

Fingolimod (FTY720) improves postoperative cognitive dysfunction in mice subjected to D-galactose-induced aging

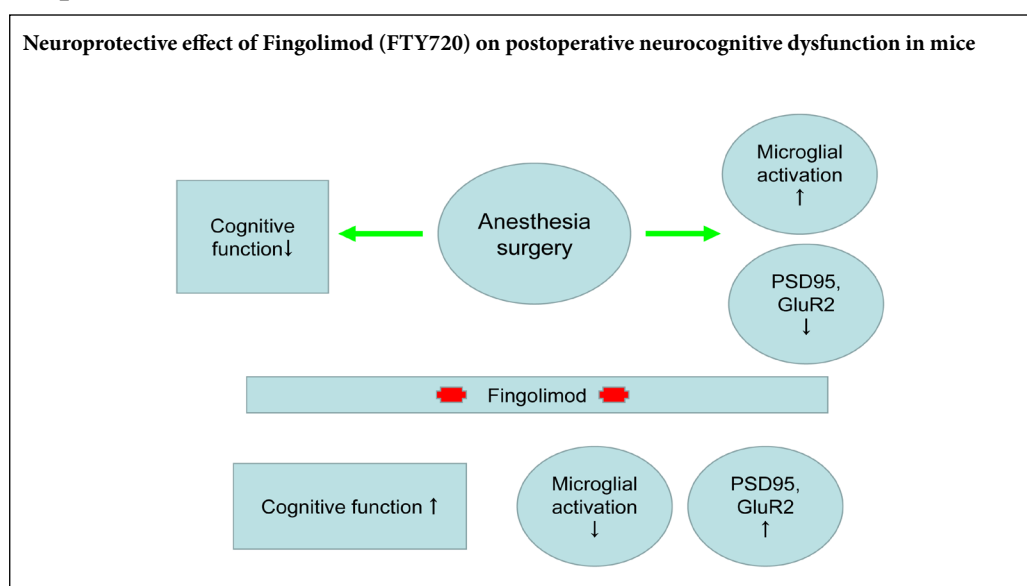
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Graphical Abstract



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Abstract

Neurocognitive dysfunction is a common postoperative complication, especially in older adult patients. Fingolimod (FTY720) is a sphingosine-1-phosphate receptor modulator that has been found to be neuroprotective in several animal models of central nervous system disease. However, few reports have examined whether FTY720 could mitigate postoperative cognitive dysfunction. In this study, we investigated whether FTY720 could prevent postoperative neurocognitive impairment in mice subjected to D-galactose-induced aging. We induced an accelerated model of aging by administering an intraperitoneal injection of D-galactose. Subsequently, we performed a partial hepatectomy under sevoflurane anesthesia. FTY720 (1 mg/kg) was administered intraperitoneally 3 hours before and 24 hours after anesthesia and surgery. Our results indicated that anesthesia and surgery significantly impaired spatial memory in the Y-maze test 6 hours after surgery. We also found that problem solving ability and long-term memory in the puzzle box test on postoperative days 2–4 were significantly improved by FTY720 treatment. Immunohistochemical staining and western blot assay demonstrated that FTY720 significantly inhibited microglial activation in the hippocampal CA1 region of mice 6 hours and 3 days after anesthesia, and down-regulated the expression of synaptic-related proteins postsynaptic density protein 95 and GluR2 in the hippocampus. These results indicate that FTY720 improved postoperative neurocognitive dysfunction in mice subjected to D-galactose-induced aging. This study was approved by the Experimental Animal Ethics Committee of the Third Xiangya Hospital of Central South University of China (approval No. LLSC (LA) 2016-025) on September 27, 2016.

