Role of NR2B/ERK signaling in the neuroprotective effect of dexmedetomidine against sevoflurane induced neurological dysfunction in the developing rat brain

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Research

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Abstract

Background

Dexmedetomidine (DEX) is a new-generation, and has been widely applied in clinic. The present study confirmed the protective effect of DEX on sevoflurane induced learning and cognitive impairment and examined its underlying mechanism.

Methods

Sprague-Dawley rats were exposed to 0.85% sevoflurane for 6 h and injected with DEX in different dose. Morris water maze (MWM) test was performed to evaluate the learning and memory function of rats. Western blot was used for the measurement of protein levels.

Results

MWM results indicated that sevoflurane treatment increased the escape latency but reduced the time spent in original quadrant of rats. The protein levels of NR2B, phosphorylated ERK were significantly influenced by sevoflurane. Ifenprodil administration alleviated sevoflurane induced neurological impairment. DEX treatment reversed the effect of sevoflurane on both escape latency and time in original quadrant in a dose manner, and pretreatment with 75 μg/kg DEX had the most dramatic effect. DEX regulates the NR2B/ERK signaling in sevoflurane treated rats.

Conclusion

NR2B/ERK signaling is involved in sevoflurane induced neurological impairment. DEX may protect against sevoflurane induced neurological dysfunction in the developing rat brain via regulating the NR2B/ERK signaling.

Background

Sevoflurane is one of the most comment used inhalational anesthetics, which is characterized by rapid induction and recovery properities [1]. It is widely used in cesarean delivery and pediatric clinical application [2]. Sevoflurane has some neurotoxicity for the nervous system, especially for developing brain [3–5]. The animal experiments have proved that volatile anesthetics at clinical concentrations can lead to neuronal apoptosis and impair the learning ability and cognitive function in neonatal and aged rodents [6–8]. Previous retrospective studies have determined that application of anesthesia in children may influence the progression of behavioral and cognitive function, especially children younger than 3 years old [9–11]. As a result of the commonly used of sevoflurane in anesthetics application for

childbirth and surgeries, it is necessary to explore effective therapy to prevent anesthesia induced neurotoxicity.

Dexmedetomidine (DEX) is a new-generation, highly selective α2 adrenergic receptor agonist. It has been widely applied in clinic, as a result of its ability to provide sedation without risk of respiratory depression [12]. In recent years, DEX catches researchers' attention for its neuroprotective effects [13]. In a study of subarachnoid hemorrhage (SAH), DEX is determined to play a neuroprotective effect in the hippocampus of vasospastic SAH rabbits [14]. Additionally, DEX is also suggested to prevent against propofol exposure induced neurotoxicity in the fetal brains, and play neurocognitive protection in the offspring rats [15]. In recent years, with the deepening of clinical research on DEX, the drug is commonly used in patients undergoing general anesthesia [16]. Although DEX is previously suggested to have neuroprotective effect in various brain injury, it is still unknown whether DEX can directly reduce cognitive dysfunction induced by sevoflurane exposure, especially in developing brain. Notably, in the depression model of rats treated by chronic unpredictable mild stress (CUMS), DEX is suggested to protect against learning and memory impairment caused by electroconvulsive shock in depressed rats, with the involvement of the NMDA receptor subunit 2B (NR2B)-ERK signaling pathway [17]. According to the above results, we suspected that whether NR2B-ERK signaling is involved in the neuroprotective effect of DEX against sevoflurane induced cognitive dysfunction.

Therefore, the present study was designed and confirmed the protective effect of DEX on sevoflurane induced learning and cognitive impairment. Furthermore, the underlying mechanism of the neuroprotective effect of DEX against sevoflurane induced cognitive dysfunction was examined, particularly, involving the NR2B-ERK signaling pathway.

Materials And Methods

Animals and Ethic statement

Postnatal day 7 (P7) Sprague-Dawley rats were purchased from the Shanghai animal center. All rats were housed in an environment with a temperature of 20–25℃, a humidity of 40%-70%, a light/dark cycle of 12/12 h, and free access to food and water. The study design was conducted with approval of the Animal Ethics Committee of the Second Affiliated Hospital of Shandong First Medical University.

Animal grouping

In addition to the control group, rats in other groups were exposed to 0.85% sevoflurane for 6 h. 30 minutes before sevoflurane treatment, rats were injected with 25, 50, 75 μg/kg Dex or normal saline intraperitoneally.

