IBRO Neuroscience Reports 17 (2024) 389–397

Contents lists available at ScienceDirect

IBRO Neuroscience Reports

journal homepage: www.sciencedirect.com/journal/IBRO-Neuroscience-Reports

# Electroacupuncture alleviates paradoxical sleep deprivation-induced postoperative hyperalgesia via a7nAChR mediated BDNF/TrkB-KCC2 signaling pathway in the spinal cord

Yi-yang Cui, (MMed) [a,1], Zi-qing Xu, (MMed) [b,c,1], Xiao-yu Qin, (MMed) [d], Huai-jing Hou, (MMed) [b,c], Jie Zhang, (MMed) [b,c], Jian-jun Xue, (MD) [b,c,e,*]

[a] Department of Anesthesiology, Zhongshan Hospital of Fudan University, China
[b] Department of Anesthesiology, Gansu Province Hospital of Traditional Chinese Medicine, Lanzhou 730050, China
[c] Gansu Clinical Research Center of Integrative Anesthesiology, Lanzhou 730050, China
[d] Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China
[e] Evidence-based Medicine Center, School of Basic Medical Science, Lanzhou University, Lanzhou, Gansu 730000, China

ARTICLE INFO

*Keywords:*
Paradoxical sleep deprivation
Hyperalgesia
Electroacupuncture
α7nAChR
BDNF/TrkB-KCC2 signaling pathway

ABSTRACT

Perioperative Paradoxical sleep deprivation (PSD) is associated with postoperative hyperalgesia. However, the clinical therapeutic strategies for PSD-induced postoperative hyperalgesia are limited. Electroacupuncture (EA) has been used for attenuating many types of pain, including neuropathic pain and inflammatory pain, but its effect on PSD-induced postoperative hyperalgesia is still unclear, and its analgesia mechanism should be further explored. In this study, we designed to investigate the possible mechanism of PSD-induced postoperative hyperalgesia and the effect of EA on PSD-induced postoperative hyperalgesia, and whether the mechanism is related to the BDNF/TrkB signaling pathway mediated by α7nAChR in the spinal cord. The paw withdrawal thermal latency (PWTL) and paw withdrawal mechanical threshold (PWMT) of rats were used to detect PSD-induced hyperalgesia. The expression of α7nAChR, BDNF, TrkB and KCC2 in the spinal cord were evaluated by Western blot and immunofluorescence. The results showed that preoperative 24 h PSD significantly decreased the PWTL and PWMT. The expression of α7nAChR and KCC2 significantly downregulated in the spinal cord of PSD-induced postoperative hyperalgesia rats, the opposite was observed for BDNF and TrkB expression. Moreover, intrathecal injection of alpha-bungarotoxin (α-BGT), a selective antagonist for α7nAChR, not only aggravated the pain hypersensitivity, but also demonstrated a further decrease of α7nAChR and KCC2 expression and a further increase of BDNF and TrkB expression. EA stimulation increased the PWTL and PWMT values of PSD-induced postoperative hyperalgesia rats, significantly upregulated α7nAChR and KCC2 expression, and significantly downregulated BDNF and TrkB expression. Moreover, intrathecal injection of α-BGT suppressed the analgesic effect of EA, inhibited the enhancement of α7nAChR and KCC2 expression and the reduction of BDNF and TrkB expression induced by EA. In conclusion, our study indicated that 24 h PSD can cause postoperative hyperalgesia, and the mechanism may be related to the disorder of α7nAChR mediated BDNF/TrkB-KCC2 signaling pathway. EA can alleviate postoperative hyperalgesia induced by PSD, which may be related to its effect in activating α7nAChR, inhibiting the expression of BDNF/TrkB, and up-regulating the expression of KCC2 in the spinal cord.

## 1. Introduction

Adequate high-quality sleep is essential for rapid postoperative recovery of patients. Perioperative sleep deprivation, especially paradoxical sleep deprivation (PSD), is one of the main risk factors for chronic post-surgical pain (Guo et al., 2022). Studies have shown that sleep deprivation can causes hyperalgesia (Kourbanova et al., 2022). Hyperalgesia can not only cause agitation and hyperstress in patients,

* Correspondence to: Department of Anesthesiology, Gansu Province Hospital of Traditional Chinese Medicine, Lanzhou, China.
*E-mail addresses:* 1527769732@qq.com (Y.-y. Cui), xuejjebm0419@163.com, xjjfei419@126.com (J.-j. Xue).
[1] Yi-yang Cui and Zi-qing Xu are co-first author.

https://doi.org/10.1016/j.ibneur.2024.10.002
Received 24 June 2024; Received in revised form 1 October 2024; Accepted 24 October 2024
Available online 24 October 2024

but also significantly reduce the analgesic effect of opioids, thereby increasing the use of opioids and leading to more serious complications (Maldonado et al., 2024). However, PSD, as one of the main factors inducing postoperative hyperalgesia, is often underestimated in postoperative pain. Therefore, it is necessary to explore the mechanism of PSD-induced postoperative hyperalgesia.

Our previous study has shown that postoperative hyperalgesia induced by PSD is closely related to brain-derived neurotrophic factor (BDNF), and PSD aggravates and prolongs the incision through descending facilitation mediated by BDNF signaling in rats (Xue et al., 2018). Many studies have found that the activation of α7 nicotinic acetylcholine receptor (α7nAChR) in the spinal cord can inhibit central sensitization by regulating the descending signaling pathway (BDNF/TrkB/KCC2) (Gu et al., 2017; Jia et al., 2023). Therefore, activation of α7nAChR in the spinal cord to inhibit the expression of descending pathways such as BDNF may be a key target for the prevention and treatment of postoperative hyperalgesia induced by PDS.

Electroacupuncture (EA) is a combination of traditional acupuncture and electrical stimulation, which provides stronger and more sustained stimulation intensity and amount to acupoints to effectively relieve pain (Liang et al., 2023). Many studies have shown that EA has been effective in treating cancer pain, neuropathic pain and incisional pain, but the analgesic effect on PSD-induced hyperalgesia is still unclear (Wang et al., 2023; Su et al., 2023; Wang et al., 2021; Zhang et al., 2012). Emerging evidence indicated that the pain-related BDNF signaling pathway is closely related to the analgesic effect of EA (Su et al., 2023; Xue et al., 2020). Additionally, 2 Hz EA treatment can significantly upregulated the expression of α7nAChR (Wang et al., 2018). These results suggest that nicotinic signaling probably mediate the modulation of pain in EA treatment. The aim of this study is to investigate the role of BDNF/TrkB signaling pathway mediated by α7nAChR in PSD-induced postoperative hyperalgesia in the spinal cord in rats. And to explore the therapeutic effect of EA on PSD-induced postoperative hyperalgesia and its underlying mechanism.

## 2. Materials and methods

### 2.1. Animals

Sixty SPF male Sprague-Dawley (SD) rats, aged 7–8 weeks, weighing 180 ± 20 g, were provided and fed by the Animal Experimental Research Center of Gansu University of Traditional Chinese Medicine. The ambient temperature was (23 ± 2) ℃, relative humidity was 50 %-60 %, and 12 h/12 h light/dark was cycled. Behavioral tests were performed between 8:30 am and 4:30 PM. All experimental procedures have been reviewed by the Animal Experiment Ethics Committee of Gansu University of Traditional Chinese Medicine. This experiment was performed in strict accordance with the International Association for the Study of Pain (IASP) guidelines for animal experiments.

