Activation of CB1R within the rostral ventromedial medulla alleviates central sensitization induced by paradoxical sleep deprivation in a plantar incision rat model

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Research Article

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Abstract

Background

Cannabinoid receptor 1 (CB1R) in the rostral ventromedial medulla (RVM) of rats has been shown to exert antinociceptive effects by altering synaptic plasticity in inflammatory and neuropathic pain models. However, the role of CB1R in the central sensitization due to paradoxical sleep deprivation (PSD) is unclear.

Methods

Male Sprague-Dawley rats were subjected to PSD for 24 hours one day before surgery, and then a plantar incision model was established. Mechanical threshold was used to evaluate hyperalgesia. The mRNA and protein levels of CB1R in RVM were detected by qRT-PCR and western blotting. WIN-55,212-2(a CB1R agonist) and SR141716(a CB1R antagonist) were injected into RVM, the expression of CB1R was detected by immunofluorescence, the mRNA and protein levels of CB1R in the RVM were again detected, and the pain degree was assessed by mechanical threshold. To investigate the role of CB1R in the process of central sensitization.

Results

We found that a 24-hour period of PSD before surgery significantly increased pain sensitivity and duration of postoperative pain in rats after incisional surgery. Compared with rats that received only plantar incision, preoperative PSD for 24 h increased CB1R expression in the RVM of rats after incision. Furthermore, the injection of CB1R agonist into the PSD and incisional model rat RVM 1 day after surgery significantly reduced pain, while the CB1R inhibitor increased nociceptive hypersensitivity.

Conclusions

These data reveal that activation of CB1R in the RVM alleviates preoperative PSD-induced incision pain sensitization. Activation of CB1R might have a positive effect on the prevention of pain sensitization after PSD by reducing central sensitization.

1. Introduction

Most patients who undergo surgery experience acute postoperative pain, but less than half experience adequate relief after surgery[1]. Within the first seven days following surgery, 63% of patients with acute postoperative pain report stable and persistent high or moderate pain[2]. Postoperative pain is injurious pain that can develop into chronic postoperative pain or persistent postoperative pain if it is not adequately managed in the initial state[3, 4]. With in-depth research on the mechanism of pain, perioperative paradoxical sleep deprivation (PSD) was found to be one of the important risk factors for postoperative pain[5]. There is a close correlation between sleep quality and pain, and insufficient sleep

may lead to pain sensitization, while improving sleep quality can help reduce pain sensitivity[6]. However, the role of PSD in postoperative pain is often underestimated, and its impact on postoperative pain has not been paid attention to and studied.

It is well known that the rostral ventromedial medulla (RVM) is central structure in the downstream facilitative and inhibitory modulatory system of pain, receiving projections from the hypothalamus and periaqueductal gray matter of the midbrain upward and exerting facilitative or inhibitory modulatory effects on pain downward through the spinal cord[7]. In inflammatory pain, nerve and visceral pain model, has confirmed that the RVM downward promoting effect on hyperalgesia[8–10]. In addition to the input of nociceptive stimuli, sleep disturbances have also been shown to activate the descending facilitation pathway of RVM and cause hyperalgesia[11].

The RVM region contains a large number of cannabinoid receptor 1 (CB1R), which induces antinociceptive sensitization, and acute painful stimuli and stress increase their levels in this region [12, 13]. CB1R acts by regulating the ratio of synaptic plasticity to excitability and inhibition[14]. It can inhibit or excite the central nervous system, thereby achieving analgesic or sensitizing effects[14]. Reverse signal transduction is the main mechanism of synapse plasticity mediated by CB1R[15]. AEA and 2-AG are the most representative endocannabinoids, which are produced in the body on demand and play an important role in pain regulation[15, 16]. Endocannabinoids are a class of signal molecules produced and released in the postsynaptic membrane, and are used in the presynaptic membrane CB1R[17]. When cannabinoids bind to CB1R, the release of corresponding neurotransmitters will be reduced, thus regulating various physiological functions such as pain perception and emotion regulation[17].However, no study has confirmed whether CB1R is involved in PSD-induced nociceptive hypersensitivity.

2. Materials and methods

2.1 Animals

We purchased adult male Sprague-Dawley rats (8–10 weeks, 270–300 g) from the animal laboratory of Gansu University of Traditional Chinese Medicine (Gansu, China). The use of animals was approved by the Animal Protection and Use Committee of the Research and Experimental Center of Gansu University of Traditional Chinese Medicine. Animals were housed in an environment with a temperature of 23°C ± 2°C, humidity of 40–50%, and a 12-hour light/dark cycle, and had free access to food and water. All experimental protocols were approved by the Ethics Committee of Gansu University of Traditional Chinese Medicine and complied with the ethical guidelines of the International Association for the Study of Pain.

The rats were divided into six groups, including group C (normal rats), group P (rats with 24-h preoperative PSD), group I (plantar incision rats), group P-I (rats with 24-h preoperative PSD followed by a plantar incision), group P-I-S (rats with 24-h preoperative PSD followed by a plantar incision and

injected with the CB1R agonist SR141716 into the cerebral RVM on the day after the operation) and group P-I-W (rats with 24-h preoperative PSD followed by a plantar incision and injected with the CB1R agonist Win 55, 212-2 into the cerebral RVM on the day after the operation).