All rats were randomly divided into 8 groups: 1) control group: rats received regular air inhalation for 6 h; 2) Sevo group: rats were exposed to 0.85% sevoflurane for 6 h; 3) Sevo + vehicle group, 30 minutes before sevoflurane treatment, rats were injected with 75 μg/kg Dex and 0.5 ml/kg vehicle (dimethylsulfoxide

(DMSO)), then exposed to 0.85% sevoflurane for 6 h; 4) Sevo + Ifen group, 30 minutes before sevoflurane treatment, rats were injected with 5 mg/kg ifenprodil (Ifen), then exposed to 0.85% sevoflurane for 6 h; 5) Sevo + NS group: 30 minutes before sevoflurane treatment, rats were injected with normal saline (NS), then exposed to 0.85% sevoflurane for 6 h; 6) Sevo + DEX50 group: 30 minutes before sevoflurane treatment, rats were injected with 50 μg/kg Dex, then exposed to 0.85% sevoflurane for 6 h; 7) Sevo + DEX75 group: 30 minutes before sevoflurane treatment, rats were injected with 75 μg/kg Dex, then exposed to 0.85% sevoflurane for 6 h. After experiment finished, rats were euthanized by decapitation, and the hippocampi tissues were collected for further experiments

Neurological function test

After experiment, a modified neurological severity score (mNSS) was performed to assess the neurological severity of rats in each group through evaluating the behavior and motor changes, including balance, touch, vision, abnormal behavior, sensation, and movement, according to Loga et al protocol [18]. The score ranged from 0 to 18, and 0 point indicated a normal brain system while 18 points indicated the most severe neurological dysfunction.

Morris water maze test

The P7 rats were fed until day 14 (P14), then their learning and memory function were evaluated by Morris water maze (MWM) test. The water maze consisted of a 120 cm circular pool with the depth of 50 cm filled with warm water. A hidden circular platform was placed 1.5 cm below the water surface. Each rat was placed in the maze from four random points of the tank, and released to find the hidden platform for 2 min. The time of rat taken to reach the hidden platform (latency) was recorded. If the rat did not reach the platform within 2 min, the rat was gently placed on the platform and left for 20 s. All rats were received training trials for four consecutive days. At the fifth day, the platform was removed from the circular toll and the probe trial was performed, the time spent in the original quadrant and escape latency were recorded.

Western blot

The total proteins were lysed by using RIPA lysates with protease and phosphatase inhibitors in ice, then the protein concentration was detected by the BCA protein assay kit. Then protein samples were mixed with a 10% SDS buffer in a 1:1 ratio and boiled at 95℃ for 5 min. The protein was then concentrated and separated by 10% Gel-electrophoresis, and the separated protein was transferred to the PVDF membrane. Followly, the PVDF membrane was sealed with a 5% BSA solution for 2 h, and the primary antibody was incubated at 4 ℃ overnight. After cleaning with TBST 3 times, the proteins were detected with antirabbit or anti-mouse secondary antibody for 2 h at room temperature for 1 h. Finally, the results are displayed by the imaging system.

Statistical analysis

All data were compared between groups by using student's t-test and one-way ANOVA analysis. All data analysis was carried out by using GraphPad Prism 7.0 software. The data was expressed as the mean ±

standard deviation (SD). *P* values less than 0.05 were considered as statistically significant.

Results

Sevoflurane impaired the neurological function in rats

As shown in the Fig. 1, the learning and memory function of rats were assessed by MWM test. During the training time, the escape latency of rats in each group decreased gradually. On the 4th day, the latency of rats in sevoflurane group was significantly higher than that in control group ($P < 0.001$, Fig. 1a). But there was no significant difference for swimming speed between the two groups ($P > 0.05$, Fig. 1b). At the fifth day, the learning and memory was evaluated by a probe trial. As shown in Fig. 1c-d, sevoflurane treatment increased the escape latency but reduced the time spent in original quadrant ($P < 0.001$). Consistent with the MWM test results, sevoflurane treatment significantly increased the neurological function score (Fig. 1e).