Key Words: D-galactose; fengomod (FTY720); hepatectomy; microglia; nerve regeneration; postoperative neurocognitive dysfunction; sevoflurane

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Introduction

Postoperative neurocognitive dysfunction (POCD) is a complication related to the central nervous system. It is more common in older vs. younger patients following surgery (Li et al., 2019; Song et al., 2019), and includes postoperative delirium, delayed neurocognitive recovery (up to 30 days after the procedure), and postoperative neurocognitive disorder (up to 12 months) (Evered et al., 2018). Although this postoperative complication usually persists for a period of days to weeks after surgery, it can last for decades in severe cases, and even develop into dementia and affect mortality. The incidence rate of POCD can range from 17% to 43%, depending on the patient characteristics (Evered et al., 2011). To date, the prevention and treatment of POCD are not optimal, and are still mainly focused on adjusting the risk factors, enhancing cognitive reserves, and administering symptomatic treatment (Feinkohl et al., 2017; Pappa et al., 2017; Kotekar et al., 2018; Liu et al., 2018). Among the many risk factors for neurocognitive dysfunction, including patient characteristics, surgery type, anesthesia, and environment (Kotekar et al., 2014; Kulason et al., 2017; Kubota et al., 2018), age was identified as an independent risk factor (Kotekar et al., 2014; Kubota et al., 2018). A prospective study by Kotekar et al. (2014) found that the incidence of POCD in individuals aged 60 years, 61–70 years, and 71–80 years was 12.5%, 20.5%, and 40.9%, respectively, suggesting that POCD is strongly associated with age. Therefore, as many societies face aging populations, new and effective methods for preventing POCD in older adult patients are urgently needed.

Fingolimod (FTY720) is a new immunosuppressant that is primarily used to treat relapsing-remitting multiple sclerosis (Kappos et al., 2010; Calabresi et al., 2014). FTY720 has been found to have neuroprotective and anti-inflammatory effects in several pre-clinical animal models of central nervous system diseases, such as Alzheimer's disease (Hemmati et al., 2013; Aytan et al., 2016), ischemic stroke (Kraft et al., 2013; Nazari et al., 2016), cerebral hemorrhage (Lu et al., 2014), hyperoxia (Serdar et al., 2016) and Parkinson's disease (Moyl et al., 2018). FTY720 has a carbon backbone, making it a highly lipophilic compound. Accordingly, it can easily traverse the blood-brain barrier where it becomes localized in the white matter in the central nervous system (Foster et al., 2007). FTY720 also modulates the sphingosine-1-phosphate receptor (S1PR), which is highly present in the central nervous system (Cruz et al., 2014; Martin et al., 2014; Healy et al., 2016). Cannon et al. (2012) found that FTY720 combined with S1PR1 and quickly but reversibly reduced P-glycoprotein activity. As P-glycoprotein activity facilitates the entry of small-molecule drugs into the central nervous system through the blood-brain barrier, it appears that FTY720 can influence the blood-brain barrier. Therefore, FTY720 may enter the central nervous system and exert a neuroprotective effect on S1PR in central nervous system cells, including microglia (Noda et al., 2013; Cipriani et al., 2015), astrocytes (Dusaban et al., 2017; Rothhammer et al., 2017), oligodendrocytes (Segura-Ulate et al., 2017), and neurons (Di et al., 2013). Many animal and clinical studies have confirmed that central nervous system cells and neuroinflammation play an indispensable role in the pathogenesis of POCD (Berger et al., 2019; Safavynia et al., 2019). Therefore, we hypothesized

that FTY720 may be useful as a preventive drug that could alleviate postoperative cognitive impairment. Zhou et al. (2013) evaluated the effects of FTY720 on sevoflurane-induced neurotoxicity in rat pups. They found that 1 mg/kg of FTY720 before exposure to sevoflurane significantly inhibited neuronal apoptosis, and that this could be abrogated by VPC23019 (S1P antagonist). Unfortunately, few studies have examined the impact of post-surgical administration of FTY720 in aged animals. Thus, the neuroprotective mechanisms of FTY720 remain unknown.

Because animals injected with D-galactose exhibit a number of aging-related features, this technique has been extensively applied to the study of aging-related diseases (Ali et al., 2015; Sadigh-Eteghad et al., 2017; Shwe et al., 2018). Therefore, in the present study, we induced aging in mice via an intraperitoneal injection of D-galactose (1000 mg/kg). We then evaluated whether FTY720 could improve POCD in mice subjected to D-galactose-induced aging and explored the underlying mechanisms.