### 2.2. Grouping and interventions

The rats were randomly divided into 6 groups, including the normal group (N group), incision group (C group), PSD-incision group (P group), PSD-incision+α-BGT (Pα group), EA+ PSD-incision (E group), and EA+ PSD-incision+α-BGT (Eα group), 10 rats in each group. The detailed experimental design was shown in Table 1. The schematic of experimental procedures was shown in Fig. 1

### 2.3. PSD procedure

The Modified multi-platform method (MMPM) was used to establish the PSD model (Xue et al., 2018; Xie et al., 2015), and the water tank with the size of 85×50×10 mm was used for PSD. A small platform with a diameter of 5 cm and a height of 8 cm was placed inside the tank, making a total of 5 small platforms. The platform spacing was 10 cm to ensure that rats could not fall asleep leaning against both platforms or the edge of the tank. The rats were placed on the platform, and the water level of the tank was 2 cm below the height of the platform (Supplementary Materials Fig.S1). The water temperature was maintained at 18–22℃. When the rat enters paradoxical sleep, the skeletal muscles relax and possibly the nose contacts the water or falls into the water, and the rat wakes up immediately from paradoxical sleep. The 24 h PSD was performed from 8:00 am on the first day to 8:00 am on the second day.

**Table 1**
Experimental design.

| Group | Intervention |
|---|---|
| N group | No intervention was performed |
| C group | The right plantar incision was performed |
| P group | A right plantar incision was performed after 24 h PSD |
| Pα group | On the basis of P group, intrathecal injection with α-BGT was performed before the plantar incision |
| E group | 30 min EA was performed after 24 h PSD before the right plantar incision surgery. |
| Eα group | On the basis of E group, intrathecal injection with α-BGT was performed before the plantar incision |

### 2.4. Plantar incision

The rat model of postoperative pain was developed as previously described (Brennan et al., 1996). The rats were anesthetized with sodium pentobarbital (35 mg/kg), the limbs were fixed in the supine position, and the right hind paw was sterilized with iodophor. The 11# blade was used to make a longitudinal incision of about 1 cm in length from 0.5 cm to the toe of the right heel. After light pressure to stop bleeding, the incision was sutured using Fs-2 needles with 5–0 silk thread (Supplementary Materials Fig.S2). The incision was covered with erythromycin ointment to prevent infection.

### 2.5. EA stimulation

"Baihui" (GV 20) and bilateral "Zusanli" (ST 36) were selected, and the acupoints were selected according to the "Atlas of Positioning of Commonly Used Acupoints in Laboratory Animals Part 2: Rats" formulated by the Experimental Acupuncture Branch of Chinese Acupuncture and Moxibustion Society. The acupoint of "Baihui" (GV 20) is located in the middle of the parietal bone. The acupoint of "Zusanli" (ST 36) was located at the medial forelimb of rats, about 5 mm away from the anterior tubercle of tibia. The rats were fixed on the board in a prone position, and a small amount of anesthetic drugs were given to avoid the shedding of acupuncture needles due to the non-cooperation of rats during EA stimulation. A disposable sterile acupuncture needle of 0.3×25 mm size was used for backward twirling and oblique needling at "Baihui" for 2 mm and straight needling at "Zusanli" for 5 mm. Then the EA instrument was connected, and the wave shape was set as disperse-dense wave, the frequency was 2/100 Hz (Silva et al., 2011), and the current intensity was 2 mA, and the electricity was switched on for 30 min (Supplementary Materials Fig.S3).

### 2.6. PWTL

The PWTL was measured using a plantar radiant thermal instrument. The rats were placed in a transparent plexiglass box (size: 12×22×18 cm, plate thickness: 2 mm) 30 min before measurement to allow them to acclimate to the environment and temperature (Supplementary Materials Fig.S4). During the formal measurement, when the rats were in a quiet state, the skin in the central area of the hind foot of the rats was irradiated with the radiation light source of the hot stabbing instrument. The hindfoot response of the rats was observed. In case of rapid paw withdrawal, licking, or shaking, the stimulus light

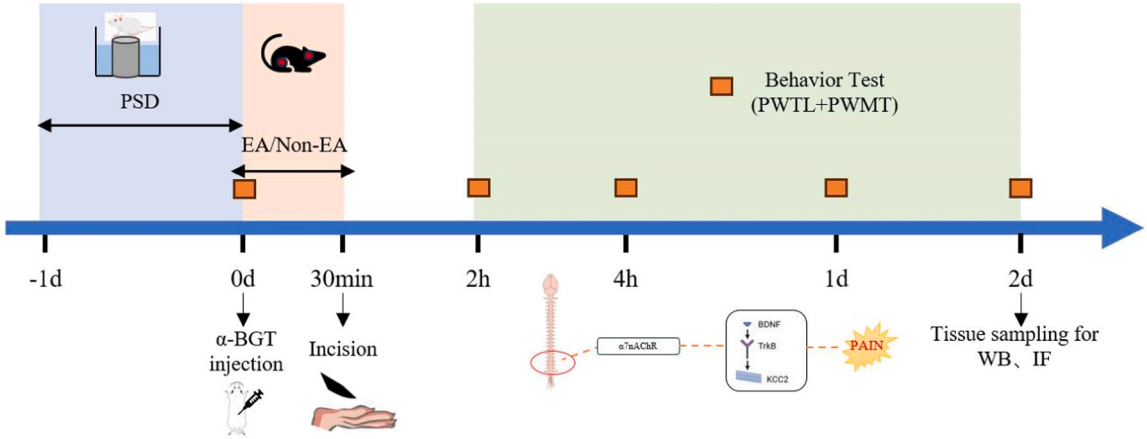


**Fig. 1.** Schematic diagram of experimental protocols.

source of the instrument was manually cut off, and the corresponding PWTL was recorded. The temperature of the stabbing instrument was set to 55℃ during the whole test process, and the parameters were kept consistent. The automatic cut-off time of the radiation light source of the heat stabbing instrument was set to 30 s to avoid causing foot tissue damage during the test. Five repeated measurements were performed for each rat, and the interval between each stimulus was not less than 5 min. The average value of the last three PWTL was calculated as the final thermal pain threshold of rats.

### 2.7. PWMT

PWMT was measured using a set of Von Frey filaments. Rats were placed in a transparent plexiglass box (size: 20×20×25 cm, with a wire mesh at the bottom of the box) 30 min before measurement to allow them to acclimate to the environment and temperature (Supplementary Materials Fig.S5). During the formal measurement, the mid-plantar region of the hindfoot of the rats was slowly and gently stimulated with Von Frey filament (avoiding the incision of the hindfoot) in a quiet state. The filament was bent for a few seconds, and the paw withdrawal reaction was observed. If the rats were observed to have rapid paw withdrawal, licking or flicking, it was marked as a positive response. If there were 3 positive responses in 5 consecutive stimuli (note that the interval between each stimulus was not less than 5 min), it was marked as X, otherwise it was marked as O. The pressure value marked as X was recorded, and then the threshold was calculated according to the up-down method.

### 2.8. Intrathecal injection

The rats were anesthetized with sodium pentobarbital (35 mg/kg), their surgical sites were skin-prepared, and the rats were fixed to the animal operating table in the prone position. The L5–6 spinous process space was marked according to the position of the rat hip joint and sterilized with iodophor. A small longitudinal incision was made at the positioning position with a scalpel, and the skin was open for fixation to facilitate the puncture needle. The injection was performed with a glass micro-syringe. When there was a feeling of frustration and a rapid tail flick reaction, the needle accurately reached the subarachnoid space. The micro-syringe was pushed slowly while the drug was injected, and the injection time was controlled at about 20–25 seconds. After the injection, the needle stayed in place for about 1 min, and then slowly pulled out to avoid the drug from being pulled out with the syringe. The injection volume of α-BGT (MedChemExpress, USA) was 1 μg/kg.

