

Original Research

Sleep Deprivation Increases the Anesthetic Potency of Sevoflurane Regardless of Duration

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Abstract

Background: Sleep deprivation reduced the time to induce anesthesia by propofol and isoflurane and prolonged the time to recovery. However, it is unknown whether sleep deprivation affects the potency of inhaled anesthetics. In this study, the effect of sleep deprivation on sevoflurane anesthetic potency was explored. **Methods:** Ten animals received the following behavioral interventions in turn (ad libitum activity, 24 h sleep deprivation, 48 h sleep deprivation, 72 h sleep deprivation). After each behavioral intervention, the 50% effective dose for loss of righting reflex (LORR ED₅₀) was determined to evaluate the potency of sevoflurane in inducing unconsciousness in mice. Repeated-measures analysis of variance was used to compare our behavioral interventions statistically, post hoc multiple comparisons were made using the Bonferroni test. **Results:** Sleep deprivation decreased the sevoflurane LORR ED₅₀ significantly ($p = 0.0003$). However, the effect of duration of sleep deprivation on LORR ED₅₀ was not statistically significant ($p > 0.9999$). **Conclusions:** Sleep deprivation can increase the anesthetic potency of sevoflurane regardless of duration of sleep deprivation.

Keywords: sleep deprivation; anesthetic potency; sevoflurane

1. Introduction

Sleep exists in all organisms despite evolutionary pressure, from *C. elegans*, to *Drosophila*, to human, they all need to sleep or in a sleep-like state, and poor sleep can have deleterious effects on development cognitive abilities and life span [1]. Sleep is a resting behavioral state characterized by reduced response to weak stimuli and rapid reversal of strong stimuli [2]. General anesthesia is a state of unresponsiveness which is like sleep to a certain extent, although sleep and anesthesia have similar behavioral characteristics, there are obvious behavioral and physiological differences between the two states. For example, unlike the natural occurrence of sleep, general anesthesia is induced by anesthetics and does not respond to external stimuli. After considering the similarities and differences between anesthesia and sleep, a hypothesis was proposed that anesthesia and sleep might share a part of neuronal network [3].

Sleep deprivation, as a manipulation for studying the function of sleep, has been widely used in various sleep-related experiments. It has been shown that sleep deprivation reduced the time to loss of righting reflex for propofol and isoflurane and prolonged the time to recovery [3]. Nevertheless, these related studies did not evaluate the 50% effective dose for loss of righting reflex (LORR ED₅₀). In addition, the effect of duration of sleep deprivation on anesthetic potency is also unknown. LORR ED₅₀ was often used to explore the potency of anesthetics in inducing unconsciousness in mice, since loss of righting reflex resembled sleep compared with the minimal alveolar concentration (MAC).

The purpose of this study was to determine the effects of sleep deprivation on the potency of sevoflurane in inducing unconsciousness in mice. We hypothesized that sleep deprivation can increase the potency of sevoflurane and the enhancement was positively correlated with the duration of sleep deprivation.

2. Methods

In this study, we first determined the basal sevoflurane LORR ED₅₀ in mice as control, followed by three behavioral interventions in the order of 24-h, 48-h, and 72-h sleep deprivation in the same mice. Sevoflurane LORR ED₅₀ was tested immediately after each behavioral intervention and the mice rested for three days to recover (Fig. 1). The sleep deprivation model was established by using modified multiple platform method. The selection of three days for recovery was based on previous research with slight modification [3], we also tested LORR ED₅₀ in another group of mice after 3-day recovery from sleep deprivation.

2.1 Animals

C57BL/6 male mice (7 weeks; weight range, 22–25 g) were ordered from Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China. Mice were bred in a temperature- and humidity-controlled room with a 12-h light/dark cycle. All mice had free access to standard mouse chow and tap water before the experiments. The animals used for the behavioral experiments were housed in the room for a week.



