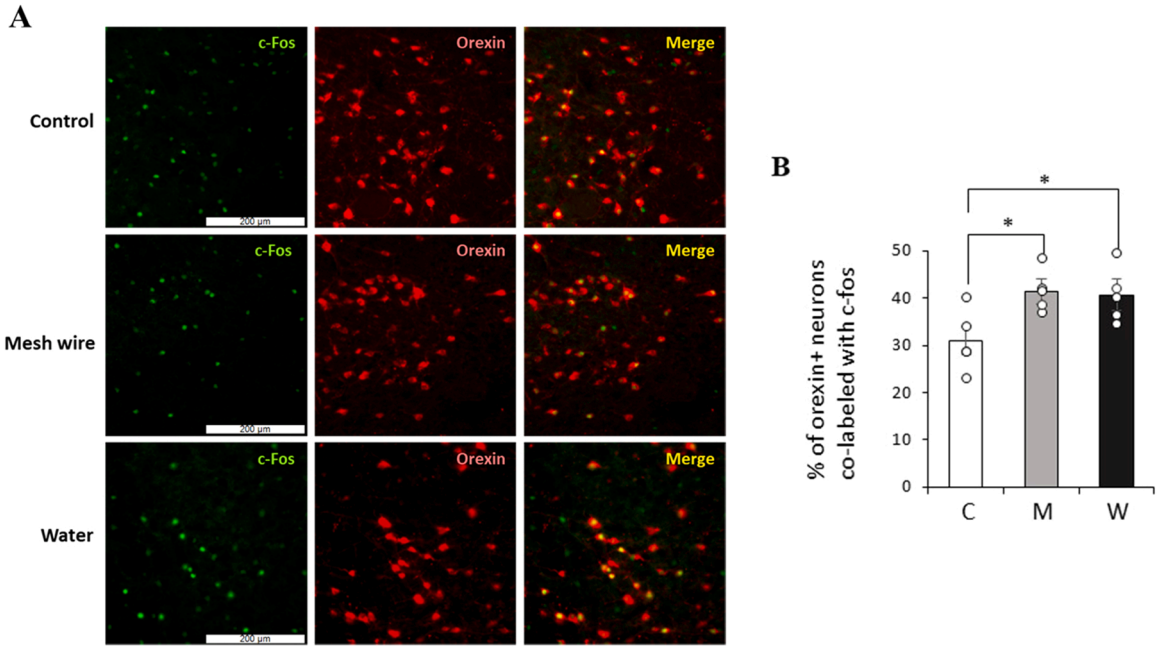

Fig. 1. Fluorescence double staining of orexin and c-fos after 21 days of predictable chronic mild stress (PCMS). (A) The co-expression of orexin (red) and c-Fos (green) in the control (C), mesh wire (M), and water (W) groups. **(B)** The percentage of orexin-positive neurons co-labeled with c-Fos. The values shown are the means ± SEM. (n = 5/group) (* $P < 0.05$).