Materials and Methods

Animals

All experiments were performed in accordance with the National Institutes of Health guidelines. The protocol was approved by the Animal Ethics Committee of the Third Xiangya Hospital of Central South University, China on September 27, 2016 (approval No. LLSC (LA) 2016-025). We purchased 2-month-old male C57BL/6J mice that weighed 20–25 g from the Central South University of China [license No. SCXK (Xiang) 2016-0002]. All mice were housed for 7 days before the experiments in a controlled environment (22–25°C, 12-hour light/dark cycle). The mice were allowed free access to water and food.

Experimental groups

The C57BL/6J mice received 1000 mg/kg of D-galactose (Sigma-Aldrich Co., St. Louis, MO, USA) via intraperitoneal injection, once daily for 60 consecutive days. The same person conducted injections at the same time every day. This accelerated aging model was successfully established in a previous study in our laboratory (Duan et al., 2018). All mice subjected to D-galactose-induced aging were randomly divided into four groups ($n = 12/\text{group}$): (1) The C group received no anesthesia, surgery, or FTY720; (2) the C + FTY720 group received FTY720 (1 mg/kg, intraperitoneally), but no anesthesia or surgery; (3) the S + vehicle group received 2% sevoflurane anesthesia for 2 hours, underwent a partial hepatectomy, and received injections of vehicle (0.5 mL, intraperitoneally) 3 hours before and 24 hours after surgery, and (4) the S + FTY720 group received 2% sevoflurane anesthesia for 2 hours, underwent a partial hepatectomy, and received injections of FTY720 (1 mg/kg, intraperitoneally) 3 hours before and 24 hours after surgery.

Drug administration

FTY720 is sparingly soluble in aqueous buffers. As per the instructions that accompanied FTY720, we first dissolved FTY720 (Cayman Chemical Co., Ann Arbor, MI, USA) in ethanol with a concentration of 20 $\mu\text{g}/\mu\text{L}$ for maximum sol-

ubility in aqueous buffers, and then diluted it with saline. FTY720 was freshly prepared for each intervention. The dose of FTY720 (1 mg/kg, administered intraperitoneally) was selected according to previous studies in neonatal rats (Zhou et al., 2013; Serdar et al., 2016). FTY720 was administered 3 hours before and 24 hours after surgery. The vehicle solution was the same as the FTY720 solution except we did not add FTY720. The injection volume and age of the vehicle solution were the same as those for the FTY720 solution.

Anesthesia and partial hepatectomy

The anesthesia and surgery were conducted in accordance with previous studies (Tang et al., 2017; Duan et al., 2018). Mice were placed into an anesthesia induction chamber that was pre-filled with 5% sevoflurane (Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) mixed with high-flow oxygen (5 L/min). After the mice lost the righting reflex, they were given 3% sevoflurane and oxygen (80–85%) for 2 hours through a mask over the mouth and nose. Sevoflurane and oxygen concentrations were monitored using a multifunctional detector (Datex-Ohmeda, Helsinki, Finland). During anesthesia, the mice were subjected to a partial hepatectomy. After skin antiseptic and disinfection, a 2-cm incision was made just below the xiphoid process. Cutting the muscle layer exposed the abdominal cavity. The left lobe of the liver was then visualized and isolated, ligated, and carefully resected. Subsequently, the incision was sutured using 5-0 thread. Finally, to relieve pain caused by the incision, lidocaine cream (2.5% lidocaine and 2.5% prilocaine) was applied to the skin incisions immediately after the surgery and three times per day for the following 2 days. Anesthesia was induced for 2 hours, after which we removed the mask and placed the mice in a warm environment to recover naturally.

Behavioral tests

We used the Y-maze test and puzzle box test to assess whether the anesthesia and surgery impaired cognitive function and whether FTY720 could reverse this change. Spatial memory was evaluated via the Y-maze test 6 hours after surgery, and executive function was assessed via the puzzle box on postoperative days 2–4.

