

Original Article

Dexmedetomidine alleviated isoflurane-induced neurotoxicity in aged rats

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Abstracts: Backgrounds: Age is a major risk factor for Alzheimer's disease. Previous studies have shown that patients with Alzheimer's syndrome suffered greater risk when undergoing general anesthesia. As an inhalation anesthetic, isoflurane (ISO) has a convenient, rapid induction effect, and is suitable for induction and maintenance of general anesthesia. Dexmedetomidine (DEX) can be combined with anesthetics to improve the anesthetic effect. Methods and Results: In this study, 24-month old rats were randomly divided into three groups and were given intraperitoneal injection of saline, ISO combined with DEX, or ISO, respectively. Subsequently, rats performed the water maze test, and Golgi staining, RT-PCR, and Western blot analysis were performed to evaluate neuronal damage caused by ISO and the alleviating action caused by DEX. The results show that, when compared with the C group, learning and memory abilities in the ISO group were reduced, and levels of the amyloid precursor protein (App), cAMP-response element binding protein (CREB), and brain-derived neurotrophic factor (BDNF) decreased, whereas levels of β -amyloid increased. No significant differences were observed with the ISO + DEX group. Conclusion: DEX can improve the activity of the CREB pathway, inhibit the degradation of APP, and alleviate nerve damage caused by ISO.

Keywords: Alzheimer's disease, anesthetic, isoflurane, dexmedetomidine, CREB pathway

Introduction

Alzheimer's disease (AD) is a type of dementia in which patients develop progressive loss of nervous system cells. The main features include harmful changes in direction, identification, judgment and personality. Age is the main risk factor of the development of AD. United Nations (UN) digital standards indicate that an age of 60 is critical [1]. The report estimated that in the United States individuals diagnosed with AD over the age of 65 years old involves 5.3 million cases, which is 25-fold higher when compared to individuals younger than 65 [2]. Administration of anesthesia is a significant risk factor of the development of AD. Studies have shown that an increased incidence of elderly patients undergoing anesthesia, increased the risk of developing AD [3, 4]. Several studies have shown that beta-amyloid protein (β -amyloid) plays an important role in the pathogenesis of AD [5, 6]. However, APP is a precursor protein of A β , indicating that the neurotoxicity caused by β -amyloid aggregation re-

quired the participation of enzymatic hydrolysis of APP. In addition, APP knockout mice show abnormal development, synaptic function, and learning and memory function [7]. Cleavage of APP by the aspartyl protease beta-site APP-cleaving enzyme (BACE) is an important step [8]. BACE-1 is a key aspartase that forms a myelin sheath in peripheral nerve cells. It is a transmembrane protein that contains two active sites and can form two dimers outside the cell. β -amyloid is usually formed by the activity of several enzymes, including BACE-1. Clinical trials have demonstrated that most patients with AD show elevated levels of BACE-1 expression, resulting in an increased β -amyloid production [9].

ISO is a commonly used clinical anesthetic and its advantages include its fast-acting capacity, with little induced irritability, and it stabilizes the hemodynamic status of patients. Recent evidence has suggested that ISO can damage the development of neurons and cause cognitive dysfunction in a dose-and time-

Table 1. Primer pairs of genes

| Genes | Primer pairs | |
|--------|--------------|----------------------|
| APP | Forward | GCCTCTGGATCTTCACTTGG |
| | Reverse | GTCTGGGCATAAGTGCCAAT |
| BACE-1 | Forward | CTGGTCCAAGAGGATTTCCA |
| | Reverse | TCATTGCCTTGACGTAGAG |
| CREB | Forward | AATTGCCTCCACACCTTCAC |
| | Reverse | TCACCAAGCTGCTCATCAAC |
| BDNF | Forward | AGACCAGTCTGTGGCTGAT |
| | Reverse | GCGGTCTTTGACGTAGGAAG |
| GAPDH | Forward | AGGCCGAGAATGGGAAGCTT |
| | Reverse | TGGAGTCTACTGGTGTCTTC |

dependent manner [10]. DEX, a α_2 adrenergic agonist, is an anesthetic and sedative that has been reported to have neuroprotective effects [11].

