



Novel benzodiazepine remimazolam tosylate delays neurodegeneration of aged mice via decreasing tau phosphorylation

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ABSTRACT

Benzodiazepines like midazolam were generally considered one of the possible causes affecting postoperative cognitive recovery. As a new kind of rapid-effect benzodiazepine, remimazolam tosylate was widely used in clinical anesthesia for its pharmacological advantage, but few studies reported its effects on cognitive function in the elderly. Here, we aimed to research the effects of remimazolam tosylate on cognitive function in aged mice and its underlying biological mechanisms. We measured the memory function of aged mice immediately and one month after intraperitoneal injection of remimazolam tosylate compared to the saline control. The brain metabolism level was detected by Positron Emission Tomography/Computed tomography (PET/CT). Compared with the control, we observed a decrease in memory ability, as well as an increase in tau phosphorylation level and a decrease in phosphatase level in the short term; however, one month later, contrary to the previous results, we observed better memory and brain metabolism and lower tau phosphorylation levels in the experimental group compared to the control. Therefore, we concluded that remimazolam tosylate did not cause long-term damage to the cognitive function of aged mice and even delayed the decline of memory function in the aging process to some extent.

1. Introduction

Postoperative cognitive dysfunction (POCD) is a common post-operative complication, which can delay postoperative recovery, prolong hospital stay, increase the economic burden of patients and even raise postoperative mortality. Age is an important factor influencing the incidence of POCD. One study reported that the incidence of POCD was 20.5% and 40.9% in patients within 61–70 and 71–80 years old, respectively, while the incidence of POCD in patients over 80 years old could reach 100% (Kotekar et al., 2014). In addition, anesthetic is another important precipitating factor, such as benzodiazepines have caught more attention to their effect on cognitive function (Duprey et al., 2022; Stewart, 2005; Li et al., 2019). It is of great clinical significance to illuminate the effect of anesthetic on POCD and explore its mechanism to guide the selection of anesthetic.

As a kind of new ultrashort-acting benzodiazepines, remimazolam tosylate (RT) can be rapidly metabolized by plasma esterase hydrolysis in vivo, independent of liver and kidney function. Its metabolite has no

pharmacological activity, so RT has the characteristics of fast onset and offset, short sedation time, and rapid recovery (Pambianco et al., 2016). Compared with midazolam, RT has better pharmacological properties, so it is increasingly widely used in clinical practice, and the new indication of maintenance during general anesthesia for remimazolam tosylate has been released in November 2021. However, the effect of RT on the short-term and long-term cognitive function of elderly patients has not been clarified.

In this article, we found that RT could cause short-term cognitive function decline in aged mice but had a protective effect on their long-term degenerative cognitive function changes. We believed that by inducing delayed activation of phosphatase, RT could reduce the tau phosphorylation levels in aged mice, maintain normal neural activity and delay the decline of cognitive function.

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2. Materials and methods

2.1. Anesthetic and chemical reagents

Remimazolam tosylate was purchased from Hengrui Pharmaceuticals Co., Ltd. (Jiangsu, China), and 0.9% normal saline was purchased from Otsuka Pharmaceutical Co. Ltd. (Guangdong, China).

2.2. Animal experimental protocols

C57BL/6 aged female mice (12 months old) purchased from B&K Universal Group Limited were used for the present study. The mice were raised to 26 months of age in the Department of Experimental Animals at Central South University with standard feed and sterilized water. The ambient temperature was controlled at 22–24 °C, and the circadian rhythm was 12 h. All experimental mice were randomly assigned to each group before treatment. Behavioral tests were double-blind and conducted between 8 a.m. and 12 a.m. All experiments were carried out following the recommendations of national and international animal care and ethical guidelines and were approved by the Ethics Committee for Animal Research of Xiangya Hospital of Central South University (permit code: 2021 sydw0165).

2.3. Establishment of drug sedation model

The sedation required to achieve and maintain an appropriate depth of sedation for 2 h to simulate clinical scenarios, which is, the gait of mice is mild to moderately affected, and the acting ability is reduced, but the righting reflex is retained. In the preliminary experiment, we used 10, 12, 14, 16, and 20 mg/kg remimazolam tosylate i.p. as the induction dose and timely additional administration in half of the corresponding dose. To achieve the appropriate depth of sedation, the sedation of the mice was assessed every ten minutes using a modified sedation assessment scale, in which the degree of sedation was classified into five grades: “0” represents normal behavioral activity, “1” represents a slight decrease in activity and sitting quietly, “2” represents no spontaneous activity, only activity when touched, “3” represents no spontaneous activity when touched and “4” represents the disappearance of the righting reflex, reduced activity without loss of the righting reflex was the level of sedation we need to achieve (Kawamata et al., 2003). According to the results of the preliminary experiment, the formal experimental dosing regimen was an intraperitoneal injection of an induction dose of 20 mg/kg RT for the first time and administration of an additional dose every 10 min or when the depth of sedation became shallow. In this study, all mice in the experimental group received 4–5 additional injections after the first dose of remimazolam tosylate injection in order to maintain the 2-hour sedation time and there was no statistical difference in the total amount of drug between the two groups (RT₁ mice: 1.52 ± 0.12 mg vs RT₂ mice: 1.55 ± 0.10 mg, *P* = 0.814). (Supplemental Fig. 1).

We performed behavioral tests, PET/CT imaging, and subsequent detection of total tau and phosphorylated tau (p-tau) in hippocampal and cortical tissues by Western blotting and immunofluorescence. The specific methods and materials used can be seen in the Supplementary Materials.

2.4. Statistical analysis

Standard uptake value (SUV) and immunoblot relative band intensities between the groups were analyzed using the unpaired t-test. Analysis of the Morris water maze (MWM) acquisition testing variables was performed using a two-way repeated-measures ANOVA. Statistical calculations were performed using Prism 9 software (GraphPad Software, Inc., San Diego, CA, USA). All data are reported as the mean ± standard deviation (SD), with a value of *P* < 0.05 considered statistically significant.

