

Original Article

Effect of sleep deprivation on general anesthesia in rats

Shangping Fang¹, Jiabao Dai¹, Wenjun Guo², Tongjun Ma¹

¹Anesthesia College of Wannan Medical College, Wuhu, Anhui, China; ²Department of Anesthesiology, Yijishan Hospital of Wannan Medical College, Wuhu, Anhui, China

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Abstract: Objective: To explore the effects of sleep deprivation on perioperative general anesthesia in rats. Methods: 45 healthy male Sprague-Dawley (SD) rats were randomly divided into 3 groups, the control group (Group A), the anesthesia group (Group B) and the sleep deprivation anesthesia group (Group C), 15 in each group. The sleep deprivation model was established by improving multi-platform water environment method. The group B and C were received propofol 80 mg/kg by intraperitoneally, the group A was given the same dose of normal saline. The EEG in each group was measured. The GABA_A R-β3 protein in cerebral cortex was detected by Western Blot. The rats were treated with Brennan incision, and the changes of thermal pain sensitive (PWL) and open field behavior were measured in each group. Results: In group C, the δ band of brainwave of EEG increased significantly, the disappearance time of righting reflex shortened significantly, the recovery time prolonged significantly, the GABA_A R-β3 protein was significantly increased, and the time of passing through the central area before operation was significantly decreased. Conclusion: Sleep deprivation can significantly inhibit the electrical activity of rat cerebral cortex induced by propofol, up-regulating the GABA_A R-β3 protein in cortex.

Keywords: Sleep deprivation, propofol, electroencephalogram, GABA_A R-β3, pain, anxiety

Introduction

Sleep is a normal physiological phenomenon of human beings and animals, which is characterized by the temporary disappearance of consciousness and the recovery of energy and physical strength. Human beings spend about 1/3 of their time in sleeping. A quality sleep keeps the respiratory system, immune system, endocrine system and other body systems functioning well. However, once deprived of sleep will bring serious irritability, fatigue, stress and dysfunction of many systems. The latest researches showed that the decrease of sleep led to significant damage of the immune system, cardiovascular system, endocrine system and central nervous system [1-3].

Anesthesia is the use of medications and close monitoring to provide comfort and maintain vital life functions during surgery or other medical procedures [4, 5]. Propofol is the most commonly used sedative-hypnotic drug for noxious procedures in clinics [6, 7]. It has the advantages of rapid onset of action, rapid recovery

rate and none accumulative effect after continuous infusion, so it is widely used for clinical anesthesia induction and maintenance [8]. The main mechanism is that the propofol stimulates GABA_A receptors in presynaptic and postsynaptic membranes to inhibit excitatory synaptic transmission [9] and produce inhibitory effect [10]. The main target is GABA_A receptor β subunit. Jonsson et al. found that the action of the general anesthetic propofol on GABA_A receptors was β2 and β3 subunits [11]. It is suggested that β2 and β3 subunits are the key parts of propofol. Propofol is used to alleviate the incidence of neurological disorders caused by sleep deprivation, directly binding of GABA_A receptor β2 and β3 subunits to activating the neural network that regulates sleep and restore balance between excitatory and inhibitory neurotransmitters [12].

Clinically, patients with preoperative psychological reactions and psychological problems have varying degrees of sleep deprivation. Sleep deprivation lead to the change of the cell nuclei and the neural activity of the brain, and the

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level of sleep awakening and the response to external environmental stimuli are decreased. Are these patients also affected to varying degrees during anesthesia? There have been few studies investigating the effect of sleep deprivation on general anesthesia. This present paper aims to explore possibilities for the effects of sleep deprivation on perioperative anesthesia, especially on the depth of anesthesia and postoperative pain and anxiety in rats.

Materials and methods

Animals

A total of 45 rats which were healthy adult SD rats, aged 8 weeks, of clean grade, weighing 250-350 g were provided by Nanjing Qinglongshan Animal Experimental Center. All rats maintained under conditions of normal biorhythm, and before the study, animals were housed in proper enriched cages, in a room with controlled temperature (20°C), ventilation, humidity (50%). All rats were adaptively fed for one week.