NR2B/ERK signaling is involved in sevoflurane induced neurological impairment in rats

NR2B/ERK signaling related proteins were detected via western bolt to investigate whether NR2B/ERK signaling is involved in sevoflurane induced neurological impairment. As shown in Fig. 2a, the protein levels of NR2B, phosphorylated ERK were significantly influenced by sevoflurane compared with the control group ($P < 0.001$). To further verify the role of NR2B/ERK signaling, ifenprodil, the NMDAR antagonist, was selected to inhibit the NR2B subunit. It was found that ifenprodil administration reversed the effect of sevoflurane on neurological impairment induced by sevoflurane. The MWM results indicated that during the training time, the escape latency of rats decreased gradually (Fig. 2b). The swimming speed showed no significant difference among different groups ($P > 0.05$, Fig. 2c). On the fourth day, ifenprodil administration decreased the escape latency of sevoflurane treated rats compared with the sevoflurane group ($P < 0.001$, Fig. 2b). According to the probe trial results, ifenprodil administration decreased the escape latency time but reduced the time spent in original quadrant of rats compared with sevoflurane group ($P < 0.001$, Fig. 2d-e). Moreover, ifenprodil administration also decreased the neurological function score significantly ($P < 0.001$, Fig. 2f).

Effect of DEX on sevoflurane induced neurological impairment in rats

Rats were given DEX with different concentration to explore the role of DEX in sevoflurane induced neurological impairment. According to the MWM results, the escape latency of rats in each group decreased gradually among different groups, and DEX treatment reduced the escape latency in a dose-dependent manner (Fig. 3a). And rats pretreated with 75 μg/kg DEX had the lowest escape latency time than other concentrations on the 4th day (Fig. 3a). But there was no significant difference for swimming

speed among different groups ($P > 0.05$, Fig. 3b). At the fifth day, the learning and memory was evaluated by a probe trial. The results indicated that DEX treatment reversed the effect of sevoflurane on both escape latency and time in original quadrant, and pretreatment with 75 μg/kg DEX had the most dramatic effect (Fig. 3c-d). Additionally, the neurological function score was also detected in different groups. Consistent with the MWM test results, sevoflurane treatment significantly increased the neurological function score, which was decreased by DEX pretreatment in a dose-dependent manner (Fig. 3e). It was concluded that DEX alleviated sevoflurane induced neurological impairment in rats.

DEX regulates the NR2B/ERK signaling in sevoflurane treated rats

To further investigate the mechanism of the protective effect of DEX against sevoflurane induced neurological impairment, NR2B/ERK signaling related proteins were detected via western bolt. As shown in Fig. 4, the protein levels of NR2B, phosphorylated ERK were significantly influenced by sevoflurane, but the effect was reversed by DEX treatment ($P < 0.001$).

Discussion

Sevoflurane is an inhalational anesthetics, which is commonly used in cesarean delivery and pediatric clinical application [2]. Previous evidence has suggested that sevoflurane exposure has certain harm on learning and cognitive functions, especially for children [5, 11]. In the current study, the P7 rats were recruited for animal experiments to investigate the role of sevoflurane in developing brain. It was found that sevoflurane exposure impaired the neurological function in rats. Additionally, western blot results suggested that sevoflurane treatment influenced the NR2B/ERK signaling related proteins. Furthermore, ifenprodil, NR2B subunit antagonist, administration reversed the effect of sevoflurane on neurological function in rats. It was concluded that NR2B/ERK signaling is involved in sevoflurane induced neurological impairment in rats.

The N-methyl-D-aspartate receptor (NMDAR) is a subtype of ionotropic glutamate receptors which mediate the neuronal plasticity and memory or learning function in the mammalian central nervous system [19]. It is observed that continued NMDAR activation can lead to neuronal injury [20]. Unbalance of NMDARs is widely suggested to be associated with the occurrence and development of various central nervous system diseases, such as ischemic stroke, Alzheimer disease, Parkinson disease and so on [19, 21]. NR2B is a subtype of NMDAR, which is associated with the over activation of NMDAR, followed by the neuronal damage [22]. NR2B overexpression can trigger cell apoptotic pathways, which may be the pathology of the neuroprotective effect of NR2B antagonists [23]. Notably NR2B activation is also closely associated with the activation of ERK signaling [24]. Moreover, NR2B-ERK signaling has been suggested to be involved in the regulation of neuronal survival and participate in the development of neurological diseases [25–27]. In the present study, sevoflurane treatment was suggested to increase the protein level of NR2B and decrease the level of phosphorylated ERK in the hippocampal tissues of rats, indicating that NR2B/ERK signaling might be involved in sevoflurane induced neurological impairment in rats.