Learning and memory abilities depend on structures within the medial temporal lobe, including the hippocampal region [12]. The development of neurons in the hippocampus is closely related to the learning and memory abilities of the developing brain. DEX increases expression of BDNF astrocytes by extracellular signal-regulated kinase-dependent pathways, and induces subsequent neuroprotective effects [13]. BDNF has also been shown to trigger fast action potentials that affect the excitability of neurons. Furthermore, BDNF has a significant effect on activity-dependent synaptic plasticity events, including long-term enhancement, learning, and memory [14].

In this study, we investigated the mechanism of cognitive impairment induced by general anesthesia in aged rats by evaluating the levels of APP, BACE-1, CREB and BDNF in ISO- and DEX-anesthetized aged rats.

Materials and methods

Animals

Wistar rats, 24 weeks old, weighing 250 ± 40 g, were used in this study. All experiments were performed in accordance with the ethical committee.

Drug administration

A total of 36 Wistar rats were divided into three groups ($n=12$ per group): control group (C), ISO

and DEX group (ISO + DEX), and an ISO group (ISO). Rats in the ISO + DEX and ISO groups were exposed to 1.5% ISO and 100% oxygen for a duration of 4 h, whereas rats in the C group were exposed to a similar amount of saline and received 100% oxygen only. The dose of DEX used was 15 $\mu\text{g}/\text{kg}$, and was administered via intramuscular injection.

Morris water maze task

The Morris water maze task included a hidden platform trial and a probe trial. To evaluate the learning and memory ability in aged rats that received ISO with or without DEX, navigation trials and spatial probe tests were used. Specific experimental methods are showed in the references section [15].

Sample collections

After completion of the learning and memory test, rats were euthanized through dislocation of the cervical spine, and the brain was collected. The hippocampus was separated, and immediately frozen in liquid nitrogen.

Golgi staining

A Golgi-Cox staining was performed to evaluate the hippocampal dendritic spine density and was conducted with the FD Rapid Golgi Stain™ Kit (pk401, FD Neuro Technologies, Inc.) following the manufacturer's instructions.

Real-time PCR and western blot analysis

Total mRNA was extracted from Hippocampus using the RNA out reagent (Beijing Tiandz, Inc., China) according to the manufacturer's instructions. The first cDNA strand was synthesized using Oligo (dT) primers and transcript reverse transcriptase (Beijing TransGen Biotech Co. Ltd., China). The primers for real-time amplification of relative cDNAs were designed using Oligo 7.22 and oligo 6.0, primer pairs as shown in **Table 1**. Protein extracts were subjected to SDS-polyacrylamide gel electrophoresis under reducing conditions on 15% gels. Primary antibodies against APP, BACE-1, β -Amyloid, CREB and BDNF proteins were 1:1000 dilution. To verify equal sample loading, membranes were incubated with a monoclonal GAPDH antibody, followed by an HRP-

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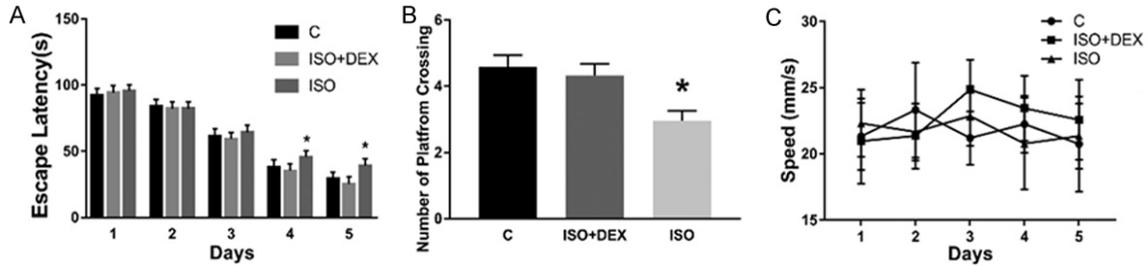


Figure 1. Behavioral experiment results of aged rats treated with ISO and/or DEX. *indicates significant difference compared with the C group.