2.2 PSD

The sleep deprivation model in rats was created using the modified multiple platform method (MMPM). A polypropylene tank of 50 cm by 50 cm by 30 cm with six wooden columns with the same spacing, each measuring 6.5 cm in diameter and 8 cm in height. The tank was filled with warm water at 24°C, with the level 2 cm below the tops of the columns. The platforms of the columns allowed the rats to walk freely between them, but they were unable to rest between the two columns. Because their leg muscles relaxed and their muscular tension decreased during REM sleep, the rats on the platforms were compelled to stay awake by contacting the water or falling into it. The top of the apparatus allowed the rats to freely consume food and water while they were sleep-deprived. Rats in the control group were placed in the same device as the experimental group, but the device was not filled with water, and the rats were allowed to move around and rest freely for the entire 24 hours of acute PSD from 8:30 am to 8:30 am the following day.

2.3 Plantar incision

Under sevoflurane general anesthesia, the plantar surface of the right hind paw of rats was sterilized using the rat plantar incision pain paradigm developed by Brennan's research team[18]. With a No. 11 blade, a 1-cm longitudinal incision was made, encompassing the skin, fascia, and metatarsal muscles, and a 1-cm longitudinal incision was made 0.5 cm from the heel's edge. The skin was then stitched together with two rows of 4 − 0 antimicrobial absorbable suture after the excised metatarsal muscles were longitudinally incised and cleaned with sterile gauze until bleeding ceased. After surgery, the rats were kept in a thermostatic cage for two hours to aid in healing.

2.4 Behavioral Testing

Cumulative pain scores

Unrestrained animals were placed on a raised plastic mesh floor in a clear plastic chamber and allowed to acclimate for 30 minutes. The foot of the incision was visualized using an angled magnifying glass. Each animal was closely observed for 1 min at 5-min intervals for 1 hour. A score of 0, 1, or 2 was assigned depending on where the foot was found during the scoring period. If the plantar portion of the hind paw was white or distorted by the grid, the foot was scored as fully weight bearing (score = 0). If the foot was completely off the grid, the score was 2. If the plantar portion of the hind paw was in contact with the mesh and not whitened or distorted, a score of 1 was assigned. The sum of the 12 scores (0–24) was calculated and recorded as the cumulative pain score.

Paw withdrawal threshold

The rats were placed in a plastic chamber on a raised mesh floor (grid 8 × 8 mm) and allowed to acclimatize to the environment for 30 min, until the rats were quiet (no longer looking around). A calibrated von Frey wire (Rayward Life Technologies LTD., Nanshan District, Shenzhen, China) was used to slowly and gently stimulate the mid-sole of the hind limb of the rat to be tested, causing the fiber wire to bend for a few seconds, and the rat's foot-contraction response was observed. Fiber filaments were applied through an opening in the mesh floor under the plastic chamber, starting with 2.0-size filaments. Apply the filament vertically to the area near the medial heel wound for 6s or until the animal retracts its hind paw. Rapid lifting, shaking, or licking of the hind paw is considered a positive response and is labeled "X"; if no response is made, it is considered negative and is labeled "O".

2.5 Microinjection of CB1R agonist or antagonist within RVM

The rats in the PSD-incision + CB1R antagonist group and the PSD-incision + CB1R agonist group were anesthetised with a mixture of 5% isoflurane and oxygen 1 day after the plantar incision model was established, and 1.2–1.5% isoflurane was delivered to the animals through the nose cone to maintain anesthesia, and the hair on the top of the head was shaved. Fixed on a brain stereotaxometer (one-arm brain stereotaxometer), iodophor was used to disinfected the skin on the top of the head, cut the skin along the middle of the top of the skull with a scalpel, expose the skull, separate the periosteum of the skull with a cotton swab, fully expose the anterior fontanel and posterior fontanel, and flatten the fontanel (the anterior fontanel and posterior fontanel of rats are on the same plane with an error of no more than 0.03mm), and determine the RVM region according to the stereotaxic map of the rat brain: 10–12 mm below the bregma, 0–2 mm on the left and right side of the midline of the brain. After drilling, a 5μl microsyringe was vertically inserted into the brain tissue, and the depth was 9–10 mm from the periosteum of the skull. Slow injection of drug CB1R antagonists (30 μg/ piece; SR141716) or CB1R agonist (30 μg/ piece; Win 55, 212-2) and 0.2 μl methylene Blue injection. After the injection is completed, the microsyringe stays for 10 minutes and slowly pulls out, seals the hole with bone wax, and sutured the skin. All rats were infiltrated subcutaneously with 1 ml ropivacaine (0.5%) around the incision for postoperative analgesia. The accuracy of injection site was determined according to the location of methylene blue dye during anatomy, and rats with incorrect injection location were excluded from the study.