3. Results

3.1. Remimazolam tosylate administration could induce a decline in cognitive function in aged mice in the short term

Aged mice were randomly divided into an experimental group and a control group. Mice in the experimental group were injected intraperitoneally with remimazolam tosylate to establish a sedation model (RT mice, *n* = 16), while mice in the control group were injected intraperitoneally with the same volume of normal saline (NS mice, *n* = 16). Then, the mice were randomly divided into two groups, with one group receiving cognitive tests starting the next day (RT₁, *n* = 8; NS₁, *n* = 8) and the other group remaining in standard conditions until one month later (RT₂, *n* = 8; NS₂, *n* = 8) (Fig. 1A).

We used the Morris water maze (MWM) to determine the spatial reference memory of aged mice after remimazolam tosylate administration. In the short term, there was no significant difference between RT₁ and NS₁ mice in the escape latency (Supplemental Fig. 2A), and we also didn't find the difference in body weight and swimming speed between the two groups (Supplemental Figs. 2E, 2F). On the last day, no significant differences in platform crossing times or target quadrant entry time were recorded in the probe trial (Supplemental Figs. 2C, D). Trajectory charts are presented in Supplemental Fig. 2G.

Considering the physical problems of aged mice, we further used object recognition tests between RT₁ and NS₁ mice to assess the memory ability following a 24-h delay (Fig. 1B). In the novel object recognition (NOR) task, the discrimination ratio was similar between the two groups (Fig. 1C). While in the object place recognition (OPR) task, the discrimination ratio of the RT₁ mice was lower than that of the NS₁ mice, which revealed that the RT₁ mice had worse visual episodic memory (Fig. 1D). Overall, these results showed that remimazolam tosylate administration partly induced deficits in memory of the aged mice in the short term.

3.2. Remimazolam tosylate administration affected the ¹⁸F-FDG SUV of the mice brain in the short term

To explore the effects of remimazolam tosylate administration on brain metabolic activity, we measured the brain glucose uptake of aged mice in multiple areas using PET/CT examinations. The standard uptake value (SUV) was obtained by digitizing the image signal to represent ¹⁸F-FDG uptake. In the short term after remimazolam tosylate administration, the mean brain ¹⁸F-FDG SUV of RT₁ mice (*n* = 7) was generally lower than that of NS₁ mice (*n* = 7) in multiple brain areas, but the difference was not statistically significant (Fig. 1E).

3.3. Remimazolam tosylate administration increased tau phosphorylation of the cortex in the short term

Tau protein was thought to be associated with both cognitive function and impaired glucose metabolism in nerve cells. Therefore, we executed the mice after completing PET/CT and obtained hippocampal and frontal cortical tissues (Fig. 1A) from aged mice on the seventh day of administration to detect the level of total tau and p-tau in the short term, the spatial expression of total tau protein in the hippocampus and frontal cortex was observed in immunofluorescent images (Fig. 2A–H). Further, we used protein quantitative analysis and found there was no significant difference in total tau levels between RT₁ (*n* = 6) and NS₁ (*n* = 6) in the hippocampus and frontal cortex (Fig. 2I, O). However, increases in tau phosphorylation were observed at pSer202 (Fig. 2P) and pThr231 (Fig. 2S) in the frontal cortex of RT₁, while there was no difference in other p-tau levels (pSer396/pSer199/pThr205) between the two groups. Overall, our results indicated that remimazolam tosylate not only impaired memory ability and brain metabolism, but also caused changes in the level of p-tau in the short term.