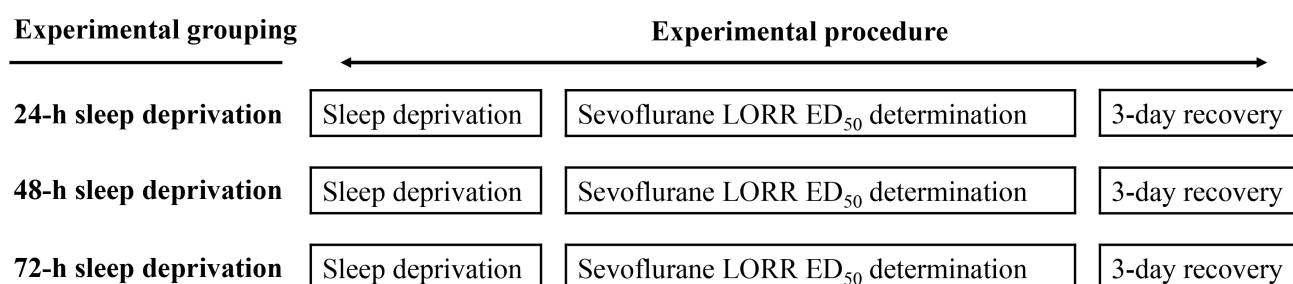


Fig. 1. Flowchart showing the study design and the temporal order (from top to bottom) of behavior interventions.

2.2 Sleep Deprivation

Sleep deprivation in mice was achieved by modified multiple platform method [4]. The mice were placed on 9 circular and 3.5 cm-diameter platforms in a ventilated transparent cage (50 × 30 × 17 cm, available food and water, 4 mice/cage) filled by water up to 1 cm of the platforms' surface enabling the mice to jump between the platforms. Whenever the mice reached the rapid eye movement (REM) sleep, they fall into the water because of muscle atonia and then wake up and tried to climb back to the platforms to avoid being drowned. The water in the cage was refreshed every day due to the excreta of mice. The sleep deprivation periods were timed to begin and end at 8:00 AM. The non-sleep deprived mice were maintained under normal feeding conditions.

2.3 LORR ED₅₀ Determination

The LORR ED₅₀ of sevoflurane in mice was determined according to our previously described method [5] with slight modifications. Briefly, all the behavioral interventions LORR ED₅₀ of each mouse was determined, and all the values were compared. For either the control group (ad libitum activity) or the sleep deprivation group LORR ED₅₀ determination, mice were individually placed in independent plastic grid V-shaped trough fixed in a transparent plastic chamber (205 × 134 × 69 mm) with an electrical fan to mix gases. One side of the chamber was connected to a sevoflurane vaporizer (Aika, Ichikawa Shiseido, Tokyo, Japan). The other side was connected to an infrared gas monitor (BeneView T5, Mindray Bio-Medical Electronics, Shenzhen, China) to measure the sevoflurane, oxygen, and carbon dioxide concentrations in real time. The monitor can monitor the sevoflurane concentration with a precision of 0.01%.

When a mouse was placed in the chamber, pure oxygen was immediately supplied at a rate of 600 mL min⁻¹. When the chamber's oxygen concentration increased to 99%, sevoflurane gas mixed in pure oxygen was provided by the vaporizer. The initial sevoflurane concentration in the chamber was 1.00%, which was maintained for 15 minutes to equilibrate the mouse with sevoflurane gas. Then, the chamber was rotated 180° to place the mouse on its back in the V-shaped trough, and its righting reflex was observed.

LORR was defined as the supine mouse unable to turn itself onto all 4 paws three times within 1 min. According to the mouse's righting reflex, a stepwise increase or decrease of 0.10% sevoflurane in the chamber was applied. Specifically, if the mouse's righting reflex disappeared, the sevoflurane concentration was decreased 0.10%; otherwise, it was increased 0.10%. After 15 minutes of equilibration at each sevoflurane concentration, the mouse's righting reflex was observed again. The LORR ED₅₀ was the average of the two critical sevoflurane concentrations at which the mouse either lost or regained its righting reflex. All determinations were made between 8:00 and 18:00. For the sleep deprivation group, LORR ED₅₀ was determined immediately after sleep deprivation.

2.4 Statistical Analysis

Sample sizes were predetermined according to our previous study [5]. GraphPad Prism software (version 8.0.2 for Windows, GraphPad Software Inc., San Diego, CA, USA) was used for statistical analyses. The acquired values of LORR ED₅₀ were expressed as mean ± SD, repeated-measures analysis of variance (RMANOVA) was used to determine the significance of our behavioral interventions. Post hoc multiple comparisons were made using the Bonferroni test. A *p* value less than 0.05 (two-tailed) was considered to be statistically significant.

3. Results

3.1 Effects of Sleep Deprivation of Sevoflurane LORR ED₅₀

The LORR ED₅₀ of sevoflurane was 1.08 ± 0.11% (95% CI, 1.01%–1.16%), 0.88 ± 0.12% (95% CI, 0.80%–0.96%), 0.89 ± 0.08% (95% CI, 0.83%–0.95%) and 0.88 ± 0.12% (95% CI, 0.80%–0.96%) for the control, sleep deprivation (24 h), sleep deprivation (48 h) and sleep deprivation (72 h) group, respectively. By comparing the LORR ED₅₀ of different groups using RMANOVA, we found a statistically significant effect of different durations of sleep deprivation on LORR ED₅₀ (*F* = 10.6, *p* = 0.0003).