2.6 Real-Time PCR

Total RNA was extracted from RVM tissues using the SteadyPure Universal RNA Extraction Kit, and the purity of RNA was determined by UV spectrophotometer, and its OD value of 260/280 was 1.90–2.10, which was acceptable for reverse transcription. The Evo M-MLV Reverse Transcription Kit was used for reverse transcription of total RNA, and qRT-PCR was performed according to the instructions of SYBR Green Pro Taq HS qPCR Kit. The expression of the corresponding mRNAs in each group was calculated by the 2-ΔΔCt method, using GAPDH as an internal reference and group C as a blank control.

List of qRT-PCR primer sequences

Gene name	Primer sequence 5' – 3'	Length (bp)
CNR1	F- AGGAGAACTTACTGTGAACAGGC	23
	R- GGTCTGTGGTGATGGTACGG	20
GADPH	F- GACATGCCGCCTGGAGAAAC	20
	R- AGCCCAGGATGCCCTTTAGT	20

2.7 Western blotting

Total protein was extracted from RVM tissues by cracking in RIPA buffer containing protease inhibitor, and the protein concentration was determined by BCA kit. Proteins were separated by SDS-PAGE gel electrophoresis (5–12%) according to their molecular weight and transferred to PVDF membrane. It was enclosed with 5% skim milk powder at room temperature for 2 h and incubated with primary antibody (for CB1R). After overnight in the refrigerator at 4 ℃, the diluted goat anti-rabbit secondary antibody (1:8000) was added and incubated at room temperature for 2 h. Finally, the configured ECL luminescent droplets were placed on the PVDF membrane and exposed in the automatic gel imaging analyzer to obtain WB bands. Repeat the measurement 3 times.

2.8 Immunofluorescence assay

The rats were deeply anesthetized with sevoflurane, followed by left ventricular perfusion with pre-cooled PBS to flush the blood, and then pre-fixed with pre-cooled 4% Paraformaldehyde (PFA), and then PBS perfusion again to flush away the residual PFA. The RVM region of rat brain tissue was quickly separated after perfusion and fixed in a centrifuge tube with 4% PFA. The next day replace with 20–30% sucrose solution for overnight dehydration. After dehydration is complete, spinal cord Tissue is encapsulated with tissue-TEK O.C.T. and stored in a -80°C refrigerator. Frozen microtome was used to cut the embedded tissue into slices with a thickness of 30μm and placed in PBS solution after cutting. During the experiment, sections of medulla oblongata with complete shape and symmetry were selected and placed into the hybrid tube (about 25–30 pieces in each tube). First, the membrane was permeated with 0.1% Triton X-100 solution at room temperature for 30 minutes, and then cleaned with PBS for 3 times for 15 minutes each time. It was then sealed with a sealing solution containing 5% donkey or goat serum at room temperature for 1–2 hours. After blocking, corresponding antibodies were added (Rabbit Anti-CB1R, 1:500, Abcam, USA; Rabbit resistant BDNF, 1:500, Abcam, USA) in a 4°C refrigerator overnight. On the second day, the non-specific binding of the primary antibody was eluted with 0.5% PBST solution (15 minutes, 3 times). After that, the corresponding fluorescent secondary antibody was added and incubated at room temperature for 1–2 hours, and then cleaned with 0.5% PBST (15 minutes, 3 times). In the case of immunocolocalization experiments, a primary or secondary antibody of the protein to be labeled is added at the same time when the antibody is incubated. Finally, the medulla oblongata slices were pasted on the slide, dried and then sealed with anti-fluorescence quenching sealing solution. Images were taken by confocal laser scanning microscope, and the fluorescence intensity of different proteins was quantified by Image J software.

2.9 Statistical analysis

The grouping of animals was not known to the experimenter conducting the behavioral tests. All data are expressed as mean ± standard error of the mean (SEM) unless otherwise stated. Each set of data was first tested for normal distribution, and data conforming to a Gaussian distribution were used for parametric analysis. Student's t-test (paired or unpaired) was used for comparisons between two groups and one-way ANOVA was used to analyze differences between more than two groups, followed by Tukey post hoc comparisons. When two independent variables were considered, a two-way ANOVA was used. The experiment was repeated at least three times for each group. All statistical analyses and data plotting were done by Graphpad Prism 9.0 (GraphPad Inc., San Diego, CA, USA). All tests were two-tailed, with statistical significance set at $P < 0.05$.

3. Results

3.1 Preoperative 24h PSD significantly aggravated incision-induced pain hypersensitivity

Basal pain thresholds in either group were unaffected by the preoperative 24-hour PSD ($P > 0.05$). After the plantar incision, paw withdrawal thresholds decreased and cumulative pain scores increased in rats and gradually returned to baseline values on day 9 ($P > 0.05$). Additionally, rats with preoperative 24h PSD showed lower paw withdrawal thresholds and higher cumulative pain levels at 1, 4, and 7 days post-paw incision compared to rats with paw incision alone ($P < 0.05$) (Fig. 1A, B).