Groups

The rats were randomly divided into three groups with 15 rats in each group, including control group (Group A), anesthesia group (Group B) and sleep deprivation anesthesia group (Group C). Seven rats in each group were randomly selected, and the electrode needle was inserted in the cerebral cortex. After 7 days of recovery, the sleep deprivation model was established by modified multiple platform method in Group C. After the successful establishment of the sleep deprivation model, Group B and Group C received propofol 80 mg/kg but Group A was given the same volume of normal saline intraperitoneally. After the administration, the righting reflex disappeared, and the EEG was measured immediately in each group. Rats were decapitated the corresponding time, then the cerebral cortex were separated, and the protein was collected. Western Blot was used to determine the expression of GABA α R- β 3 in cerebral cortex.

The remaining 8 rats in each group were divided into two groups according to the above method. The disappearance and recovery period of righting reflex were recorded. Brennan incision was performed on the left foot and the

skin was excised. Open field test was score the rats locomotor activity, thermal pain sensitive (PWL) was monitored before and after surgery on 12 h, on day 1, 3.

The model of electrode tip implanted into cortex

Seven rats were anesthetized with intraperitoneal injection of 1% pentobarbital sodium (30 mg/kg). Animals were then shaved and placed on a stereotactic head holder. According to the Rat Brain in Stereotaxic Coordinates, the anterior fontanelle was the origin, 2.0 mm backward, 1.5 mm on both sides as the puncture point, drilling the skull and placing two insulating lacquer-wrapped acupuncture needles at the depth of 1.2 mm under the skull [7, 8]. The third acupuncture needle was placed beside the nasal bone as the ground pole. The miniature screws were placed between two acupuncture needles and fixed with bone cement. The rats ate freely and recovered for 7 days.

Improved multiple platform water environment

The water environment deprivation box measures 150 cm by 65 cm, built in a small platform with a diameter 3.5 cm, surrounded by water, the platform exceed the water surface 1 cm, the water temperature maintained at 22°C, and the rats were unable to sleep freely and achieve the purpose of sleep deprivation for 48 hours [13].

Electroencephalogram (EEG)

Rats were anesthetized with intraperitoneal injection of propofol 80 mg/kg. After righting reflex disappeared, we measured the EEG by BW-200 physiological wireless telemetry system. After EEG measurement, the rats were decapitated. The brain tissues were taken out completely with bone nipping forceps. The cerebral cortex were separated on ice and stored in -80°C refrigerator.

Brennan's rodent paw incision model

Rats were anesthetized with intraperitoneal injection of propofol 80 mg/kg. After righting reflex disappeared, the left posterior sole of rats was disinfected. A 1 cm long middle incision on the skin starting one-half inch from the

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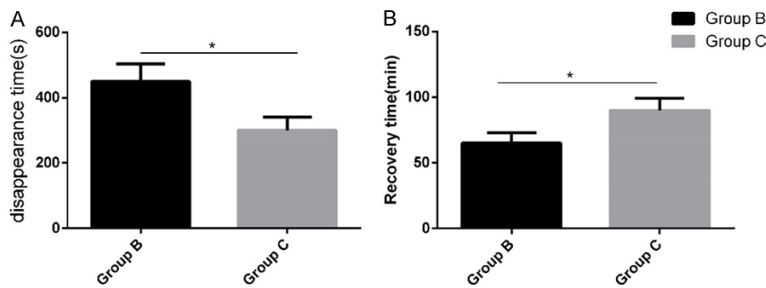


Figure 1. Disappearance time and recovery time of righting reflex in propofol anesthetized rats. A: Disappearance time of righting reflex in propofol anesthetized rats. B: Recovery time of righting reflex in propofol anesthetized rats.

proximal end of the heel. The underlying fascia and muscle were also incised. Subsequently, the wound was sutured with 4-0 nylon and knots were placed on the lateral side. Finally, the rat was transferred to a warm recovery chamber. Postincisional nociception by disappearance time and recovery time was determined at specific time intervals after incision [14].