Furthermore, ifenprodil, the NMDAR antagonist, was selected to inhibit the NR2B subunit, and further verify the role of NR2B/ERK signaling. As expect, ifenprodil administration was observed to alleviate sevoflurane induced leaning and memory dysfunction via MWM test. The results confirmed that sevoflurane exposure led to neurological impairment in rats through regulating NR2B/ERK signaling. Consistently, a study about chronic intermittent hypoxia-hypercapnia (CIHH) reported that the ameliorative cognitive deficits caused by lovastatin are due to the downregulation of NR2B expression and the increased expression of ERK signaling [28]. Besides, NR2B-ERK pathway is associated with the recall of morphine-associated contextual memory [29]. All evidences reveal the crucial role of NR2B-ERK signaling in learning and memory function, which supported our present results.

DEX is a useful anesthetic adjuvant, and has been widely used in clinical area. Several studies have reported that DEX regulates the memory formation in a dose-dependent [30]. Considering the neuroprotective role of DEX in various neurological diseases, the current study further explored the role of DEX in sevoflurane induced neurological impairment. The rats were given DEX with different concentration before sevoflurane exposure, and the neurological function was evaluated by MWM test. As expected, the results demonstrated that DEX pretreatment alleviated sevoflurane induced neurological impairment in rats in a dose-dependent manner, and pretreatment with 75 μg/kg DEX had the most dramatic effect. As previous described, DEX is demonstrated to protect against anesthesia-induced neurotoxicity by several studies [31], which was consistent with the present results. Notably, in a study about drug-resistant depression, DEX is suggested to protect against learning and memory impairments caused by electroconvulsive shock in depressed rats [17], these findings supported our present results about the protective role of DEX against sevoflurane induced neurological impairment. Additionally, as the previous study reported, DEX at dose 10 and 25 μg/kg showed neuroprotective effects, and the dose of 25 μg/kg was more effective than 10 μg/kg [17]. Therefore, the current study selected higher dose of 50 and 75 μg/kg, and DEX at a dose of 75 μg/kg had a more powerful neuroprotective effect. Moreover, NR2B-ERK signaling is also reported to participate in the neuroprotective role of DEX in depressed rats [17]. In consideration of the involvement of NR2B-ERK signaling in sevoflurane induced neurological impairment, we suspected that whether NR2B-ERK signaling is involved in the neuroprotective effect of DEX in sevoflurane treated rats. It was found that DEX regulates the NR2B/ERK signaling in sevoflurane treated rats, we concluded that DEX protects against sevoflurane induced neurological dysfunction in the developing rat brain via regulating the NR2B/ERK signaling. However, other studies are needed to verify the present results, and thorough studies should be done for the mechanism exploration.

In conclusion, the present results demonstrated that NR2B/ERK signaling was involved in sevoflurane induced neurological impairment. DEX might protect against sevoflurane induced neurological dysfunction in the developing rat brain via regulating the NR2B/ERK signaling.

Abbreviations

DEX
Dexmedetomidine;

MWM
Morris water maze;
SAH
subarachnoid hemorrhage;
CUMS
chronic unpredictable mild stress;
P7
Postnatal day 7;
DMSO
dimethylsulfoxide;
mNSS
modified neurological severity score;
SD
standard deviation;
NMDAR
N-methyl-D-aspartate receptor;
CIHH
chronic intermittent hypoxia-hypercapnia

Declarations

Ethics approval and consent to participate.

The study design was conducted with approval of the Animal Ethics Committee of the Second Affiliated Hospital of Shandong First Medical University.

Consent for publication.

Not applicable.

Availability of data and materials.

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

Competing interests.

The authors declare that they have no competing interests.

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Authors' contributions.

GL, YJ, FC, DW, and LZ conceived and designed the study and drafted the manuscript. GL, YJ, and LZ collected the clinical data, interpreted them and revised the manuscript. All authors read and approved the final manuscript.

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Not applicable.