3.2 Preoperative 24h PSD markedly elevated the expression of CB1R in the RVM region of control and incision rats

The levels of CB1R proteins in the RVM area of rats were measured in four groups: Control, PSD, Incision, and PSD and incision. Rats in groups PSD, Incision, and PSD and incision had significantly higher levels of CB1R proteins in the RVM area on postoperative day 1 compared to their levels before the incision ($P < 0.05$). These levels gradually returned to normal on day 9 in groups Incision, and PSD and incision ($P > 0.05$), as shown in Fig. 2A, B. Additionally, rats in the PSD and incision group had higher levels of CB1R protein in the RVM area on postoperative day 1 compared to rats in the Incision group ($P < 0.05$) (Fig. 2A, B). Compared with the rats before incision, the expression levels of CB1R mRNA in RVM area of group Incision and group PSD and incision were significantly increased on the 1st and 4th day after surgery, and gradually recovered to normal in group Incision on the 7th day after surgery, and gradually recovered in group PSD and incision on the 9th day after surgery (Fig. 2C). Compared with group Incision, the expression levels of CB1R mRNA in RVM area of PSD and incision group were significantly increased at 1, 4 and 7 days after surgery ($P < 0.05$) (Fig. 2C).

3.3 CB1R agonists in the RVM area reduce pain hypersensitivity, while CB1R antagonists worsen it

In the P-I group, a CB1R agonist injection was administered into the RVM area of the brain one day after surgery. This led to a decrease in cumulative pain scores and an increase in paw withdrawal thresholds observed at 1, 4, and 7 days after surgery when compared to the P-I group ($P < 0.05$) (Fig. 3A, B). these measures had gradually returned by day 9. Conversely, an injection of a CB1R antagonist into the RVM region of the P-I group resulted in increased cumulative pain scores and decreased paw withdrawal thresholds at 1, 4, and 7 days when compared to the control group ($P < 0.05$) (Fig. 3A, B). By day 9, these measures had gradually returned.

: paradoxical sleep deprivation and incision + CB1R agonist, P-I + SR: paradoxical sleep deprivation and incision + CB1R antagonist.

3.4 In the RVM, CB1R agonists upregulate the expression of CB1R, whereas CB1R antagonists downregulate the expression of these receptors

Our results showed that, when CB1R agonists were injected into the RVM area of rat brains, there was an increase in CB1R mRNA content and protein expression (Fig. 4B, C, E). Conversely, when CB1R inhibitors were injected, both CB1R mRNA content and protein expression decreased (Fig. 4B, C, E). According to our immunofluorescence findings, CB1R agonist therapy markedly increased CB1R expression in the RVM area (Fig. 4D, E). In contrast, CB1R expression was markedly reduced by CB1R inhibitors in the medullary RVM area (Fig. 4D, E).

4. Discussion

According to the findings of the current study, a 24-hour preoperative sleep deprivation (PSD) considerably increased the responsiveness of incisional pain and lengthened the time it took for pain to subside. Furthermore, the rostral ventromedial medulla (RVM) of both normal and plantarly incised rats showed elevated CB1R protein expression two days following a 24-hour sleep deprivation. Injecting a CB1R agonist into the RVM on the first postoperative day could considerably lessen the PSD-induced postoperative pain hypersensitivity, whereas injecting a CB1R inhibitor could make it worse. Taken together, these findings suggest that CB1R mediated synaptic plasticity changes in the RVM region are involved in PSD induced postoperative pain sensitization in incision rats. Activating CB1R in the RVM region can help alleviate postoperative incision pain sensitization induced by PSD.

About 30% of the world's population suffers from sleep deprivation, and sleep deprivation has become a major threat to human health[19]. Disturbance of any stage of sleep can lead to increased sensitivity to pain, and different sleep stages produce different degrees of increased sensitivity to pain[20]. Another study showed that perioperative continuous REM sleep deprivation of 6 hours/day for 3 days extended the duration of postoperative pain[21], while restored sleep can produce analgesic effects[22]. In this

study, we developed an animal model of preoperative 24-hour PSD, and our results demonstrated that PSD enhanced postoperative incisional pain hypersensitivity. These results agree with earlier data from the literature.

CB1R located in the presynaptic membrane is closely associated with functions such as stress, nociception, memory and emotion regulation[23–26]. CB1R can alter synaptic plasticity and regulate the excitation-inhibition ratio, which in turn inhibits or excites central sensitization and exerts analgesic or sensitizing effects[14]. Reverse signaling is the main mechanism by which CB1R mediates synaptic plasticity[24]. Recent studies have shown that sleep deprivation increases CB1R expression in lateral hypothalamic structures[27], and that CB1R agonists reduce abdominal pain by suppressing visceral sensation[28].

In models of pain such as neuropathic pain, inflammatory pain, visceral pain, and other acute and chronic pain, CB1R agonists have demonstrated the ability to relieve pain-like behavior in rats, while CB1R inhibitors have the potential to enhance pain sensitivity in rats[24, 28–30].We have shown that microinjections of CB1R agonists into the RVM suppress PSD-induced pain sensitization at 1, 4, or 7 days after incision. Conversely, microinjections of CB1R inhibitors into the RVM exacerbate PSD-induced pain sensitization.

In conclusion, this study suggests that PSD 24 hours before plantar incision caused increased postoperative pain hypersensitivity and increased presynaptic CB1R expression. Interfering with the CB1R is expected to offer a unique approach to the treatment of postoperative pain in an effort to prevent the transition from acute to chronic post-operative pain.