Determination of thermal sensitivity

The thermal sensitivity experiment was completed at night. Rats were placed in the test room to adapt for 30 minutes. Animals were placed in an acrylic box with glass pane floor and the plantar surface of their hind paw was exposed to a beam of infrared radiant heat (Ugo Basile, Stoelting, Chicago, IL). The paw withdrawal latencies were recorded at 2 different infrared intensities, 30 and 70, and were measured twice per session, separated by a minimum interval of 5 minutes. Minimum and maximum cut-offs were assigned at 1 and 30 seconds, respectively. Again, paw withdrawals due to locomotion or weight shifting were not counted and the trials repeated.

Open field test

An open field test utilizes a large cubic box, usually measuring 1 m long \times 1 m wide \times 1 m high. The top of the cube is typically left uncovered. An animal is placed in the middle of the bottom surface, and its movements are recorded over the course of minutes to hours as it moves around and explores its environment. After the experiment is completed, computer tracking programs analyze the movements of the animal over time. This assay can measure horizontal activity, time spent in various regions of the open field, and the total distance traveled.

Statistical analysis

SPSS 19.0 software was used for statistical analysis. The measurement data were expressed by mean \pm standard deviation ($\bar{x} \pm SD$). The comparison among the three groups was carried out by means of one-way ANOVA and SNK test. In case of enumeration data expressed as $[n (\%)]$, comparison studies were carried out through χ^2 test for intergroup comparison. The difference was statistically significant when $p < 0.05$.

Results

Sleep deprivation shorten the disappearance time of righting reflex and prolong the recovery time

As shown in **Figure 1A**, the disappearance time of righting reflex in Group C was significantly shorter than that of Group B ($P < 0.05$). The recovery time of righting reflex in Group C was significantly longer than that in Group B, as shown in **Figure 1B**. The above results suggested that sleep deprivation could significantly increase the time of propofol anesthesia, and the difference was statistically significant ($P < 0.05$).

Sleep deprivation increases the proportion of EEG δ band with propofol

The EEG waveforms and the proportion of each frequency band of rats in each group were obtained. As shown in **Figure 2A**, the proportion of α and β bands was dominant in the Group A, the slow wave was increased and the power ratio of δ band was dominant in Group B, while the power of δ band in Group C was significantly higher than that in Group B. The above results suggest that sleep deprivation can significantly enhance the inhibitory effect of propofol on cerebral cortical electrical activity in rats ($P < 0.05$).

Sleep deprivation increases GABA α R- β 3 protein in AD rats

Compared with Group A, the expression of GABA α R- β 3 in Group B was significantly higher