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Figures

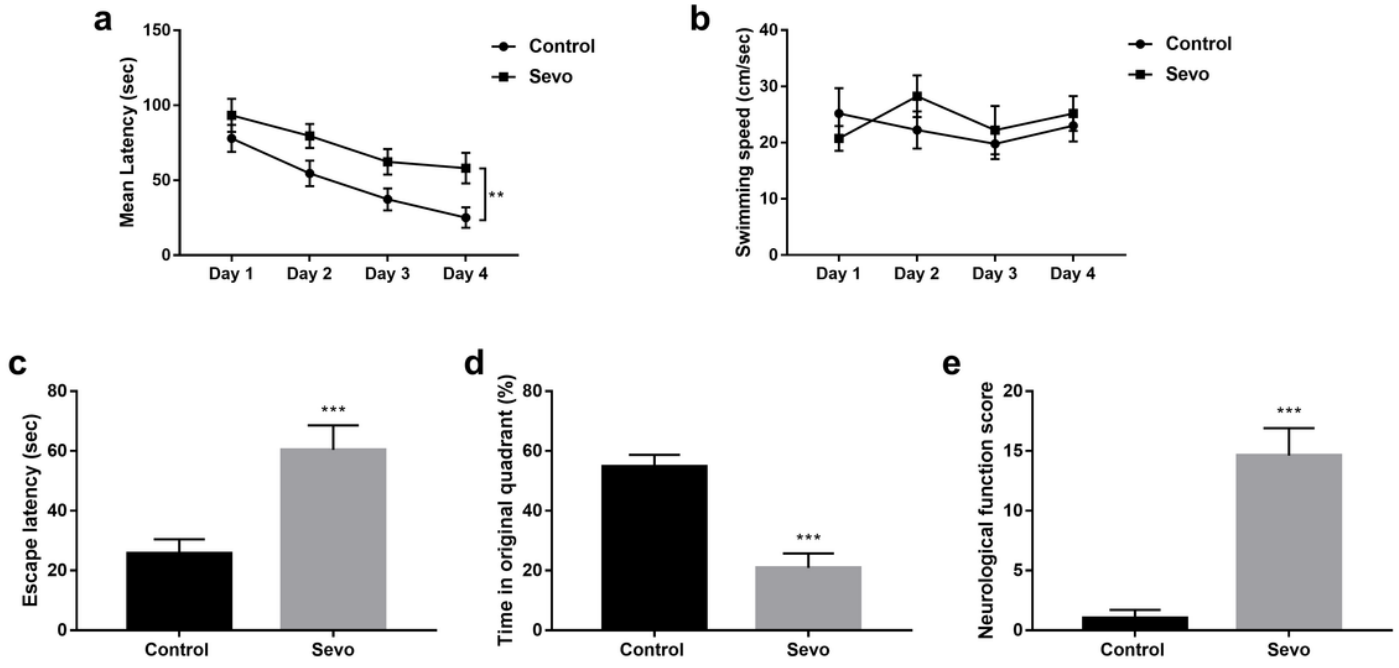

Figure 1

Sevoflurane impaired the neurological function in rats. a. During the training time, the escape latency of rats in each group decreased gradually, and the latency of rats in sevoflurane group was significantly higher than that in control group on the 4th day. b. There was no significant difference for swimming speed between the two groups. c-d. Sevoflurane treatment increased the escape latency but reduced the time spent in original quadrant. e. Sevoflurane treatment significantly increased the neurological function score. ** P < 0.01; *** P < 0.001.