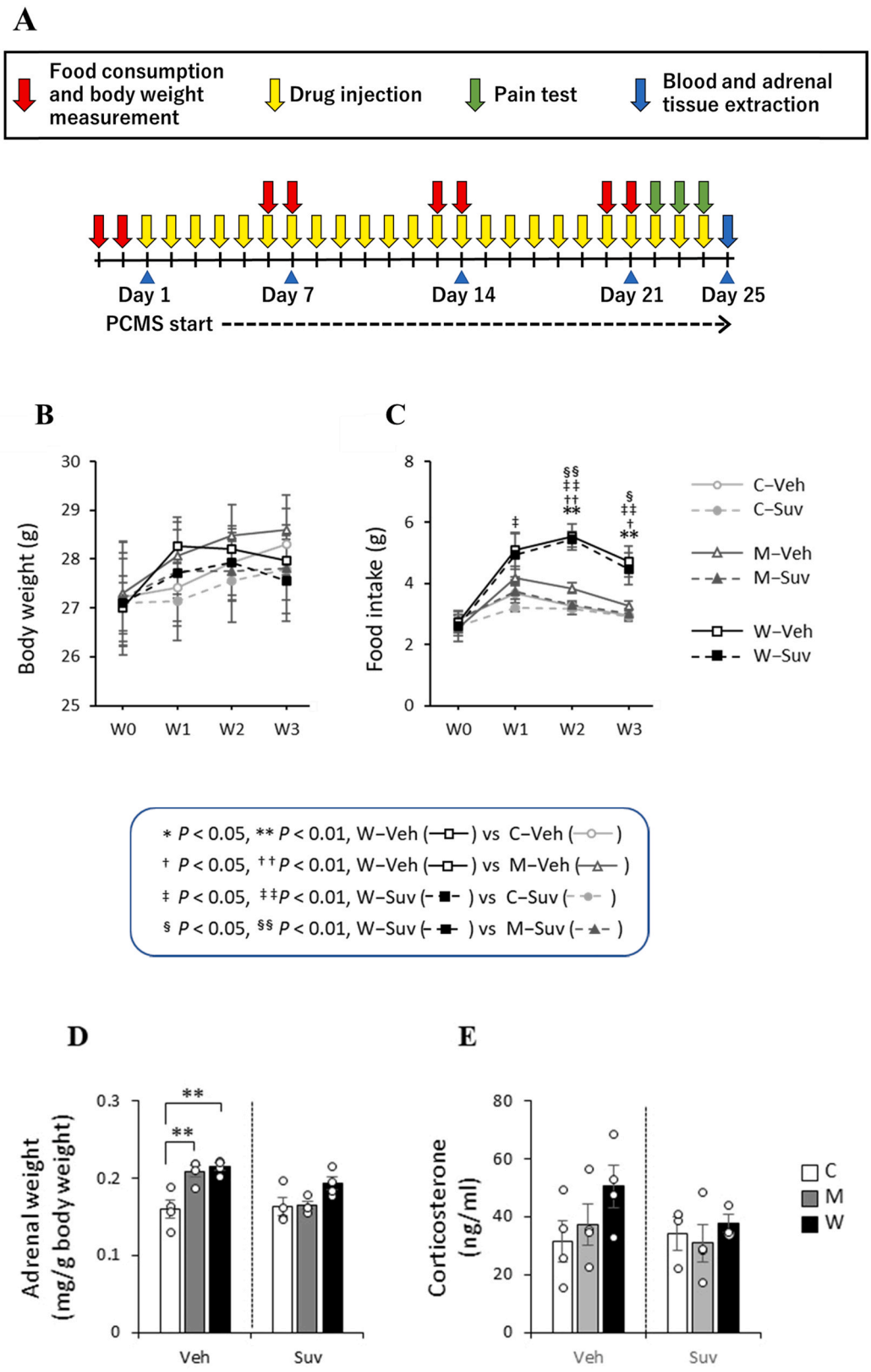

Fig. 2. The effect of suvorexant on body weight, food intake, adrenal weight, and corticosterone levels in mice under predictable chronic mild stress (PCMS). (A) Timeline of the experiment with suvorexant injection under PCMS. Effect of suvorexant on body weight (B) and food intake (C) while under PCMS for 21 days in the control group injected with vehicle (C-Veh) or suvorexant (C-Suv); in the mesh wire group (M) injected with vehicle (M-Veh) or suvorexant (M-Suv); and in the water group (W) injected with vehicle (W-Veh) or suvorexant (W-Suv) (B and C; n = 7/group). Effect of suvorexant on adrenal weights (D) and corticosterone levels (E) after 21 days of PCMS (D and E; n = 3–4/group). Data are presented as means ± SEM. * * $P < 0.01$.

$P < 0.001$). In weeks 2 and 3, food consumption also increased in the W group injected with suvorexant compared to the M and C groups injected with suvorexant ($F_{(2,18)} = 18.497$, $P < 0.001$).