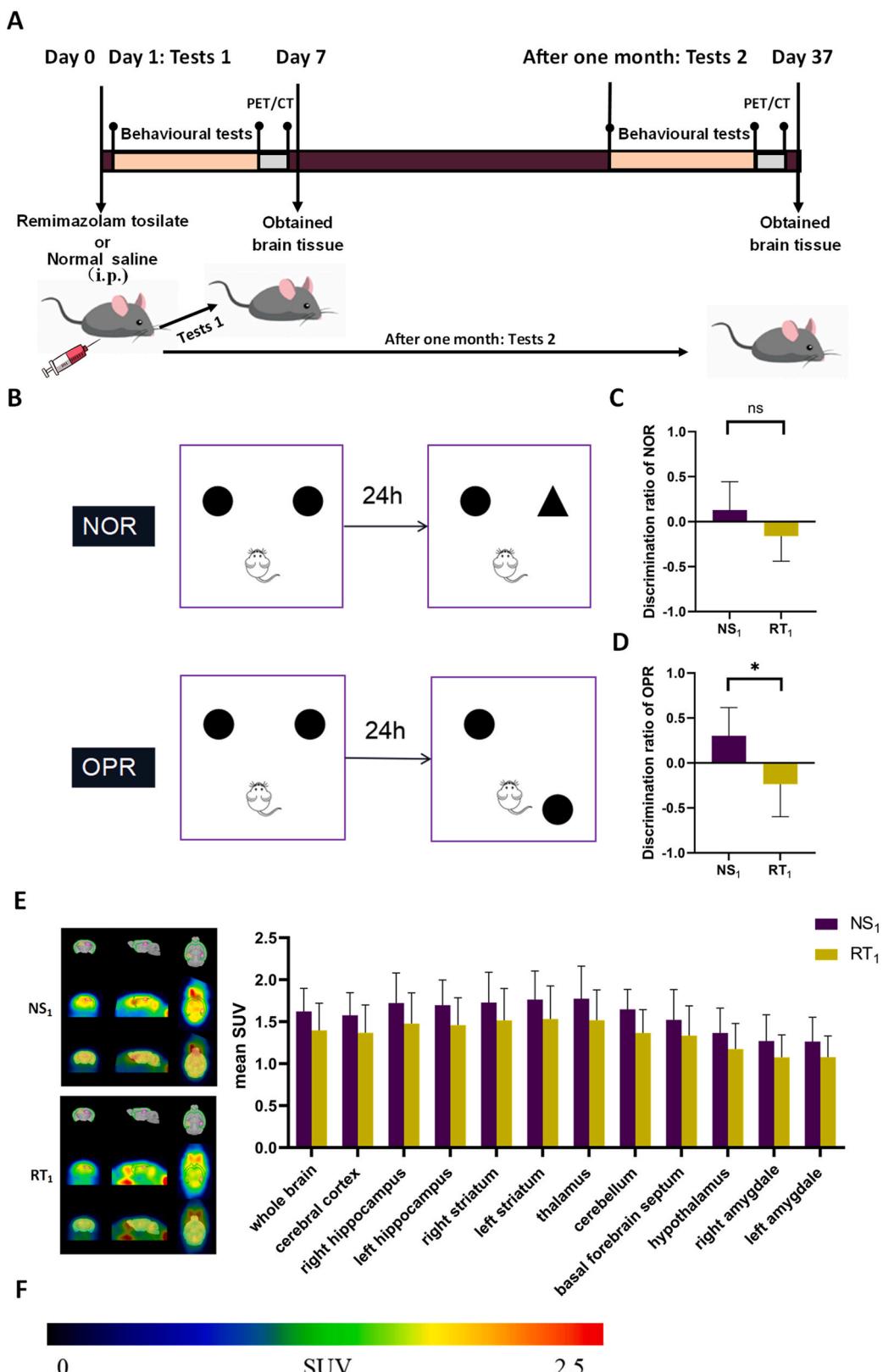


Fig. 1. The effects of intraperitoneal injection of remimazolam tosylate on aged mice in the short term. A Procedure of animal experiments. B Object recognition tasks. C The discrimination ratio of NOR ($n = 8$). D The discrimination ratio of OPR ($n = 8$). E ^{18}F -FDG SUV in multiple brain areas of NS₁ ($n = 7$) and RT₁ ($n = 7$). CT images, PET images, and PET/CT fusion images were shown separately, and specific values were obtained by image signal simulation. F Standardized uptake value. In the image, the change from cool tone to warm tone represented the increase in the uptake value of ^{18}F -FDG. Values are mean \pm standard deviation of the mean (SD). * $p < 0.05$, compared to the NS₁ group. NOR: novel object recognition, OPR: object place recognition, NS₁: aged mice whose cognitive function was tested the day after intraperitoneal injection of isometric normal saline, RT₁: aged mice whose cognitive function was tested the day after intraperitoneal injection of remimazolam tosylate.

