

# Dexmedetomidine suppresses sevoflurane anesthesia-induced neuroinflammation through activating PI3K/Akt/mTOR pathway

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## Research Article

**Keywords:** Dexmedetomidine, sevoflurane, neuroinflammation, PI3K/Akt/mTOR pathway

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# Abstract

**Background:** Sevoflurane, an inhalational general anesthetic, has become one of the most widely used inhalational anesthetics in surgery. However, previous studies have found that sevoflurane anesthesia can trigger an inflammatory response, resulting in secondary damage. Dexmedetomidine (DEX), a highly-selective  $\alpha$  adrenergic receptor agonist, is widely used as an anesthesia adjuvant drug in the clinic. Here, we investigate whether DEX can suppress sevoflurane anesthesia-induced neuroinflammation.

**Methods:** We sought to determine the mechanism of action of this suppressive effect using rats as a model. Rats were randomly divided into control group (n=10), low-dose sevoflurane group (L-Sev; n=10), high-dose sevoflurane group (H-Sev; n=10), vehicle group (n=10) and DEX group (n=10). Western blot was used to detect the expression of proinflammatory cytokines (IL-6, IL-8, TNF- $\alpha$ ) and the activity level of the phosphatidylinositol 3-hydroxy kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway.

**Results:** We find that sevoflurane anesthesia induces an increase in the levels of pro-inflammatory cytokines, while decreasing activation of the PI3K/Akt/mTOR pathway in both the cortex and hippocampus of rats. Moreover, treatment with DEX reduced pro-inflammatory cytokine levels and prevented inactivation of the PI3K/Akt/mTOR pathway.

**Conclusions:** These data suggest that the PI3K/Akt/mTOR pathway could contribute to sevoflurane-induced neuroinflammation, and that activation of PI3K/Akt/mTOR signaling by DEX could help to reduce the neuroinflammation effects of sevoflurane.

**Keywords:** Dexmedetomidine, sevoflurane, neuroinflammation, PI3K/Akt/mTOR pathway

## Background

In recent years the inhalational anesthetic sevoflurane has replaced other volatile anesthetics due to its mild irritation, quick induction, stable maintenance and rapid revival [1, 2]. However, recent evidence has shown that sevoflurane can lead to pathophysiological alterations of brain in the recovery period, such as neuronal apoptosis, tau aggregates, abnormal discharge of neurons, and neuroinflammation, which cause neurodegenerative changes in the development of mammalian brain [3-7]. Exposure to anesthesia in childhood may lead to adverse neurodevelopmental outcomes in children [8]. Despite evidence linking inhalational anesthesia to neurodegenerative effects, it is still considered safer than other anesthesia methods, and more than 3 million children undergo inhaled anesthesia every year [9, 10]. Therefore, it is urgent to search for anesthesia adjuvants to reduce the neurotoxicity of inhaled anesthetics.

Dexmedetomidine (DEX), a selective  $\alpha$ 2-adrenoceptor agonist, is used clinically as an adjuvant of local anesthetics used in peripheral nerve blocks, where it prolongs the duration of sensory and motor blockades without causing toxicity [11, 12]. In addition, DEX is able to cross the blood brain barrier and stimulate  $\alpha$ 2-adrenoceptors centrally and has been shown to exert neuroprotective effect [13]. Wang et al.

found that DEX treatment could inhibit the expression of inflammatory cytokines and their mediators, resulting in reduced focal cerebral ischemia-reperfusion injury in rats. This data suggested that inhibition of the nuclear factor- $\kappa$ B pathway (NF- $\kappa$ B) may be a mechanism underlying the neuroprotective action of DEX [14]. Treatment with DEX after subarachnoid haemorrhage (SAH) attenuated SAH-induced early brain injury, partially through suppression of the toll like receptor 4 (TLR4)/NF- $\kappa$ B pathway and the NLRP3 inflammasome [15]. Additionally, DEX has been shown to improve post-operative cognitive dysfunction in aged mice by inhibition of the hippocampal inflammatory response and reduction of neuronal apoptosis [16]. Taken together, these data suggest that DEX has anti-inflammatory properties in the central nervous system (CNS).

Given the inflammation induced by sevoflurane and the anti-inflammatory properties of DEX, previous research has been conducted to determine whether DEX could provide protection from sevoflurane anesthesia-induced inflammation. In the present study, we investigated the molecular mechanism responsible for the protective effect of DEX against sevoflurane-induced inflammation in the CNS. We find that this inflammation is closely associated with the activity of the phosphatidylinositol 3-hydroxy kinase/ protein kinase B/ mammalian target of rapamycin (PI3K/Akt/mTOR) pathway, and that treatment with DEX can prevent disruption of PI3K/Akt/mTOR pathway signaling.