Declarations

Funding

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Data availability

Due to sensitivity reasons, the data supporting the results of this study cannot be publicly provided and can be obtained from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This research protocol was reviewed and approved by the ethical review of Animal Experiments at Gansu University of Chinese Medicine

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Source of funding

This study was supported by the Natural Science Foundation of Hubei Province (2025AFC063) and Science and Technology Project of Gansu Province - Construction of Clinical Medical Research Center (20JR10RA435).

Conflict of interest and Compliance with ethical standards

The authors declare that they have no conflict of interest.

Author Contribution

ZT and LWJ participated in the conception of the study, study design, data abstraction, data analysis, and manuscript preparation. ZSB, XJJ and WP participated in the data abstraction, data analysis, and manuscript preparation. HL and PMY participated in data analysis, and manuscript preparation. All authors read and approved the final manuscript.

Data Availability

Data is provided within the manuscript or supplementary information files

References

1. Chou R, Gordon DB, de Leon-Casasola OA, et al. Management of Postoperative Pain: A Clinical Practice Guideline From the American Pain Society, the American Society of Regional Anesthesia and Pain Medicine, and the American Society of Anesthesiologists' Committee on Regional Anesthesia, Executive Committee, and Administrative Council[J]. J Pain. 2016;17(2):131–57.
2. Vasilopoulos T, Wardhan R, Rashidi P, et al. Patient and Procedural Determinants of Postoperative Pain Trajectories[J]. Anesthesiology. 2021;134(3):421–34.
3. Macrae WA. Chronic post-surgical pain: 10 years on[J]. Br J Anaesth. 2008;101(1):77–86.
4. Ishida Y, Okada T, Kobayashi T, et al. Pain Management of Acute and Chronic Postoperative Pain[J]. Cureus. 2022;14(4):e23999.
5. Small C, Laycock H. Acute postoperative pain management[J]. Br J Surg. 2020;107(2):e70–80.

6. Krause AJ, Prather AA, Wager TD, et al. The Pain of Sleep Loss: A Brain Characterization in Humans[J]. J Neurosci. 2019;39(12):2291–300.
7. Peng B, Jiao Y, Zhang Y, et al. Bulbospinal nociceptive ON and OFF cells related neural circuits and transmitters[J]. Front Pharmacol. 2023;14:1159753.
8. Mills EP, Di Pietro F, Alshelh Z, et al. Brainstem Pain-Control Circuitry Connectivity in Chronic Neuropathic Pain[J]. J Neurosci. 2018;38(2):465–73.
9. Salas R, Ramirez K, Tortorici V, et al. Functional relationship between brainstem putative pain-facilitating neurons and spinal nociceptfive neurons during development of inflammation in rats[J]. Brain Res. 2018;1686:55–64.
10. Wan J, Ding Y, Tahir AH, et al. Electroacupuncture Attenuates Visceral Hypersensitivity by Inhibiting JAK2/STAT3 Signaling Pathway in the Descending Pain Modulation System[J]. Front Neurosci. 2017;11:644.
11. Tomim DH, Pontarolla FM, Bertolini JF, et al. The Pronociceptive Effect of Paradoxical Sleep Deprivation in Rats: Evidence for a Role of Descending Pain Modulation Mechanisms[J]. Mol Neurobiol. 2016;53(3):1706–17.
12. Milligan AL, Szabo-Pardi TA, Burton MD. Cannabinoid Receptor Type 1 and Its Role as an Analgesic: An Opioid Alternative?[J]. J Dual Diagn. 2020;16(1):106–19.
13. Starowicz K, Finn DP. Cannabinoids and Pain: Sites and Mechanisms of Action[J]. Adv Pharmacol. 2017;80:437–75.
14. Hempel B, Xi ZX. Receptor mechanisms underlying the CNS effects of cannabinoids: CB(1) receptor and beyond[J]. Adv Pharmacol. 2022;93:275–333.
15. Sideris A, Lauzadis J, Kaczocha M. The Basic Science of Cannabinoids[J]. Anesth Analg. 2024;138(1):42–53.
16. Rezende B, Alencar AKN, de Bem GF, et al. Endocannabinoid System: Chemical Characteristics and Biological Activity[J]. Pharmaceuticals (Basel). 2023;16(2):148.
17. Farah SI, Hilston S, Tran N, et al. 1-, 2- and 3-AG as substrates of the endocannabinoid enzymes and endogenous ligands of the cannabinoid receptor 1[J]. Biochem Biophys Res Commun. 2022;591:31–6.
18. Zahn PK, Pogatzki EM, Brennan TJ. Mechanisms for pain caused by incisions[J]. Reg Anesth Pain Med. 2002;27(5):514–6.
19. Liew SC, Aung T. Sleep deprivation and its association with diseases- a review[J]. Sleep Med. 2021;77:192–204.
20. Alexandre C, Latremoliere A, Ferreira A, et al. Decreased alertness due to sleep loss increases pain sensitivity in mice[J]. Nat Med. 2017;23(6):768–74.
21. Li L, Zhang H, Zheng Z, et al. Perioperative sleep deprivation activates the paraventricular thalamic nucleus resulting in persistent postoperative incisional pain in mice[J]. Front Neuroanat. 2022;16:1074310.