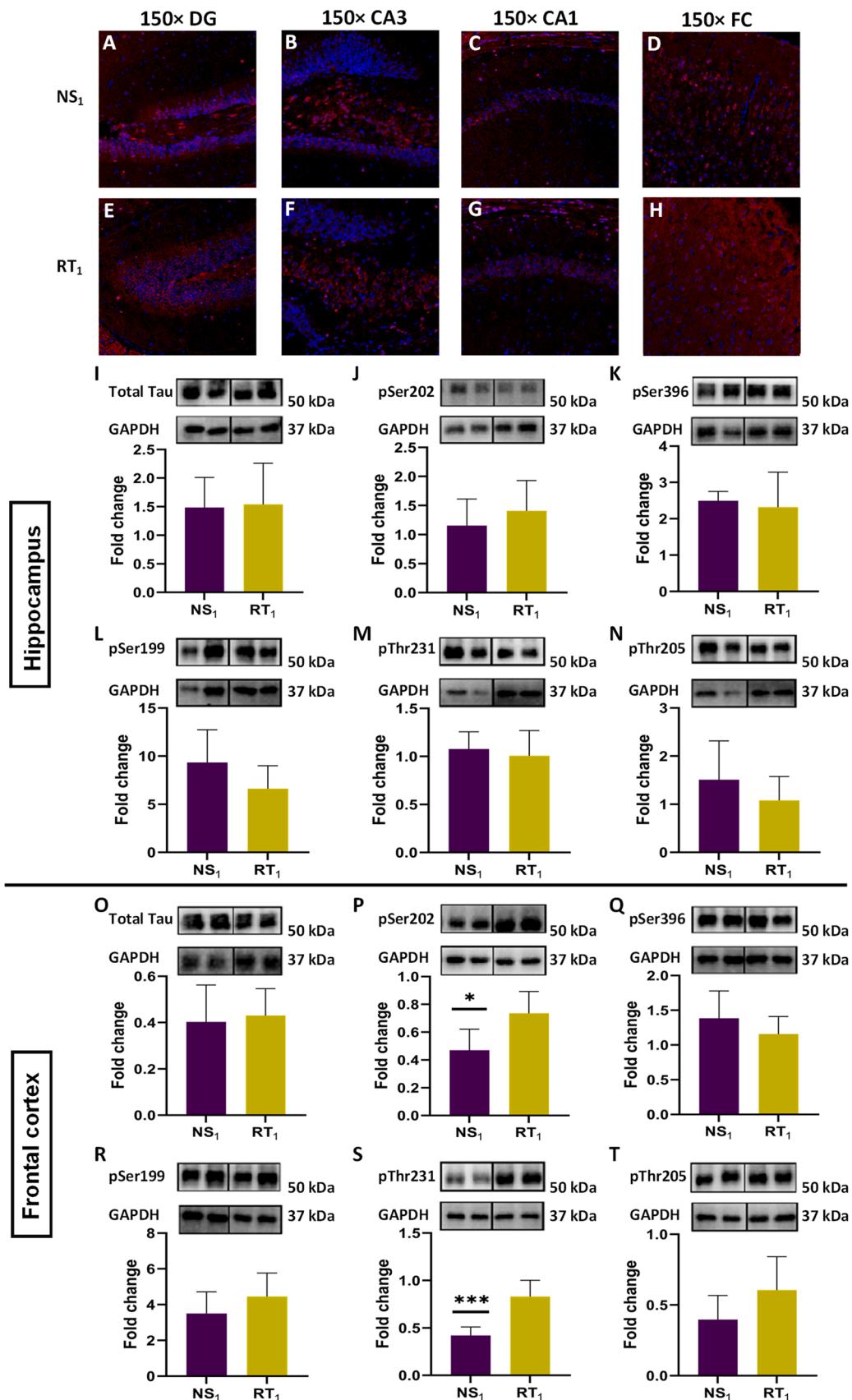


Fig. 2. Intraperitoneal injection of remimazolam tosylate induced an increase in tau phosphorylation proteins of aged mice in the short term. Immunofluorescence staining: A-C Hippocampus (DG, CA3, CA1) for total tau (red) in NS₁ (n = 1). D Frontal cortex for total tau (red) in aged mouse after treatment with normal saline in the short term (NS₁, n = 1). E-G Hippocampus (DG, CA3, CA1) for total tau (red) in RT₁ (n = 1). H Frontal cortex for total tau (red) in aged mouse after treatment with remimazolam tosylate in the short term (RT₁, n = 1). (DAPI, blue). The images of immunofluorescence staining were not quantified. Relative quantification of total tau and phosphorylated tau (p-tau) at the pSer202, pSer396, pSer199, pThr231 and pThr205 phosphoepitopes in NS₁ (n = 6) and RT₁ (n = 6). I-N Total tau and p-tau levels in hippocampal tissues. O-T Total tau and p-tau levels in frontal cortical tissues. GAPDH was selected as an internal reference to control sample loading. Values are expressed as the mean ± SD, *p < 0.05, **p < 0.01, ***p < 0.001, compared to the NS₁ group. FC: frontal cortex, DG: dentate gyrus.

3.4. Remimazolam tosylate administration had a protective effect on the cognitive function of aged mice in the long term

To determine the effect in the long term, aged mice were kept in a standard environment one month after remimazolam tosylate administration (RT_2 , $n = 8$, and NS_2 , $n = 8$). In the MWM, there were also no differences between the two groups in average escape latency and platform crossing times (Supplemental Figs. 3A, 3C). Trajectory charts are presented in Supplemental Fig. 3G. Although the time cost in the first quadrant of RT_2 was longer than the time cost of NS_2 , there was no difference in the time cost in the second quadrant where the original platform was (Supplemental Fig. 3D). Then, in the NOR task, it was similar in discrimination ratio between the two groups (Fig. 3A), while in the OPR task, the discrimination ratio of RT_2 mice was significantly greater than that of NS_2 mice, which indicated RT_2 mice showed a better memory than NS_2 mice (Fig. 3B). Overall, these results indicated that the short-term deficits in cognition induced by remimazolam tosylate didn't last one month, and remimazolam tosylate even protected the memory of aged mice in the long term.

3.5. Remimazolam tosylate administration induced increased ^{18}F -FDG SUV of the mice brain in the long term

Further, when we measured the brain metabolism one month after remimazolam tosylate administration, we found the mean ^{18}F -FDG SUV

of RT_2 mice ($n = 8$) was significantly higher than that of NS_2 mice ($n = 8$) in multiple areas, especially in the cerebral cortex and hippocampus, which were closely associated with cognition (Fig. 3C). These results indicated that remimazolam tosylate not only affected cognitive function but also changed the brain metabolic activity of aged mice in the long term.

3.6. Remimazolam tosylate administration decreased tau phosphorylation of the cortex in the long term

We also detected the total tau and p-tau levels in the hippocampus and frontal cortex of RT_2 ($n = 7$) and NS_2 ($n = 6$) mice on the thirty-seventh day after administration (Fig. 1A). In the hippocampus, there was also no difference in total tau and p-tau levels between the two groups. However, a decrease in tau phosphorylation was found at pSer396 (Fig. 4Q) and pThr205 (Fig. 4T) in the frontal cortex of RT_2 mice, with no change in total tau or other p-tau levels (pSer202/pSer199/pThr231). This result showed remimazolam tosylate had different effects on p-tau of the frontal cortex from the effects of one month ago, and it could reduce p-tau level, which was consistent with the changes in cognition and brain metabolism.