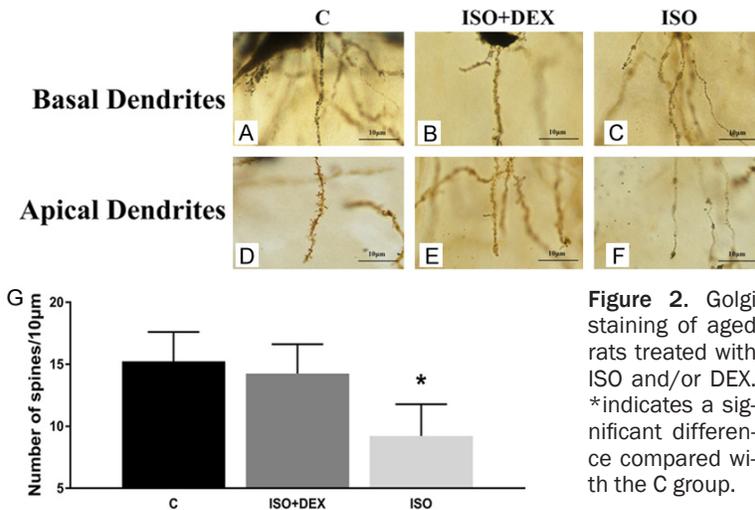


Figure 2. Golgi staining of aged rats treated with ISO and/or DEX. *indicates a significant difference compared with the C group.

Statistical analysis

Statistical parameters in this study were calculated using GraphPad Prism 6.2. Data were analyzed using one-way analysis of variance. Values are expressed as mean \pm S.D. $P < 0.05$ was considered significant.

Results

DEX alleviates cognitive impairment of ISO in aged rats

As shown in **Figure 1**, in the Morris water maze task, all rats could find the platform, and no differences were observed between rats in the ISO + DEX group and the C group. On Day 4 and Day 5, rats in the ISO group were slower in finding the platform when compared to rats in the C group, and this difference was significant ($P < 0.05$). In the space probe test, the number of platform crossings in the ISO group on Day 5 was significantly lower when compared to that in the C group. Moreover, no significant differences were observed in the swimming speed of the animals between rats in the C, ISO + DEX and ISO groups.

Golgi staining

As shown in **Figure 2**, when compared with the C group, the density of the hippocampal den-

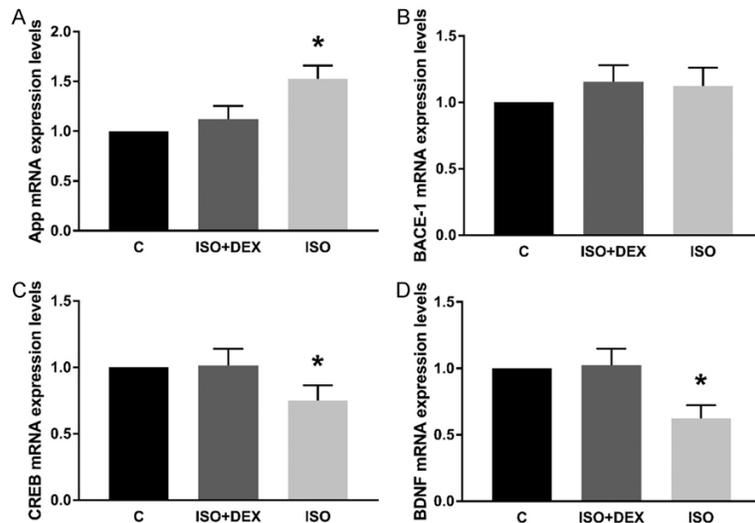


Figure 3. The mRNA expression of APP, BACE-1, CREB and BDNF in hippocampus. *indicates a significant difference compared with the C group.

conjugated goat anti-mouse IgG (1:1000, Santa Cruz Biotechnology, USA).