The results of Bonferroni multiple comparisons test showed a decrease in LORR ED₅₀ after 24-h (*p* = 0.0174), 48-h (*p* = 0.0043) and 72-h (*p* = 0.0023) sleep deprivation compared with the control group. Nevertheless, there

are no significant statistical differences in LORR ED₅₀ between sleep deprivation groups of different durations ($p > 0.9999$) (Fig. 2). In other words, the sleep deprivation duration did not affect the LORR ED₅₀. Because a decreased ED₅₀ means increased potency of anesthetic, these findings indicated that sleep deprivation can increase anesthetic potency.

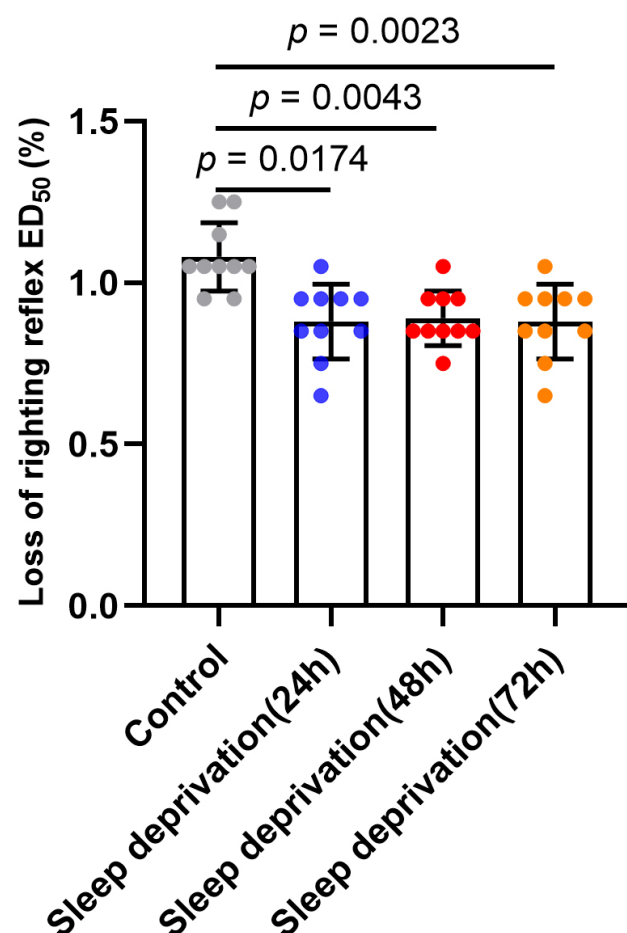


Fig. 2. Effects of sleep deprivation on the LORR ED₅₀ of sevoflurane in mice. The LORR ED₅₀ of sevoflurane was 1.08% (95% CI, 1.01%–1.16%), 0.88% (95% CI, 0.80%–0.96%), 0.89% (95% CI, 0.83%–0.95%) and 0.88% (95% CI, 0.80%–0.96%) for the control, sleep deprivation (24 h), sleep deprivation (48 h) and sleep deprivation (72 h) group, respectively. Data are shown as mean ± SD (n = 10/group).

3.2 LORR ED₅₀ Determination after 3-Day Recovery from Sleep Deprivation

The LORR ED₅₀ of sevoflurane was $1.12 \pm 0.08\%$ (95% CI, 1.03%–1.20%), $1.12 \pm 0.08\%$ (95% CI, 1.03%–1.20%), $1.15 \pm 0.09\%$ (95% CI, 1.06%–1.24%) for the control, 3-day recovery from sleep deprivation (24 h) and 3-day recovery from sleep deprivation (48 h) group, respectively (Fig. 3). By comparing the LORR ED₅₀ of different

groups using RMANOVA, we found there was no statistically significant difference in LORR ED₅₀ after 3-day recovery from sleep deprivation ($F = 0.6250$, $p = 0.5549$).