## Methods

### Animals

Fifty healthy Sprague-Dawley (SD) rats, weighing 150-200 g were purchased from the Animal Experiment Center of Institute of Radiation Medicine of the Chinese Academy of Medical Sciences, China. The rats were fed food and water under specific pathogen-free conditions and kept in a 12 h light/dark cycle at 22-24°C. All animal procedures were approved by The Institute of Radiation Medicine of the Chinese Academy of Medical Sciences and conducted in accordance with the ethical principles for Experiments on Animals as well as international standards.

### Groups and treatments

Rats were randomly divided into five groups: control group (n=10), low-dose sevoflurane group (L-Sev; n=10), high-dose sevoflurane group (H-Sev; n=10), vehicle group (n=10) and DEX group (n=10). All rats were placed in the anesthesia box and received anesthesia using an inhalation machine. Oxygen concentration and anesthesia doses were continuously monitored. The control group was treated with 60% O<sub>2</sub> for 2 h, L-Sev group was treated with 1.5% sevoflurane inhalation for 2 h, and the remaining groups (L-Sev group, vehicle group, and DEX group) were treated with 3% sevoflurane inhalation for 2 h [2]. One hour before sevoflurane treatment the DEX group received intraperitoneal injection of 4  $\mu$ g/kg and the vehicle group received intraperitoneal injection of saline [17]. The anesthesia time commenced when the sevoflurane concentration reached the maximum for each group. Gas flow in the anesthesia chamber was maintained at a rate of 4L/min.

## Tissue preparation

At the end of the experiment, rats were sacrificed under anesthesia using 50 mg/kg sodium pentobarbital by intraperitoneal injection. The brain tissue was removed and a subset of brain samples was used to make 10  $\mu\text{m}$  sections for use in immunofluorescence staining. The remaining brains were placed on tin foil and the cortical and the hippocampal tissues were separated and stored at  $-80\text{ }^{\circ}\text{C}$  for western blot.

## Immunofluorescence staining

Frozen brain sections were treated with ice cold acetone for 15 minutes, followed by incubation in 1% Triton-X for 20 min at room temperature. After blocking with 10% goat serum (Beyotime, China) for 30 min at  $37\text{ }^{\circ}\text{C}$ , sections were incubated with rabbit anti-PI3K (1:100, CST, USA), rabbit anti-Akt (1:100, CST, USA), rabbit anti-mTOR (1:50, CST, USA) and mouse anti-NeuN (1:200, Millipore, USA) overnight at  $4\text{ }^{\circ}\text{C}$ . Sections were washed with PBS and incubated with goat anti-rabbit Alexa Fluor cy3 (1:50, Beyotime, China) and goat anti-mouse Alexa Fluor 488. After washing with PBS, cell nuclei were then stained with DAPI (1:100, Beyotime, China). Fluorescence images were captured via laser scanning confocal microscopy (Nikon).

## Western blot analysis

Cortical and hippocampal tissue samples were lysed and total protein was extracted using whole-cell protein extraction kits (Beyotime, China) according to the manufacturer's protocols. A bicinchoninic acid (BCA) protein quantification kit (Beyotime, China) was used to measure the protein concentration of the samples. The proteins were separated with 10% SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. PVDF membranes were blocked in 2% BSA in TBST at room temperature for 2 hours. Membranes were then incubated with primary antibodies overnight at  $4^{\circ}\text{C}$ . The primary antibodies used in our study include rabbit anti-IL-6 (1:1000, Abcam, USA), IL-8 (1:1000, Abcam, USA), TNF $\alpha$  (1:1000, Abcam, USA), PI3K (1:1000, CST, USA), p-PI3K (1:1000, CST, USA), Akt (1:1000, CST, USA), p-Akt (1:1000, CST, USA), mTOR (1:1000, CST, USA), p-mTOR (1:1000, CST, USA) and GAPDH (1:4000, Proteintech, USA). Following staining with primary antibody, three TBST washes were performed and membranes were incubated with a horseradish peroxidase-conjugated goat anti-rabbit IgG (1:4000, Proteintech, USA) for 2 h at room temperature. PVDF membranes were then washed three times with TBST and developed using ECL Plus reagent.

## Statistical Analysis

All values are presented as the mean  $\pm$  SEM. Comparisons between groups were made using t-tests and one-way ANOVA followed by a post hoc Tukey test. Differences were considered to be statistically significant at the level of  $P < 0.05$ .

## Results

### Effect of sevoflurane on neuroinflammation

To determine the effect of sevoflurane on neuroinflammation, expression levels of the inflammatory markers IL-6, IL-8 and TNF- $\alpha$  were measured in the cortex and hippocampus of rats in the control, L-Sev and H-Sev groups by western blot. IL-6, IL-8 and TNF- $\alpha$  expression was significantly increased in the cortex and hippocampus after low-dose or high-dose sevoflurane anesthesia compared with that of the control group (Figure 1,  $p < 0.05$ ). Moreover, the high-dose sevoflurane group showed significantly higher levels of IL-6, IL-8 and TNF- $\alpha$  than the low-dose group, indicating that the induction of inflammation was dose dependent (Figure 1,  $p < 0.05$ ).

