Article

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Investigating the Mechanisms of Esketamine in Treating Propofol-Induced Cognitive Impairment in Elderly Rats

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Background: Common cognitive impairment in the elderly may be aggravated by the anesthetic propofol, whereas the mammalian target of rapamycin protein (*mTOR*)-brain-derived neurotrophic factor (*BDNF*) pathway and autophagy regulation play a key role in neuroprotection. In this study, we investigated whether esketamine can improve propofol-induced cognitive impairment in aged rats by affecting these mechanisms and revealed potential new therapeutic strategies.

Methods: A propofol-induced age-related cognitive dysfunction model was used in the experiments. Behaviours were evaluated by the sugar-water preference test and the water maze, neuronal damage by Nissl staining, and neuronal apoptosis was detected by flow cytometry. Neuronal autophagy-related proteins phospho-mammalian target of rapamycin (p-mTOR), mTOR, BDNF, phospho-Unc-51 like autophagy activating kinase 1 (p-ULK1), Unc-51 like autophagy activating kinase 1 (ULK1), autophagy related 5 (ATG-5), and microtubule-associated protein 1 light chain 3-I/microtubule-associated protein 1 light chain 3-II (LC3-II/LC3-I) were detected by western blotting (WB); immunohistochemistry was used to detect the deposition of β-amyloid (amyloid-beta, Aβ) in the hippocampal region and the positivity rate of caspase-3; postsynaptic density protein 95 (PSD95) and synapsin I (SYN1) levels were detected by WB.

Results: Water maze and sugar-water preference tests showed that the propofol group had longer escape latency, more platform crossings, lower platform quadrant time ratio, and reduced sugar-water preference, all improved by esketamine ($p < 0.05$). Nissl staining and immunohistochemistry revealed sparser neurons, darker staining, wrinkled morphology, and increased Aβ in the propofol group, all improved by esketamine ($p < 0.05$). WB showed increased phosphorylated Tau (p-Tau) and Aβ, higher apoptosis and caspase-3 positivity, and decreased BDNF, and ATG-5 in the propofol group, all reversed by esketamine. Propofol increased inflammatory markers and decreased SYN1, PSD95, and SYN expression, all of which were improved by esketamine ($p < 0.05$).

Conclusion: By inhibiting the mTOR-BDNF pathway with esketamine, the inhibition of neuronal autophagy ultimately improves the cognitive dysfunction induced by propofol.

Keywords: esketamine; mTOR-BDNF pathway; neuronal autophagy; cognitive dysfunction in the elderly; propofol; postoperative cognitive dysfunction

Introduction

With the acceleration of global aging, the issue of cognitive dysfunction in old age is attracting increasing attention. Cognitive dysfunction in old age includes a variety of disorders, such as Alzheimer's disease and vascular cognitive impairment, which are commonly characterized by memory loss, reduced learning ability and executive dysfunction [1]. These symptoms have a serious impact on the daily lives of elderly patients and constitute a significant burden on both families and society.

In the pathophysiology of cognitive dysfunction in old age, alterations in neuronal structure and function play a key role [2]. Neuronal autophagy is a process of cellular self-degradation that maintains cellular homeostasis by breaking down harmful or aged organelles and proteins within the cell [3]. However, in old age, neuronal autophagy may be disturbed, accumulating intracellular junk and affecting the normal function of neurons [4]. Therefore, the study of how to regulate neuronal autophagy to maintain the normal function of the nervous system has become an important direction for research on the treatment of cognitive dysfunction in old age.

Mammalian target of rapamycin protein (mTOR) is a highly conserved protein kinase found in two distinct intracellular complexes, mammalian target of rapamycin protein Complex 1 (mTORC1) and mTORC2 [5]. mTORC1 plays a vital role in regulating cellular metabolism, growth, and autophagy [2]. Its ability to regulate protein synthesis makes mTORC1 essential for neuronal function maintenance and

repair [6]. The mTOR pathway associated with neuronal autophagy plays a crucial role in the normal function of the nervous system [7]. Studies have shown that aberrant activation of the mTOR pathway may lead to inhibition of neuronal autophagy, which affects the clearance of intracellular waste products and, consequently, neuronal function [8,9]. Therefore, modulation of the mTOR pathway may potentially improve cognitive dysfunction in old age.

Brain-derived neurotrophic factor (BDNF) is a growth factor widely distributed in the nervous system and is essential for neuronal survival, growth, and functional maintenance [10]. BDNF activates multiple signaling pathways, including the mTOR pathway, by binding to its receptor Tropomyosin receptor kinase B (TrkB) [11]. mTOR-BDNF pathway interactions in neuronal survival and functional maintenance have become a hot topic in the therapeutic study of neurological diseases [12]. Excessive activation of mTORC1 may lead to protein synthesis disorders and impair neuronal structure and function [13]. In addition, reduction in BDNF is closely related to the decline of neuronal survival and synaptic plasticity in old age [14]. Therefore, regulation of the mTOR-BDNF pathway promotes a balance of neuronal autophagy and thus protects neurons from aging and cognitive decline.

Propofol is a drug widely used for anaesthesia and sedation; however, its use has also been found to be associated with cognitive dysfunction in old age [15]. Studies have shown that propofol may affect the nervous system through multiple pathways, including activation of cell death pathways in neurons and interference with synaptic transmission, leading to cognitive decline [16,17]. Against this background, it is particularly important to find effective treatments to ameliorate propofol-induced cognitive dysfunction.

Esketamine, a new type of antidepressant drug, has been approved for the treatment of refractory depression [18]. Unlike conventional antidepressants, esketamine exerts rapid antidepressant effects by acting as a noncompetitive NMDA-receptor antagonist. In recent years, studies have shown that esketamine not only can quickly relieve depressive symptoms, but also may play a neuroprotective role by regulating the mTOR and BDNF pathways [19,20]. The mTOR pathway plays an important role in cell growth, proliferation, and autophagy, whereas BDNF has a key role in neuronal survival, differentiation, and plasticity. By activating the mTOR-BDNF pathway, esketamine is expected to promote neuronal autophagy, thereby improving neuronal function, especially in the response to cognitive dysfunction caused by anesthetic drugs such as propofol. An improved understanding of the mechanism has important research and clinical value.