3.3. The weight of the adrenal glands increased in the PCMS groups injected with vehicle

The weight of the adrenal glands (Fig. 2D) increased in both the PCMS (M and W) groups injected with the vehicle compared to the C group injected with the vehicle ($F_{(2,9)} = 12.456$, $P = 0.003$). All animals in the vehicle-treated M ($P < 0.01$) and W ($P < 0.01$) groups showed adrenal gland hypertrophy when compared with the C group. However, the PCMS groups injected with suvorexant did not show adrenal gland hypertrophy (Fig. 2D). There was no significant difference in adrenal weight between the C-vehicle and C-suvorexant groups.

The plasma corticosterone levels (Fig. 2E) of the W groups, which were calculated after the administration of PCMS, tended to increase when compared with those of the C group; however, the difference was not statistically significant. This tendency was not observed in the groups injected with suvorexant. There was also no significant difference in plasma corticosterone levels between the C-vehicle and C-suvorexant groups.

3.4. Suvorexant decreased thermal pain sensitivity in the tail-immersion test

The M ($P < 0.05$) and W ($P < 0.05$) groups treated with suvorexant showed an increased reaction time to a thermal stimulus when the tail immersion test was performed after 21 days of PCMS (Fig. 3A) ($F_{(2,18)} = 5.950$, $P = 0.010$), although the W group already had lower pain sensitivity to hot water than the C group, even after vehicle administration. For comparisons between non-stress (C-vehicle and C-suvorexant groups), the results showed that C-suvorexant had a significantly greater latency than C-vehicle in the tail immersion test.

3.5. Suvorexant attenuated the increased mechanical pain sensitivity in the tail-pinch test under PCMS

The reaction time to the tail-pinch was decreased in the vehicle-treated M and W groups when compared with the vehicle-treated C group (Fig. 3B) ($F_{(2,18)} = 5.244$, $P = 0.016$), suggesting that mechanical hyperalgesia was observed under PCMS. However, the PCMS groups injected with suvorexant did not show heightened mechanical pain sensitivity compared to the control group. There was also no significant difference in the tail pinch test between the C-vehicle and C-suvorexant groups.

3.6. Suvorexant attenuated the increased thermal pain sensitivity in the hot plate test under PCMS

Decreased latency in paw-licking behavior was observed in the vehicle-treated W group compared to the vehicle-treated C group (Fig. 4A) ($F_{(2,18)} = 4.668$, $P = 0.023$). Similarly, reduced latency in rearing behavior was observed in both the M ($P < 0.05$) and W ($P < 0.01$) groups treated with vehicle ($F_{(2,18)} = 7.782$, $P = 0.004$). These results suggest that both PCMS (M and W) groups treated with vehicle experienced increased sensitivity to thermal pain. Interestingly, the suvorexant-treated PCMS groups did not show any significant decreases in the latency in paw-licking (Fig. 4A) and rearing behavior (Fig. 4B) results when compared with the suvorexant-treated C group, suggesting that inhibition of orexin receptors reduces PCMS-induced hyperalgesia in the hot plate test. There was no significant difference observed in the paw-licking (Fig. 4C) and rearing (Fig. 4D) frequencies in all groups treated with suvorexant and vehicle after 21 days of PCMS. There was also no significant difference in the hot plate test between the C-vehicle and C-suvorexant groups.

4. Discussion

In our study, we found that mice subjected to PCMS showed increased activation of orexin neurons in the lateral hypothalamic region and increased pain sensitivity. The hyperalgesia was significantly reduced by suvorexant administration. This is the first study to show that orexin plays an important role in hyperalgesia induced by PCMS. Orexin receptor antagonists such as suvorexant may be used to mitigate these effects.

4.1. Hypothalamic-pituitary-adrenal axis

Previous studies have shown marked overactivity of the hypothalamic-pituitary-adrenal (HPA) axis as a result of stress (Herman et al., 2016; Martí and Armario, 1998). Chronic activation of the HPA axis leads to neural adaptation in stress-related brain circuits, which has consequences for an organism's behavioral responses to stress exposure. In the present study, PCMS mice (W and M groups) treated with the vehicle also showed marked adrenal hypertrophy and a tendency to have increased blood corticosterone levels. This suggests that the PCMS model used in this experiment successfully induced a stress response via the HPA axis.