22. Stroemel-Scheder C, Kundermann B, Lautenbacher S. The effects of recovery sleep on pain perception: A systematic review[J]. Neurosci Biobehav Rev. 2020;113:408–25.
23. Llorente-Berzal A, Terzian AL, di Marzo V, et al. 2-AG promotes the expression of conditioned fear via cannabinoid receptor type 1 on GABAergic neurons[J]. Psychopharmacology. 2015;232(15):2811–25.
24. Zeng X, Mai J, Xie H, et al. Activation of CB1R alleviates central sensitization by regulating HCN2-pNR2B signaling in a chronic migraine rat model[J]. J Headache Pain. 2023;24(1):44.
25. Pourrahimi AM, Abbasnejad M, Raoof M, et al. The involvement of orexin 1 and cannabinoid 1 receptors within the ventrolateral periaqueductal gray matter in the modulation of migraine-induced anxiety and social behavior deficits of rats[J]. Peptides. 2021;146:170651.
26. Li M, Lv X, Li T, et al. Basolateral Amygdala Cannabinoid CB1 Receptor Controls Formation and Elimination of Social Fear Memory[J]. ACS Chem Neurosci. 2023;14(19):3674–85.
27. Belali R, Mard SA, Khoshnam SE, et al. Anandamide improves food intake and orexinergic neuronal activity in the chronic sleep deprivation induction model in rats by modulating the expression of the CB1 receptor in the lateral hypothalamus[J]. Neuropeptides. 2023;101:102336.
28. Yu Y, Tsang QK, Jaramillo-Polanco J, et al. Cannabinoid 1 and mu-Opioid Receptor Agonists Synergistically Inhibit Abdominal Pain and Lack Side Effects in Mice[J]. J Neurosci. 2022;42(33):6313–24.
29. Tran H, Feng Y, Chao D, et al. Descending mechanism by which medial prefrontal cortex endocannabinoid signaling controls the development of neuropathic pain and neuronal activity of dorsal root ganglion[J]. Pain. 2024;165(1):102–14.
30. Ford NC, Barpujari A, He SQ, et al. Role of primary sensory neurone cannabinoid type-1 receptors in pain and the analgesic effects of the peripherally acting agonist CB-13 in mice[J]. Br J Anaesth. 2022;128(1):159–73.

Figures

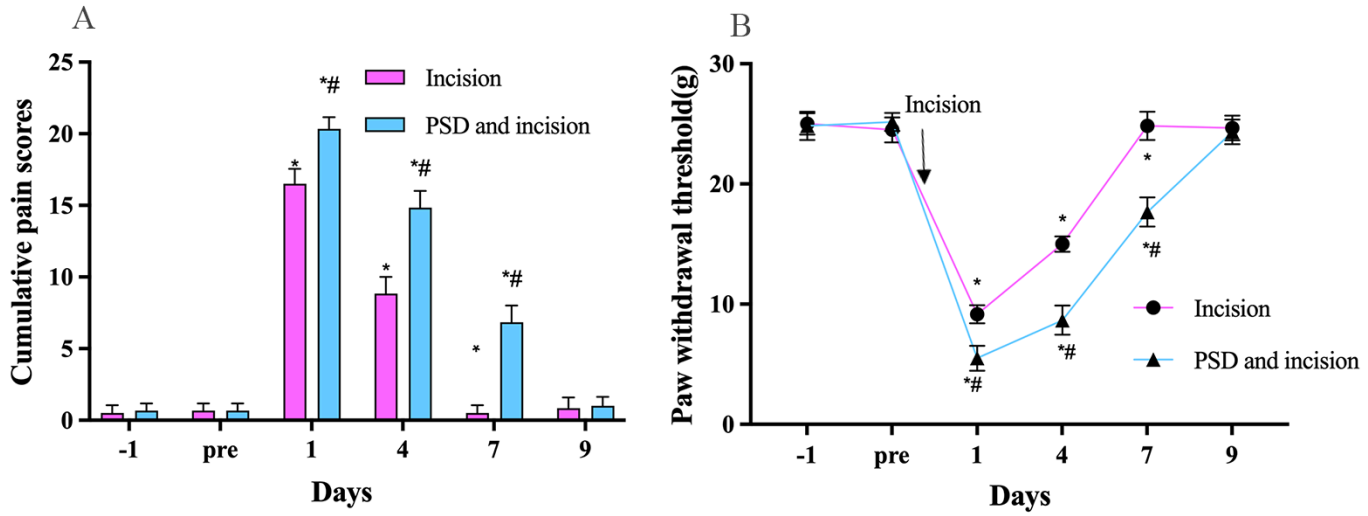

Figure 1

(A) Cumulative pain scores before surgery and at 1, 4, 7 and 9 days after surgery; (B) paw withdrawal thresholds before surgery and at 1, 4, 7, and 9 days after surgery. *P < 0.05, compared with baseline values; #P < 0.05, compared with incision only, P < 0.05; n = 6 rats per group. Bonferroni's test of two-factor ANOVA was used for statistical analysis. PSD: paradoxical sleep deprivation; CPS: Cumulative pain score; PWT: paw withdrawal thresholds; pre: Before plantar incision; Days: Days before and after.