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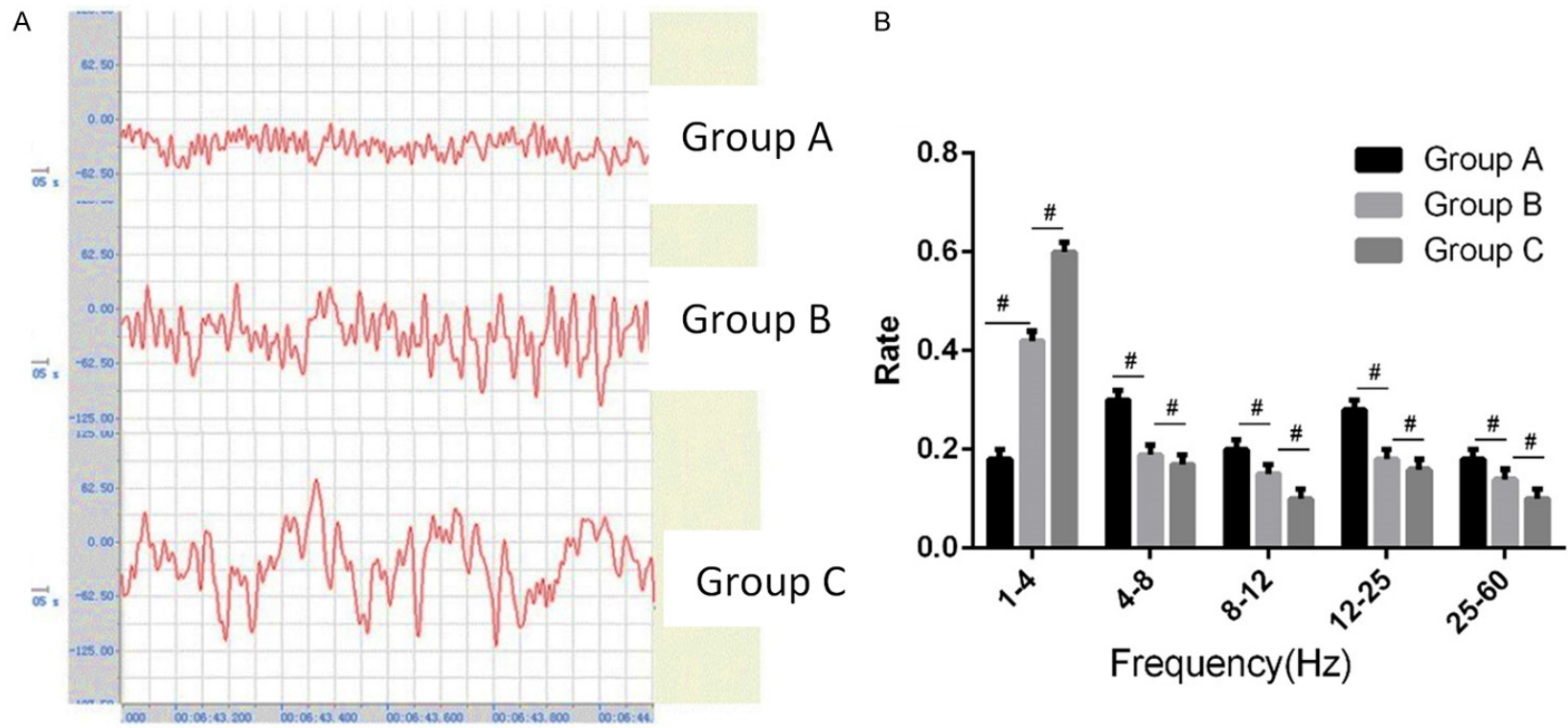


Figure 2. Comparison of sleep deprivation on brain waves in rats. A: The measurement of cortical electroencephalogram, the electroencephalogram. B: The ratio of different frequency bands. Compared with Group B, *P < 0.05, #P < 0.01.

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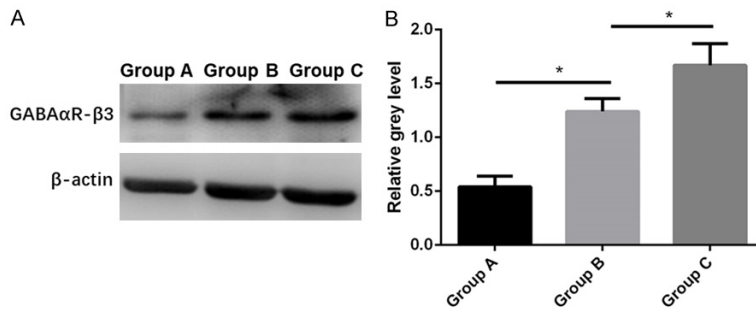


Figure 3. Effects of sleep deprivation on the expression of GABA α R- β 3 protein. A: The bands of GABA α R- β 3 protein expression in cortical homogenate; B: The gray analysis diagram GABA α R- β 3 protein expression. Compared with Group B, *P < 0.05.

Table 1. Effects of sleep deprivation on PWL in rats

	before	12 h	24 h	48 h
Group A	6.79±3.36	7.02±2.45	7.63±3.12	6.94±2.26
Group B	6.91±1.24	7.94±2.17	6.84±1.42	6.56±1.84
Group C	3.05±1.05*	5.56±2.01 [Ⓟ]	6.61±2.20	6.94±3.05
F	2.175	3.525	1.149	2.760
p	0.036	0.029	0.379	0.617

Note: Compared with group A *P < 0.05; Compared with before, [Ⓟ]P < 0.05.

than that of Group A, while Group C was significantly higher than that of Group B (P < 0.05), as shown in **Figure 3**.

Sleep deprivation reduces thermal hyperalgesia in rats anesthetized with propofol

The preoperative thermal pain threshold of rats in Group C was lower than that of the other two groups, and the rats in Group C were sensitive to pain stimulation. It began to increase at 12 hours after operation, but it was still lower than that in Group B and returned to normal level 24 hours after operation. See **Table 1**.