Based on the above background, the goal of the present study was to investigate whether esketamine can modulate neuronal autophagy by mediating the mTOR-BDNF pathway, thereby ameliorating propofol-induced cognitive dysfunction in aged rats. By thoroughly investigating this novel therapeutic strategy, we expect to provide new ideas and potential drug targets for the treatment of cognitive dysfunction in old age. By understanding the role of esketamine in neuronal autophagy, through effects on the mTOR-BDNF pathway, we will provide for the development of more effective treatments for cognitive dysfunction in aging.

Materials and Methods

Laboratory Animals

Forty-five Specific Pathogen Free (SPF)-grade Sprague Dawley male rats, aged 22-month-old and weighing (525.12 ± 15.12) g, were selected and purchased from Hunan Slaughter Kingda Laboratory Animal Co., Ltd., Laboratory Animal Production License No.: SCXK (Hunan) 2023-0009.

Animal Modeling and Group Drug Administration

Continuous injection of propofol (5 mg/kg, 6 h, Y0000016, Sigma Aldrich, Shanghai, China) and intraperitoneal injection of sodium pentobarbital (0.3%, 40 mg/kg, 11715, Sigma Aldrich, Shanghai, China) were used to construct a model of cognitive depression in rats. Rectal temperature, breathing, and heart rate were monitored during anesthesia. Body temperature was maintained using a heat lamp. After the modeling was completed, water maze and sugar water preference experiments were performed, and the modeling was judged to be successful if the rats showed a decrease in sugar water preference rate, avoidance latency, number of times of crossing the platform, and target quadrant residence time compared to control rats [21]. Then, one group of model rats was treated with intraperitoneally injected esketamine (15 mg/kg, Pfizer, Shanghai, China) [22], whereas the normal control group and the other group of model rats were injected with the same dose of saline. The experimental groups were: Normal, propofol (Prop), esketamine + Prop; there were 15 mice per group, and the injection cycle was 21 days. After hippocampus tissue extraction, rats were injected with sodium pentobarbital (3%, 150 mg/kg) and euthanized.

Sucrose Preference Test and Water Maze

After the modeling was completed, sucrose preference test data were collected. The methods were as follows: for each experiment, rats were acclimatized to sugar-containing drinking water for 1 h. Two bottles were placed in each cage, one with 0.8% sucrose aqueous solution and one with pure water, the position of the water bottles was changed every half an hour, and the amount of water consumed by the two bottles was recorded. Sugar-water preference values were calculated based on the percentage of overall fluid consumption observed [23].

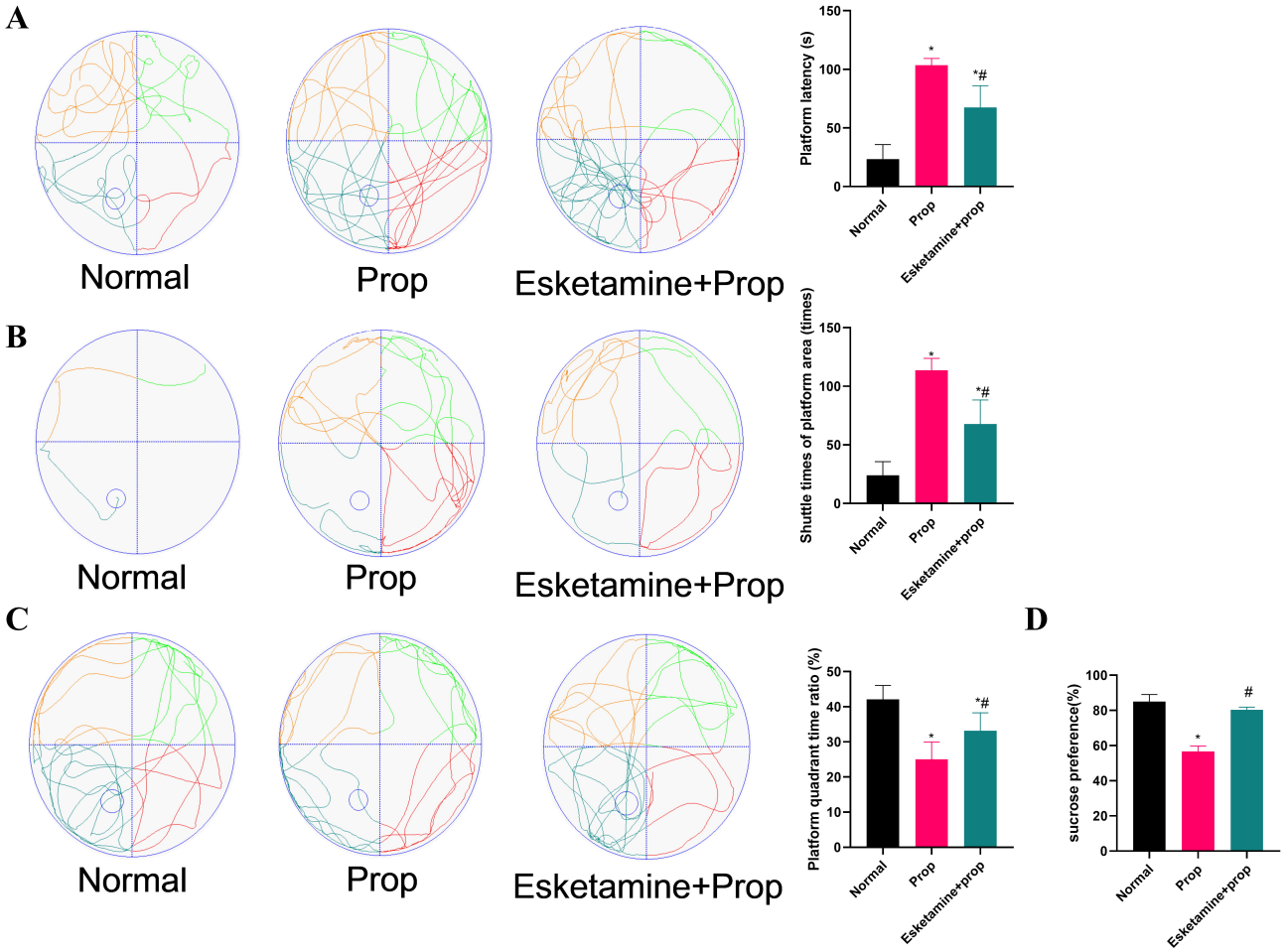

Fig. 1. Behavioural effects of esketamine treatment on aged cognitively impaired rats after propofol anaesthesia. (A) Platform latency. (B) Shuttle times of platform area. (C) Platform quadrant time ratio. (D) Sucrose preference. $^{*}p < 0.05$ vs Normal. $^{\#}p < 0.05$ vs Prop. n = 15. Prop, propofol.