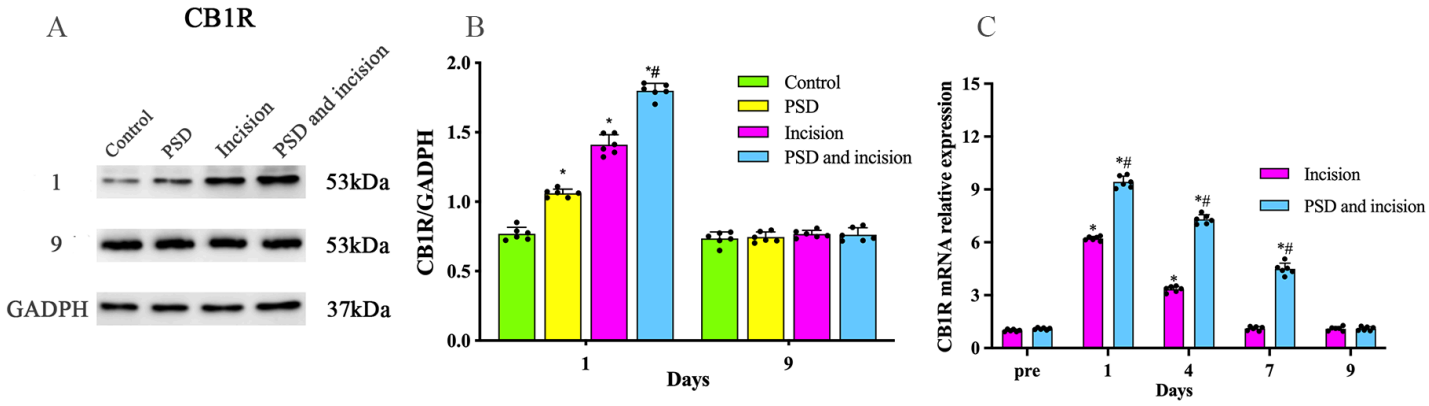

Figure 2

(A) Band map of CB1R protein expression in the RVM on day 1 and 9 after surgery; (B) The expression level of CB1R protein in the RVM on the 1st and 9th day after surgery. (C) Relative expression levels of CB1R and BDNF mRNA in the RVM of rats in each group. *P < 0.05, compared with baseline values; #P <

0.05, compared with control group; n = 6 rats per group. PSD: paradoxical sleep deprivation; Days: Days before and after.

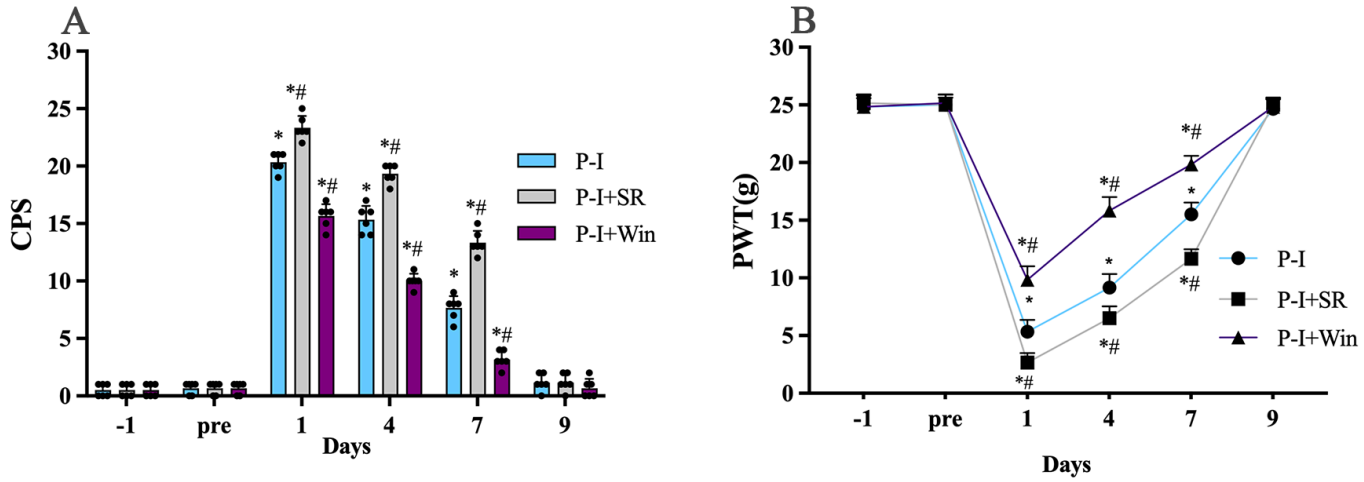

Figure 3

(A) Cumulative pain scores before surgery and at 1, 4, 7 and 9 days after surgery; (B) paw withdrawal thresholds before surgery and at 1, 4, 7, and 9 days after surgery. *P: Compared with the preoperative baseline value, $P < 0.05$; #P: Compared with only plantar incision group, $P < 0.05$; n = 6 rats per group. Bonferroni's test of two-factor ANOVA was used for statistical analysis. PSD: paradoxical sleep deprivation; CPS: Cumulative pain score; PWT: paw withdrawal thresholds; pre: Before plantar incision; Days: Days before and after; P-I+Win

: paradoxical sleep deprivation and incision + CB1R agonist, P-I+SR: paradoxical sleep deprivation and incision + CB1R antagonist.