The effects of chronic stress on the orexin system vary depending on

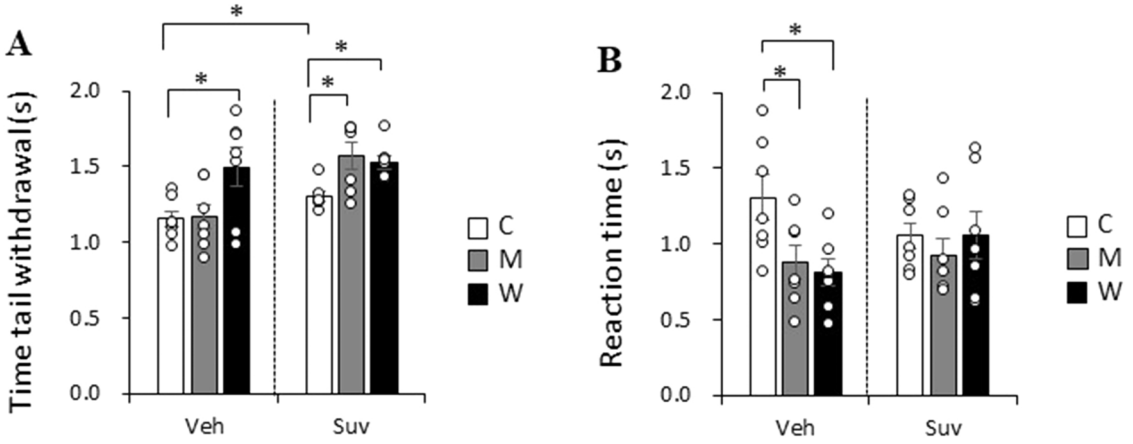

Fig. 3. Effect of suvorexant on pain sensitivity after 21 days of predictable chronic mild stress (PCMS). Latency results of tail withdrawal in the tail-immersion test (A) and reaction time results in the tail-pinch test (B) of the control group (C), mesh wire (M), and water (W) groups injected with vehicle (Veh) or suvorexant (Suv). Data are presented as the mean ± SEM (A and B, n = 7/group). * $P < 0.05$.