The water maze tank was a 1.6 m diameter, 0.6 m high cylinder filled with approximately 0.4 m of warm water. The rats were placed with their heads toward the wall of the pool, and one of the four starting positions, east, west, south, and north, was randomly selected. The time to find the platform (latency), the number of times the platform was crossed, and the ratio of time spent in the target quadrant (the ratio of time in the platform quadrant to that in the quadrant opposite the platform) was recorded within 60 seconds. The data were analyzed using a data analysis system. The data analysis system evenly divided the screen into clockwise quadrants I, II, III, and IV, and analyzed the swimming trajectory. All rats were handled for 5 days before surgery to familiarize them with the maze environment.

Nissl Staining

Rat brain tissue was removed and fixed in 4% paraformaldehyde solution (441244, Sigma, Shanghai, China) for 24 h. Paraffin sections of rat hippocampal tissues, 4 µm thick, were prepared by gradient alcohol dehydration, stained with Nippon's stain (R30434, Yuanye, Shanghai, China), permeabilized with xylene (534056, Sigma, Shanghai, China), blocked with neutral gum, and observed under a microscope (XSP-11CF, SHGX, Shanghai, China).

Western Blotting

The hippocampal tissue was cut on ice, RIPA tissue lysate (R0278, Sigma, Shanghai, China) and protease inhibitor were added, and the supernatant was centrifuged. The protein concentration was detected using a BCA kit (A55861, ThermoFisher, Shanghai, China), and proteins were heat denatured and aliquoted. Proteins were separated by SDS-PAGE gel electrophoresis and transferred to a membrane blocked with 5% skimmed milk for 1 h. Proteins were incubated with primary antibodies phosphorylated Tau (p-Tau) (AP0170, 1:500, Abclonal, Wuhan, China), t-Tau (AP1378, 1:1000, Abclonal, Wuhan, China), β-amyloid (Aβ) (sc-28365, 1:1000, SCBT, Santa Cruz, CA, USA), Glycogen Synthase Kinase-3 Beta (GSK-3β)

Table 1. Primer sequences.

Gene	F (5′-3′)	R (5′-3′)
TNF-α	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
IL-1β	ATGAGCTGAAAGCTCTCCACCT	GAGGTGCTGATGTACCAGTTGGG
IL-6	TCCAGTTGCCTTCTTGGGACTG	TTGCCGAGTAGACCTCATAGTGACC
β-actin	AGAGCTACGAGCTGCCTGAC	AGCACTGTGTTGGCGTACAG

TNF-α, tumor necrosis factor alpha; *IL-1β*, interleukin-1 beta.

(A2081, 1:2000, Abclonal, Wuhan, China), Phosphorylated GSK-3β (pGSK-3β) (AP1088, 1:2000, Abclonal, Wuhan, China), phospho-mammalian target of rapamycin (p-mTOR) (AP0115, 1:1000, Abclonal, Wuhan, China), mTOR (A11355, 1:2000, Abclonal, Wuhan, China), BDNF (A1307, 1:1000, Abclonal, Wuhan, China), phospho-Unc-51 like autophagy activating kinase 1 (p-ULK1) (29006-1-AP, 1:1000, proteintech, Wuhan, China), Unc-51 like autophagy activating kinase 1 (ULK1) (A8529, 1:1000, Abclonal, Wuhan, China), autophagy related 5 (ATG-5) (A19677, 1:1000, Abclonal, Wuhan, China), microtubule-associated protein 1 light chain 3-I/microtubule-associated protein 1 light chain 3-II (LC3-II/LC3-I) (A5618, 1:1000, Abclonal, Wuhan, China), postsynaptic density protein 95 (PSD95) (A7889, 1:1000, Abclonal, Wuhan, China), synapsin (SYN) (A9165, 1:1000, Abclonal, Wuhan, China) and β-actin (AC038, 1:10,000, Abclonal, Wuhan, China) overnight at 4 °C, and washed with TBST for 8 min, repeating 3 times. Proteins were then incubated with Horseradish Peroxidase (HRP) secondary antibody (AS014, 1:10,000, Abclonal, Wuhan, China; sc-525409, 1:1000, SCBT, Santa Cruz, CA, USA) for 1 h at room temperature, washed, and the substrate was colour developed on a machine (iBright CL750, Thermo Fisher Scientific, Shanghai, China). The analysis of grey values was performed using ImageJ (V1.8.0.112, NIH, Madison, WI, USA).

Immunohistochemistry

The prepared hippocampal area tissues were paraffin sectioned, deparaffinized with xylene, treated with gradient ethanol, and retrieved using (pH 6.0, 10 mM) citrate buffer. Sections were washed with phosphate buffered saline (PBS), and incubated in 2% fetal bovine serum protein solution for 2 h. The sections were then incubated with anti-Aβ_{1-42} (AB5078P, 1:100, Sigma-Aldrich, Shanghai, China), anti-caspase-3 (AB3623, 1:100, Sigma-Aldrich, Shanghai, China), anti-SYN1 (HPA000397, 1:100, Sigma-Aldrich, Shanghai, China) overnight. On the following day, samples were rinsed with PBS for 3 min, repeating 3 times. Samples were incubated with the secondary antibody (AS014, 1:10,000, Abclonal, Shanghai, China) at room temperature for 1 h. After PBS rinsing, 50 µL of Streptomyces anti-biotin-peroxidase solution was added to sections, incubated, and rinsed. DAB display reagent was added, samples were stained with hematoxylin (G1120, Solarbio, Shanghai, China), and the sections were sealed with neutral gum after being rendered transparently by xylene. Samples were visualized by a microscope and photographed. ImageJ was used to analyze the area proportion of the positive signal.