### **Effect of sevoflurane on the PI3K/Akt/mTOR pathway**

We first used immunofluorescence to determine the expression pattern of PI3K, Akt, and mTOR in the brain. We found that PI3K/Akt/mTOR pathway components were widely expressed in both neurons and other non-neuronal cells (Figure 2), indicating that the PI3K/Akt/mTOR pathway might play an important role in the physiological control of numerous functions of the central nervous system. Following this initial characterization, we assayed the levels of PI3K, Akt, and mTOR expression, as well as their phosphorylation status, in the cortex and hippocampus of rats from the control, L-Sev, and H-Sev groups to assess the effect of sevoflurane anesthesia on PI3K/Akt/mTOR signaling. As shown in Figure 3, both low-dose and high-dose sevoflurane treatment groups showed significantly decreased levels of PI3K/Akt/mTOR pathway phosphorylation ( $p < 0.05$ ). Moreover, the level of PI3K/Akt/mTOR pathway activity was significantly lower in the cortex and hippocampus after high-dose sevoflurane anesthesia compared with that of L-Sev group (Figure 3,  $p < 0.05$ ).

### **Effect of dexmedetomidine on sevoflurane anesthesia-induced neuroinflammation**

To determine if dexmedetomidine could reduce neuroinflammation induced by sevoflurane anesthesia we measured the levels of IL-6, IL-8 and TNF- $\alpha$  in the cortex and hippocampus of rats in the vehicle and DEX groups. Rats treated with DEX exhibited decreased levels of IL-6, IL-8, and TNF- $\alpha$  compared to the vehicle control group, suggesting that DEX can inhibit sevoflurane anesthesia-induced neuroinflammation (Figure 4,  $p < 0.05$ ).

### **Effect of dexmedetomidine on the PI3K/Akt/mTOR pathway**

Using western blot, we assayed the effects of DEX on PI3K/Akt/mTOR pathway activation. As shown in figure 5, the levels of PI3K, Akt, and mTOR phosphorylation were significantly increased in the cortex and hippocampus in the DEX group compared with vehicle group, indicating DEX could activate the PI3K/Akt/mTOR pathway under sevoflurane anesthesia ( $p < 0.05$ ).

## **Discussion**

In the present study, we observed that sevoflurane anesthesia treatment induced an increase in the expression of proinflammatory factors and led to a decrease in PI3K/Akt/mTOR pathway activity in both the cortex and hippocampus of rats. Additionally, we found that DEX treatment could restore

PI3K/Akt/mTOR activity in rats treated with sevoflurane anesthesia. Thus, we propose that DEX can suppress sevoflurane anesthesia-induced neuroinflammation by modulating [PI3K/Akt/mTOR pathway](#) activity.

Postoperative neuroinflammation is a common pathological phenomenon in the CNS, and can lead to secondary damage, such as delirium, cognitive dysfunction, Alzheimer's disease, and other detrimental effects [18, 19]. A healthy immune response is crucial for proper wound healing and repair of tissue damage, as well as to combat infection without harming the host's own cells or tissue. In contrast, excessive inflammatory responses are harmful, and can result in severe tissue damage, and even death [18, 20]. Inhalational anesthetics, such as isoflurane and sevoflurane, can trigger pathological immune responses during surgery. Isoflurane, which has been used since the 1980s, is metabolized slowly leading to reduced induction of anesthesia during surgery and shorter recovery times [21]. Sevoflurane began to be used a decade later and has a lower blood-gas partition coefficient than the other anesthetics, leading to rapid induction of anesthesia and faster recovery times after anesthesia [22, 23]. Several decades of research have been conducted on the toxicity and side effects of these volatile anesthetics. Here, we investigated the relationship between sevoflurane anesthesia and neuroinflammation. We used low- or high-doses sevoflurane to treat rats, and then detected the expression of proinflammatory cytokines (IL-6, IL-8, TNF- $\alpha$ ). We confirmed that sevoflurane could increase the levels of IL-6, IL-8 and TNF- $\alpha$  in the cortex and hippocampus of anesthetized rats, in agreement with previous research which indicated that either isoflurane or sevoflurane can increase IL-6 levels via activation of NF- $\kappa$ B signalling [24].