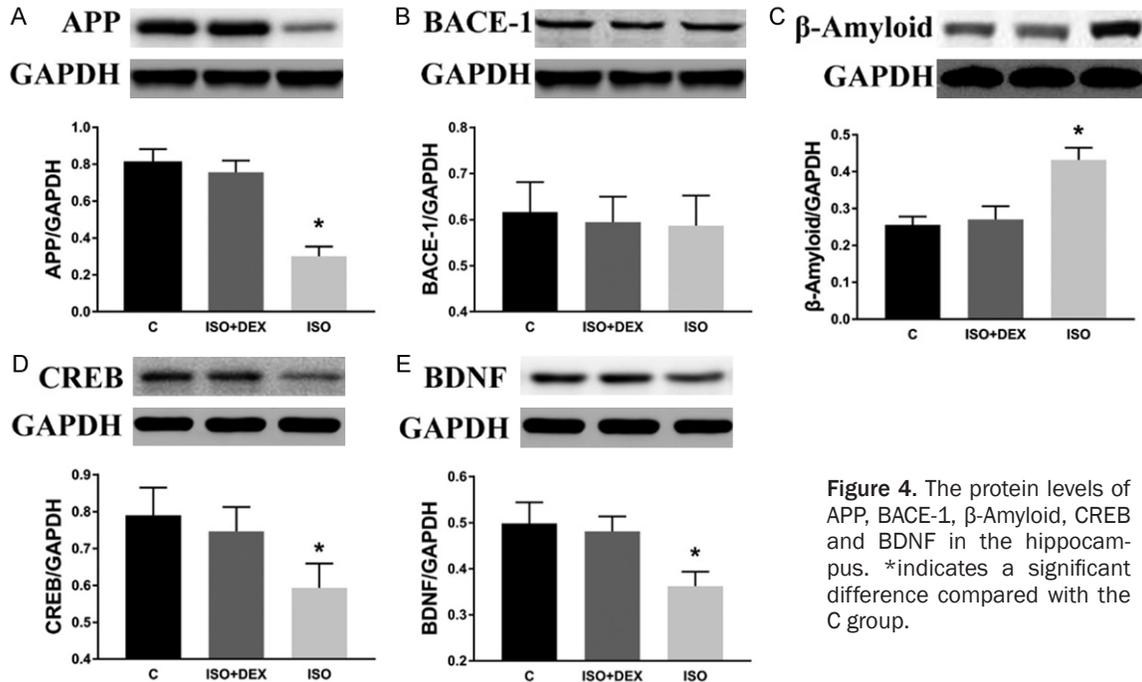


Figure 4. The protein levels of APP, BACE-1, β -Amyloid, CREB and BDNF in the hippocampus. *indicates a significant difference compared with the C group.

driftic spine decreased by 39.6% in the ISO group ($P < 0.05$).

Expression of mRNAs in the hippocampus

Figure 3 shows that, when compared with the C group, App levels increased to 152.2% in the ISO group, whereas CREB and BDNF levels decreased by 25.8% and 37.6%, respectively, in the ISO group. No significantly changes were observed in the ISO + DEX group, and no differences BACE-1 levels were observed among the groups.

The expression of proteins in hippocampus

As shown in **Figure 4**, when compared with the C group, App, CREB and BDNF levels decreased to 37.2%, 75.1% and 72.7%, respectively. In addition, protein levels of β -amyloid increased to 169% in the ISO group when compared with the C group. Moreover, no significant changes were observed in the ISO + DEX group, and no differences in BACE-1 levels were observed among the groups.