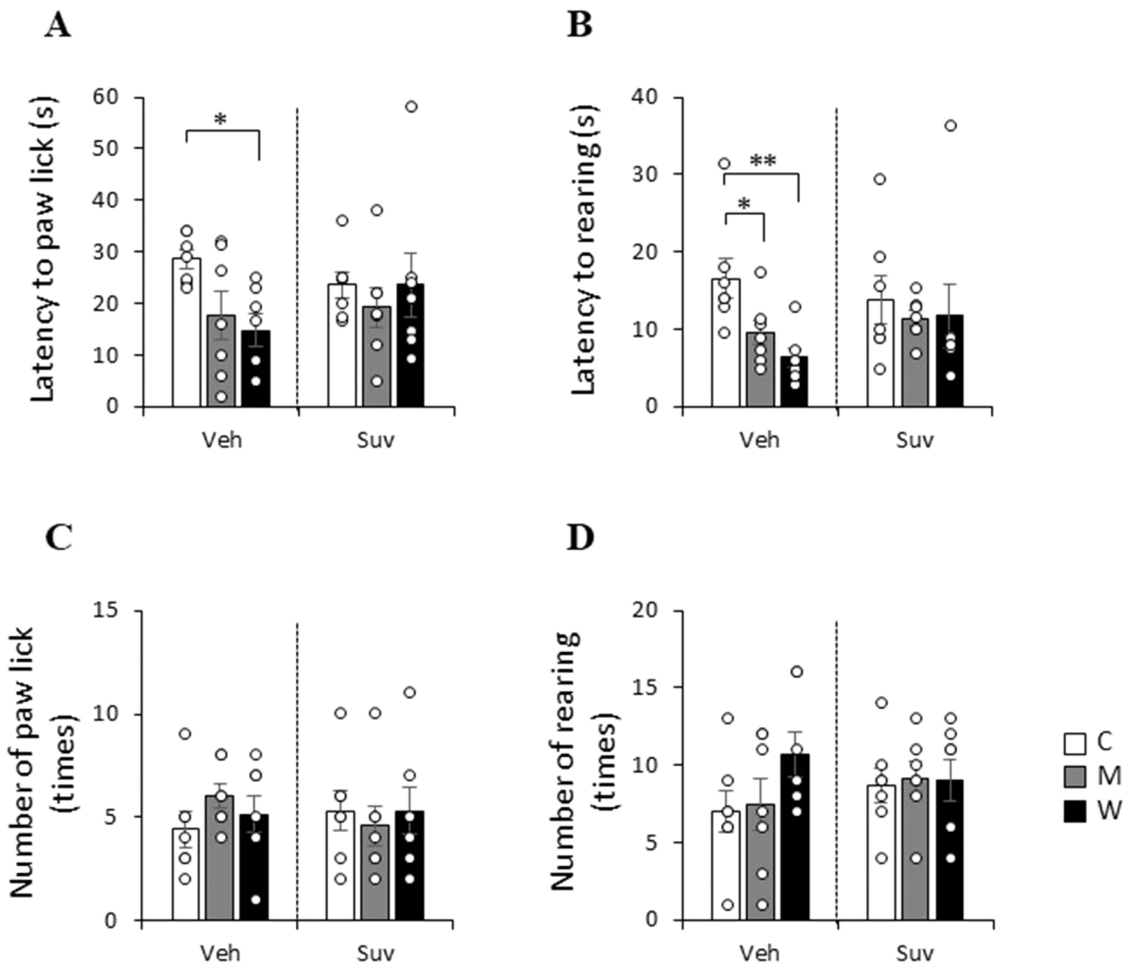

Fig. 4. Effect of suvorexant on hot plate test results after 21 days of predictable chronic mild stress (PCMS). Latency of paw lick (A) and rearing (B) and the number of paw licks (C) and rearing behaviors (D) in the control (C), mesh wire (M), and water (W) groups injected with vehicle (Veh) or suvorexant (Suv). Data are presented as means ± SEM. (A-D, n = 7/group). *P<0.05, **P< 0.01.

the type of stressor and brain region involved. In general, chronic stress leads to changes in the number of orexin neurons (Mikrouli et al., 2011), orexin mRNA levels (Chung et al., 2014), and orexin neuronal activation (Nollet et al., 2011). Consistent with these previous studies, we observed increased activation of orexin neurons in the hypothalamus of mice subjected to PCMS. In contrast, the administration of an orexin receptor antagonist alleviated PCMS-induced adrenal hypertrophy and suppressed the increase in blood corticosterone levels in our study. Considering the previous literature and our results, it seems that orexin neurons are also activated by PCMS and contribute to the long-term regulation of the HPA axis. This phenomenon will certainly be interesting for future studies pertaining to the involvement of the orexin system in chronic stress and its effects on the HPA axis.

4.2. Orexin and pain

The orexin and pain regulatory systems are known to interact with each other. For example, the findings of a study performed in 2005 suggest that both persistent pain and stress activate the orexin neurons of the hypothalamus (Watanabe et al., 2005). Furthermore, stimulation of the lateral hypothalamus has been shown to produce antinociceptive effects in rats with thermal hyperalgesia from neuropathic pain (Wardach et al., 2016). On the other hand, a study by Inutsuka A. et al. in 2016 showed that noxious stimuli induced correlated activation of orexin neurons, and pharmacogenetic activation of orexin neurons attenuated pain perception under heat and cold stimuli (Inutsuka et al., 2016).

In our experiments, the PCMS group, which had an increased number of activated orexin neurons, also had increased pain sensitivity, as observed in both the tail pinch and hot plate tests. Interestingly, suvorexant treatment led to decreased sensitivity to mechanical and thermal pain stimuli caused by PCMS. In the tail immersion test using hot water, the thermal pain response of the W group treated with the vehicle was slower than that of the control, likely because the W group was accustomed to the water. However, in this test, suvorexant administration reduced pain sensitivity, at least in group M, which was unchanged by vehicle administration. Although the specific neural projections involved in PCMS-induced hyperalgesia are not yet clear, our results indicate that orexin neurons contribute significantly to this mechanism.

