

Isovitexin restores sevoflurane-induced cognitive dysfunction by mediating autophagy through activation of the PGC-1 α /FNDC5 signaling pathway

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Postoperative cognitive dysfunction is a severe neurological complication. How to improve the cognitive impairment caused by sevoflurane, a most common inhaled anesthetic, remains a question worthy of studying. Isovitexin (IVX) is a trihydroxyl flavonoid that is a naturally bioactive ingredient found in various medicinal plants and has antioxidant, anti-inflammatory and neuroprotective properties. The role of IVX in anesthetic-induced nerve injury is rarely reported and the mechanisms are unclear. In this study, we found that IVX improved the cognitive dysfunction induced by sevoflurane in rats. It inhibited sevoflurane-induced cell apoptosis. In addition, IVX increased sevoflurane-induced autophagy in rat brain. Mechanically, IVX activated the PGC-1 α /FNDC5 pathway in rat brain, and depletion of FNDC5 could inhibit the neuroprotective function of IVX. In conclusion, our results suggested that IVX restored sevoflurane-induced cognitive dysfunction by mediating autophagy through the activation of the PGC-1 α /FNDC5 pathway.

Key words: postoperative cognitive dysfunction, isovitexin, sevoflurane, autophagy, PGC-1 α /FNDC5 pathway

INTRODUCTION

Postoperative cognitive dysfunction (POCD) is a severe neurological complication characterized by subtle declines in memory, attention, and information processing speed following anesthesia and surgery, and is more common in older adults (Li et al., 2020). Sevoflurane is the most common inhaled anesthetic in surgery (Li et al., 2021). A growing number of studies have found that sevoflurane-induced cytotoxicity may contribute to early cognitive impairment in humans and animals (Smucny et al., 2021). The strategy of reducing sevoflurane-induced cognitive impairments remains unresolved and requires further study.

Sevoflurane-induced stress can lead to neuroinflammation, neuronal apoptosis, oxidative stress and abnormal protein deposition, resulting in cognitive impairment (Huang et al., 2021). In addition, autophagy

plays an important role in the prevention of neurodegenerative diseases (Hahm et al., 2021). Several studies have shown that the occurrence and progression of neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease, are associated with autophagy dysfunction. Appropriate autophagy may also protect against anaesthetic-induced neurotoxicity. Previous studies have demonstrated that promoting SIRT1-regulated autophagy can restore sevoflurane-induced cognitive dysfunction in mice (Zhang et al., 2021). Rapamycin ameliorates sevoflurane-induced cognitive dysfunction in elderly rats by regulating autophagy via TLR4/MyD88/NF- κ B signaling pathway (Wang et al., 2021). Screening of effective drugs to inhibit the cognitive impairment caused by sevoflurane remains a top priority (Zhao et al., 2021).

Isovitexin (IVX) is a trihydroxyl flavonoid that is a naturally bioactive ingredient found in various me-

dicinal plants and has antioxidant, anti-inflammatory and neuroprotective properties (Duong et al., 2008). For example, IVX improved SEV 25-35 peptide induced neuronal toxicity in an in vitro model. IVX could also induce autophagy in AD rat models, thus alleviating neurotoxicity via PI3K/Akt/mTOR pathway (Zhou et al., 1997). However, the role of IVX in anesthetic-induced nerve injury is rarely reported and the mechanisms are unclear. Therefore, we hypothesized that IVX could induce the activation of PGC-1 α /FNDC5 pathway and ameliorate anaesthetic induced cognitive dysfunction by regulating autophagy.

In this study, we found that IVX activates the PGC-1 α /FNDC5 pathway, reduces neuronal apoptosis and improves sevoflurane-induced cognitive impairment by regulating autophagy. Our study confirms IVX as a potential treatment for POCD.