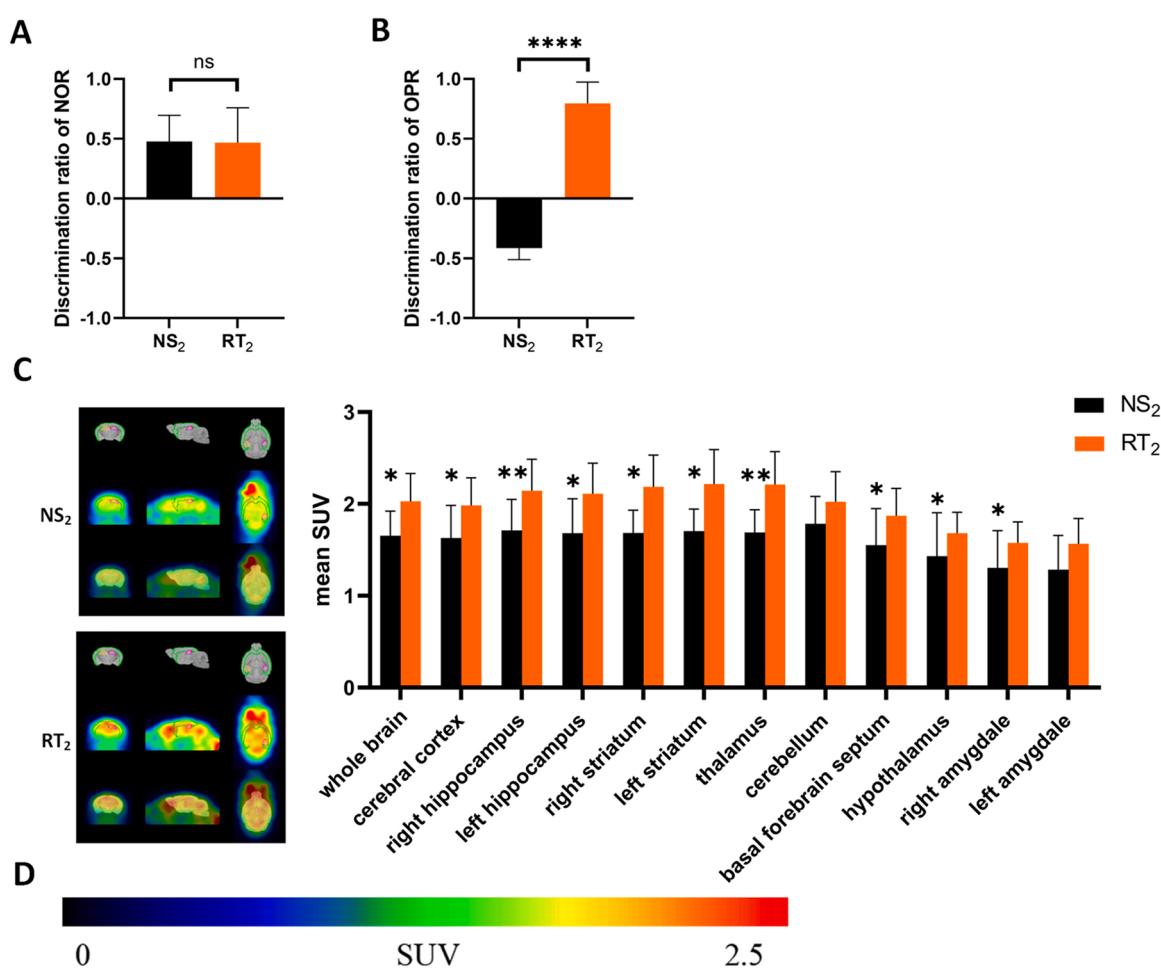


Fig. 3. The effects of intraperitoneal injection of remimazolam tosylate on aged mice in the long term. Object recognition tasks: A The discrimination ratio of NOR ($n = 8$). B The discrimination ratio of OPR ($n = 8$). C ^{18}F -FDG SUV in multiple brain areas of NS_2 ($n = 8$) and RT_2 ($n = 8$). D Standardized uptake value. Values are mean \pm SD, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, compared to the NS_2 group. NOR: novel object recognition, OPR: object place recognition, NS_2 : aged mice whose cognitive function was tested one month after intraperitoneal injection of isometric normal saline, RT_2 : aged mice whose cognitive function was tested one month after intraperitoneal injection of remimazolam tosylate.