Y-maze test

We used the Y-maze test to evaluate spatial memory ability, as previously described (Peng et al., 2016). The Y-maze consisted of a start arm (always open), second arm (always open), and novel arm (blocked during the first trial, open during the second trial). The angle between each arm was 120 degrees. The Y-maze test consisted of two trials separated by a 2-hour interval. In the first trial (training), which was 10 minutes long, the mouse freely explored the two arms (start arm and second arm) of the maze with the novel arm blocked. After a 2-hour interval, we conducted a second trial (retention) in which the mouse was placed in the maze at the start arm and allowed to explore all three arms for 5 minutes. A logitech video camera was placed directly above the Y-maze such that it captured activity in all three arms. The number of entries and the time spent in each arm were recorded and analyzed. More entries in the novel arm (%) and a longer duration of time spent in the novel arm (%)

indicated better spatial recognition memory. At the end of each experiment, a 75% ethanol solution was sprayed on the bottom and inner wall of the maze to remove odors, feces, and urine.

Puzzle box test

In accordance with a previous study (Zurek et al., 2016), we used the puzzle box test to assess executive function, including problem solving and cognitive flexibility. The puzzle box was composed of a light box ($58.0 \times 28.0 \times 27.5 \text{ cm}^3$) and dark goal box ($14.0 \times 28.0 \times 27.5 \text{ cm}^3$), which were connected by a door and also by a covered tunnel. The mouse was required to travel from the light box to the dark box (the goal box). The puzzle box test consisted of four trials: the 1st trial (door open, tunnel open), 2nd trial (door closed, tunnel open), 3rd trial (door closed, bedding in tunnel), and 4th trial (door closed, obstacle in tunnel). Thus, the task difficulty was gradually increased from the 1st trial to the 4th trial. The mouse was placed in the middle of the bright box and allowed to explore freely until it reached the dark box. If the mouse did not reach the dark box within 5 minutes, it was then gently guided to the dark box. The whole experiment was conducted over 3 days with three steps on each day. On the 1st day (trials 1-2-2), we conducted trial 1 (door open, tunnel open), in which the mouse could reach the dark box through the open door or the tunnel. After a 2-minute interval, we conducted trial 2 (door closed, tunnel open), in which the mouse could get to the dark box only through the tunnel (problem-solving). After a 2-minute interval, we tested short-term memory for this task by repeating trial 2. On the 2nd day (trials 2-3-3), we conducted trial 2 to assess long-term memory for the task. After a 2-minute interval, we conducted trial 3 (door closed, bedding in tunnel), in which the mouse was required to burrow into the clean bedding material to find the entrance to the tunnel and enter the dark box (more difficult problem-solving). After another 2-minute interval, we repeated trial 3 to assess short-term memory for this task. On the 3rd day (trial 3), we repeated trial 3. We found that trial 4 was too difficult for the mouse, so we excluded this task. We recorded and analyzed the time required to solve each task. The mice were given 5 minutes for each trial, which was considered complete if the mouse had all four paws inside the goal box. Shorter task latency indicated better problem-solving, short-term memory, and long-term memory.

Immunohistochemistry

After anesthetizing the mice, they were perfused with 4% paraformaldehyde from the heart to the brain until the body was stiff. The brain tissue was then removed from the skull and fixed in 4% paraformaldehyde. After 2 days, the brain tissue was dehydrated with different concentrations of sucrose (15%, 30%, 30%, and 35%) at 4°C. When the tissue sank to the bottom of the container, the brains were embedded in OCT compound and stored at –80°C.

We used a pre-freezing sliding microtome (Leica CM1950, Wetzlar, Germany) to continuously cut brain tissue containing the hippocampus into 20- μm sections. These were washed three times using phosphate buffered saline and then exposed to 3% hydrogen peroxide for 10 minutes at room temperature.