METHODS

Experimental animals and group assignment

Male Sprague-Dawley rats (30 rats about 2 months, 260–280 g) were bought from the Animal Laboratory Center of China Medical University. The research project was performed with the guidance of Guide for the Care and Use of Laboratory Animals. All animal experiments were approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University. Rats were housed in sterile atmosphere at the temperature 24 \pm 2°C and 50% humidity. Rats had free access to standard pelleted chow and drinking water. The rats were randomly divided into 5 groups: 1) sham group; 2) SEV group: Rats were anesthetized with 2% sevoflurane for 5 h; 3) SEV+IVX (20 mg/kg), rats were pretreated with IVX with 20mg/kg before anesthesia for 5 h in 2% sevoflurane; 4) SEV+IVX (40 mg/kg), rats were pretreated with IVX with 40 mg/kg before anesthesia for 5 h in 2% sevoflurane; 5) SEV+IVX (80 mg/kg), rats were pretreated with IVX with 80 mg/kg before anesthesia for 5 h in 2% sevoflurane. IVX was obtained from Sigma-Aldrich. IVX suspended in water containing 12% Tween 80 (from day 1 to day 21) was administrated *via* intragastric intubation.

Morris water maze (MWM) tests

The MWM tests were carried out in a swimming pool with four quadrants in two diagonals to examine spatial learning and memory 3 weeks after indicated treatment. The water maze was set in a dim room,

and rats were immersed in water and forced to find the submerged platform in water. Before the experiment, the rats were trained for 5 consecutive days to find the location of the platform. Maze was divided into 4 quadrants and rats were forced to finish swim in the four quadrants of MWM during training. Rats were placed in the center of one of the three quadrants without the hidden platform. At the end of each swim, rats were towel dried and rested for 30 min. The time with a maximum of 60 s was set for each mouse to find the platform. The time of rats to find the platform was recorded. After the final training, the platform was removed and the time and number that rat reached the platform were recorded. Then, the speed of swimming and escape latency were recorded. The average of 4 results of the experimental result for each animal was recorded.

TUNEL assay

We detected apoptotic cell proportion in SEV-induced cognitive impaired rats through the TUNEL assays. Cell apoptosis was detected with TUNEL assay kit in accordance with its instruction (Roche Diagnostics). Briefly, 5- μ m paraffin embedded sections were dewaxed, permeabilized and sealed. The sections were incubated with 50 μ l TUNEL reaction solution in a wet dark box at 37°C for 1 h. Nuclei were stained with DAPI and observed under a fluorescence microscope. After taking images, the number of total nuclei and TUNEL-positive nuclei were analyzed.

Immunoblot assay

We detected the expression of several proteins *via* immunoblot in SEV-induced cognitive impaired rats. Proteins were extracted with RIPA buffer (Beyotime). The BCA assay method was used for protein concentration determination, after which proteins were separated (20 μ g per lane) by 10% SDS-PAGE, and transferred onto PVDF membranes, followed by blocked with 5% fat-free milk in TBST buffer. Subsequently, membranes were conjugated with primary antibodies targeting Bax (1:1000, Abcam, Cambridge, UK), Bcl-2 (1:1000, Abcam), cleaved caspase-3 (1:1000, Abcam), LC3 (1:1000, Abcam), p62 (1:1000, Abcam), beclin 1 (1:1000, Abcam), PGC-1 α (1:1000, Abcam), FNDC5 (1:1000, Abcam), BDNF (1:1000, Abcam), and beta-actin (1:10000, Abcam) for 2 h at room temperature. Subsequently the membranes were incubated with specific secondary antibodies at room temperature for 1 h. The blots were analyzed with ECL kit.

FND5 knockdown

FND5 shRNA lentivector and the scrambled control vector plus VSV-G, pRSV-REV, and pMDLg/pRRE packaging plasmids were co-transfected in HEK293T cells. The cells were transfected using 10 μ l Lipofectamine[®] 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) in each well. Cells were cultured for 4 h with Lipofectamine[®]/plasmid mix at 37°C and the transfection was completed. Subsequent assays were performed after 24 h. Viral particles were collected after transfection for 36, 48, and 60 h from the supernatants. Then, the virus was purified with PEG8000 and diluted to 10⁹ transducing units per ml. FND5 shRNAs knockdown yield over 70% knockdown efficiency with the following sequences:

5'-GCCATCTCTCAGCAGAAGAAGGATGTGCG-3';

5'-CTGGAGGAGGACACAGAATATATCGTCCA-3';

FND5 shRNA lentivirus or scramble shRNA lentivirus (6 \times 10⁸ transducing units per rats) were delivered by IP injection.

Statistical analysis

Data was displayed as mean \pm SD. Statistical analysis was performed using GraphPad. One-way ANOVA followed by Dunnett's *post hoc* test was used for multiple comparisons was used. $p < 0.05$ was considered as statistically significant.