Discussion

In this study, aged rats were subjected to anesthesia and subjected to different tests. The Morris water maze task showed that although all aged rats could eventually find the platform,

rats in ISO group were slower in finding the platform when compared with the C group. No significant differences were found between the ISO + DEX group and the C group, indicating that, DEX had a positive effect on spatial learning and memory ability of the aged rats. In the collected hippocampal tissue, Golgi staining showed that the number of dendritic ridges in ISO treated rats was reduced when compared with the C group, which was consistent with the decrease of CREB and BDNF protein expression levels. In this study, β -amyloid protein increased and APP decreased, while no significant changes were observed in BACE-1 levels. Similar to our findings, the use of ISO anesthesia in rats at 14 days of gestation can cause significant obstructive behavior in the offspring [16]. Other narcotic drugs, such as ketamine, can cause similar damage, resulting in a reduction in rat learning and their memory ability [15, 17]. In general, anesthetics can play a dual role in neuroprotection and neurotoxicity, and is related to the dose, mode of use, and exposure time. In this study, aged rats were exposed to 1.5% of ISO for 4 h, which resulted in a significant impairment in learning and memory. These findings are different from the results reported by Li et al. [18]. The key factor may be the difference in dose of ISO used. In this study, no significant changes were observed in the ISO + DEX group when compared with the C group,

indicating that the damage caused by ISO was weakened by DEX. It has been reported that DEX relieved damage caused by bupivacaine [19]. Pancaro et al. hypothesized that ketamine caused significant cellular degeneration and apoptosis in limbic brain regions when compared with DEX treatment [20].

In the water maze experiment, no significant differences in swimming speed were observed between groups, indicating that the motor function of rats in each group was not affected. These findings also indicate that exposure to ISO and DEX did not cause anxiety in aged rats. Although the rats in each group learned how to use the water maze, the rate of learning in the ISO group was significantly slower, and there was a significant difference between Day 4 and Day 5 when the ISO group was compared with the C group. Moreover, the spatial probe test indicated that the number of times the ISO-treated rats passed through the platform area, after this was removed, was significantly reduced, which was consistent with the findings reported by Wang et al. [21]. Interestingly, no significant changes were observed in the ISO + DEX group when compared to the C group. A study performed by Si et al. indicated that DEX stimulated the JAK2/STAT3 signaling pathway and protected spatial learning and memory impairment of isolemic mice when mice were aged, which was consistent with the results of our study [22].

Exposure to ISO can lead to inactivation of CREB transcription [23]. Through Golgi staining, we showed that the number of dendrites in ISO-exposed rats was significantly reduced, whereas the number of dendritic ridges in the ISO + DEX group was not different from that of rats in the C group. The number of dendrites directly reflected the ability to conduct signals between nerve cells, and CREB phosphorylation is very important in the process of producing new dendrites [24]. DEX can effectively phosphorylate CREB, thereby improving learning and memory disorders in aged rats [25, 26]. Studies have shown that DEX alleviated propofol-induced hippocampal neuronal damage and increased CREB and BDNF expression [27, 28].

During aging, APP plays an important role in maintaining synaptic function, and APP has been extensively studied in AD. It has previously

been reported that ISO caused decomposition of APP and accelerated the process of senile dementia-related neurodegenerative diseases [3]. In this study, we demonstrate that mRNA expression of APP in rats that received ISO alone was significantly higher, however its protein content was significantly lower compared to the other two groups, which may be due to decomposition of APP caused by ISO. The mRNA and protein expression of APP was inconsistent. Moreover, the relation between mRNA and protein levels involved many factors, for example, to maintain the balance of intracellular protein expression, cells are self-regulating [29]. Further, APP protein is easily produced by both the non-amyloidogenic and amyloidogenic pathway and processed into downstream products [30]. Our data suggest that aged rats exposed to 1.5% ISO caused degradation of APP and induced overexpression of β -amyloid, which was attenuated by DEX. Expression of mRNA and protein levels of BACE-1 did not change, which was in line with the findings presented by Liu et al. [31]. In this study, although no changes were observed in the expression level of BACE-1, we speculate that BACE-1 degraded APP to β -amyloid by amyloidosis, however this requires further experimental validation.

In summary, DEX prevented degradation of APP to β -amyloid, and improved the level of CREB and BDNF, to effectively alleviate ISO-caused nerve damage to aged rats.

Disclosure of conflict of interest

None.