Terminal Deoxynucleotidyl Transferase (TdT)-Mediated dUTP Nick-End Labeling (TUNEL)

Using TdT (4810-30-02, R&D Systems, MN, USA) to mediate the incorporation of fluorescein-labeled dUTP (6571, R&D Systems, MN, USA) at the DNA break ends, apoptotic cells in rat brain tissue were identified and quantified through the TUNEL assay. Rat brain tissue samples were first fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned into 5-µm slices. The sections were deparaffinized, rehydrated, and treated with proteinase K for permeabilization. TdT-mediated labeling was carried out by incubating the sections with fluorescein-labeled dUTP in a humidified chamber at 37 °C for 1 hour. After terminating the reaction and washing with PBS, the sections were counterstained with DAPI to visualize all cell nuclei. Fluorescent signals were observed under a fluorescence microscope, where apoptotic cells were recognized by bright green fluorescein signals in the nuclei. Quantification was performed by counting the fluorescein-positive nuclei in randomly selected fields, with results expressed as a percentage of total cells.

RT-qPCR

Rat brain tissue homogenate was collected, total RNA was extracted, and qPCR analysis of target genes was performed after reverse transcription. Each group of experiments was repeated 3 times, and *β-actin* was used as an internal reference to calculate the relative expression levels of tumor necrosis factor alpha (*TNF-α*), interleukin-1 beta (*IL-1β*), and interleukin-6 (*IL-6*) by the $2^{-\Delta\Delta Ct}$ formula. The primer sequences are shown in Table 1.

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 software (IBM Corp., Armonk, NY, USA). All experiments were repeated three times, and the groups were compared using the independent-sample *t*-test or analysis of variance with Bonferroni correction for multiple comparisons. $p < 0.05$ indicates a statistically significant difference.

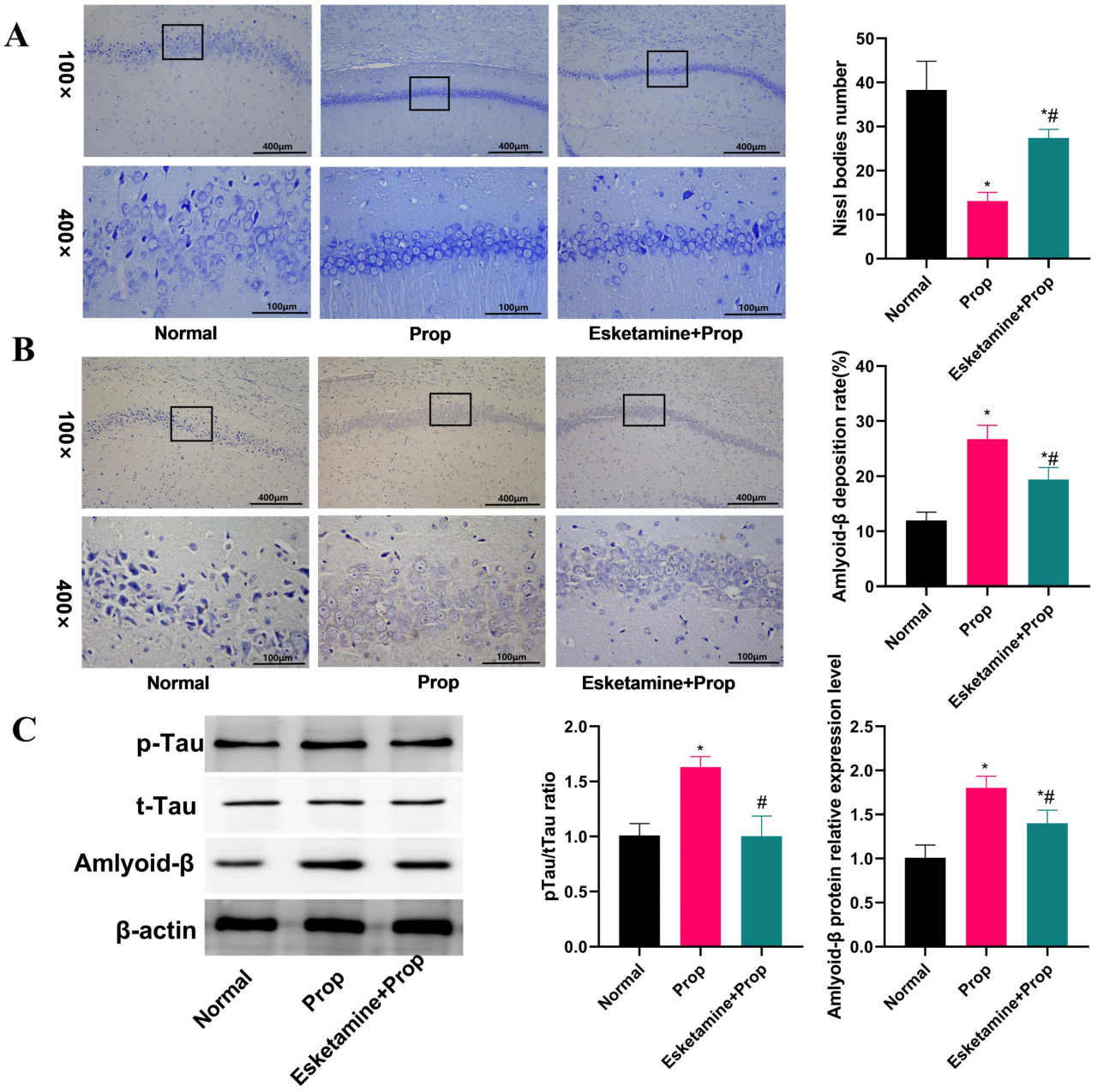

Fig. 2. Effects of esketamine treatment on hippocampal neuronal damage in aged cognitively impaired rats after propofol anaesthesia. (A) The neurons in the hippocampus area were observed by Nissl staining. (B) β-amyloid (Aβ) deposition was observed by immunohistochemistry. (C) Western blotting to detect the expression of p-Tau, t-Tau, and Aβ proteins in the hippocampus. The 400× magnification images of each group are enlarged sections of the black rectangular area in the corresponding 100× magnification images. *$p < 0.05$ vs Normal. $^{\#}p < 0.05$ vs Prop. n = 15. p-Tau, phosphorylated Tau.