RESULTS

IVX reduces sevoflurane-induced cognitive impairment in aged rats

To detect the effect of IVX in SEV-induced cognitive impaired rats, we performed the Morris water maze (MWM) tests in different groups. As displayed in Fig. 1A the cognitive ability in SEV group was significantly impaired. IVX treatment improved the performance of rats in MWM test in a dose-dependent manner (Fig. 1A, B, C, D). The time spent in the target quadrant, target zone transitions and distance covered in the target quadrant were reduced in SEV group. IVX treatment improved the performance in MWM (Fig. 1C, D, E). These results indicated the impaired cognitive ability can be improved by IVX treatment.

IVX alleviates SEV-induced neuron apoptosis

Then brain tissues were subjected to apoptosis detection. We noticed the increased apoptotic cell proportion in SEV-induced cognitive impaired rats. After IVX treatment, the cell apoptosis was significantly reduced (Fig. 2A). The levels of Bax and cleaved caspase-3 were examined by Immunoblot. The levels of Bax and cleaved caspase-3 in the SEV treated group were higher than control group while Bcl-2 was reduced (Fig. 2B). However, Bax and cleaved caspase-3

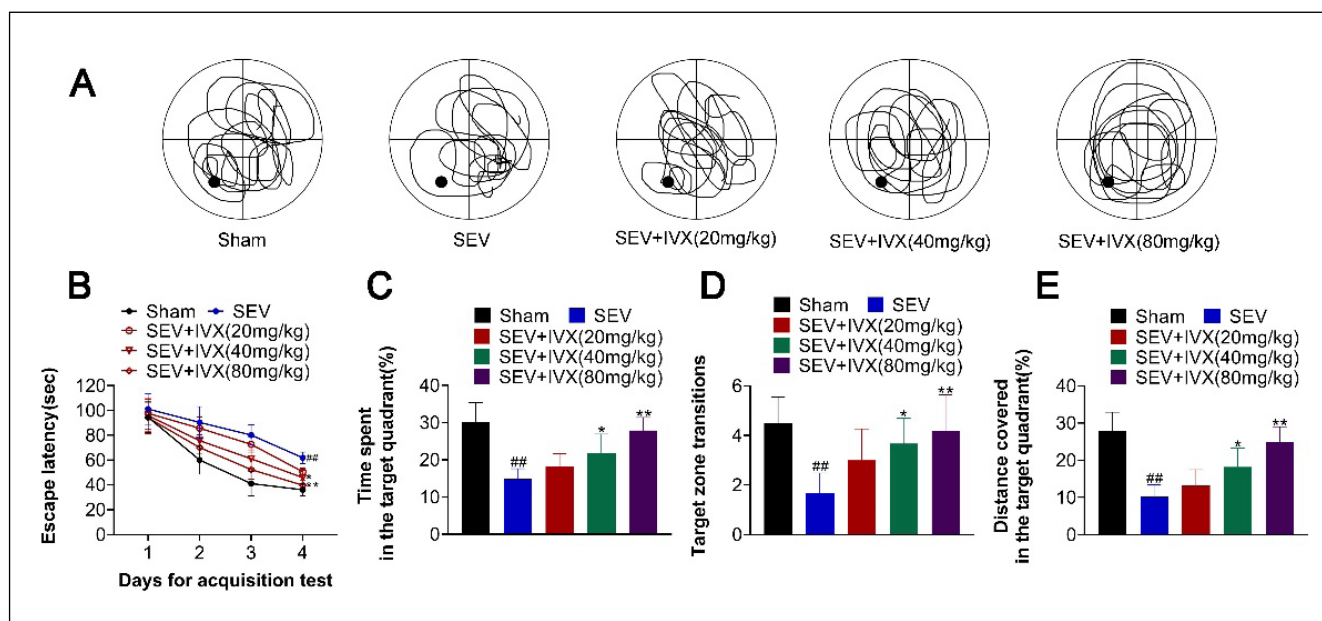


Fig. 1. IVX reduces sevoflurane-induced cognitive impairment in aged rats. (A) The MWM assay in different groups. (B, C, D) The escape latency, time spent in the target quadrant, target zone transitions and distance covered in the target quadrant in different groups. ##, $p < 0.01$ vs. sham, *, $p < 0.05$, **, $p < 0.01$ vs. SEV.