After washing the slices again with phosphate buffered saline, the sections were sealed with 5% bovine serum albumin (Sigma, St. Louis, MO, USA) for 1 hour, and then incubated in a rabbit anti-Iba-1 dilution (1:1000; Wako, Osaka, Japan; marker for microglial cells, polyclonal antibody) at room temperature overnight. We conducted Iba1 staining according to the instructions for use of the 3,3'-diaminobenzidine reagent (Beijing Zhongshan Jinqiao Biological Technology Co., Ltd., Beijing, China). The sections were dried, de-waxed, dehydrated with different concentrations of alcohol (70%, 95%, 100%, and 100%), sealed, and dried overnight.

Activated microglia cells were counted using a microscope (Nikon, Tokyo, Japan). The procedure for counting activated microglia cells in CA1 was consistent with that used in a previous study (Cerbai et al., 2012). First, we established criteria for determining whether microglia cells were "resting" or "reactive". According to the literature, resting microglia cells were defined as small, round, thin, and branched with protuberations around the cell body. Reactive microglia cells were defined as multipolar (bipolar, tripolar, or spindle/rod shaped), short branched, wound, or asymmetrically distributed, with larger cell bodies compared with resting cells.

Western blot assay

We used a western blot assay to assess the expression of synaptophysin (SYN), postsynaptic density protein 95 (PSD95), and GluR2 in the hippocampus. After anesthesia and perfusion with 4% paraformaldehyde, the hippocampal tissues were extracted and stored in liquid nitrogen. The hippocampal samples were then treated with histone lysate, which contained NP40 lysate, a 1% phosphatase inhibitor, and a 1% protease inhibitor (Sigma-Aldrich, St. Louis, MO, USA). The tissue samples were homogenized via a probe, centrifuged at $12,000 \times g$ at 4°C , and the supernatant was obtained. Total protein concentration was assessed according to the instructions that accompanied the bicinchoninic acid protein assay kit (CWBio, Beijing, China). We added the marker and protein sample directly to sodium dodecylsulfate-polyacrylamide electrophoresis gel. We then conducted electrophoresis at a constant voltage of 80 V until the marker separated, at which point we increased the voltage to 110 V. After transferring the sample onto a polyvinylidene fluoride membrane (BioRad, Hercules, CA, USA), the membrane was placed in Tris-buffered saline Tween + 5% non-fat milk, and sealed at room temperature for 60 minutes. Subsequently, the membrane was incubated with rabbit anti-SYN (1:500; Proteintech, Chicago, IL, USA; a marker for presynaptic terminals, polyclonal antibody), rabbit anti-PSD95 (1:1000; Abcam, Cambridge, MA, USA; a marker for postsynaptic terminals, polyclonal antibody), rabbit anti-GluR2 (1:1000; Proteintech; a glutamate receptor subunit, polyclonal antibody), and rabbit anti- β -actin (1:2000; Proteintech; polyclonal antibody) at 4°C overnight. After three washes with Tris-buffered saline Tween, the membrane was treated with the diluted secondary antibody (1:8000; anti-rabbit, 926-32211, Li-CORr, polyclonal antibody) and slowly shaken at room temperature for 60 minutes. The immunoblot bands were detected using Odyssey-CLX infrared imaging visualizer (Li-CORr). The relative protein levels of SYN, PSD95, and Glu R2 compared with β -actin were analyzed using ImageJ software (National

Institutes of Health, Bethesda, MD, USA).

Statistical analysis

We used GraphPad Prism 6.0 software (GraphPad Software Inc., La Jolla, CA, USA) for statistical analysis. All data are presented as the mean \pm SEM. The results of the Y-maze test, microglia activated ratio, and western blots were analyzed using a one-way analysis of variance followed by Tukey's *post hoc* test. The results of the puzzle box test were analyzed using a two-way analysis of variance followed by Tukey's *post hoc* test. *P*-values < 0.05 were considered statistically significant.