Results

Behavioural Effects of Esketamine Treatment on Aged Cognitively Impaired Rats After Propofol Anaesthesia

The water maze and sucrose preference tests showed that, compared to the normal group, the propofol group had longer escape latency, more platform crossings, lower platform quadrant time ratio, and reduced sucrose preference. Compared to the propofol group, the esketamine + propofol group improved on all these measures; they had shorter escape latency, fewer platform crossings, higher platform quadrant time ratio, and increased sucrose preference ($p < 0.05$, Fig. 1A–D).

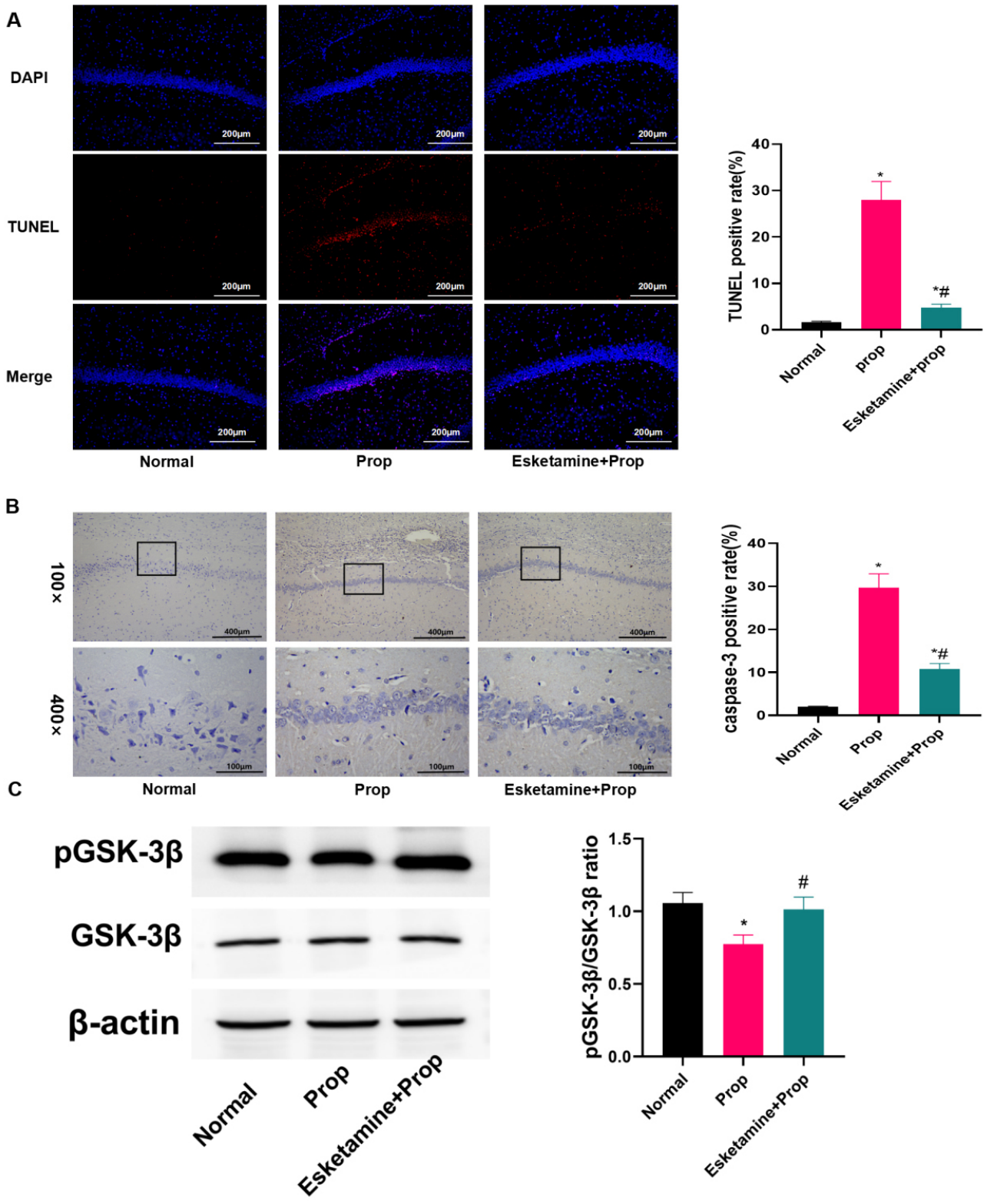

Fig. 3. Effect of esketamine treatment on hippocampal neuronal apoptosis in aged cognitively impaired rats after propofol anaesthesia. (A) Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) was used to detect hippocampal neuronal cell apoptosis. (B) The expression of caspase-3 protein was detected by immunohistochemistry. (C) Western blotting was used to detect the expression of Glycogen Synthase Kinase-3 Beta (GSK-3β) and Phosphorylated GSK-3β (pGSK-3β) in the hippocampus. The 400× magnification images of each group are enlarged sections of the black rectangular area in the corresponding 100× magnification images. *$p < 0.05$ vs Normal. $^{\#}p < 0.05$ vs Prop. n = 15.