The PI3K/Akt/mTOR cascade is important in mediating the release of proinflammatory cytokines. PI3K is a ubiquitous lipid kinase which plays a crucial role in signal transduction through receptor tyrosine kinases. PI3K phosphorylates phosphatidylinositol-4,5-bis-phosphate (PIP2) to form phosphatidylinositol-4,5-tri-phosphate (PIP3); PIP3 recruits other downstream molecules, such as serine-threonine kinases, including the major effector of PI3K activation, Akt [25]. Activated Akt can then in turn activate mTOR, leading to the phosphorylation of two downstream effectors, p70 ribosomal protein S6 kinase 1 (S6K1) and eIF4E binding protein (4E-BP1), which promotes translational initiation and elongation [26-29]. In addition to its role in protein synthesis, mTOR also regulates the ubiquitin-proteasome system (UPS) [29, 30]. The UPS is an important regulator of NF- $\kappa$ B signaling, as the inhibitory regulator of NF- $\kappa$ B must be degraded by the proteasome in order for NF- $\kappa$ B to become active. [Rebecca et al.](#) suggested that inhibition of mTOR by rapamycin could increase polyubiquitination and ubiquitin-mediated degradation of I $\kappa$ B- $\alpha$ , leading to NF- $\kappa$ B-induced transcriptional activation. This result suggested that the activation of mTOR could prevent the occurrence of inflammation through suppression of NF- $\kappa$ B activation [31], as NF- $\kappa$ B activity promotes the expression of proinflammatory cytokines [32, 33]. We observed an increase in pro-inflammatory cytokine release after treatment with sevoflurane anesthesia, and this release of cytokines was accompanied by the inactivation of the PI3K/Akt/mTOR pathway. In addition, we observed widespread expression of PI3K, Akt, and mTOR in neurons and non-neuronal cells, which indicated that PI3K/Akt/mTOR pathway might be involved in the physiological control of numerous functions of the central nervous system. In sum, these data suggest that the inactivation of PI3K/Akt/mTOR pathway

may be associated with sevoflurane anesthesia-induced neuroinflammation. Thus, increasing PI3K/Akt/mTOR pathway activity may be a promising and novel therapeutic strategy against postoperative neuroinflammation.

Recent studies have suggested that DEX has anti-inflammatory properties. Chen et al., for example, found that DEX treatment could suppress retinal ischemia/reperfusion injury, and showed effective anti-inflammatory effects through inhibition of toll-like receptor 4 (TLR4)/NF- $\kappa$ B expression [34]. Additionally, Huang et al. also suggested that DEX could inhibit the nuclear translocation and binding activity of activated NF- $\kappa$ B, thus reducing inflammatory cytokines [34]. Given the anti-inflammatory properties of DEX and its effects on the PI3K/Akt/mTOR signaling pathway, we hypothesized that DEX could suppress sevoflurane anesthesia-induced neuroinflammation. Surprisingly, we found that treatment with DEX could down-regulate pro-inflammatory cytokines (IL-6, IL-8 and TNF- $\alpha$ ) and up-regulate phosphorylated levels of PI3K/Akt/mTOR, as a previous study indicated that DEX exerts an anti-inflammatory effect via the activation of PI3K/Akt/mTOR signaling in rats with traumatic brain injury [35]. Thus, we propose that DEX can ameliorate sevoflurane anesthesia-induced neuroinflammation through activation of the PI3K/Akt/mTOR signaling pathway.

## Conclusions

Sevoflurane anesthesia induces neuroinflammation in the CNS, and this inflammation may be the result of decreased signaling through the PI3K/Akt/mTOR pathway. The administration of DEX can reduce neuroinflammation caused by sevoflurane, providing an important reference basis for clinical anesthesia.

## Abbreviations

DEX	Dexmedetomidine
PI3K/Akt/mTOR	Phosphatidylinositol 3-hydroxy kinase/protein kinase B/mammalian target of rapamycin
NF- $\kappa$ B	Nuclear factor- $\kappa$ B pathway
SAH	Subarachnoid haemorrhage
TLR4	Toll like receptor 4
PIP2	Phosphatidylinositol-4,5-bis-phosphate
PIP3	Phosphatidylinositol-4,5-tri-phosphate
S6K1	S6 kinase 1
UPS	Ubiquitin-proteasome system

# Declarations

## Ethics approval and consent to participate

All animal procedures were approved by the Institute of Radiation Medicine of the Chinese Academy of Medical Sciences and conducted in accordance with the ethical principles for Experiments on Animals as well as international standards.

## Consent for publication

Not applicable

## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on a reasonable request.

## Competing interests

The authors declare that they have no competing interests.

## Funding

No funding was received.

## Authors' contributions

Research idea and project design: MW. Data collection and dataset setup: NW. Data analysis: NW. All authors contributed to the development of the manuscript, and approved the final version.