Results

Perioperative FTY720 administration ameliorates postoperative cognitive impairment in mice subjected to D-galactose-induced aging

In the Y-maze test, the percentage of entries in the novel arm and the percentage of time spent in the novel arm were statistically different among the groups (entries in novel arm% $F_{(3, 43)} = 3.470$, $P = 0.024$; time spent in novel arm% $F_{(3, 43)} = 4.485$, $P = 0.008$; **Figure 1A and B**). Tukey's *post hoc* analysis confirmed that the percentage of entries in the novel arm was significantly lower in the S + vehicle group compared with the C group, and that the percentage of time spent in the novel arm was significantly lower in the S + vehicle group compared with the C + FTY720 group (entries in novel arm%: S + vehicle vs. C, $P < 0.05$; time spent in novel arm%: S + vehicle vs. C + FTY720: $P < 0.05$; **Figure 1A and B**). There was no statistical difference between the S + FTY720 group and S + vehicle group (entries in novel arm%, $P = 0.498$; time spent in novel arm%, $P = 0.788$). These data suggest that anesthesia and surgery impaired spatial memory in mice subjected to D-galactose-induced aging.

Finally, we assessed changes in problem solving and memory, as revealed by performance in the Puzzle Box test. Problem solving ability was assessed according to the time taken to enter the dark box. The difficulty of the task was progressively increased. As shown in **Figure 1**, in terms of problem solving ability, the mice in all groups took longer to reach the dark box when they were required to burrow into the tunnel, compared with when the tunnel was open and when both the door and tunnel were open (two-way analysis of variance: group, $F_{(3, 99)} = 6.117$, $P < 0.001$; interaction, $F_{(6, 99)} = 4.611$, $P < 0.001$; **Figure 1C**). Tukey's multiple comparisons showed that the mice in the S + vehicle group spent significantly more time to solve the burrowing task than those in the other groups (S + vehicle vs. C, $P = 0.0001$; S + vehicle vs. C + FTY720, $P < 0.0001$; S + vehicle vs. S + FTY720, $P < 0.0001$; **Figure 1C**). We assessed short-term memory and long-term memory by retesting the mice 2 minutes and 24 hours, respectively, after first exposing the mice to the task. In terms of short-term memory, we found no significant difference among the four groups (two-way analysis of variance; group, $F_{(3, 66)} = 1.067$, $P = 0.369$; interaction, $F_{(3, 66)} = 0.297$, $P = 0.828$; **Figure 1D**). In terms of long-term memory, the four groups of mice exhibited a similar latency in the open tunnel task. However, the time required to complete the burrowing task was longer in the S + vehicle group compared with the other groups (two-way analysis of

variance; group, $F_{(3,66)} = 3.504$, $P < 0.05$; interaction, $F_{(3,66)} = 2.374$, $P = 0.078$. Tukey's multiple comparisons: S + vehicle vs. C, $P < 0.01$; S + vehicle vs. C + FTY720, $P < 0.01$; S + vehicle vs. S + FTY720: $P < 0.05$; **Figure 1E**).

These data suggest that anesthesia and surgery impaired problem-solving ability and long-term memory and that while mice subjected to D-galactose-induced aging had more difficulty completing the tasks, FTY720 ameliorated these impairments.

Perioperative FTY720 administration inhibits microglial activation after anesthesia and surgery in mice subjected to D-galactose-induced aging

Microglial over-activation has been reported to drive neuroinflammation via positive feedback mechanisms, which is an important pathological mechanism of postoperative cognitive impairment (Hovens et al., 2014). Next, we examined the degree to which microglia were activated in the hippocampal CA1 area.

Our results showed that the percentage of activated microglia was statistically different among the four groups (6 hours: $F_{(3,12)} = 17.24$, $P = 0.0001$; 3 days: $F_{(3,12)} = 12.82$, $P = 0.0005$). Tukey's *post hoc* analysis showed that microglia in the S + vehicle group were significantly activated in the CA1 area compared with those in the C group and C + FTY720 group at 6 hours and 3 days after surgery (6 hours: S + vehicle vs. C, $P < 0.001$; S + vehicle vs. C + FTY720, $P < 0.001$; 3 days: S + vehicle vs. C, $P < 0.01$; S + vehicle vs. C + FTY720, $P < 0.001$; **Figure 2**). Compared with that in the S + vehicle group, the ratio of activated microglia in the S + FTY720 group was clearly lower at 6 hours and 3 days after surgery (6 hours: S + vehicle vs. S + FTY720, $P < 0.05$; 3 days: S + vehicle vs. S + FTY720, $P < 0.05$; **Figure 2**). These results suggest that FTY720 inhibited microglial activation after anesthesia and surgery in mice subjected to D-Gal-induced aging.