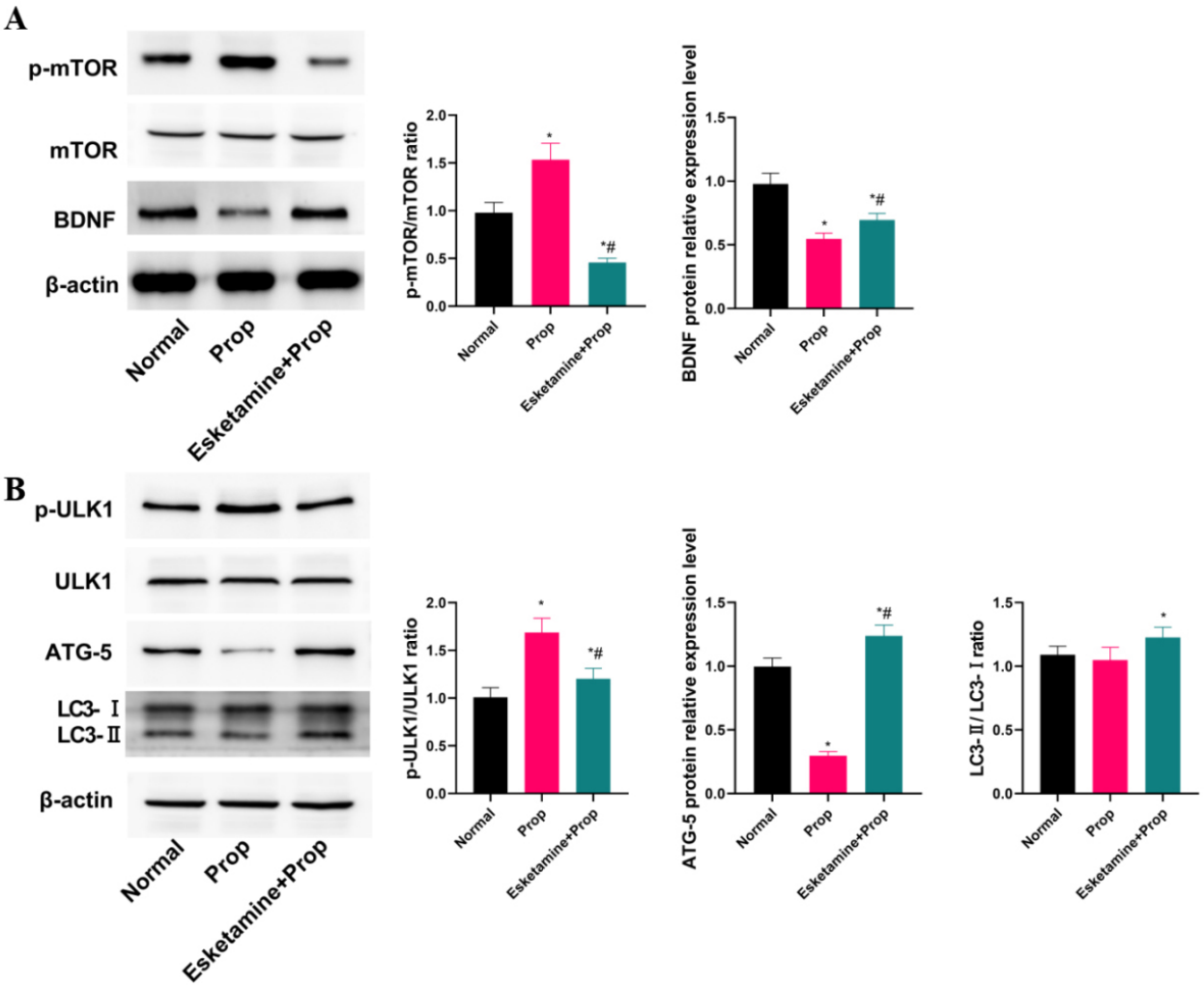

Fig. 4. Effects of esketamine treatment on the mTOR-BDNF pathway and neuronal autophagy in the hippocampus of aged cognitively impaired rats after propofol anaesthesia. (A,B) Western blotting detection of neuronal autophagy-associated protein expression in the hippocampus. *$p < 0.05$ vs Normal. $^{\#}p < 0.05$ vs Prop. n = 15. mTOR, mammalian target of rapamycin protein; BDNF, brain-derived neurotrophic factor; p-ULK1, phospho-Unc-51 like autophagy activating kinase 1; ATG-5, autophagy related 5; LC3-II/LC3-I, microtubule-associated protein 1 light chain 3-I/microtubule-associated protein 1 light chain 3-II.

Effects of Esketamine Treatment on Hippocampal Neuronal Damage in Aged Cognitively Impaired Rats After Propofol Anaesthesia

Nissl staining and immunohistochemistry revealed that, compared to the normal group, the propofol group had sparse hippocampal neurons with darker staining, wrinkled morphology, and ruptured cytosol, along with significantly increased $A\beta$ deposition. In contrast, the esketamine + propofol group had neatly arranged neurons with lighter staining, spherical morphology, and clearer cell membrane outlines, with reduced Aβ deposition ($p < 0.05$, Fig. 2A,B). Western blotting (WB) analysis showed that p-Tau and Aβ protein levels were elevated in the propofol group compared with the normal group. The p-Tau and Aβ protein levels were reduced in the esketamine + propofol group compared with the propofol group ($p < 0.05$, Fig. 2C).

Effect of Esketamine Treatment on Hippocampal Neuronal Apoptosis in Aged Cognitively Impaired Rats After Propofol Anaesthesia

The results showed that, compared to the normal group, the propofol group had significantly higher rates of hippocampal neuron apoptosis and caspase-3 positivity, with decreased GSK-3β expression. Compared to the propofol group, neuron apoptosis and caspase-3 positivity were reduced, and GSK-3β phosphorylation was increased in the esketamine + propofol group ($p < 0.05$, Fig. 3A–C).