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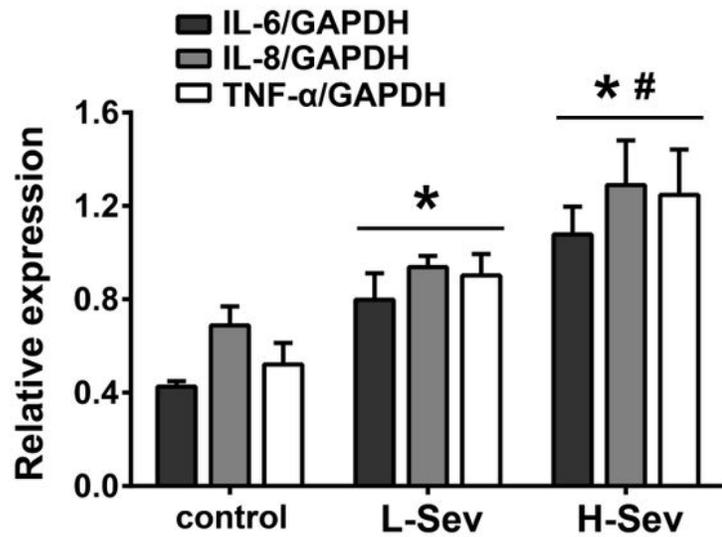
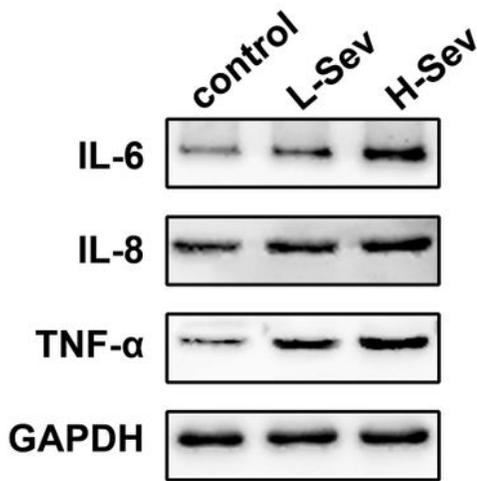
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## Figures

## cortex



## hippocampus

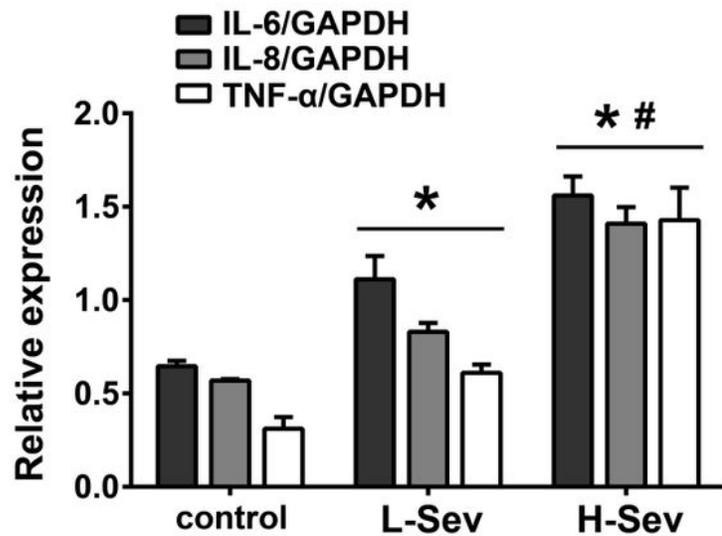
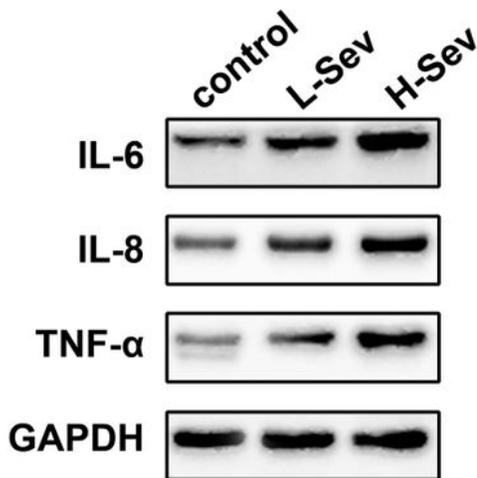
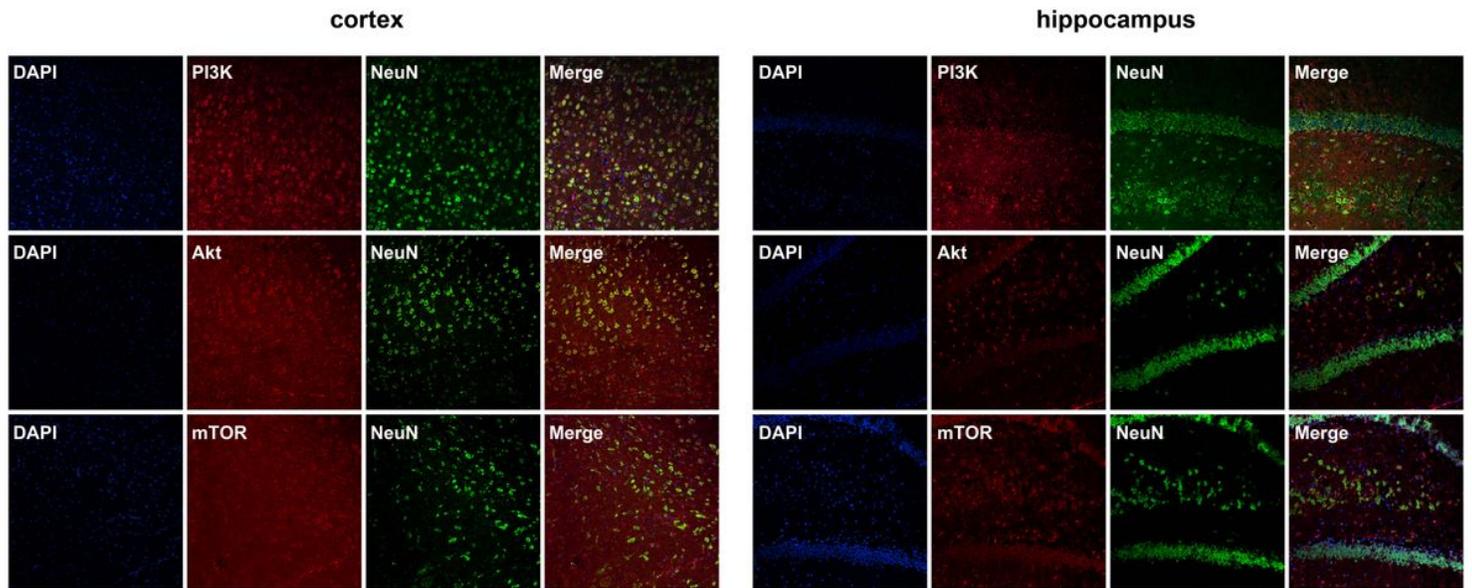