Perioperative FTY720 administration increases synaptic protein expression in mice subjected to D-Gal-induced aging mice

Synaptic plasticity-associated proteins including presynaptic protein SYN, PSD95, and AMPAR are involved in postoperative cognition (Jiang et al., 2018; Zhang et al., 2018; Zhou et al., 2018). Here, we measured the expression of SYN, PSD95, and the AMPAR subunit GluR2 in the hippocampus at 6 hours and 3 days after anesthesia and surgery (**Figure 3**). The expression of PSD95 and GluR2 proteins in the hippocampus was statistically different among the four groups at 6 hours and 3 days after surgery (PSD95: 6 hours: $F_{(3,8)} = 7.707$, $P = 0.010$; 3 days: $F_{(3,8)} = 66.24$, $P < 0.0001$; GluR2: 6 hours: $F_{(3,8)} = 33.36$, $P < 0.0001$; 3 days: $F_{(3,8)} = 28.63$, $P = 0.0001$). Tukey's *post hoc* tests showed that, compared with that in the C group, C + FTY720 group, and S + FTY720 group, hippocampal expression of PSD95 and GluR2 proteins in the S + vehicle group was significantly lower at 6 hours and 3 days after surgery (all $P < 0.05$; **Figure 3**). There were no significant differences in SYN expression among the four groups at 6 hours and 3 days after surgery (all $P > 0.05$; **Figure 3**). These results showed that anesthesia and surgery induced significant decreases in GluR2 and PSD95 expression in the hippocampus, which were then alleviated by perioperative FTY720 treatment.

Discussion

In this study, we sought to determine whether FTY720 could improve POCD in mice subjected to D-Gal-induced aging, and to examine the possible mechanisms underlying this phenomenon. Our results demonstrated that FTY720 treatment alleviated postoperative decreases in problem solving ability and long-term memory in the puzzle box test on postoperative days 2–4. Corresponding with this behavioral improvement, FTY720 also alleviated postoperative microglial activation and the loss of synaptic plasticity-associated proteins (PSD95, GluR2). These results suggest that FTY720 is neuroprotective and thus represents a potential preventive reagent for POCD.

POCD is a common central nervous system complication after surgery. Age, frailty, surgery-induced inflammation, anesthetic toxicity, sleep disturbances, and pain all are closely associated with the occurrence and development of POCD (Callaway et al., 2015; Hovens et al., 2016; Gu et al., 2018). To date, the main preventive strategies have addressed various risk factors involving the patient, surgery methods, and anesthesia. Pharmacological agents such as acetylcholine esterase inhibitors, COX-2 inhibitors, dexmedetomidine, and statins have been studied in terms of their potential to relieve the symptoms of POCD (Safavynia et al., 2019). However, the pathogenesis of POCD is not fully understood, and so an optimal solution for preventing and treating POCD has yet to be established. The high prevalence of POCD remains a clinical challenge.