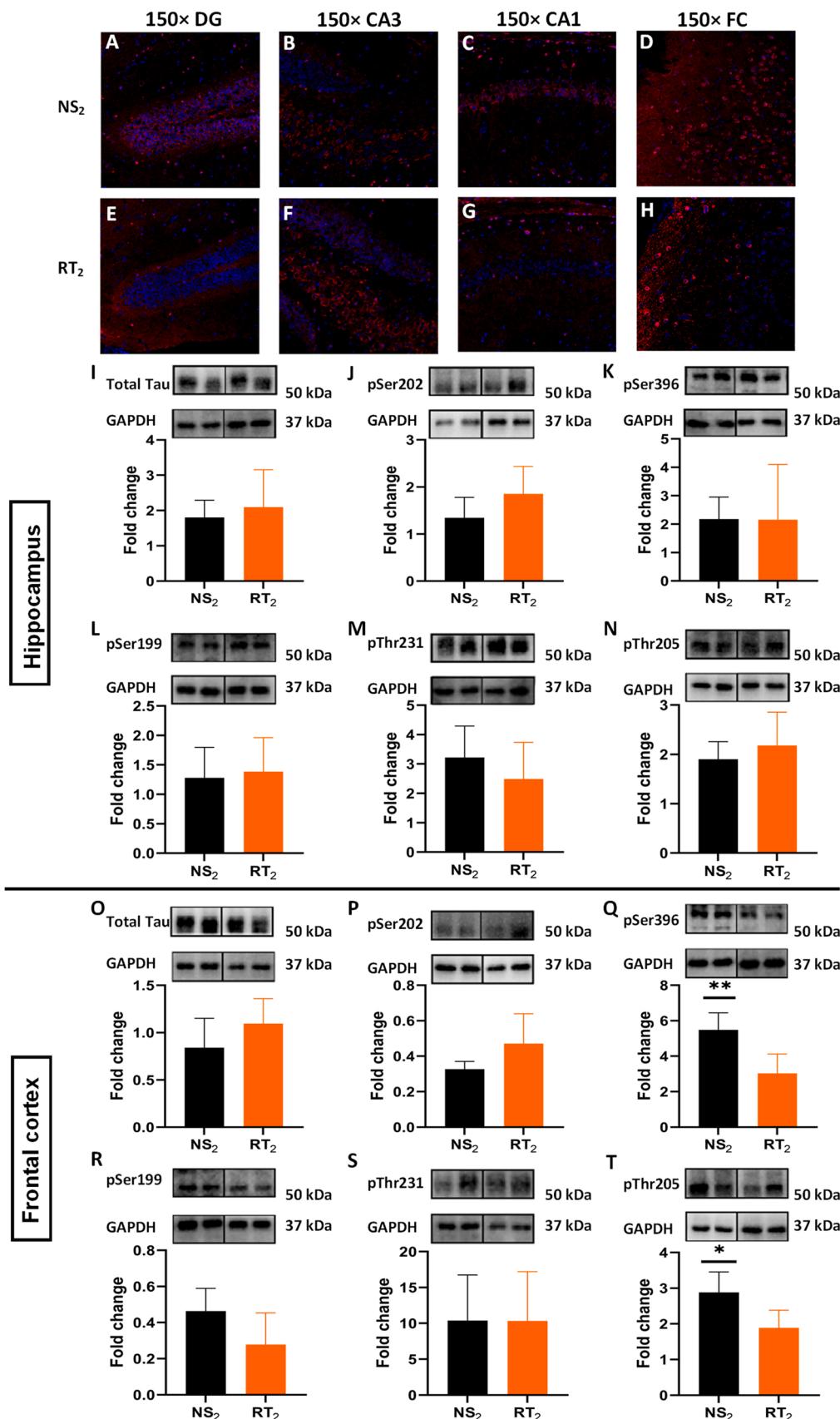


Fig. 4. Intraperitoneal injection of remimazolam tosylate induced a decrease in tau phosphorylation proteins of aged mice in the long term. Immunofluorescence staining: A–C Hippocampus (DG, CA3, CA1) for total tau (red) in NS₂ (n = 1). D Frontal cortex for total tau (red) in aged mouse one month after treatment with normal saline (NS₂, n = 1). E–G Hippocampus (DG, CA3, CA1) for total tau (red) in RT₂ (n = 1). H Frontal cortex for total tau (red) in aged mouse one month after treatment with remimazolam tosylate (RT₂, n = 1). (DAPI, blue). The images of immunofluorescence staining were not quantified. Relative quantification of total tau and phosphorylated tau (p-tau) at the pSer202, pSer396, pSer199, pThr231 and pThr205 phosphoepitopes in NS₂ (n = 6) and RT₂ (n = 7). I–N Total tau and p-tau levels in hippocampal tissues. O–T Total tau and p-tau levels in frontal cortical tissues. GAPDH was selected as an internal reference to control sample loading. Values are expressed as the mean ± SD, *p < 0.05, **p < 0.01, compared to the NS₂ group. FC frontal cortex, DG dentate gyrus.

3.7. Mechanisms of tau phosphorylation change after remimazolam tosylate administration

The tau phosphorylation state was dependent on the activity of several protein phosphatases, including protein phosphatase 1 (PP1), protein phosphatase 2B (PP2B), protein phosphatase 2A (PP2A), and protein phosphatase 5 (PP5). To explore the cause of the changes in tau protein phosphorylation, we further measured the level of phosphatase in the frontal cortex. In the short term, the level of PP2B in RT₁ mice was lower than that in NS₁ mice (Fig. 5D), and the other phosphatase levels were not significantly different between the two groups. While in the long term, we observed higher levels of PP2A in RT₂ mice than that in NS₂ mice (Fig. 5F), with no difference in other phosphatases. These results suggested that phosphatase might be the reason for the change in tau phosphorylation levels in the short and long term.

4. Discussion

In this study, we demonstrated that single-dose RT application had different effects on short-term and long-term cognitive functions in aged mice by affecting the expression of different kinds of protein phosphatase and inducing different phosphorylation profiles of tau at different time points. Though in the short term, mice with RT injection showed impaired cognitive function, one month after single-dose RT administration, behavioral tests and cerebral PET/CT all proved that the aged mice have better object memory ability and metabolic activity than controls, which could attribute to a high level of PP2A expression and

relatively low level of tau phosphorylation at pSer396 and pThr205. It was the first research to study the short-term and long-term effects of RT on cognitive function in aged mice and its mechanism.

Tau belongs to a large family of microtubule-associated proteins (Liu and Götz, 2013). P-tau can result in extensive destruction of neuronal microtubule structure and damage normal axon function (Neitzel et al., 2019; Taleski and Sontag, 2018). Studies have shown that the increase of tau phosphorylation level is significantly related to the decline of cognitive function in the elderly and the disorders of neuronal cell function can reduce the glucose metabolism level of the brain (Lloret et al., 2019; Jagust, 2018). Therefore, through a comprehensive literature review, we selected these representative phosphorylation sites (pSer202, pThr231, pSer396, pSer199, pThr205), which were closely associated with cognitive impairment, for further research. pSer202 is important for regulating microtubule assembly in neurons (Duka et al., 2006), CP13 (monoclonal antibody of pSer202) was also found to have a significant effect on improving tau pathology and reducing insoluble Tau protein (d'Abromo et al., 2015). pThr231-Tau is highly neurotoxic and is considered to initiate a cascade of tau hyperphosphorylation as an early driver of tau pathological changes in a variety of neurodegenerative diseases (Martin et al., 2011). In addition, phosphorylation at this site is seen in mild cognitive change and is not confined to the pathological process of AD (Tournissac et al., 2017). pSer396 plays an important role in mediating tau pathology and the formation of NFT. pSer396 is believed to reduce the affinity between tau and microtubules (Ohene-Nyako et al., 2021). A Higher pSer396-tau level was found in cases of mild cognitive impairment (MCI) (Neddens et al., 2018). Among