Effects of Esketamine Treatment on the mTOR-BDNF Pathway and Neuronal Autophagy in the Hippocampus of Aged Cognitively Impaired Rats After Propofol Anaesthesia

WB results showed that, compared to the normal group, the propofol group had significantly down-regulated

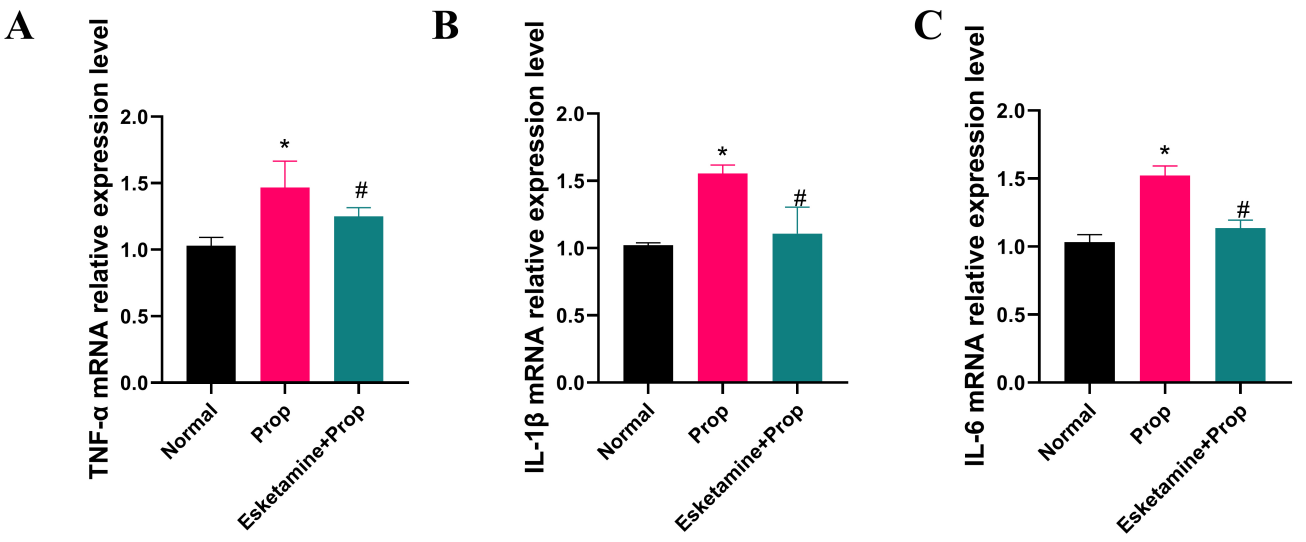

Fig. 5. Effect of esketamine treatment on mRNA expression of genes for inflammatory factors in the hippocampus of aged cognitively impaired rats after propofol anaesthesia. (A–C) RT-qPCR was used to detect mRNA expression levels of *TNF-α, IL-1β*, and *IL-6* genes. $^{*}p < 0.05$ vs Normal. $^{\#}p < 0.05$ vs Prop. n = 15.

BDNF and ATG-5 protein levels, with elevated mTOR and ULK1 phosphorylation levels. In contrast, compared to the propofol group, the esketamine + propofol group had significantly up-regulated BDNF, LC3-II/LC3-I, and ATG-5 proteins, and decreased mTOR and ULK1 phosphorylation levels ($p < 0.05$, Fig. 4A,B).

Effect of Esketamine Treatment on mRNA Expression of Genes for Inflammatory Factors in the Hippocampus of Aged Cognitively Impaired Rats After Propofol Anaesthesia

The data showed a significant increase in the expression level of mRNA of *TNF-α, IL-1β* and *IL-6* genes in the Prop group compared to the control group, and a decrease in the expression level of mRNA of *TNF-α, IL-1β* and *IL-6* genes in the esketamine + Prop group compared to the Prop group ($p < 0.05$, Fig. 5A–C).

Effects of Esketamine Treatment on the Plasticity of Synaptic Structures in Aged Cognitively Impaired Rats After Propofol Anaesthesia

The results of WB and immunohistochemical staining experiments showed that the SYN1 positivity rate, and PSD95 and SYN protein expression levels, were significantly lower in the Prop group compared to the normal group. However, compared with the Prop group, the esketamine + Prop group displayed a greater SYN1 positivity rate, and higher levels of PSD95 and SYN protein expression ($p < 0.05$, Fig. 6A,B).

Discussion

Age-related cognitive dysfunction (ACD) is an increasingly prominent problem with the aging of the population, and this study aimed to investigate whether esketamine ameliorates propofol-induced cognitive dysfunction in aged rats through the regulation of neuronal autophagy via the mTOR-BDNF pathway. Through a series of experimental approaches, we observed that esketamine attenuated propofol-induced hippocampal neuronal damage by reducing neuronal injury and Aβ deposition, thereby improving cognitive function. Regarding the mTOR-BDNF pathway and neuronal autophagy, we found that the esketamine-treated group showed a positive modulation of both pathways. The mTOR-BDNF pathway plays a vital role in neuronal autophagy and synaptic plasticity, which is closely related to cognitive function.

Neuronal autophagy plays a vital role in the health of the nervous system [24]. Autophagy is an intracellular process of self-degradation and reuse that helps maintain the stability of the intracellular environment by removing damaged or abnormal organelles and proteins [25]. However, overactivation and autophagy inhibition may negatively affect neuronal function [26]. Abnormal regulation of neuronal autophagy is thought to be an important factor in the pathogenesis of ACD [3].

Cognitive dysfunction in aged rats is a complex pathological process usually accompanied by changes in brain structure and function [27]. In the present study, we used a propofol-induced ACD model in aged rats, which has been shown to mimic propofol-induced cognitive dysfunction.