### 2.9. Western Blot assay

Rats were deeply anesthetized with sodium pentobarbital (35 mg/kg), and L4-L6 lumbar enlargement segments were removed rapidly and stored in liquid nitrogen. Tissue samples were homogenized in lysis buffer. The homogenate was centrifuged at 12,000 r/min for 10 min at 4℃, and supernatant was removed. The protein concentration was determined by the BCA Protein Assay Kit, following the manufacturer's instructions. Proteins (70 μg) were separated on SDS-PAGE (6 %–12 %) and transferred onto a polyvinyl difluoride (PVDF) membrane. The filter membranes were blocked with 5 % nonfat milk for 1 h at room temperature and incubated with the primary antibody α7nAChR (1:1000, Genetex, USA), anti-BDNF (1:1000, Genetex, USA), anti-trkB (1:1000, Genetex, USA) and anti-KCC (1:1000, Genetex, USA) for overnight at 4℃. The membrane was washed with tris-buffered saline + tween 20 (TBST) buffer and incubated for 1 h with the secondary antibody conjugated with horseradish peroxidase (1:1000, Genetex, USA) for 1 h at room temperature. Next, the immune complexes were detected using the ECL system. GAPDH was used as an internal control for total protein.

### 2.10. Immunofluorescence staining

L4-L6 lumbar enlargement segments were immediately removed and putted into 4 % paraformaldehyde solution for overnight, and then followed by cryoprotection in 30 % sucrose, at 4 °C until it submerged. Transverse sections (20 μm) were cut in a − 20 °C cryostat and washed with PBS 30 min, and then blocked with 3 % BSA for 60 min at room temperature. After washed with PBS for 30 min and incubated with 1 % BSA for overnight at 4 °C, the sections were incubated in the primary antibody solution. After washed with PBS for 30 min, the sections were incubated with the corresponding secondary antibody for 60 min at room temperature. All stained sections were detected and analyzed with a confocal laser scanning fluorescence microscope and panoramic tissue cell quantitative analysis system.

### 2.11. Statistical analysis

SPSS 22.0 software (IBM Corporation, Armonk City, USA) was used for statistical analysis and GraphPad Prism 9.0 software was used for mapping. If the data conformed to normal distribution, they were expressed as Mean ± standard deviation (SD). When the homogeneity of variance test did not show significance (α=0.05), one-way or two-way ANOVA and Tukey's post hoc test were used to compare the differences between more than two groups. Otherwise, Dunnett's T3 was used for between-group analysis. If the data did not meet the normal distribution, they were expressed as Median and Inter-quartile (IQR), and the comparison between groups was performed by the multiple sample rank

sum test. A $P$ value < 0.05 was considered statistically significant.

## 3. Results

### 3.1. 24 h PSD aggravates postsurgical incision-induced pain

In the present study, a continuous 24 h PSD was performed prior to the unilateral hind paw plantar incision, to verify the impact of pre-treatment with short-term PSD on postsurgical hyperalgesia. Both PWTL and PWMT of rats in each group were measured before incision, 2 h, 4 h, 1d and 2d after incision. The results are shown in Table 2. Baseline measurements were comparable in N group, C group, and P group ($P<0.05$). Compared with the C group, 24 h PSD aggravated the post-surgical mechanical and thermal hypersensitivities of rats in group P ($P<0.05$).

### 3.2. α7nAChR regulates PSD-induced postoperative hyperalgesia

To verify whether α7nAChR regulates PSD-induced postoperative hyperalgesia, rats with PSD-induced postoperative hyperalgesia were intrathecal injected with α-BGT. As shown in Tables 2 and 3, compared with the P group, Pα group had a worsening of both PWMT and PWTL decline at each time point after incision ($P<0.05$)."

### 3.3. EA significantly attenuated 24 h PSD-induced postoperative hyperalgesia

We examined the effects of EA on mechanical and thermal allodynia behaviors in 24 h PSD rats. As shown in Tables 2 and 3, compared with the P group, E group showed a significant increase in both PWMT and PWTL ($P<0.05$). These results suggested that EA relieved 24 h PSD-induced mechanical and thermal hyperalgesia.

### 3.4. α7nAChR is related to the effect of EA on PSD-induced postoperative hyperalgesia rats

To further explore whether α7nAChR is related to the effect of EA on PSD-induced postoperative hyperalgesia, an α7nAChR antagonist α-BGT was intrathecally injected in 24 h PSD rats. As shown in Tables 2 and 3, compared with the E group, Eα group showed a significant decrease in both PWMT and PWTL ($P<0.05$), indicating that α-BGT reversed the effect of EA.

**Table 2**
The time course of PWTL (s).

| Group | Baseline | 2 h | 4 h | 1d | 2d |
|---|---|---|---|---|---|
| N group | 13.84 ±1.43 | 14.23 ±0.63 | 13.13 ±1.00 | 12.66 ±1.60 | 13.73 ±1.85 |
| C group | 13.22 ±1.19 | 9.26 ±0.76[a] | 7.58 ±0.96[a] | 7.08 ±0.47[a] | 9.21 ±0.43[a] |
| P group | 14.15 ±0.44 | 5.59 ±0.90[b] | 5.24±0.3[b] | 4.78 ±0.54[b] | 6.53 ±0.80[b] |
| Pα group | 13.09 ±1.94 | 4.33±0.54 | 3.98 ±0.47[c] | 3.51 ±0.54[c] | 5.20±0.52 |
| E group | 13.35 ±1.03 | 8.34 ±0.76[d] | 8.36 ±0.38[d] | 8.74 ±0.44[d] | 9.49 ±0.90[d] |
| Eα group | 13.35 ±1.42 | 6.35 ±0.34[e,f] | 6.35 ±0.69[e,f] | 6.54 ±0.51[e,f] | 7.26 ±0.36[e,f] |

Data are expressed as mean± standard deviation (n=10 in each group).
[a] $P<0.05$, C group compared with N group;
[b] $P<0.05$, P group compared with C group;
[c] $P<0.05$, Pα group compared with P group;
[d] $P<0.05$, E group compared with P group;
[e] $P<0.05$, Eα group compared with E group
[f] $P<0.05$, Eα group compared with Pα group.

**Table 3**
The time course of PWMT (g).

| Group | Baseline | 2 h | 4 h | 1d | 2d |
|---|---|---|---|---|---|
| N group | 21.54 ±1.91 | 20.61 ±1.25 | 21.73 ±2.73 | 21.71 ±1.53 | 21.80 ±1.69 |
| C group | 22.90 ±0.75 | 9.58 ±1.36[a] | 8.77 ±1.27[a] | 9.24 ±0.78[a] | 8.74 ±1.00[a] |
| P group | 22.70 ±0.99 | 5.20 ±0.64[b] | 6.51 ±0.80[b] | 6.16 ±0.72[b] | 6.18 ±0.74[b] |
| Pα group | 22.69 ±0.67 | 4.33±0.54 | 4.24 ±0.70[c] | 3.10 ±0.78[c] | 4.30 ±0.63[c] |
| E group | 21.71 ±1.36 | 13.57 ±1.21[d] | 15.28 ±2.10[d] | 14.00 ±1.67[d] | 13.83 ±1.03[d] |
| Eα group | 23.65 ±1.03 | 7.15 ±0.67[e,f] | 6.59 ±0.86[e,f] | 7.32 ±0.91[e,f] | 7.67 ±1.04[e,f] |

Data are expressed as mean± standard deviation (n=10 in each group).
[a] $P<0.05$, C group compared with N group;
[b] $P<0.05$, P group compared with C group;
[c] $P<0.05$, Pα group compared with P group;
[d] $P<0.05$, E group compared with P group;
[e] $P<0.05$, Eα group compared with E group
[f] $P<0.05$, Eα group compared with Pα group.

### 3.5. 24 h PSD induced the downregulation of α7nAChR in the spinal cord of rats

To further confirm the effect of EA on PSD-induced hyperalgesia through α7nAChR signaling, Western blot and immunofluorescence were used to determine the protein levels of α7nAChR in the spinal cord of 24 h PSD rats. As shown in Figs. 2 and 3, compared with the C group, the protein level of α7nAChR in the spinal cord of 24 h PSD rats was significantly downregulated ($p<0.05$). Compared with the P group, intrathecal injection of α7nAChR antagonist α-BGT showed a further decline ($P<0.05$). These findings indicated that 24 h PSD down-regulated α7nAChR protein levels in the spinal cord.