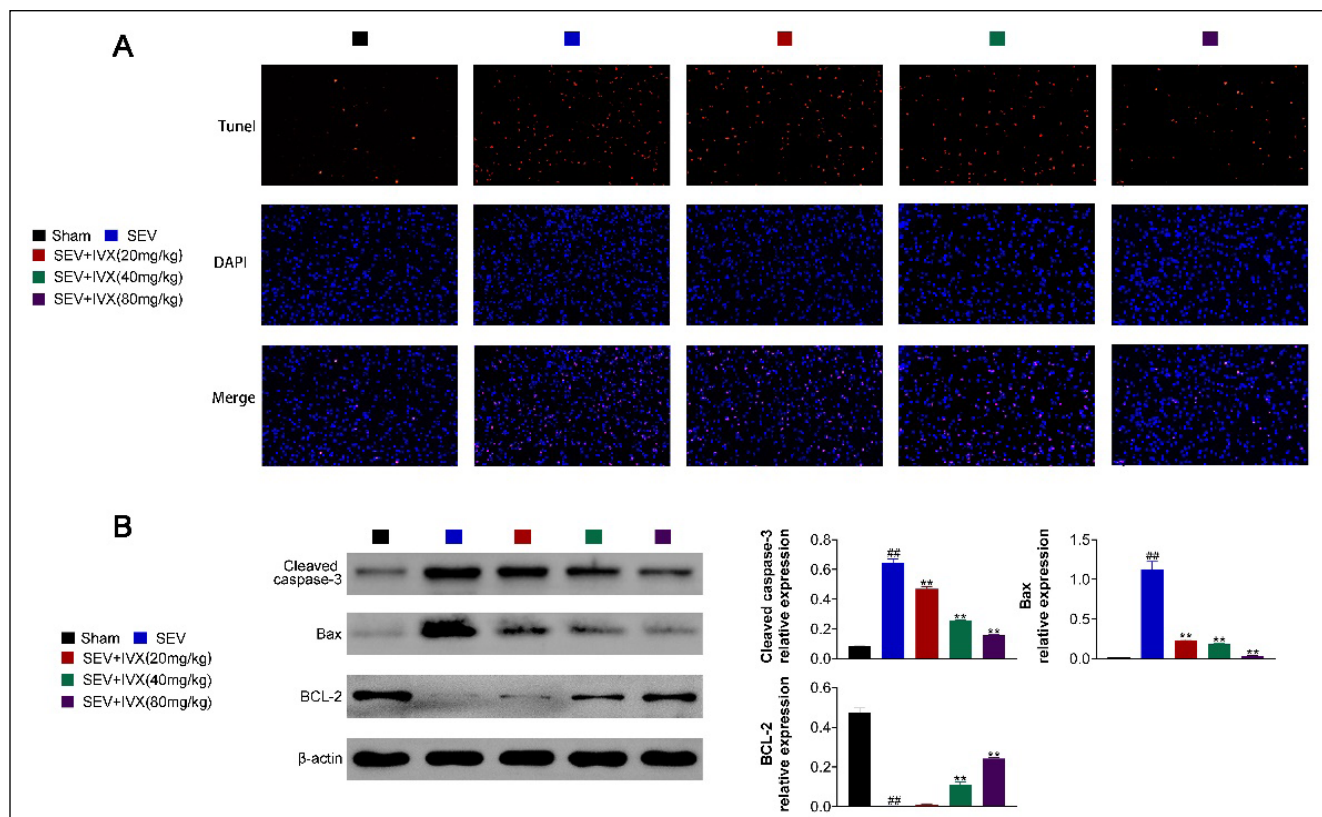


Fig. 2. IVX alleviates SEV-induced neuron apoptosis. (A) TUNEL assay in different groups. (B) The level of cleaved caspase-3, Bax, and Bcl-2 in different groups. ##, $p < 0.01$ vs. sham, **, $p < 0.01$ vs. SEV.

were reduced and Bcl-2 was markedly enhanced in IVX-treated rats compared with SEV-treated group (Fig. 2B). Therefore, we thought IVX could alleviate SEV-induced neuron apoptosis.

LC3II and Beclin 1 were reduced and p62 was increased in SEV-induced rats, whereas the level of LC3II and Beclin 1 were enhanced and p62 was reduced by IVX treatment (Fig. 3). These data implied that IVX could elevate autophagy in SEV-induced rat models.