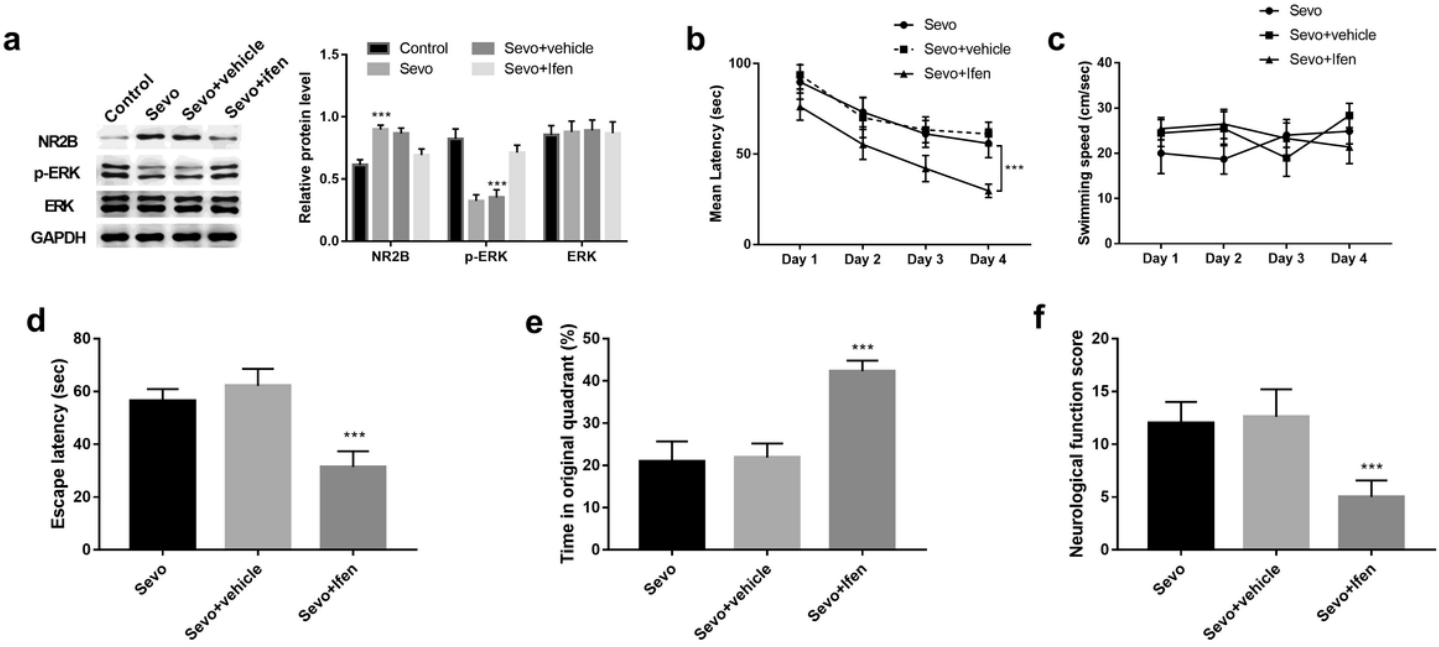

Figure 2

The role of NR2B/ERK signaling in sevoflurane induced neurological impairment in rats. a. The protein levels of NR2B, phosphorylated ERK were significantly influenced by sevoflurane compared with the control group, but the influences were reversed by ifenprodil administration. b. during the training time, the escape latency of rats decreased gradually, and on the fourth day, ifenprodil administration decreased the escape latency of sevoflurane treated rats compared with the sevoflurane group. c. The swimming speed showed no significant difference among different groups. d-e. On the fifth day, ifenprodil administration decreased the escape latency time but reduced the time spent in original quadrant of rats compared with sevoflurane group. f. ifenprodil administration also decreased the neurological function score significantly. *** P < 0.001.