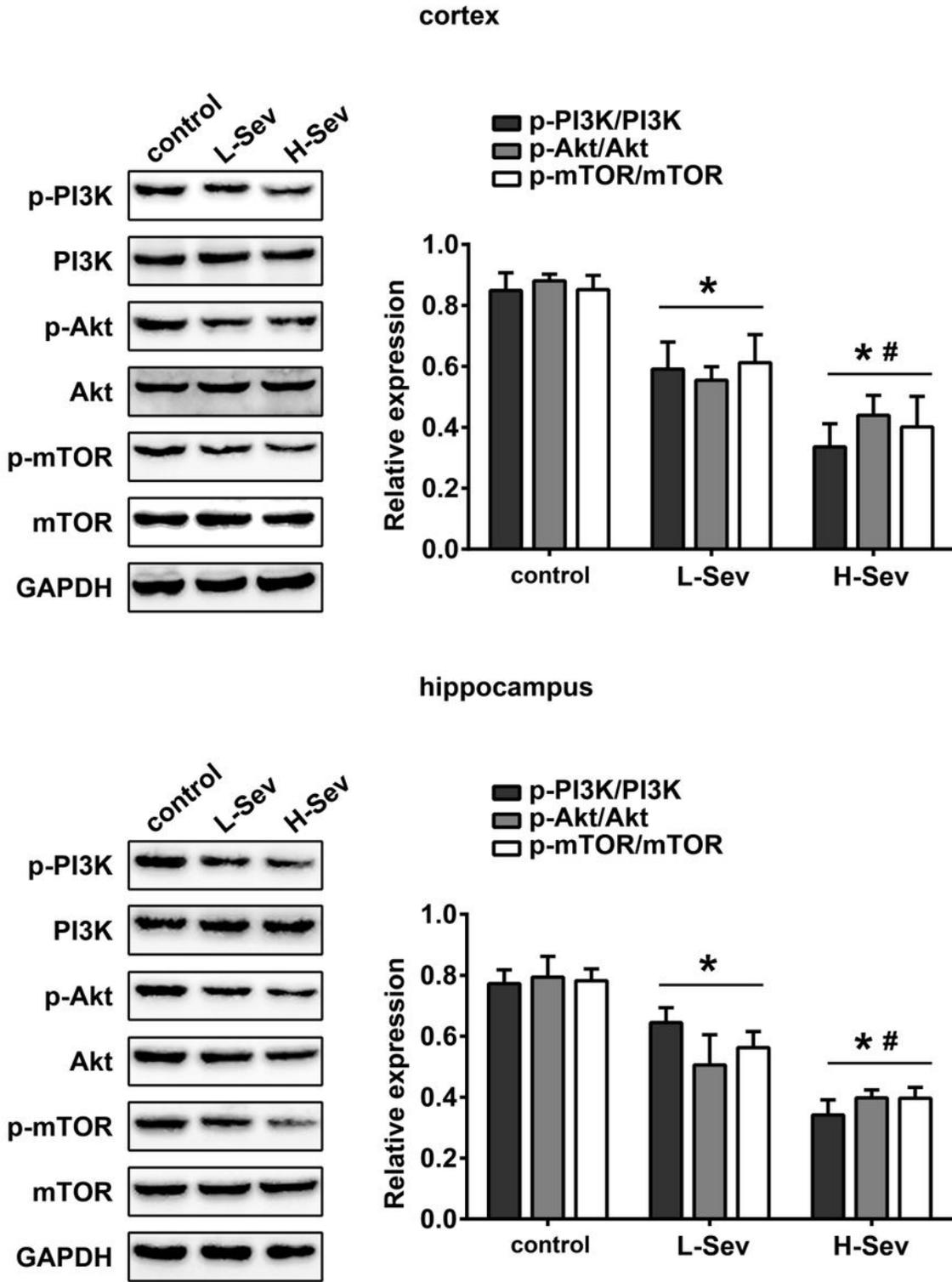
Figure 1

Sevoflurane treatment increases inflammatory markers in the hippocampus. Expression levels of IL-6, IL-8 and TNF- $\alpha$  in the cortex and hippocampus of rats in the control, L-Sev, and H-Sev groups were measured by