IVX enhances SEV-induced autophagy

To measure the autophagy in SEV-induced cognitive impairment rat, the levels of LC3II and Beclin 1 were examined and the results indicated that the levels of

IVX activates PGC-1 α /FNDC5 symptoms

To delineate the related mechanism in IVX related symptom, we detected the PGC-1 α /FNDC5 pathway in

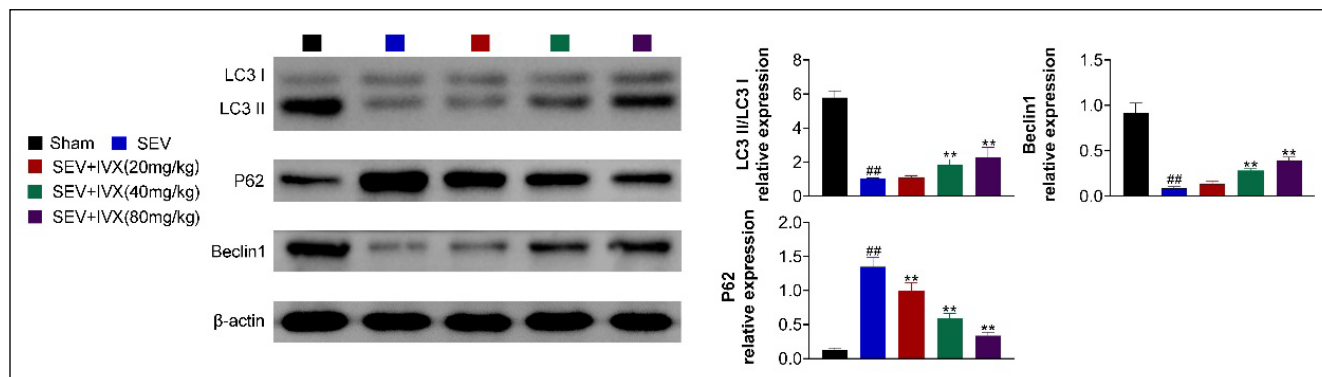


Fig. 3. IVX enhances SEV-induced autophagy. The level of LC 3 II, p62, and beclin 1 in different groups. ##, $p < 0.01$ vs. sham, **, $p < 0.01$ vs. SEV.

this animal model. The levels of PGC-1 α , FNDC5 and BDNF were significantly reduced in SEV-induced rats. IVX treatment markedly enhanced PGC-1 α , FNDC5 and BDNF level after treatment with SEV (Fig. 4). Therefore, these data confirmed that IVX could activate PGC-1 α /FNDC5-mediated symptoms.

IVX alleviates SEV-induced cognitive injury by PGC-1 α /FNDC5 pathway

To uncover the potential mechanism of SEV-mediated cognitive damage, the role of PGC-1 α /FNDC5 pathway was assessed in each group. IVX treatment-induced cognitive ability improvement was inhibited by FNDC5 knockdown in rats (Fig. 5A, B). Moreover, the cell apoptosis relieved by IVX was reversed by FNDC5 ablation (Fig. 5C). The enhanced cell autophagy in IVX group was weakened by FNDC5 depletion (Fig. 5D). These data suggested that FNDC5 ablation mediates IVX induced cognitive improvement.

DISCUSSION

Sevoflurane is an inhaled anesthetic commonly used in surgery. Several studies have demonstrated the association between sevoflurane and cognitive dysfunction (Bahr et al., 2021). Cognitive dysfunction has also become an important reason to limit the clinical application of sevoflurane (Yang et al., 2021). To more effectively improve the symptoms of cognitive impairment, effective drugs need to be found. Sevoflurane-induced stress can lead to neuroinflammation, neuronal apoptosis, oxidative stress and abnormal protein deposition, resulting in cognitive impairment (Tian et al., 2018; Mao et al., 2021). In this study, we found that IVX can improve anaesthetic induced cognitive dysfunction by regulating autophagy. We further investigated the possible mechanisms.