### 3.6. EA induced the upregulation of α7nAChR in the spinal cord of 24 h PSD rats

To further confirm the effect of EA on the spinal α7nAChR in 24 h PSD rats, Western blot and immunofluorescence were used to determine the protein levels of α7nAChR in the spinal cord of PSD rats after EA stimulation. As shown in Figs. 2 and 3, compared with the P group, EA upregulated the level of α7nAChR in the spinal cord ($p<0.05$). Compared with the E group, intrathecal administration of α-BGT distinctly downregulated the protein expression of α7nAChR in the spinal cord of PSD rats with EA ($P<0.05$). The findings demonstrated that EA may exert an analgesic effect by upregulating the expression of α7nAChR in the spinal cord.

### 3.7. 24 h PSD induced the upregulation of BDNF and TrkB in the spinal cord of rats

To verify that α7nAChR mediates BDNF/TrkB/KCC2 signaling pathway is involved in PSD-induced postoperative hyperalgesia, we used Western blot to determine BDNF and TrkB protein levels. As shown in Figs. 4 and 5, compared with the C group, the expression of BDNF and TrkB in the spinal cord of PSD rats was significantly increased after 24 h PSD ($p<0.05$). Compared with the P group, intrathecal administration of α-BGT showed a further upregulation of the expression of BDNF and TrkB in the spinal cord ($P<0.05$). The findings demonstrated that α7nAChR mediates BDNF/TrkB/KCC2 signaling pathway is involved in PSD-induced postoperative hyperalgesia.

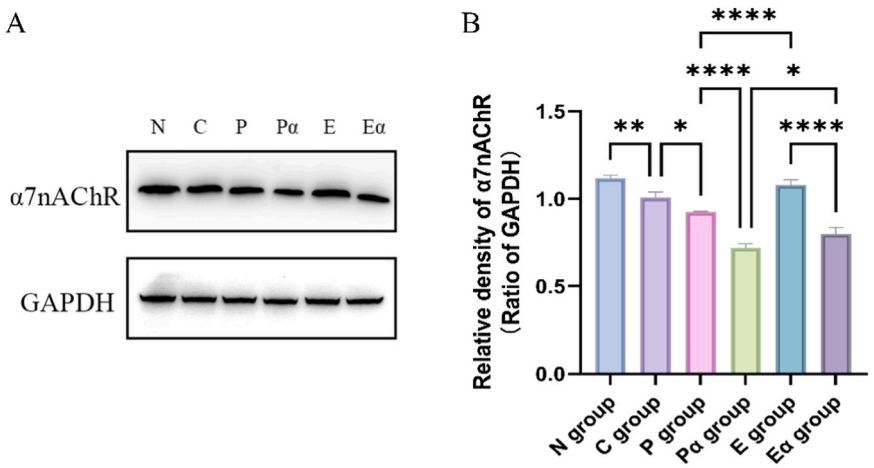


**Fig. 2.** The protein expression of α7nAChR (n=10 in each group). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

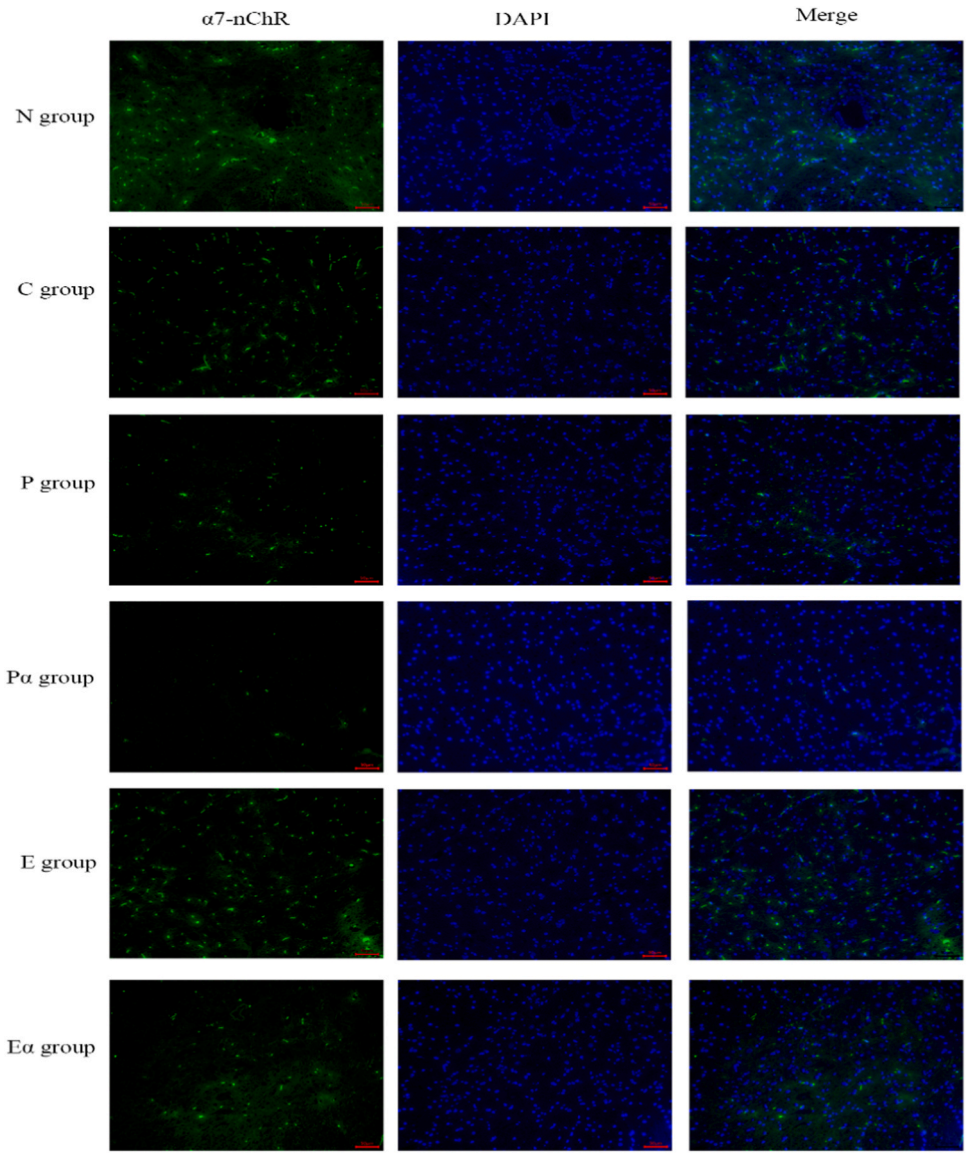


**Fig. 3.** Schematic diagram of the fluorescence of α7nAChR in the spinal cord (n=10 in each group). Scale bars are 50 μm.