Sleep deprivation enhances anxiety in rats anesthetized with propofol

The activity time of central area in Group C was lower than that in the other two groups before operation, and the rats in Group C showed obvious anxiety-like behavior due to sleep deprivation. On the first day after operation, there was no significant difference in the activity time of central area between Group B and Group C (P > 0.05). The anxiety factors caused by sleep deprivation basically disappeared, but it was still lower than that in Group A, and the anxiety caused by incision pain still existed. Three days

after operation, all groups basically returned to normal, and there was no significant difference among the three groups (P > 0.05). See **Figure 4**.

Discussion

Sleep disorders lead to anxiety, which are considered as the most frequently occurring category of mental disorder in the general population. Estimates of the lifetime prevalence of anxiety disorders have ranged between 10% and 25%. Epidemiological studies have also showed the high prevalence of sleep complaints. As much as one third of the adult population reports difficulty sleeping and sleep disturbance is considered as the second most common symptom of mental distress.

In this study, it was found that sleep deprivation shortened the disappearance time of righting reflex and prolonged the recovery time of righting reflex. The electrical activity of cortical nerve cells reflected by EEG is directly related to the depth of sleep or anesthesia [15]. Wang YJ et al. confirmed that sleep deprivation decreased the entropy of EEG in the whole brain region in varying degrees [16], and Gao DR et al. also showed that slow wave components increased and fast wave components decreased in sleep deprivation group [17].

In patients with sleep deprivation, the activity of central neurotransmitters is out of balance due to the disorder of sleep-awakening rhythm [18]. It was found that the content of γ -aminobutyric acid (GABA) in the brain of patients with sleep deprivation was significantly decreased, while the content of glutamate was significantly increased, and the excitation and inhibition of neurotransmitters in the brain were out of balance [19]. The β 3 subunit of GABA α receptor is the key site for the anesthetic effect of propofol. Propofol plays its role through the following aspect. It can enhance the response of GABA α receptor to GABA, increase the expression level of GABA α receptor

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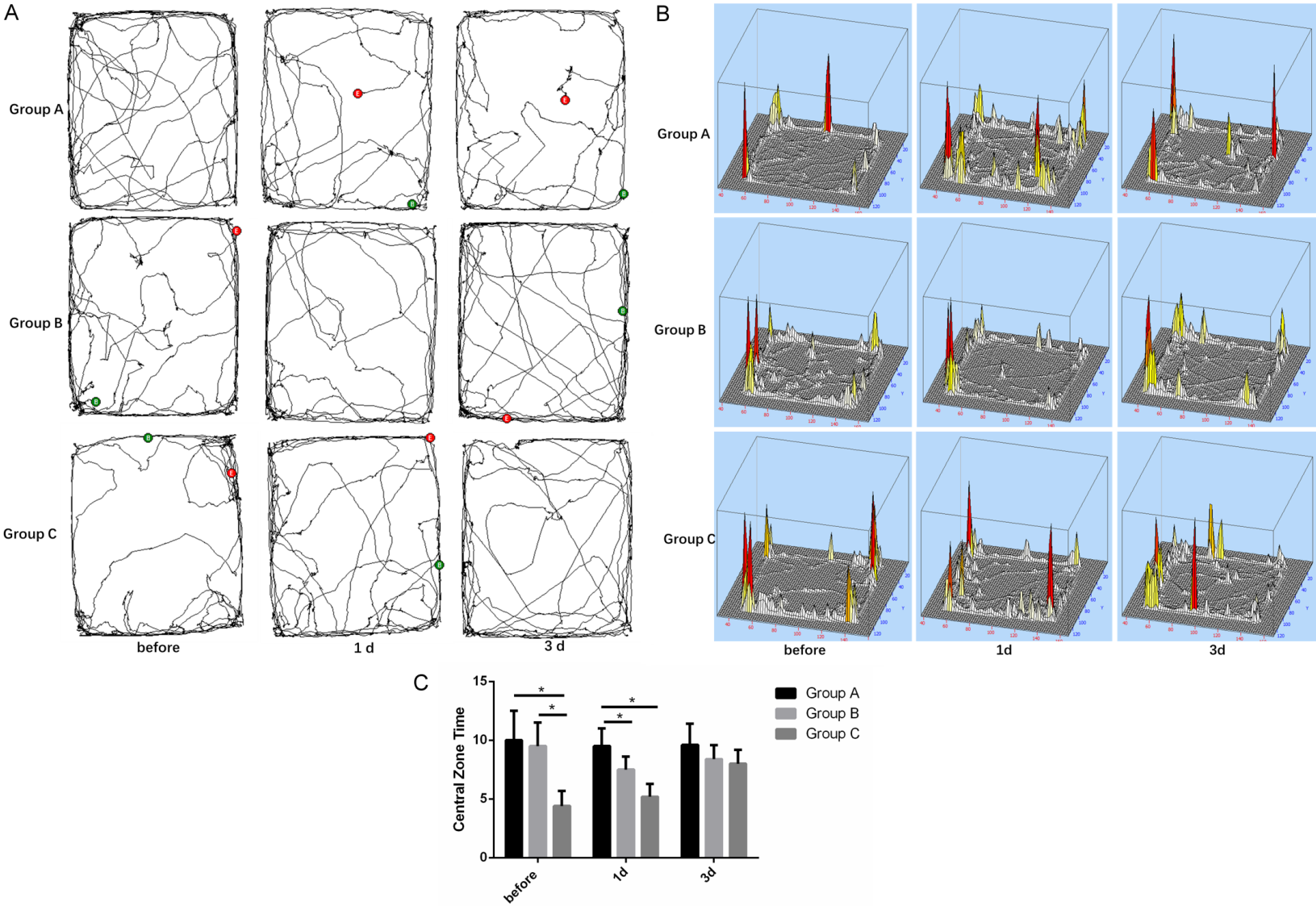