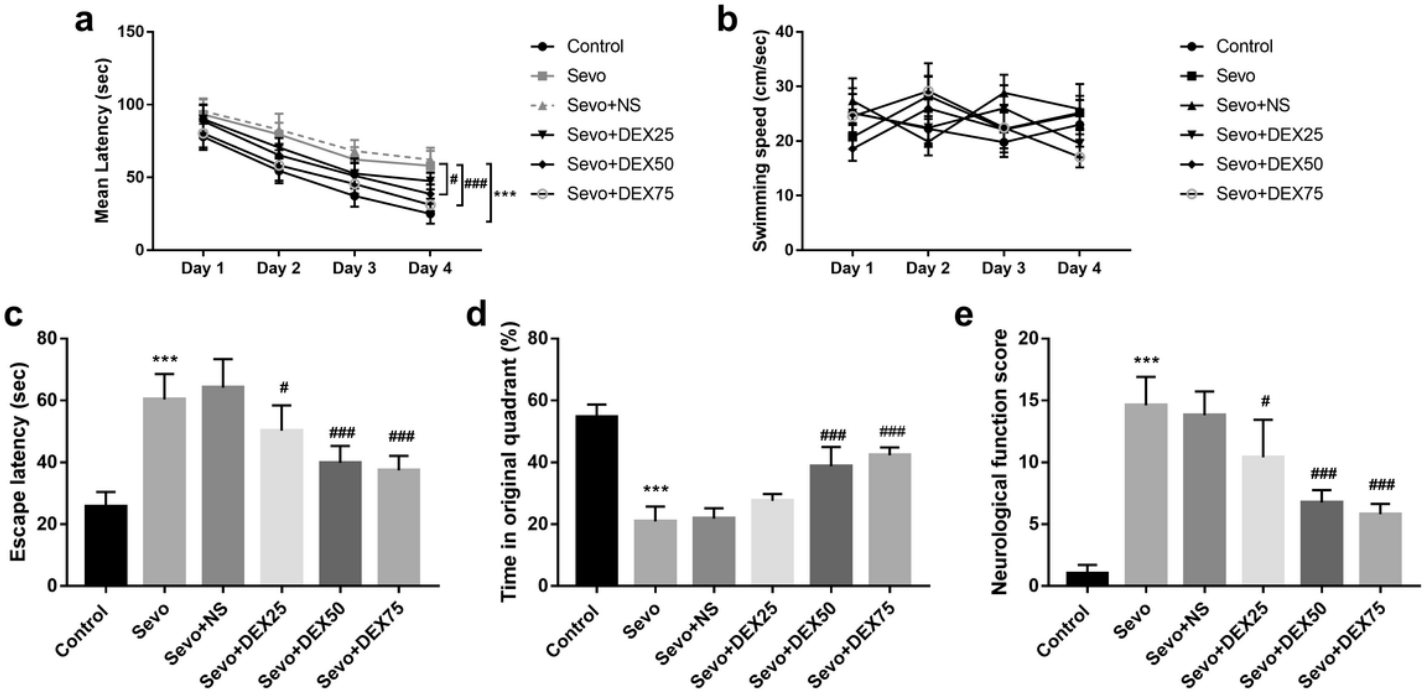

Figure 3

Effect of DEX on sevoflurane induced neurological impairment in rats. a. the escape latency of rats in each group decreased gradually among different groups, and DEX treatment reduced the escape latency in a dose-dependent manner. b. There was no significant difference for swimming speed among different groups. c-d. DEX treatment reversed the effect of sevoflurane on both escape latency and time in original quadrant, and pretreatment with 75μg/kg DEX had the most dramatic effect. e. Sevoflurane treatment significantly increased the neurological function score, which was decreased by DEX pretreatment in a dose-dependent manner. *** P < 0.001, compared with control group; # P < 0.01, ### P < 0.001, compared with sevoflurane group.

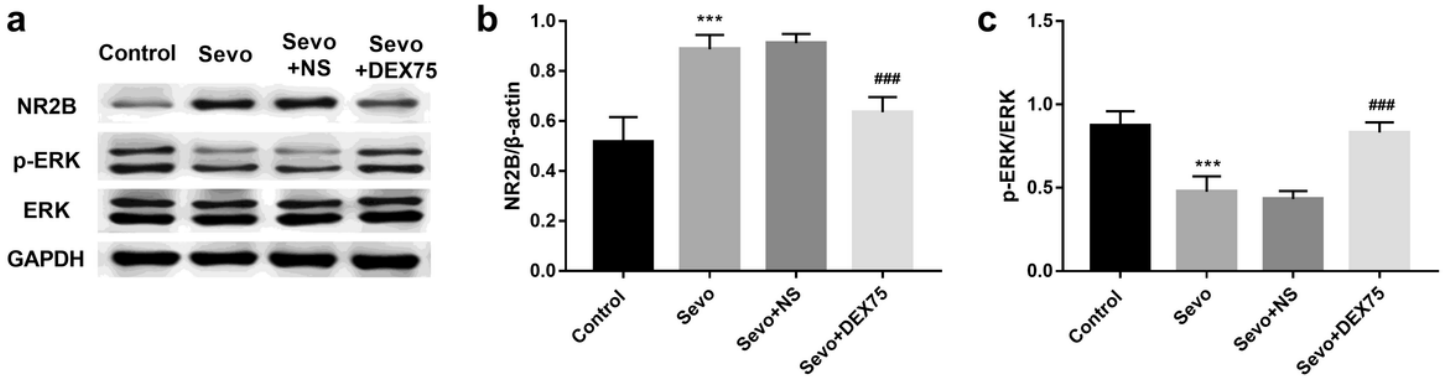

Figure 4

DEX regulates the NR2B/ERK signaling in sevoflurane treated rats. a. Western blot results. b. Sevoflurane treatment upregulated the protein level of NR2B, which was reduced by pretreatment with 75μg/kg DEX. c. phosphorylated ERK level was decreased by sevoflurane treatment, which was increased by pretreatment with 75μg/kg DEX. *** $P < 0.001$, compared with control group; ### $P < 0.001$, compared with sevoflurane group.