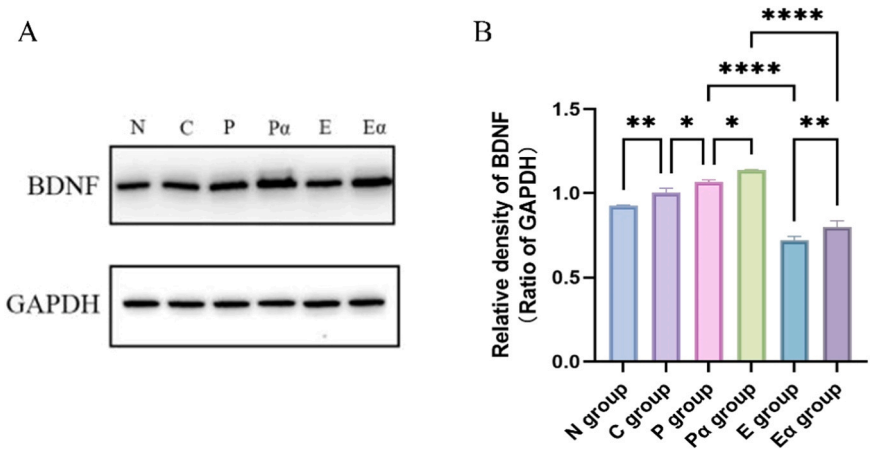


**Fig. 4.** The protein expression of BDNF (n=10 in each group). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

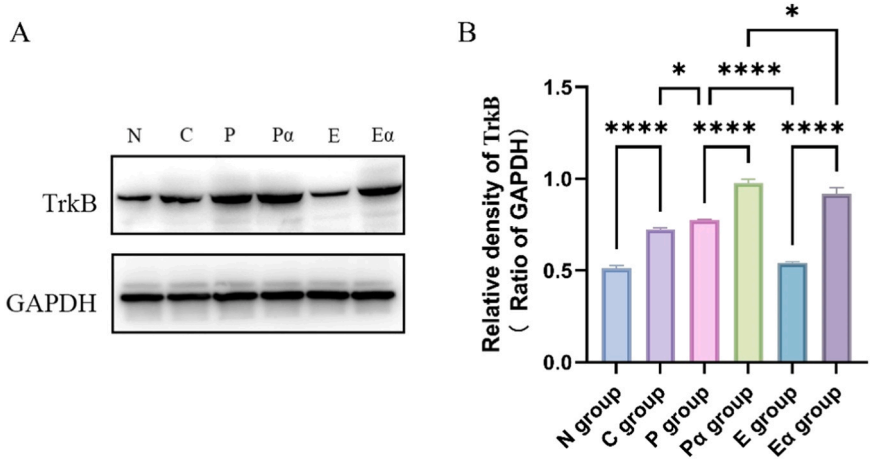


**Fig. 5.** The protein expression of TrkB (n=10 in each group). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

### *3.8. EA induced the downregulation of BDNF and TrkB in the spinal cord of 24 h PSD rats*

To further confirm the effect of EA on the spinal α7nAChR mediates BDNF/TrkB/KCC2 signaling pathway in 24 h PSD rats, Western blot was used to determine the protein levels of BDNF and TrkB in the spinal cord of PSD rats after EA stimulation. As shown in Figs. 2A and 3, compared with the P group, EA downregulated the level of BDNF and TrkB in the spinal cord ($p<0.05$), while intrathecal administration of α-BGT reversed those changes ($P<0.05$). The findings demonstrated that EA may exert an analgesic effect by downregulating the expression of BDNF and TrkB in the spinal cord

### *3.9. 24 h PSD induced the downregulation of KCC2 in the spinal cord of rats*

To verify that α7nAChR mediates BDNF/TrkB/KCC2 signaling pathway is involved in PSD-induced postoperative hyperalgesia, we used Western blot to determine KCC2 protein levels. As shown in Figs. 2A and 3, compared with the C group, the protein level of KCC2 in the spinal cord of 24 h PSD rats was significantly downregulated ($p<0.05$). Compared with the P group, intrathecal injection of α-BGT showed a further decline ($P<0.05$). Indicating that α7nAChR mediates BDNF/TrkB/KCC2 signaling pathway is involved in PSD-induced postoperative hyperalgesia.

### *3.10. EA induced the upregulation of KCC2 in the spinal cord of 24 h PSD rats*

To further confirm the effect of EA on the spinal α7nAChR mediates BDNF/TrkB/KCC2 signaling pathway in 24 h PSD rats, Western blot was used to determine the protein levels of KCC2 in the spinal cord of PSD rats after EA stimulation. As shown in Fig. 6, compared with the P group, EA upregulated the level of KCC2 in the spinal cord ($p<0.05$), while intrathecal administration of α-BGT reversed those changes ($P<0.05$). Indicating that EA may exert an analgesic effect by upregulating the expression of KCC2 in the spinal cord.

## 4. Discussion

Sleep deprivation (SD) refers to the lack of sleep or poor sleep quality caused by various reasons. Ample evidence suggests that sleep and pain are closely related (Finan et al., 2013; Moldofsky, 2001). Perioperative sleep deprivation is one of the main risk factors for postoperative hyperalgesia. Rapid eye movement (REM) sleep is also known as paradoxical sleep, and Hicks et al (Onen et al., 2000). were the first to demonstrate that sleep deprivation during this phase can induce hyperalgesia in healthy rats. Roehrs et al (Roehrs et al., 2006). found that less than 4 h of sleep or PSD significantly reduced the threshold of finger withdrawal response to thermal stimulation in patients, suggesting that partial sleep deprivation may induce hyperalgesia. Varallo et al (Varallo et al., 2022). confirmed through systematic review that perioperative SD can reduce the tolerance to pain in surgical patients, and

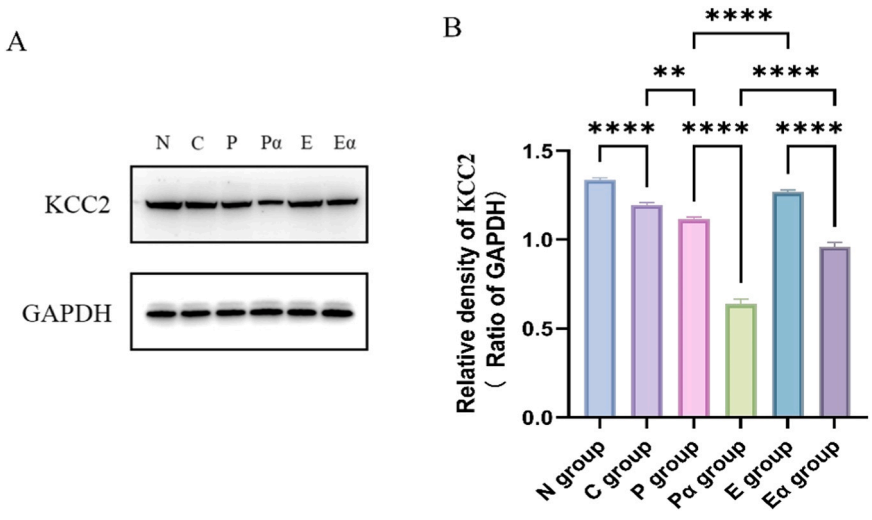


**Fig. 6.** The protein expression of KCC2 (n=10 in each group). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

lead to poor postoperative pain control, increased opioid use, and then develop into chronic postoperative pain. Chronic pain and sleep disorders have a bidirectional relationship. Pain leads to sleep disorders, and sleep disorders can also aggravate pain. Because the pathogenesis of postoperative hyperalgesia caused by SD is complex and not fully understood, the current clinical treatment options are mainly to improve sleep quality and relieve pain, but the existing treatment effects are not satisfactory, and may be accompanied by drug-related adverse reactions or addiction (Varallo et al., 2022). Therefore, exploring new effective and safe treatment methods and therapeutic targets is an urgent problem to be solved in the medical community.

In this study, rats were chosen to undergo PSD for 24 h, a duration that has been shown to increase the sensitivity of rats to mechanical, thermal, and noxious stimuli. The PSD model was established using MMPM, which is simple, feasible, controllable and applicable, and has a low mortality rate of animals (HQ et al., 2023). The behavioral results showed that compared with the rats without PSD, the rats with PSD 24 h showed significantly reduced tolerance to thermal and mechanical stimulation and prolonged paw withdrawal time from 2 hours after operation, and this pain sensitivity was most obvious from 4 hours to 1 day after operation. It gradually recovered 2 days after surgery and was more sensitive to mechanical stimulation than to thermal stimulation. These results demonstrated that PSD 24 h model can significantly affect rat pain threshold.

α7nAChR is one of the major subtypes of the nicotinic acetylcholine receptor family. It is widely distributed in the peripheral and central nervous system and can regulate a wide range of cellular functions. In recent years, α7nAChR have been shown to be involved in pain regulation (Abbas et al., 2019). Plenty of evidence has shown that α7nAChR expression is significantly downregulated in the sciatic nerve, dorsal root ganglion, and spinal dorsal horn in rodent models of pain (Zhou et al., 2022). A number of studies in rodents have confirmed that therapeutic strategies targeting α7nAChR, such as intrathecal or intraperitoneal injection of α7nAChR agonists or allosteric activators, can exert good analgesic effects. Zhang et al (Zhang et al., 2015). found that activation of α7nAChR can alleviate postoperative hyperalgesia response to remifentanil by inhibiting proinflammatory cytokines IL-6, TNF-α and p-NR2B.