IVX has a variety of biological activities, including anti-tumor, antioxidant, anti-inflammatory and neuroprotective effects (Duong et al., 2008). IVX induces AMPK/PGC-1 α activation and changes the polarity of glial cells (Gravandi et al., 2021). IVX could also inhibit tumor growth in human colon cancer cells (Zhu et al., 2021). It also suppressed the stemness of lung cancer stem-like cells via the blocking AMPK pathway and inhibition of glycolysis (Lv et al., 2016). Similarly, here we also showed the effects of IVX on the autophagy of brain in rats upon the treatment of sevoflurane. Our study further confirmed that IVX activated PGC-1 α /FNDC5 pathway, reduced neuronal apoptosis and improved sevoflurane-induced cognitive impairment by regulating autophagy.

IVX induced AMPK/PGC-1 α activation and altered glial polarity (Babaei et al., 2021). Activated PGC-1 α can activate downstream FNDC5/BDNF expression, thus preventing senile cognitive impairment caused by aging (Azimi et al., 2018). More importantly, PGC-1 α /FNDC5 is also involved in the regulation of autophagy (Zhao et al., 2020). We hypothesized that IVX could induce the activation of PGC-1 α /FNDC5 pathway and improve anesthetic-induced cognitive dysfunction by regulating autophagy (Belviranli et al., 2018). Interestingly, our results confirmed this hypothesis. Our data and these findings therefore confirmed that PGC-1 α pathway could serve as a target for the treatment of cognitive dysfunction.

It was noticed that FNDC5 affected the cognitive dysfunction in different disease models. High-fat diet induced obesity regulates the PGC-1 α /FNDC5/BDNF axis to exacerbate IVX flurane-induced cognitive dysfunction in older mice (Hu et al., 2018). Importantly, IVX induces AMPK/PGC-1 α activation and changes the polarity of glial cells (Gravandi et al., 2021). In this study, we also found that IVX prevented sevoflurane-induced cognitive impairment via targeting FUNDC5, and next we should confirm whether IVX affects the expression of BDNF, and explore the possible mechanisms.

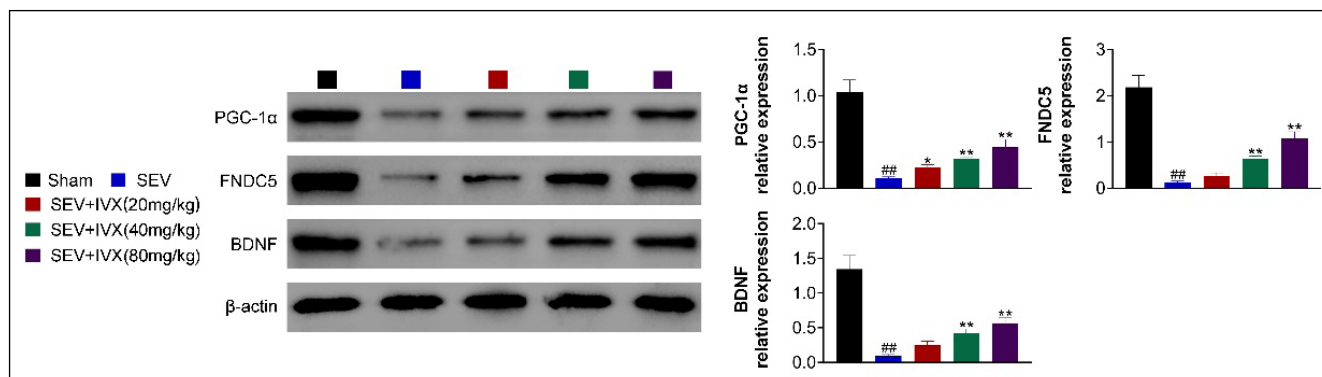


Fig. 4. IVX activates PGC-1 α /FNDC5 symptoms. The level of PGC-1 α , FNDC5 and BDNF in different groups. ##, $p < 0.01$ vs. sham, *, $p < 0.05$, **, $p < 0.01$ vs. SEV.