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References

- [1] Organization WH. MDS: definition of an older person. 2006.
- [2] Association AS. 2015 Alzheimer's disease facts and figures. *Alzheimers Dement* 2015; 11: 332-84.
- [3] Planel E, Bretteville A, Liu L, Virag L, Du AL, Yu WH, Dickson DW, Whittington RA and Duff KE. Acceleration and persistence of neurofibrillary pathology in a mouse model of tauopathy fol-

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- lowing anesthesia. *FASEB J* 2009; 23: 2595-604.
- [4] Xie Z, Dong Y, Maeda U, Alfille P, Culley DJ, Crosby G and Tanzi RE. The common inhalation anesthetic isoflurane induces apoptosis and increases amyloid β protein levels. *Anesthesiology* 2006; 104: 988-94.
- [5] Selkoe DJ and Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med* 2016; 8: 595-608.
- [6] Magara F, Müller U, Li ZW, Lipp HP, Weissmann C, Staglar M and Wolfer DP. Genetic background changes the pattern of forebrain commissure defects in transgenic mice underexpressing the β -amyloid-precursor protein. *Proc Natl Acad Sci U S A* 1999; 96: 4656-4661.
- [7] Senechal Y, Kelly PH, Dev KK. Amyloid precursor protein knockout mice show age-dependent deficits in passive avoidance learning. *Behav Brain Res* 2008; 186: 126-32.
- [8] Cole SL and Vassar R. BACE1 structure and function in health and Alzheimer's disease. *Curr Alzheimer Res* 2008; 5: 100-20.
- [9] Ruderisch N, Schlatter D and Kuglstatter A. Potent and selective BACE-1 peptide inhibitors lower brain $a\beta$ levels mediated by brain shuttle transport. *Ebiomedicine* 2017; 24: 76-92.
- [10] Luo F, Yan H, Zhao W, Zuo Z, Qi Y, Liu Z, Lin J, Feng Y, Li B and Wu L. Maternal exposure of rats to isoflurane during late pregnancy impairs spatial learning and memory in the offspring by up-regulating the expression of histone deacetylase 2. *PLoS One* 2016; 11: e0160826.
- [11] Kim SE, Ko IG, Kim CJ, Chung JY, Yi JW, Choi JH, Jang MS and Han JH. Dexmedetomidine promotes the recovery of the field excitatory postsynaptic potentials (fEPSPs) in rat hippocampal slices exposed to oxygen-glucose deprivation. *Neurosci Lett* 2016; 631: 91-96.
- [12] Squire LR, Stark CE and Clark RE. The medial temporal lobe. *Annu Rev Neurosci* 2004; 27: 279-306.
- [13] Degos V, Charpentier TL, Chhor V, Brissaud O, Lebon S, Schwendimann L, Bednareck N, Passemard S, Mantz J and Gressens P. Neuroprotective effects of dexmedetomidine against glutamate agonist-induced neuronal cell death are related to increased astrocyte brain-derived neurotrophic factor expression. *Anesthesiology* 2013; 118: 1123-32.
- [14] Nacmias B, Piccini C, Bagnoli S, Tedde A, Cellini E, Bracco L and Sorbi S. Brain-derived neurotrophic factor, apolipoprotein E genetic variants and cognitive performance in Alzheimer's disease. *Neurosci Lett* 2004; 367: 379-83.
- [15] Li X, Guo C, Li Y, Li L, Wang Y, Zhang Y, Li Y, Chen Y, Liu W and Gao L. Ketamine administered pregnant rats impair learning and memory in offspring via the CREB pathway. *Oncotarget* 2017; 8: 32433-32449.
- [16] Palanisamy A, Baxter MG, Keel PK, Xie Z, Crosby G and Culley DJ. Rats exposed to isoflurane in utero during early gestation are behaviorally abnormal as adults. *Anesthesiology* 2011; 114: 521-8.
- [17] Li Y, Li X, Cen G, Li L, Wang Y, Zhang Y, Yu C, Liu W and Li G. Long-term neurocognitive dysfunction in offspring via NGF/ERK/CREB signaling pathway caused by ketamine exposure during the second trimester of pregnancy in rats. *Oncotarget* 2017; 8: 30956-30970.
- [18] Li Y, Liang G, Wang S, Meng Q, Wang Q and Wei H. Effects of fetal exposure to isoflurane on postnatal memory and learning in rats. *Neuropharmacology* 2007; 53: 942-950.
- [19] Memari E, Hosseinian MA, Mirkheshti A, Arhami-Dolatabadi A, Mirabotalebi M, Khandaghy M, Daneshbod Y, Alizadeh L and Shirian S. Comparison of histopathological effects of perineural administration of bupivacaine and bupivacaine-dexmedetomidine in rat sciatic nerve. *Exp Toxicol Pathol* 2016; 68: 559-564.
- [20] Pancaro C, Segal BS, Sikes RW, Almeer Z, Schumann R, Azocar R and Marchand JE. Dexmedetomidine and ketamine show distinct patterns of cell degeneration and apoptosis in the developing rat neonatal brain. *J Matern Fetal Neonatal Med* 2016; 29: 3827-33.
- [21] Wang HL, Ma RH, Fang H, Xue ZG and Liao QW. Impaired spatial learning memory after isoflurane anesthesia or appendectomy in aged mice is associated with microglia activation. *J Cell Death* 2015; 8: 9-19.
- [22] Si Y, Zhang Y, Han L, Chen L, Xu Y, Sun F, Ji M, Yang J and Bao H. Dexmedetomidine acts via the JAK2/STAT3 pathway to attenuate isoflurane-induced neurocognitive deficits in senile mice. *PLoS One* 2016; 11: e0164763.
- [23] Sen T and Sen N. Isoflurane-induced inactivation of CREB through histone deacetylase 4 is responsible for cognitive impairment in developing brain. *Neurobiol Dis* 2016; 96: 12-21.
- [24] Bourtchuladze R, Frenguelli B, Blendy J, Cioffi D, Schutz G and Silva AJ. Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell* 1994; 79: 59-68.
- [25] Yan M, Dai H, Ding T, Dai A, Zhang F, Yu L, Chen G and Chen Z. Effects of dexmedetomidine on the release of glial cell line-derived neurotrophic factor from rat astrocyte cells. *Neurochem Int* 2011; 58: 549-57.
- [26] Gao X, Zhuang FZ, Qin SJ, Zhou L, Wang Y, Shen QF, Li M, Villarreal M, Benefield L and Gu SL. Dexmedetomidine protects against learning and memory impairments caused by electroconvulsive shock in depressed rats: involve-