Esketamine, an NMDA receptor antagonist, has shown potential benefits in the treatment of a variety of neurological disorders [28]. In the present study, we found that esketamine treatment significantly improved propofol-induced cognitive dysfunction, which was associated with its modulation of neuronal autophagy. Specifically, we ob-

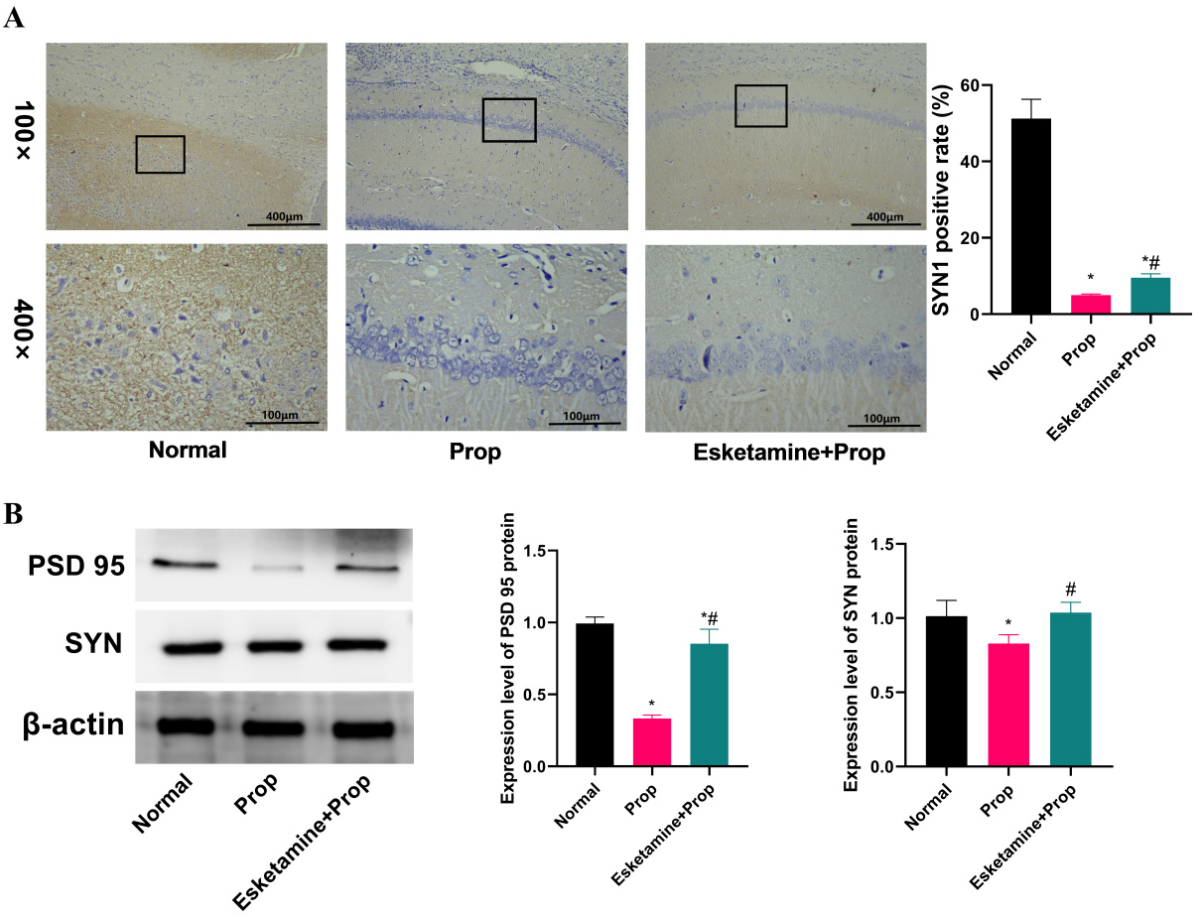

Fig. 6. Effects of esketamine treatment on the plasticity of synaptic structures in aged cognitively impaired rats after propofol anaesthesia. (A) The positive rate of SYN1 was observed by immunohistochemistry. (B) Western blotting (WB) was used to detect the expression of PSD95 and SYN. *$p < 0.05$ vs Normal. The 400× magnification images of each group are enlarged sections of the black rectangular area in the corresponding 100× magnification images. $^{\#}p < 0.05$ vs Prop. n = 15.

served that esketamine treatment increased mTOR activity and promoted the expression of BDNF. These results further emphasize the importance of the mTOR-BDNF pathway in the regulation of neuronal autophagy.

mTOR is a key cell signaling pathway that plays an important role in cell growth, metabolism and autophagy [29]. By modulating the mTOR signaling pathway, esketamine may affect the degree of autophagy and thus play a positive role in ACD treatment [30]. In addition, BDNF is considered a key molecule for neuronal survival and functional maintenance [31]. By increasing the expression of BDNF, esketamine may provide a mechanism for neuronal protection and repair, which in turn improves cognitive function [32].

Esketamine treatment also increased the expression of PSD95 and Syn proteins. PSD95 and Syn are two specific protein markers used to measure synaptic plasticity. PSD95 is abundantly expressed in the postsynaptic region and interacts with membrane receptors, ion channels, and cell adhesion factors, which are involved in the regulation of synaptic plasticity and learning and memory. Syn is a key presynaptic vesicle membrane protein, and its expression accurately reflects synaptic distribution, number, and density; it is the most direct index of the changes in synaptic plasticity.

There are some limitations of this study that need to be noted. First, our experiments were mainly focused on rat models, so more animal experiments and clinical studies are needed before generalizing the results to humans. Second, we have not fully elucidated the regulatory mechanism of the mTOR-BDNF pathway, and future studies could further delve into the molecular mechanisms in this regard.

Taken together, the present study reveals the potential mechanism by which esketamine regulates neuronal autophagy by mediating the mTOR-BDNF pathway, thereby ameliorating propofol-induced cognitive dysfunction in aged rats. This finding provides a new avenue for further exploration of ACD therapeutic strategies, as well as an experimental basis for studies related to neuronal autophagy and the mTOR-BDNF pathway. Future studies can further explore the applicability of this mechanism in different models and clinical practices, and delve into the thera-

peutic mechanism of esketamine to provide more theoretical and experimental support for the clinical treatment of cognitive dysfunction in the elderly.

Conclusion

Esketamine improves propofol-induced cognitive dysfunction in aged rats by inhibiting neuronal autophagy through modulation of the mTOR-BDNF pathway.

Availability of Data and Materials

The data used and/or analyzed during the current study are available from the corresponding authors.

Author Contributions

JZ, GX and XW designed the research study. XW and LQ performed the research. JZ provided help and advice on the experiments. GX and LQ analyzed the data. All authors wrote the manuscript. All authors contributed significantly to editorial changes of important content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was approved by the Animal Ethics Committee of Hunan Evidence-based Biotechnology Co., Ltd (2023XZ005).

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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