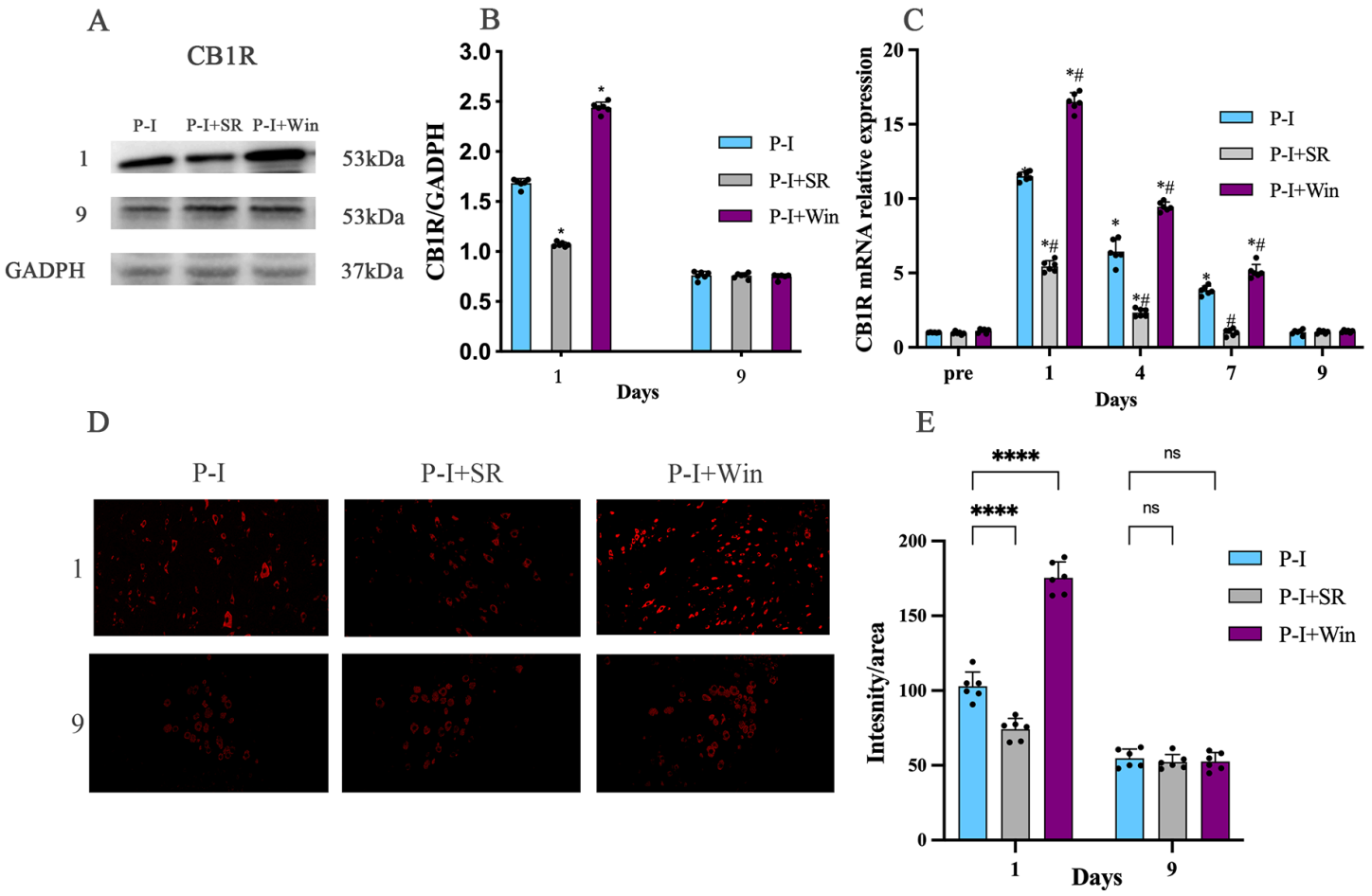

Figure 4

(A) Band map of CB1R protein expression in the RVM on day 1 and 9 after surgery; (B) The expression level of CB1R protein in the RVM on the 1st and 9th day after surgery. (C) Relative expression levels of CB1R mRNA in the RVM of rats in each group. (D) Immunofluorescence staining assay of CB1R in the RVM on the 1st and 9th day after surgery. (E) CB1R immunoreactivity in the RVM on the 1st and 9th day after surgery. *$P < 0.05$, compared with baseline values; $^{\#}P < 0.05$, compared with control group; **** $p < 0.0001$. n = 6 rats per group. Days: Days before and after. P-I: paradoxical sleep deprivation and incision, P-I-W: paradoxical sleep deprivation and incision + CB1R agonist, P-I-S: paradoxical sleep deprivation and incision + CB1R inhibitor

Supplementary Files

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- Figure2the1stdayaftersurgery.tiff
- Figure2the9thdayaftersurgery.tif
- Figure4the1stdayaftersurgery.tif

- Figure4the9thdayaftersurgery.tif