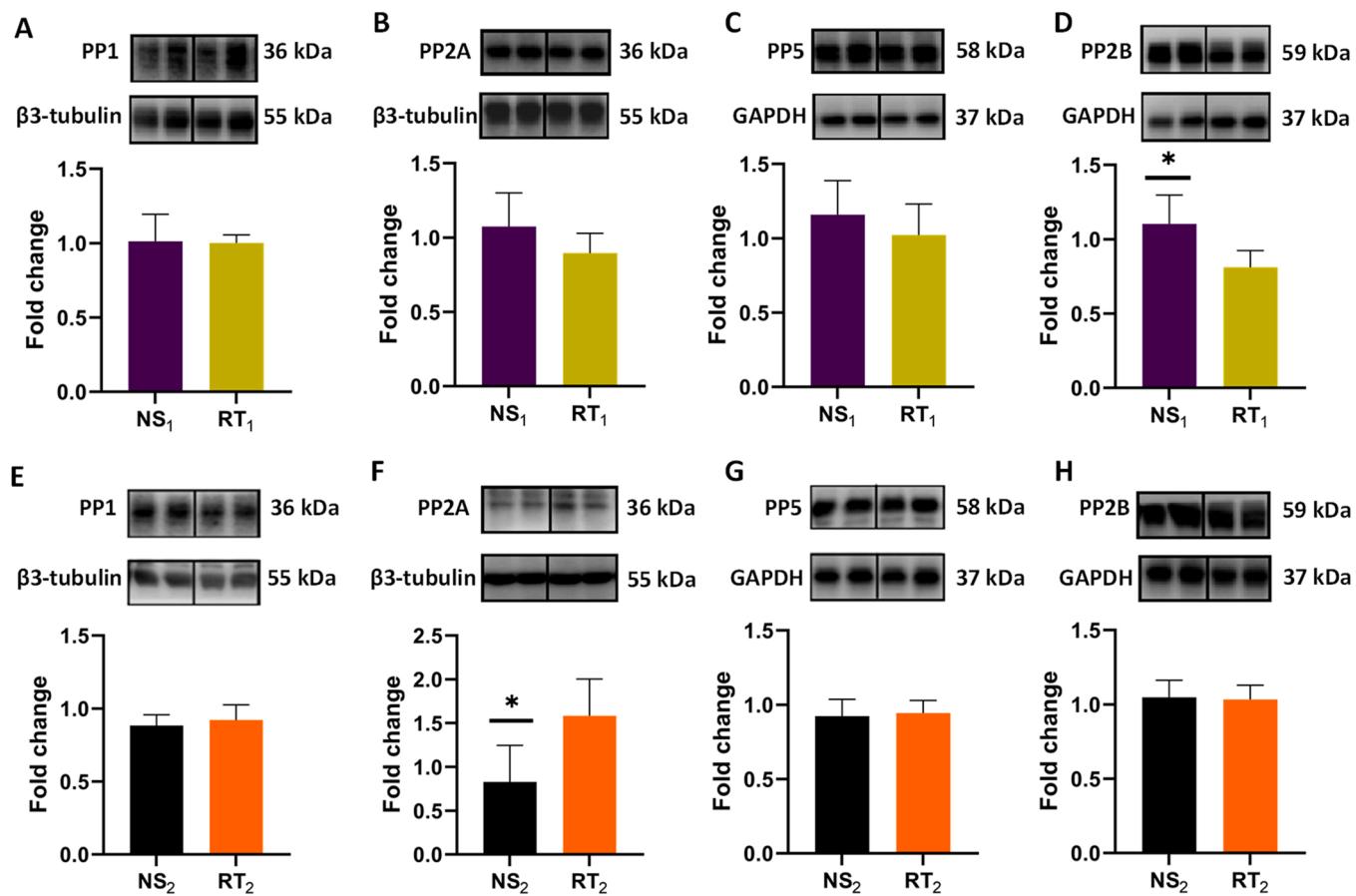


Fig. 5. Remimazolam tosylate changed the phosphatases level in cortical tissue of aged mice. A–D PP1, PP2A, PP5, and PP2B levels in the frontal cortex were measured after treatment with normal saline (NS₁, n = 6) and remimazolam tosylate (RT₁, n = 6) in the short term. E–F PP1, PP2A, PP5, and PP2B levels in the frontal cortex were measured after treatment with normal saline (NS₂, n = 6) and remimazolam tosylate (RT₂, n = 7) in the long term. β3-tubulin and GAPDH were selected as internal references to control sample loading. Values are expressed as the mean ± SD, *p < 0.05, compared to the NS₁ or NS₂ group. PP1: phosphatase 1, PP2A: protein phosphatase 2B, PP5: phosphatase 5, PP2B: protein phosphatase 2B.

multiple phosphorylation sites, pSer199 is highly correlated with the toxic function of tau protein (Bai et al., 2021). In addition, this site is also associated with the occurrence of female sex-regulated AD (Cáceres and González, 2020). pThr205 is associated with increased levels of cognitive impairment, brain edema, and inflammation in brain injury diseases (Shi et al., 2020). pThr205 is considered to be a landmark Tau phosphorylation site for the diagnosis of AD (Dávila-Bouziguet et al., 2019). Besides, pSer202 and pThr205 have been reported to be associated with anaesthesia related cognitive impairment in both midazolam and dexmedetomidine studies (Whittington et al., 2019; Sun et al., 2021). Consistent with previous research, in our study, an elevated level of p-tau caused worse cognitive function and lower glucose uptake in the brain.