Based on the above evidence, we hypothesized that spinal α7nAChR is involved in PSD-induced postoperative hyperalgesia. Immunofluorescence and Western Blot were used to observe the fluorescence and protein expression of α7nAChR in the spinal cord of rats PSD 24 h. The results showed that the average fluorescence intensity and protein expression level of α7nAChR in the spinal cord of rats with PSD at 24 hours were significantly lower than those of rats without PSD. In order to further explore the role of α7nAChR in spinal cord injury, we treated rats with α7nAChR by intrathecal injection of its specific inhibitor α-BGT. Immunofluorescence and Western Blot results showed that the average fluorescence intensity and protein expression level of α7nAChR in the spinal cord were further reduced. This indicates that intrathecal injection of the inhibitor was successful, and further validates the role of spinal α7nAChR in PSD-induced postoperative hyperalgesia.

Central sensitization plays a crucial role in the occurrence and development of hyperalgesia, which is manifested as enhanced neuronal reactivity, decreased pain threshold and amplification of pain sensitivity (Volcheck et al., 2023). BDNF is an important member of the central neurotrophic factor family and plays a crucial role in the central nervous system, especially in regulating pain transmission (Thakkar and Acevedo, 2023). When the body is subjected to noxious stimulation, the nociceptor neurons will release a large amount of BDNF, which initiates the sensitization of the central center (spinal dorsal horn) at the corresponding stage, causes the continuous excitation of the dorsal horn neurons, and further aggravates the occurrence and maintenance of pain sensitization (Zhou et al., 2021; Garraway and Huie, 2016). TrkB is a strong affinity receptor for BDNF, and the combination of the two can regulate the expression of KCC2 in spinal dorsal horn neurons (Allen et al., 2013; Harward et al., 2016). KCC2 is the main chloride transporter in the central nervous system, which is responsible for maintaining the intracellular and extracellular chloride concentration gradient and participating in the maintenance of the transmission of the main inhibitory neurotransmitter GABA in the central nervous system (Mapplebeck et al., 2019). The combination of BDNF and TrkB can reduce the expression of KCC2, weaken the effect of inhibitory synapses, increase the excitability of the nervous system, and induce hyperalgesia. These results indicate that BDNF/TrkB-KCC2 signaling pathway plays an important role in hyperalgesia (Foster et al., 2015).

Based on the above evidence, the present study hypothesized that spinal BDNF/TrkB-KCC2 signaling pathway was involved in PSD-induced postoperative hyperalgesia. The protein expressions of BDNF, TrkB and KCC2 in the spinal cord of PSD rats were detected by Western Blot. The results showed that the protein expression levels of BDNF and TrkB in the spinal cord of the model rats were significantly increased, and the expression level of KCC2 was significantly decreased after 24 hours of PSD compared with the rats without PSD. To further verify that this pathway could be regulated by α7nAChR in the spinal cord, intrathecal injection of α-BGT further increased the protein expression of BDNF and TrkB and decreased the expression of KCC2 in the spinal cord

of rats. This suggests that spinal α7nAChR mediates BDNF/TrkB-KCC2 signaling pathway and plays a role in PSD-induced postoperative hyperalgesia.

In recent years, there has been an increasing number of studies on acupuncture or EA analgesia, including animal pain models of inflammatory pain, neuropathic pain, visceral pain, and opioid-induced hyperalgesia, and they have also been widely used in clinical practice (Qiao et al., 2020). The selection of acupoints, frequency, current intensity and waveform will affect the efficacy of EA analgesia. “Zusanli” acupoint has been proved to have the effect of conditioning organs, balancing Yin and Yang, and promoting blood circulation, so as to achieve effective analgesia (Wang et al., 2023; Zhang et al., 2012). "Baihui" acupoint is the intersection acupoint of the whole body, which has the effect of improving brain and nervous system function, dredging the collaterals and relieving pain (Cao et al., 2022). Therefore, the combination of "Zusanli" and "Baihui" was selected in our study, which is the most commonly used and considered to be the most effective acupoint combination in analgesia. EA stimulation parameters are also important factors affecting the analgesic effect (Liang et al., 2023; HW et al., 2018). It has been proved that 30 minutes of EA stimulation is appropriate, which can significantly increase the pain threshold (HW et al., 2018). Disperse-dense wave is a waveform commonly used for analgesia. Compared with continuous wave, disperse-dense wave can play a stronger analgesic effect and maintenance effect, and is less prone to adaptive response. With a frequency of 2/100 Hz, disperse-dense alternating can dreg the meridians of the head to achieve analgesic effect (Liu et al., 2015; Huang et al., 2017).

Both low intensity (1 mA) and high intensity (2–3 mA) can increase the pain threshold of rats, but studies have confirmed that the analgesic and maintenance effects of high intensity EA are better than those of low intensity EA (Yu et al., 2014). Therefore, the setting of parameters in our study were, disperse-dense wave, 2/100 Hz frequency, 2 mA current intensity, 30 min stimulation.

The behavioral results showed that the thermal and mechanical pain sensitivity thresholds of the rats with PSD were significantly increased after 30 min of preoperative EA stimulation compared with the group without EA stimulation, and the PWTL and PWMT still showed an upward trend on 2d after PSD. The effect of EA on improving PWMT was more sensitive than that of PWTL, which was significantly increased at 4 h after operation, but the effect of EA on thermal stimulation was longer lasting, and the upward trend of PWTL was still very obvious at 2d after operation. It is suggested that EA can effectively relieve postoperative hyperalgesia induced by PSD, which is more sensitive to mechanical stimulation and more durable to thermal stimulation. Some studies have found that low intensity 0.5 mA can promote the expression of α7nAChR in the spleen and spinal dorsal horn, and reduce the release of inflammatory factors, so as to relieve pain (LN et al., 2022). Wang et al (Wang et al., 2018). found that 2 Hz EA can effectively increase the expression of α7nAChR in the spinal cord of rats with sciatic nerve injury, inhibit the release of pro-inflammatory factor IL-1β, and play an analgesic role. In addition, the analgesic effect of EA was suppressed by intrathecal injection of the α7nAChR inhibitor α-BGT. These findings suggest that α7nAChR is involved in the analgesic mechanism of EA and is one of the important targets of EA for analgesia. In addition, Xue et al (Xue et al., 2020). found that 2 Hz EA could effectively inhibit BDNF/TrkB signaling pathway in the spinal cord, thereby reducing central hypersensitivity in rats with sciatic nerve injury.

To explore the effect of EA on the expression of α7nAChR in PSD-induced postoperative hyperalgesia, immunofluorescence and Western Blot were used to observe the fluorescence and protein expression of α7nAChR in the spinal cord of rats. The results showed that preoperative EA stimulation significantly upregulated the mean fluorescence intensity and protein expression of α7nAChR in the spinal cord of rats with PSD-induced postoperative hyperalgesia. However, intrathecal injection of α-BGT reversed the effect of EA on the elevation of PWLT and PWMT in PSD rats. The results of immunofluorescence and Western Blot showed that the mean fluorescence intensity and protein expression of α7nAChR in the spinal cord were significantly decreased after intrathecal administration of α-BGT in rats, which obviously reversed the upregulation of α7nAChR expression induced by EA. We further verified that BDNF/TrkB-KCC2 signaling pathway is involved in the alleviating effect of EA on postoperative hyperalgesia in rats after PSD. The protein expression of BDNF, TrkB and KCC2 in the spinal cord of rats was detected by Western Blot. The results showed that after 30 min of preoperative EA stimulation, the expression of BDNF and TrkB protein in the spinal cord of PSD rats was significantly inhibited, and the expression of KCC2 was significantly increased. To further validate the analgesic effect of EA through the modulation of the BDNF/TrkB-KCC2 signaling pathway by α7nAChR in the spinal cord, we found that intrathecal administration of α-BGT also reversed the downregulation of BDNF and TrkB expression and the upregulation of KCC2 expression induced by EA.