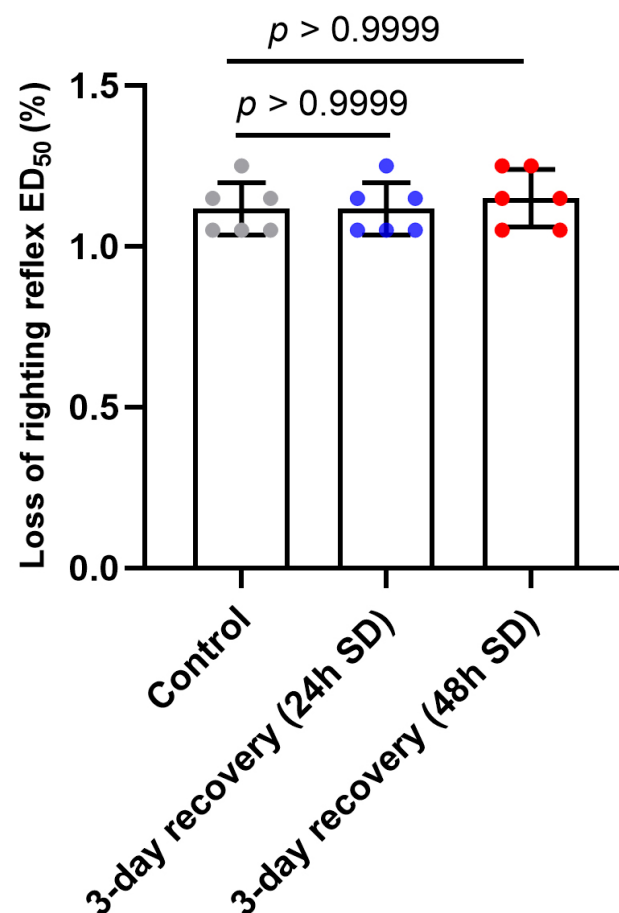


Fig. 3. LORR ED₅₀ determination after 3-day recovery from sleep deprivation. SD means sleep deprivation. There was no statistically significant difference in LORR ED₅₀ after 3-day recovery from sleep deprivation ($p > 0.9999$). Data are shown as mean ± SD (n = 6/group).

4. Discussion

In this paper, we present two main findings. First, sleep deprivation can increase the anesthetic potency of sevoflurane. Second, the duration of sleep deprivation did not affect the anesthetic potency of sevoflurane. These findings showed that sleep homeostasis affects the potency of anesthetics, suggesting that general anesthesia, sleep may share a common mechanism. So, our future work is to explore what this common mechanism is and how it works.

4.1 Sleep Homeostasis and Circadian Clock

Previous study had shown that circadian clock genes can affect sleep homeostasis [6] and there are circadian differences in the minimal alveolar concentration (MAC) for recovery of righting reflex (MAC_{RORR}) and the time

to recovery of righting reflex ($\text{Time}_{\text{RORR}}$) [7], REM sleep deprivation can induce circadian clock gene abnormalities of rats' hippocampus after sevoflurane inhalation [8]. So, there may be a shared component between sleep, anesthesia and circadian rhythms. We designed the above experiment to explore the relationship between sleep and anesthesia, in this animal experiment, we found an approximate 20% reduction in the LORR ED_{50} after a behavioral intervention of sleep deprivation, however the reduction did not change with the duration of sleep deprivation.

4.2 Sleep Deprivation and Neurotransmitters

Our findings indicated that sleep deprivation can increase the anesthetic potency of sevoflurane. Previous research has been done to evaluate the time to loss of righting reflex and recovery by propofol and isoflurane after sleep deprivation, the results also suggested that sleep deprivation can potentiate the effect of anesthetics. Then how might sleep deprivation affect the anesthetic potency? Although the underlying mechanism of sleep deprivation is still unclear, there have been some discoveries about the role of neurotransmitters. For example, the concentration of adenosine in the basal forebrain of cats increased after 6 hours of sleep deprivation and decreased after recovery [9]. However, administration of adenosine antagonist cannot completely reverse the effects of sleep deprivation on righting reflex, suggesting that the effects of sleep deprivation may not be mediated by adenosine [10]. In addition, another widely concerned neuropeptides produced by the posterior hypothalamic region, which plays a key role in the maintenance of sleep and arousal, include orexin and melanin concentrating hormone (MCH). Orexin is a neuropeptide family secreted by hypothalamus that promotes appetite and regulates sleep and wakefulness, it consists of two peptides orexin-A and orexin-B. Dong *et al.* [11] found that intrabasis microinjection of orexin-A can shorten the emergence time to sevoflurane anesthesia and the orexin receptor antagonist (SB-334867A) prolonged the emergence time to sevoflurane anesthesia. The decrease of orexin-A is also the reason for the delayed recovery of sleep deprived rats under isoflurane anesthesia [12]. Whereas MCH perform opposite roles to orexin in sleep and wakefulness [13]. In the rebound phase of REM sleep after 72 hours of REM sleep deprivation, MCH⁺ neurons were strongly active, and rapid eye movement sleep was prolonged after lateral ventricular injection of MCH [14]. Systemic application of MCHR-1 antagonist resulted in a dose-dependent reduction of slow wave (SW) sleep and REM sleep, and a corresponding increase in wakefulness [15]. Orexin and MCH were also found to be related with the effects of ketamine and propofol on sleep structure in the period of postanesthesia [16]. Other neurotransmitters include glutamate (Glu), and γ -amino butyric acid (GABA) also play an important role in the wake/sleep cycle, Xie *et al.* [17] found that 24 h sleep deprivation significantly increased the concentration of Glu