4.3. Pain and sleep

Orexin is known to be one of the most important wake-promoting and sleep-suppressing neuropeptides (España et al., 2001); therefore, suvorexant, the orexin receptor antagonist, is a drug that has been used clinically in recent years for the treatment of insomnia (Kuriyama and Tabata, 2017). A previous study also demonstrated that suvorexant in patients with fibromyalgia led to improved sleep times and reduced pain sensitivity in assessments of heat stimulus (Roehrs et al., 2020). Our previous studies have also reported that the same kinds of PCMS (M and W group) cause hyperalgesia and insomnia in these behavioral

experiments (Dalanon et al., 2021). In these experiments, there was a significant negative correlation between sleep quality and pain sensitivity; that is, poor sleep quality was associated with higher pain sensitivity. Since we used the same PCMS model in the present study as in the previous study, sleep disturbances may have been elicited in the PCMS group, although we did not measure sleep this time. In our study, we found that suvorexant relieved hyperalgesia in the tail-pinch and hot plate tests in the PCMS group. Combining these studies with the current results, it is possible that insomnia induced by PCMS may have been ameliorated by suppressing orexin receptors, leading to the mitigation of the PCMS-induced increase in pain sensitivity.

In other words, it can be suggested that suvorexant administration may improve insomnia and PCMS-induced hyperalgesia.

4.4. Food consumption and body weight

This study also showed differences in the food consumption and body weight of mice under PCMS. Food consumption was markedly higher in the W group in the first, second, and third weeks of chronic stress, whereas the body weight values did not show any significant changes. It is known that increased orexin expression has been associated with increased appetite and feeding behavior, as centrally administered orexin has been shown to stimulate food consumption in rats (Sakurai et al., 1998). This is also reflected in the results of the present study, in which the W group, in which orexin neurons were more activated, had a higher food intake. In contrast, M group mice showed the same increase in orexin neuron activity as the W group, but their food intake was similar to that of the C group. As shown in Fig. 3A and Fig. 3B, hyperalgesia was not as pronounced as in the W group in some behavioral tests. These results suggested that the stress intensity in the M group was milder than that in the W group. Thus, even within the PCMS, different types and intensities of stress had different effects on stress responses, including feeding behavior and pain sensitivity in animals.

5. Conclusion

In conclusion, PCMS activates orexin neurons in the lateral hypothalamus and causes hyperalgesia in mice, and the use of orexin receptor antagonists is effective in suppressing PCMS-induced hyperalgesia. This is the first study to show that orexin plays an important role in hyperalgesia induced by PCMS. However, the specific mechanisms and brain regions of how the orexin system reduces PCMS-induced hyperalgesia are not yet clear in this experiment. Previous studies have shown that the endogenous orexin system modulates opioid analgesia by affecting OX1R in several brain regions, including the locus coeruleus (LC) and lateral paragangial giant cells (LPGi) (Ahmadi-Soleimani et al., 2017). Future studies are expected to elucidate the detailed mechanisms of PCMS-induced hyperalgesia, which may have clinical applications in relieving pain associated with stress and insomnia.

Author contributions

P.C., J.D., S.C., and T.S. performed the experimental work. S.C., P.C., and K.O. analyzed and interpreted the data. S.C., P.C., N.S., Y.M., and H. S. designed the study., P.C. and S.C. wrote the manuscript. All authors contributed to, edited, and agreed with the published version of the manuscript.

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CRediT authorship contribution statement

Sachiko Chikahisa: Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing. **Parimal Chavan:** Conceptualization, Investigation, Formal analysis, Data curation, Writing – original draft. **Junhel Dalanon:** Visualization, Investigation, Writing – review & editing. **Hiroyoshi Sei:** Conceptualization, Writing – review & editing. **Tetsuya Shiuchi:** Methodology, Investigation, Writing – review & editing. **Noriyuki Shimizu:** Methodology, Writing – review & editing. **Kazuo Okura:** Formal analysis, Writing – review & editing. **Yoshizo Matsuka:** Conceptualization, Writing – review & editing.

Declaration of conflict of interest

The authors declare no conflict.

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