There are limitations in the current study. There are individualized differences in pain tolerance in rats, multiple measurements of thermal or mechanical pain in a short period can also cause pain sensitization, so there are some uncontrollable errors in the experiment. Moreover, the underlying mechanism of how activation of a7nAChR mediates BDNF/TrkB signaling was not further explored in the present study. There are numerous upstream and downstream targets of α7nAChR, and the mechanism of action needs to be further explored.

## 5. Conclusion

In summary, this study demonstrated that, in PSD-induced postoperative hyperalgesia rats, the expression of α7nAChR is significantly downregulated, and the downstream BDNF/TrkB signaling pathway is activated. The expression of BDNF and TrkB is significantly upregulated, and the expression of KCC2 is significantly downregulated, which contributes to the occurrence and development of postoperative hyperalgesia. EA at "Zusanli" and "Baihui" acupoints for 30 min before operation can upregulate the expression of α7nAChR in the spinal cord, thereby inhibiting the expression of BDNF/TrkB, increasing the expression of KCC2, and alleviating the hyperalgesia. This study confirmed that BDNF/TrkB-KCC2 signaling pathway mediated by α7nAChR in the spinal cord is a potential target for the prevention and treatment of postoperative hyperalgesia. This study provides a new direction for future research on PSD-induced postoperative hyperalgesia.

## Funding

The work was supported by the Natural Science Foundation Of Gansu Province (Grant number 23JRRA1251) and Gansu Province Traditional Chinese Medicine Characteristic Advantageous Specialty Construction Project-Anesthesia (Pain) Department.

## CRediT authorship contribution statement

**Huai-jing Hou:** Writing – review & editing, Software, Methodology. **Zi-qing Xu:** Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Yi-yang Cui:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Xiao-yu Qin:** Writing – review & editing. **Jian-jun Xue:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Conceptualization. **Jie Zhang:** Writing – review & editing, Software, Methodology, Formal analysis.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ibneur.2024.10.002.

## References


Guo, M., Wu, Y., Zheng, D., Chen, L., Xiong, B., Wu, J., et al., 2022. Preoperative Acute Sleep Deprivation Causes Postoperative Pain Hypersensitivity and Abnormal Cerebral Function. Neurosci. Bull. 38 (12), 1491–1507. https://doi.org/10.1007/s12264-022-00955-1.

Kourbanova, K., Alexandre, C., Latremoliere, A., 2022. Effect of sleep loss on pain-New conceptual and mechanistic avenues. Front. Neurosci. 16, 1009902. https://doi.org/10.3389/fnins.2022.1009902.

Maldonado, R.J., De Jesus, O., 2024. Hyperesthesia. StatPearls. Treasure Island (FL) ineligible companies. Disclosure: Orlando De Jesus declares no relevant financial relationships with ineligible companies. StatPearls Publishing Copyright © 2024, StatPearls Publishing LLC.

Xue, J., Li, H., Xu, Z., Ma, D., Guo, R., Yang, K., et al., 2018. Paradoxical Sleep Deprivation Aggravates and Prolongs Incision-Induced Pain Hypersensitivity via BDNF Signaling-Mediated Descending Facilitation in Rats. Neurochem. Res. 43 (12), 2353–2361. https://doi.org/10.1007/s11064-018-2660-2.

Gu, W., Zhang, W., Lei, Y., Cui, Y., Chu, S., Gu, X., et al., 2017. Activation of spinal alpha-7 nicotinic acetylcholine receptor shortens the duration of remifentanil-induced postoperative hyperalgesia by upregulating KCC2 in the spinal dorsal horn in rats. Mol. Pain. 13, 1744806917704769. https://doi.org/10.1177/1744806917704769.

Jia, D., Liu, G., Sun, Y., Hu, Z., Huang, Z., Huang, C., 2023. Trifluoro-icaritin ameliorates spared nerve injury-induced neuropathic pain by inhibiting microglial activation through α7nAChR-mediated blockade of BDNF/TrkB/KCC2 signaling in the spinal cord of rats. Biomed. Pharmacother. = Biomedecine Pharmacother. 157, 114001. https://doi.org/10.1016/j.biopha.2022.114001.

Liang, Y., Zhou, J., Sun, J., Fan, X.-z, Zheng, S.-y, Fang, J.-f, et al., 2023. The dose-effect relationship of electroacupuncture analgesia and its stimulus parameters: Progress in the last 3 decades. World J. Acupunct. - Moxibustion 33 (1), 12–19. https://doi.org/10.1016/j.wjam.2022.12.001.

Wang, Y.L., Zhu, H.Y., Lv, X.Q., Ren, X.Y., Peng, Y.C., Qu, J.Y., et al., 2023. Electroacupuncture Zusanli (ST36) Relieves Somatic Pain in Colitis Rats by Inhibiting Dorsal Root Ganglion Sympathetic-Sensory Coupling and Neurogenic Inflammation. Neural Plast. 2023, 9303419. https://doi.org/10.1155/2023/9303419.

Su, H., Chen, H., Zhang, X., Su, S., Li, J., Guo, Y., et al., 2023. Electroacupuncture ameliorates pain in cervical spondylotic radiculopathy rat by inhibiting the CaMKII/CREB/BDNF signaling pathway and regulating spinal synaptic plasticity. Brain Behav. 13 (10), e3177. https://doi.org/10.1002/brb3.3177.

Wang, J., Zhang, J., Gao, Y., Chen, Y., Duanmu, C., Liu, J., 2021. Electroacupuncture Alleviates Hyperalgesia by Regulating CB1 Receptor of Spinal Cord in Incisional Neck Pain Rats (eCAM). Evid. -Based Complement. Altern. Med. 2021, 5880690. https://doi.org/10.1155/2021/5880690.

Zhang, Z., Wang, C., Gu, G., Li, H., Zhao, H., Wang, K., et al., 2012. The effects of electroacupuncture at the ST36 (Zusanli) acupoint on cancer pain and transient receptor potential vanilloid subfamily 1 expression in Walker 256 tumor-bearing rats. Anesth. Analg. 114 (4), 879–885. https://doi.org/10.1213/ANE.0b013e318246536d.

Xue, M., Sun, Y.L., Xia, Y.Y., Huang, Z.H., Huang, C., Xing, G.G., 2020. Electroacupuncture Modulates Spinal BDNF/TrκB Signaling Pathway and Ameliorates the Sensitization of Dorsal Horn WDR Neurons in Spared Nerve Injury Rats. Int. J. Mol. Sci. 21 (18). https://doi.org/10.3390/ijms21186524.

Wang, Y., Jiang, Q., Xia, Y.Y., Huang, Z.H., Huang, C., 2018. Involvement of α7nAChR in electroacupuncture relieving neuropathic pain in the spinal cord of rat with spared nerve injury. Brain Res. Bull. 137, 257–264. https://doi.org/10.1016/j.brainresbull.2018.01.002.

Xie, F., Li, X., Bao, M., Shi, R., Yue, Y., Guan, Y., et al., 2015. Anesthetic propofol normalized the increased release of glutamate and γ-amino butyric acid in hippocampus after paradoxical sleep deprivation in rats. Neurol. Res. 37 (12), 1102–1107. https://doi.org/10.1080/01616412.2015.1114231.

Brennan, T.J., Vandermeulen, E.P., Gebhart, G.F., 1996. Characterization of a rat model of incisional pain. Pain 64 (3), 493–502. https://doi.org/10.1016/0304-3959(95)01441-1.