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- ment of the NMDA receptor subunit 2B (NR2B)-ERK signaling pathway. *Psychiatry Res* 2016; 243: 446-452.
- [27] Wei Y, Hu J, Liang Y, Zhong Y, He D, Qin Y, Li L, Chen J, Xiao Q and Xie Y. Dexmedetomidine pretreatment attenuates propofol-induced neurotoxicity in neuronal cultures from the rat hippocampus. *Mol Med Rep* 2016; 14: 3413-20.
- [28] Lv J, Ou W, Zou XH, Yao Y and Wu JL. Effect of dexmedetomidine on hippocampal neuron development and BDNF-TrkB signal expression in neonatal rats. *Neuropsychiatr Dis Treat* 2016; 12: 3153-3159.
- [29] de Sousa Abreu R, Penalva LO, Marcotte EM and Vogel C. Global signatures of protein and mRNA expression levels. *Mol Biosyst* 2009; 5: 1512-26.
- [30] Xu X. Gamma-secretase catalyzes sequential cleavages of the Aβ₄₂ transmembrane domain. *J Alzheimers Dis* 2009; 16: 211-24.
- [31] Liu W, Xu J, Wang H, Xu C, Ji C, Wang Y, Feng C, Zhang X, Xu Z and Wu A. Isoflurane-induced spatial memory impairment by a mechanism independent of amyloid-beta levels and tau protein phosphorylation changes in aged rats. *Neurol Res* 2012; 34: 3-10.