**Figure 2**

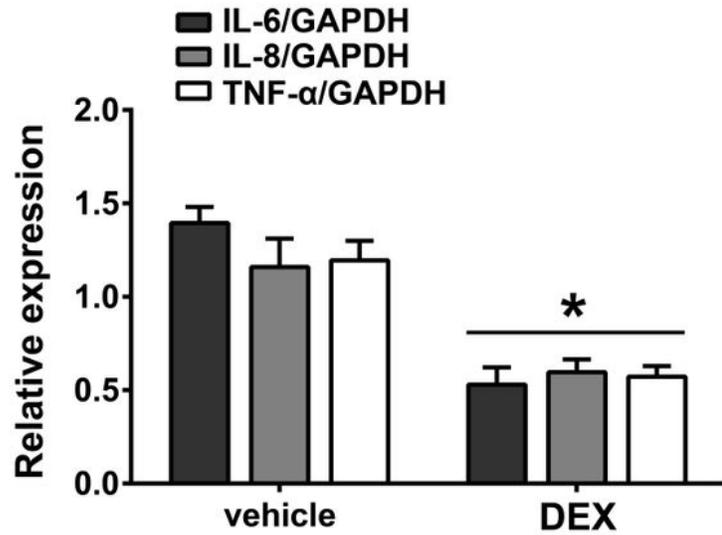
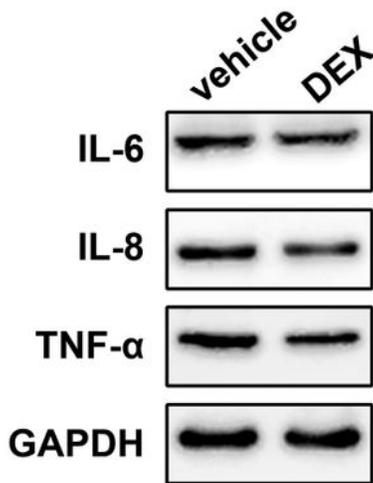
Immunofluorescence staining for PI3K/Akt/mTOR pathway components. PI3K, Akt, and mTOR are widely expressed in neurons and other non-neuronal cells in the cortex and hippocampus (magnification 200x).



**Figure 3**

Effect of dexmedetomidine on the PI3K/Akt/mTOR pathway Images and quantification of western blot measurements of PI3K, p-PI3K, Akt, p-Akt, mTOR and p-mTOR in the cortex and hippocampus of rats in the vehicle and DEX groups (mean  $\pm$  SD, n=5 per group, t test). \*p<0.05 vs vehicle group.