and GABA in the rat's hippocampus and propofol anesthesia can normalized the upregulated GABA and Glu levels like natural sleep.

4.3 Selection of Methods

We chose LORR ED_{50} as an observed index because this index was more stable compared with the time to loss of righting reflex or the emergence time from anesthesia, and it only takes 0.5–1 h to complete a LORR ED_{50} test, which is not too long. Induction time is not an index to evaluate the potency of inhaled anesthetics, for example, sevoflurane induces anesthesia faster than isoflurane, but sevoflurane is less potent than isoflurane [18]. LORR ED_{50} has been commonly used to evaluate the potency of inhaled anesthetics in inducing unconsciousness in mice [5,19]. Furthermore, although studies demonstrated that prolonged sedation with propofol can discharge the sleep debt [20,21], when exposed to the inhaled anesthetics such as sevoflurane, isoflurane and halothane, rapid eye movement sleep debt accrues in mice [22,23]. Pal *et al.* [24] also found that sevoflurane induction time was shortened after sleep deprivation and REM sleep could not be restored during sevoflurane anesthesia. Therefore, we did not need to consider the recovery of REM sleep that may be caused by the duration of LORR ED_{50} test. We did not use the minimal alveolar concentration (MAC) as a measure to evaluate the anesthetic potency for following reasons. At first, MAC involves the painful stimulation during tail-clamp, while sleep loss would increase the pain sensitivity in mice [25–27]. In addition, MAC was used to evaluate the anesthetic potency of inducing immobility, and the value was not altered after spinal cord transection in rats which means that MAC associated body movements may be in the spinal cord [28], nevertheless, the potency of anesthetics to induce hypnosis is measured by loss of righting reflex and hypothalamic nuclei and cerebral cortex may be involved in this process [29].

There are many ways to model sleep deprivation, in this study we chose the modified multiple platform method for several reasons. Above all, we want to explore the effects of REM sleep deprivation on anesthetic potency as the determination of LORR ED_{50} required a period of 0.5–1 h though it is not too long, we do not know that if the sleep debt can be discharged during anesthesia, whereas as mentioned above, inhaled anesthetics cannot satisfy the homeostatic need for REM sleep, so we can exclude the effect of anesthetic time on our results. Also, modified multiple platform method was widely used for REM sleep deprivation [4,30,31] and it is convenient to model.

4.4 Clinical Significance

Our results demonstrated that preoperative sleep disorders may affect perioperative anesthetic managements. Nowadays, sleep disorders are regarded as an independent risk factor of postoperative cognitive dysfunction (POCD) which is a severe postoperative neurological sequela. So,

for the anesthesia of such patients, we can consider reducing the dose of anesthetics during operation, this may help to reduce the incidence of POCD, however, this hypothesis needs to be further verified.

4.5 Limitations and Future Directions

This study only presents a behavioral finding and does not further investigate the mechanism. Therefore, the next step is to explore the basic mechanism of sleep deprivation leading to the increased anesthetic potency and try to uncover the relationship between sleep and anesthesia.

In addition, it can also be clinically explored whether reducing the dosage of anesthetics in patients with sleep disorders can reduce the incidence of POCD.

5. Conclusions

In summary, we report that sleep deprivation can increase the anesthetic potency of sevoflurane regardless of duration of sleep deprivation, suggested that general anesthesia may share a common mechanism with sleep. More researches are needed to explore the mechanisms that how do sleep and anesthesia interact with each other.

Author Contributions

SZ designed this study. HQ, QZ and NC performed the experiments. HQ and QZ wrote the manuscript. All authors have read and approved the final manuscript.

Ethics Approval and Consent to Participate

All animal operations and experimental protocols conformed to the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and were approved by the Institutional Animal Care and Use Committee (approval No: S164) at Tongji Medical College, Huazhong University of Science and Technology.

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Conflict of Interest

The authors declare no conflict of interest.

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