Figure 4. Effects of sleep deprivation on open-field behavior of rats. A: The open field test trajectories of each group of rats in different time periods. B: The corresponding 3D thermal imaging trajectories. C: The statistical chart of the time in the central area of each group. Compared with Group C, *P < 0.05.

on the surface of neurons [20], and activate GABA_A receptor by directly acting on the $\beta 3$ subunit of propofol receptor [21]. Goldschmied JR et al. experiment proved that sleep deprivation increased the slow wave of EEG [22]. These results suggested that sleep deprivation significantly enhances the inhibitory effect of propofol on cortical EEG activity and related to GABA_A R- $\beta 3$ protein closely. The possible mechanism is that sleep deprivation increases the expression of GABA_A R- $\beta 3$ and enhances the sensitivity of propofol and GABA_A receptor. Therefore, it is speculated that the sensitivity of propofol to GABA_A R- $\beta 3$ protein is increased, and the inhibition of EEG activity is stronger.

In this study, it was found that the preoperative thermal pain threshold of rats after sleep deprivation was lower than that of the other two groups, and was more sensitive to pain stimulation. It began to increase at 12 hours after operation, but it was still lower than that in the non-sleep deprivation group, and returned to normal 24 hours after operation. At present, animal and clinical experiments have made it clear that preoperative sleep deprivation can increase pain sensitivity and aggravate postoperative pain.

The sleep deprivation anesthesia showed obvious anxiety-like behavior. Sleep deprivation could produce obvious anxiety-like behavior [23], but there was no significant difference between propofol anesthesia group and sleep deprivation group. The possible reason is that the sample size is small, the behavior test is easy to be affected, and propofol can't cause the effect of preoperative anxiety. It may be that propofol eliminates sleep debt and reduces its adverse effects.

Conclusion

Sleep deprivation can significantly inhibit the electrical activity of cerebral cortex induced by propofol, probably by increasing the expression of GABA_A R- $\beta 3$ protein in the cortex, and sleep deprivation can aggravate postoperative pain and pain-related anxiety.

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Disclosure of conflict of interest

None.

Address correspondence to: Tongjun Ma and Shangping Fang, Anesthesia College of Wannan Medical College, No. 22, West Wenchang Road, Yijiang District, Wuhu City 241000, Anhui Province, China. E-mail: 476116295@qq.com (TJM); Fangshangping-0@163.com (SPF)

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