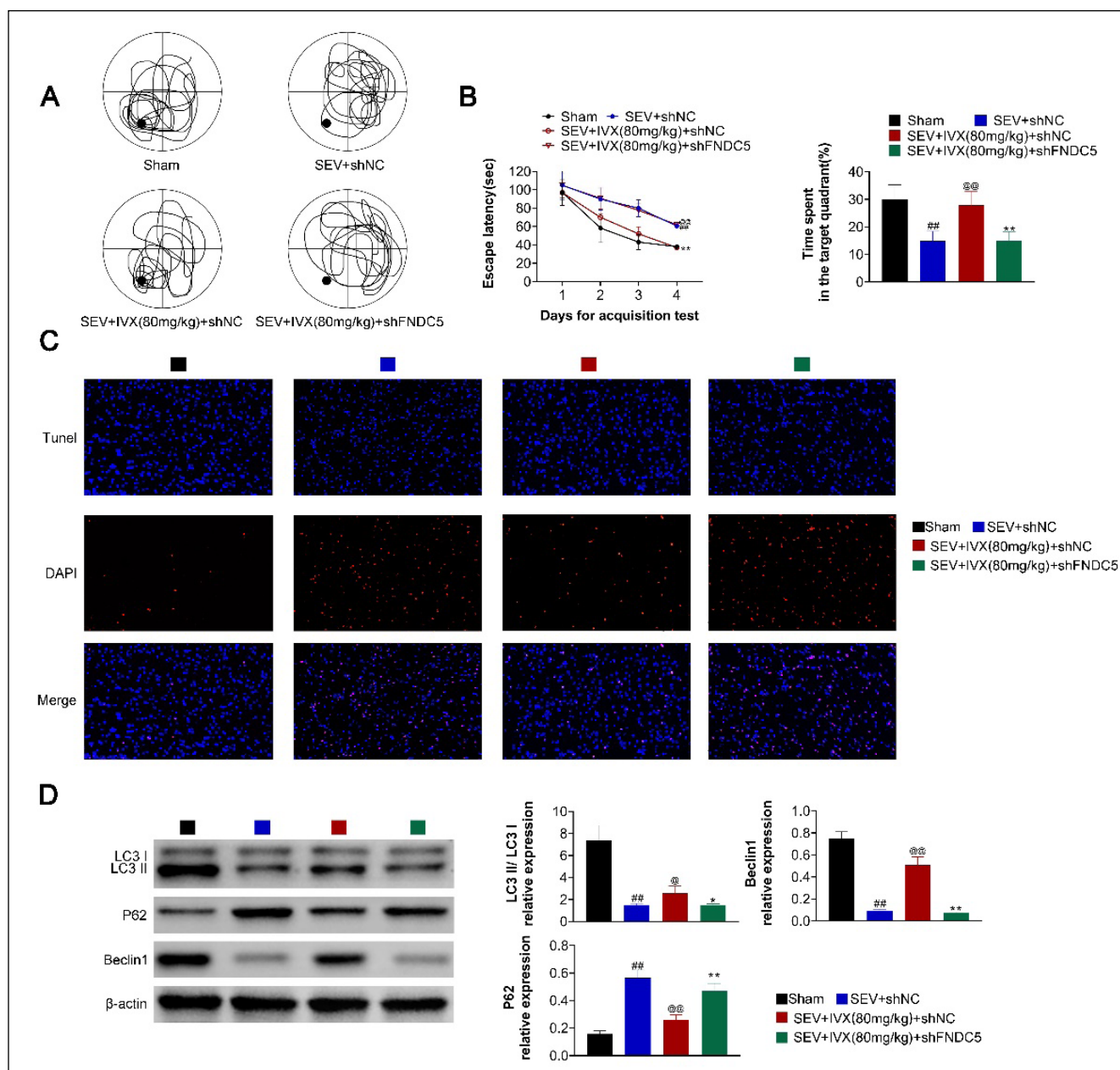


Fig. 5. IVX alleviates SEV-induced cognitive injury by PGC-1 α /FNDC5 pathway. A, B, The MWM assay in different groups. C, The TUNEL assay in different groups. D, The level of LC 3 II, p62, beclin 1 in different groups. ##, $p < 0.01$ vs. sham, *, $p < 0.05$, **, $p < 0.01$ vs. SEV+IVX+shNC, @, $p < 0.05$, @@, $p < 0.01$ vs. SEV+shNC.

CONCLUSION

In conclusion, we found that IVX improved the sevoflurane-induced cognitive dysfunction in rats. IVX could inhibit sevoflurane-induced brain apoptosis and promote autophagy in rats. Mechanically, we found that IVX restored sevoflurane-induced cognitive dysfunction via affecting autophagy through PGC-1 α /FNDC5 pathway. Our data suggest that IVX has the po-

tential to be served as a promising drug for the treatment of cognitive dysfunction.

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