Phosphoprotein Phosphatases (PPPs) family members include proteins PP1, PP2A, PP2B, and PP5 whose catalytic subunits bind to various regulatory subunits, which are all believed to be involved in tau dephosphorylation (Liu and Götz, 2013). Previous studies reported that propofol could induce hyperphosphorylation of tau by inhibiting PP2A activity (Whittington et al., 2011), and sevoflurane also could increase tau phosphorylation by specific kinases activation (Le Freche et al., 2012). Therefore, the balance between phosphorylation and dephosphorylation of tau protein may be the key link in the influence of anesthetic on cognitive function. The substrate specificity of PP2B, PP2A, PP1, and PP5 on tau phosphorylation has been systematically studied. PP1, PP2A, PP2B and PP5 all could dephosphorylate tau at Ser199, Ser202, Thr205, Thr212, Ser214, Ser235, Ser262, Ser396, Ser404 and Ser409 in vitro (Liu et al., 2005). But changes in the phosphorylated site of tau and the type of phosphoprotein phosphatase involved in disease progression varies from disease to disease. For example, metformin effectively prevented tau hyperphosphorylation at Ser202 caused by microcystin-leucine-arginine through PP2A activity (Zhang et al., 2021); PP1 and PP2B might participate in the regulation of phosphorylation at Thr231 and Ser235 epitope of tau in mild memory impairment vivo model (Chen, 2005). In our research, remimazolam tosylate downregulated PP2B expression with tau hyperphosphorylation at Ser202 and Thr 231 in the short term and upregulated PP2A expression with tau dephosphorylation at Ser396 and Thr205 in the long term, which could explain why aged mice showed different behavioral characteristics and molecular biological changes between seven days and one month after RT injection. Multiple studies have shown that the activity of PP2A in the brains of AD patients is significantly reduced (Watkins et al., 2012; Voronkov et al., 2011). However, the activity of PP2A was also significantly decreased in tau gene knockout mice, which indicated that the tau could feedback activated expression of PP2A (Tuo et al., 2015). Therefore, we believed that the phosphorylated tau protein in the short term could be the reason for the upregulation of PP2A in the long term, an elevated level of PP2A delayed the tau phosphorylation and cognitive impairment that exist in the natural aging process, which need more experiment to verify in the future work.

Postoperative cognitive decline is an important topic in perioperative management of elderly patients. Postoperative cognitive decline is associated with a 30-day mortality of 7–10%, compared with 1% in those without postoperative cognitive decline. The occurrence of postoperative cognitive decline caused significantly higher healthcare costs, estimated between £ 2000 and £ 8000 additional cost per case (Jin et al., 2020). Advanced age and anesthetic are both important risk factors for postoperative cognitive decline (Stewart, 2005; Ros-Cucurull et al., 2018; Moller et al., 1998). On the one hand, aging of the brain is accompanied by declines in cognitive and memory function (Torres et al., 2021), therefore vulnerable to damage factors. On the other hand, several have confirmed that anesthetic can affect the phosphorylation of tau protein, and may promote cognitive dysfunction (Whittington et al., 2011, 2015; Planell et al., 2004). Benzodiazepines may be one of the important reasons for the increased risk of postoperative cognitive impairment, especially for elderly patients, where the influence is more significant (Stewart, 2005; Ros-Cucurull et al., 2018). Remimazolam

tosylate (RT) is a novel short-acting benzodiazepine GABA(A) receptor agonist and has the characteristics of fast action and fast metabolism, which is expected to reduce the cognitive impairment side effects of benzodiazepines (Chen et al., 2021), at present, there was little study on the effects of RT on cognitive function in the elderly. Our study found that RT administration induced several reversible neurological impairment on aged mice, causing relatively poor object memory, a mild decrease in glucose uptake in several areas of the brain and increased tau phosphorylation of the frontal cortex at pSer202 and pThr231 in the short term. But, one month after a single-dose injection of RT, aged mice with exhibited better object memory than controls, with increased glucose uptake in the brain, and decreased tau phosphorylation at pSer396 and pThr205 in the frontal cortex. These results indicated that the short-term inhibition of cognitive function by remimazolam in elderly patients could be reversible, and it could delay the decline of cognitive function in the long term, RT would be a better choice as a part of general anesthesia for elderly patients.

It is worth discussing that in our study, NOR showed different results from OPR after one month. Increased glucose uptake in brain tissue may be the reason for the inconsistent improvement outcomes. Many studies have shown that the hippocampus and prefrontal cortex play an important role in the formation of episodic memory (Tanimizu et al., 2018; Aggleton, 2012). One study found glucose exerts a stronger influence on location memory than on object memory (Stollery and Christian, 2016). Thus, we infer that the increased glucose uptake levels may account for the more significant cognitive improvement in OPR rather than NOR of RT₂ mice.

This study also has some limitations. Firstly, the 26-month-old mice may have experienced a severe physical decline during natural aging, and we did not get an ideal learning curve during the acquisition training phase, so we conducted an object recognition experiment. In addition, the effects of drugs on aged mice in this study are still at the phenomenal level, and the internal molecular biological mechanism has not been thoroughly studied and explained, which requires further experiments to clarify the mechanism of action of RT. For the regulation of tau protein phosphorylation level, we detected the phosphatase level only, and the detection of phosphokinase level needs to be further explored in subsequent experiments to provide more basis for exploring the mechanism of tau protein phosphorylation.

We concluded that by decreasing the expression of PP2B, a single injection of remimazolam tosylate increased the level of p-tau at pSer202 and pThr232 in the cortex of aged mice in the short term, thus damaging the cognitive function and brain glucose uptake of aged mice. But remimazolam tosylate administration could delay the cognitive decline and enhance the brain glucose uptake of aged mice by promoting the expression of PP2A and reducing the p-tau protein at pSer396 and pThr205 after one month.

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CRediT authorship contribution statement

Xingyang Liu: Conceptualization, Methodology, Data curation, Writing – original draft. **Lizhe Guo:** Conceptualization, Methodology, Data curation, Writing – original draft. **Bin Duan:** Visualization, Investigation. **Jinghan Wu:** Visualization, Formal analysis. **E Wang:** Writing – review & editing, Supervision,

Declaration of Competing Interest

None.

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Author contributions

EW designed and supervised the study. XL, LG, BD, and JW performed the experiments. XL and LG wrote the manuscript draft. EW revised the manuscript. All authors read and approved the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.neuro.2022.08.003](https://doi.org/10.1016/j.neuro.2022.08.003).

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