Silva, J.R., Silva, M.L., Prado, W.A., 2011. Analgesia induced by 2- or 100-Hz electroacupuncture in the rat tail-flick test depends on the activation of different descending pain inhibitory mechanisms. J. Pain. 12 (1), 51–60. https://doi.org/10.1016/j.jpain.2010.04.008.

Finan, P.H., Goodin, B.R., Smith, M.T., 2013. The association of sleep and pain: an update and a path forward. J. Pain. 14 (12), 1539–1552. https://doi.org/10.1016/j.jpain.2013.08.007.

Moldofsky, H., 2001. Sleep and pain. Sleep. Med. Rev. 5 (5), 385–396. https://doi.org/10.1053/smrv.2001.0179.

Onen, S.H., Alloui, A., Eschalier, A., Dubray, C., 2000. Vocalization thresholds related to noxious paw pressure are decreased by paradoxical sleep deprivation and increased after sleep recovery in rat. Neurosci. Lett. 291 (1), 25–28. https://doi.org/10.1016/s0304-3940(00)01383-5.

Roehrs, T., Hyde, M., Blaisdell, B., Greenwald, M., Roth, T., 2006. Sleep loss and REM sleep loss are hyperalgesic. Sleep 29 (2), 145–151. https://doi.org/10.1093/sleep/29.2.145.

Varallo, G., Giusti, E.M., Manna, C., Castelnuovo, G., Pizza, F., Franceschini, C., et al., 2022. Sleep disturbances and sleep disorders as risk factors for chronic postsurgical pain: A systematic review and meta-analysis. Sleep. Med. Rev. 63, 101630. https://doi.org/10.1016/j.smrv.2022.101630.

HQ, D., J, Z., M, W., YF, C., QS, Z., XL, L., 2023. Application and evaluation of the modified multi-platform method in animal model of sleep deprivation. ACTA Lab. ANIMALIS Sci. Sin. 31 (1), 120–128. https://doi.org/10.3969/j.issn.1005-4847.2023.01.015.

Abbas, M., Alzarea, S., Papke, R.L., Rahman, S., 2019. The α7 nicotinic acetylcholine receptor positive allosteric modulator prevents lipopolysaccharide-induced allodynia, hyperalgesia and TNF-α in the hippocampus in mice. Pharmacol. Rep.: PR 71 (6), 1168–1176. https://doi.org/10.1016/j.pharep.2019.07.001.

Zhou, Y.Q., Liu, D.Q., Liu, C., Xu, A.J., Tian, Y.K., Mei, W., et al., 2022. Targeting α7 nicotinic acetylcholine receptors for chronic pain. Front. Mol. Neurosci. 15, 970040. https://doi.org/10.3389/fnmol.2022.970040.

Zhang, W., Liu, Y., Hou, B., Gu, X., Ma, Z., 2015. Activation of spinal alpha-7 nicotinic acetylcholine receptor attenuates remifentanil-induced postoperative hyperalgesia. Int. J. Clin. Exp. Med. 8 (2), 1871–1879.

Volcheck, M.M., Graham, S.M., Fleming, K.C., Mohabbat, A.B., Luedtke, C.A., 2023. Central sensitization, chronic pain, and other symptoms: Better understanding, better management. Clevel. Clin. J. Med. 90 (4), 245–254. https://doi.org/10.3949/ccjm.90a.22019.

Thakkar, B., Acevedo, E.O., 2023. BDNF as a biomarker for neuropathic pain: Consideration of mechanisms of action and associated measurement challenges. Brain Behav. 13 (3), e2903. https://doi.org/10.1002/brb3.2903.

Zhou, W., Xie, Z., Li, C., Xing, Z., Xie, S., Li, M., et al., 2021. Driving effect of BDNF in the spinal dorsal horn on neuropathic pain. Neurosci. Lett. 756, 135965. https://doi.org/10.1016/j.neulet.2021.135965.

Garraway, S.M., Huie, J.R., 2016. Spinal Plasticity and Behavior: BDNF-Induced Neuromodulation in Uninjured and Injured Spinal Cord. Neural Plast. 2016, 9857201. https://doi.org/10.1155/2016/9857201.

Allen, S.J., Watson, J.J., Shoemark, D.K., Barua, N.U., Patel, N.K., 2013. GDNF, NGF and BDNF as therapeutic options for neurodegeneration. Pharmacol. Ther. 138 (2), 155–175. https://doi.org/10.1016/j.pharmthera.2013.01.004.

Harward, S.C., Hedrick, N.G., Hall, C.E., Parra-Bueno, P., Milner, T.A., Pan, E., et al., 2016. Autocrine BDNF-TrkB signalling within a single dendritic spine. Nature 538 (7623), 99–103. https://doi.org/10.1038/nature19766.

Mapplebeck, J.C.S., Lorenzo, L.E., Lee, K.Y., Gauthier, C., Muley, M.M., De Koninck, Y., et al., 2019. Chloride Dysregulation through Downregulation of KCC2 Mediates Neuropathic Pain in Both Sexes. e4 Cell Rep. 28 (3), 590–596. https://doi.org/10.1016/j.celrep.2019.06.059.

Foster, E., Wildner, H., Tudeau, L., Haueter, S., Ralvenius, W.T., Jegen, M., et al., 2015. Targeted ablation, silencing, and activation establish glycinergic dorsal horn neurons as key components of a spinal gate for pain and itch. Neuron 85 (6), 1289–1304. https://doi.org/10.1016/j.neuron.2015.02.028.

Qiao, L., Guo, M., Qian, J., Xu, B., Gu, C., Yang, Y., 2020. Research Advances on Acupuncture Analgesia. Am. J. Chin. Med. 48 (2), 245–258. https://doi.org/10.1142/s0192415x20500135.

Cao, F., Xu, Y., Zhang, M., Li, X., Chen, Y., Zhi, M., et al., 2022. Baihui (DU20), Shenmen (HT7) and Sanyinjiao (SP6) target the cAMP/CREB/BDNF and PI3K/Akt pathways to reduce central nervous system apoptosis in rats with insomnia. Heliyon 8 (12), e12574. https://doi.org/10.1016/j.heliyon.2022.e12574.

HW, F., LJ, Y., YY, L., Y, G., YM, G., 2018. Summary of the Study on the Effect of Different Electroacupuncture Parameters on the Analgesic Effect. Shanghai Jounal Acupunct. Moxibustion 37 (11), 1331–1335. https://doi.org/10.13460/j.issn.1005-0957.2018.11.1331.

Liu, X., Li, S., Wang, B., An, L., Ren, X., Wu, H., 2015. Intraoperative and postoperative anaesthetic and analgesic effect of multipoint transcutaneous electrical acupuncture stimulation combined with sufentanil anaesthesia in patients undergoing supratentorial craniotomy. Acupunct. Med.: J. Br. Med. Acupunct. Soc. 33 (4), 270–276. https://doi.org/10.1136/acupmed-2014-010749.

Huang, S., Peng, W., Tian, X., Liang, H., Jia, Z., Lo, T., et al., 2017. Effects of transcutaneous electrical acupoint stimulation at different frequencies on perioperative anesthetic dosage, recovery, complications, and prognosis in video-assisted thoracic surgical lobectomy: a randomized, double-blinded, placebo-controlled trial. J. Anesth. 31 (1), 58–65. https://doi.org/10.1007/s00540-015-2057-1.

Yu, Z., Luo, L., Li, Y., Wu, Q., Deng, S., Lian, S., et al., 2014. Different manual manipulations and electrical parameters exert different therapeutic effects of acupuncture. J. Tradit. Chin. Med. = Chung i tsa chih ying wen pan 34 (6), 754–758. https://doi.org/10.1016/s0254-6272(15)30092-3.

LN, P., YY, L., ZF, W., XM, C., XM, Y., 2022. Mechanism of inflammation regulation of f α7nAChR in spleen and spinal dorsal horn of mice with paclitaxel-induced neuropathic pain by electroacupuncture on Zusanli acupoint. Rehabil. Med. 32 (5), 426–433. https://doi.org/10.3724/sp.J.1329.2022.05007.