## cortex



## hippocampus

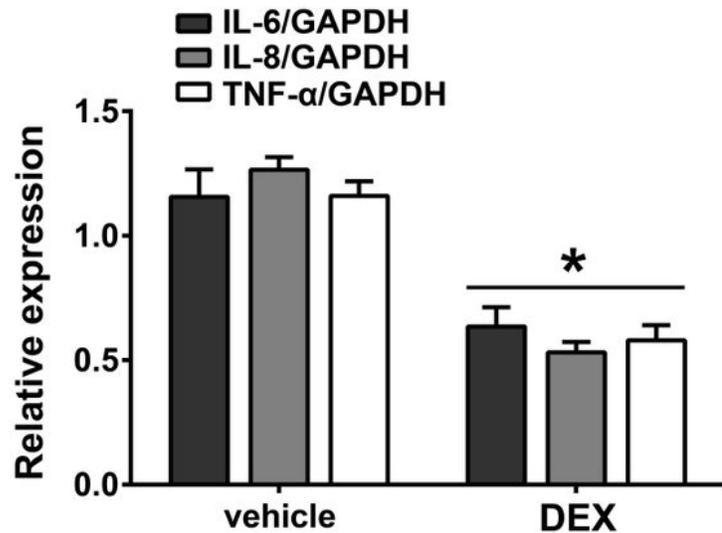
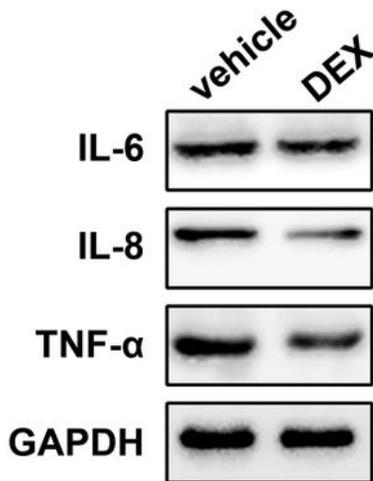
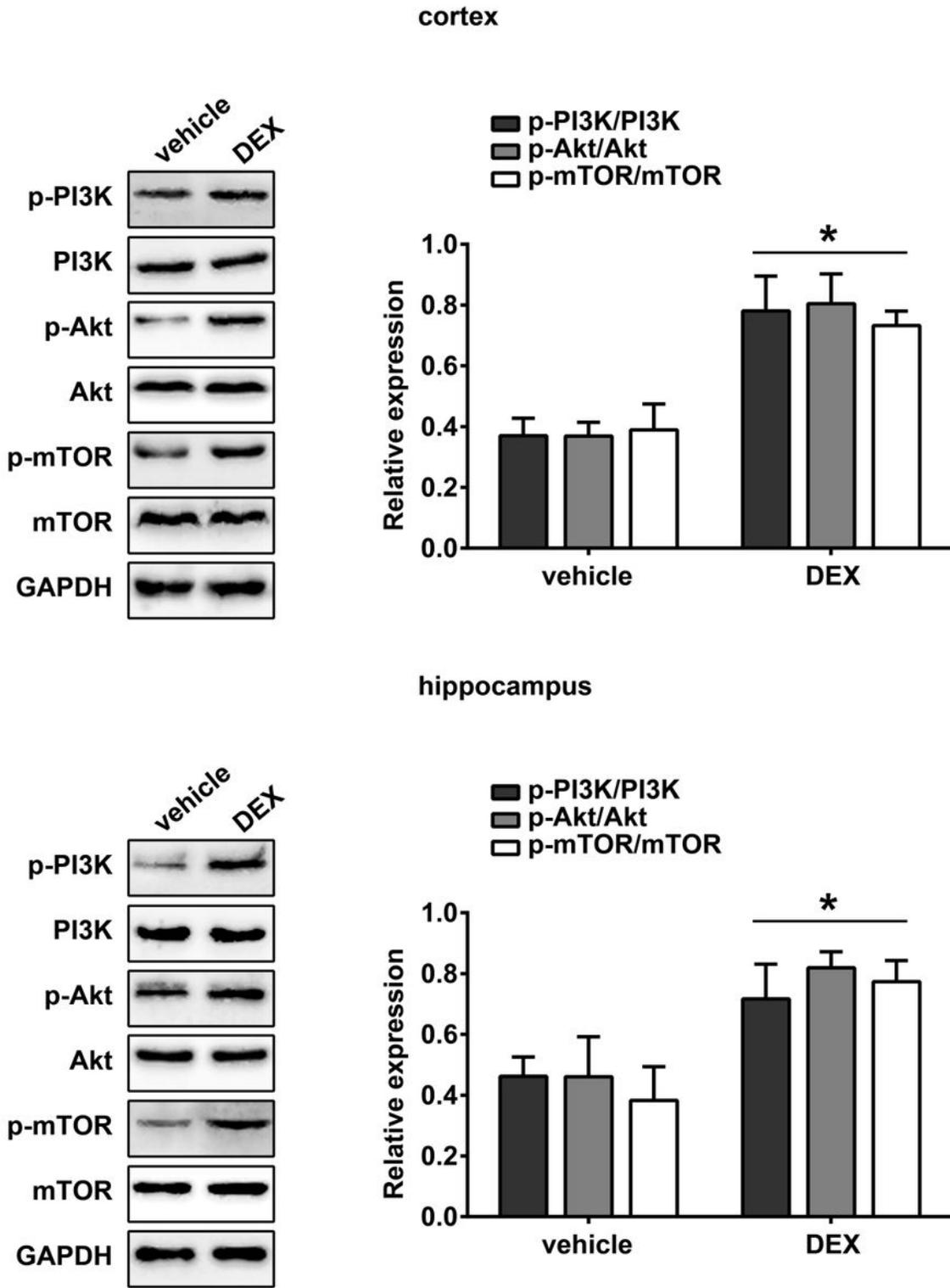


Figure 4

Effect of sevoflurane on PI3K/Akt/mTOR pathway activity. Cortical and hippocampal levels of PI3K, p-PI3K, Akt, p-Akt, mTOR and p-mTOR of rats in the control, L-Sev and H-Sev groups were measured by western blotting (mean  $\pm$  SD, n=5 per group, one-way ANOVA). \* $p < 0.05$  vs control group, # $p < 0.05$  vs L-Sev group.



**Figure 5**

Effect of dexmedetomidine on sevoflurane anesthesia-induced neuroinflammation Western blot measurements of IL-6, IL-8, and TNF- $\alpha$  from cortical and hippocampal samples of DEX and vehicle treated rats (mean  $\pm$  SD, n=5 per group, t test). \*p<0.05 vs vehicle group.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplement1.pdf](#)