S1P receptors are widely expressed in cells in the brain, heart, liver, stomach, and retina, with the exception of leucocytes and lymphocytes (Subei et al., 2015; Chaudhry et al., 2017). FTY720 is a functional S1P receptor modulator that has been used to treat patients with multiple sclerosis, immune diseases, organ transplants, myasthenia gravis, and some metastatic cancers (Mandal et al., 2017; Huwiler et al., 2018). FTY720 has been reported to exert neuroprotective and anti-inflammatory effects in the central nervous system disease model, while neuroinflammation is one mechanism of POCD (Luo et al., 2019; Safavynia et al., 2019). However, few studies have examined the use of FTY720 for treating POCD. In this study, we measured the preventive role of FTY720 in mice subjected to POCD-induced aging. We found that FTY720 treatment alleviated postoperative impairment in terms of problem solving ability and long-term memory in the puzzle box test on postoperative days 2–4. Corresponding with this behavioral improvement, FTY720 also alleviated postoperative microglia activation and synaptic plasticity-associated protein loss (PSD95, GluR2). These results are in accordance with previous studies (Zhou et al., 2013; Miguez et al., 2015; Nazari et al., 2016; Serdar et al., 2016; Xu et al., 2017). For example, Zhou et al. (2013) demonstrated that FTY720 attenuated sevoflurane-induced neurotoxicity in rat pups, whereas VPC23019 (S1P antagonist) inhibited the protective action of FTY720. These results suggest that FTY720 has a protective effect against sevoflurane-induced neurotoxicity in developing rats. In a neonatal model of hyperoxia, Serdar et al. (2016) found that FTY720 could reduce hyperoxia-induced cognitive dysfunction, microglial activation, and associated pro-inflammatory cytokine expression. Furthermore, to ascertain

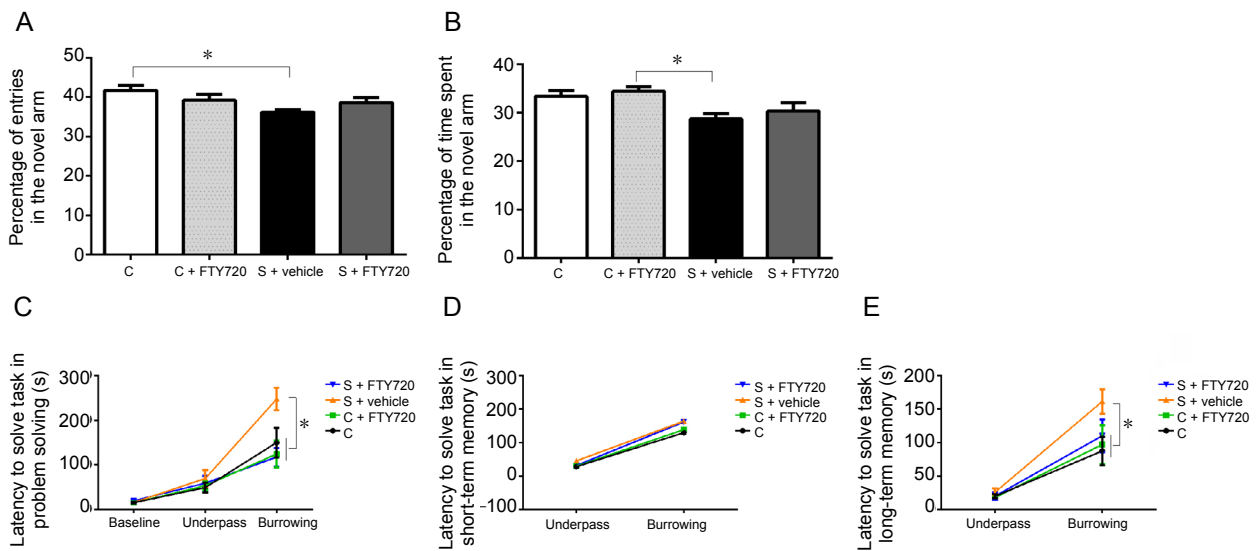


Figure 1 Effect of fingolimod (FTY720) on postoperative cognitive dysfunction in mice subjected to D-galactose-induced aging.

(A, B) Percentage of entries and percentage of time spent in the novel arm of the Y-maze test 6 hours after surgery (mean \pm SEM, $n = 12$; one-way analysis of variance followed by Tukey's *post hoc* test). (C–E) Puzzle box test performance on postoperative days 2–4 (mean \pm SEM, $n = 12$; two-way analysis of variance followed by Tukey's *post hoc* test). * $P < 0.05$. C group: Received no anesthesia, surgery, or FTY720; C + FTY720 group: received FTY720, but no anesthesia or surgery; S + vehicle group: received vehicle, anesthesia, and surgery; S + FTY720 group: received FTY720, anesthesia, and surgery.

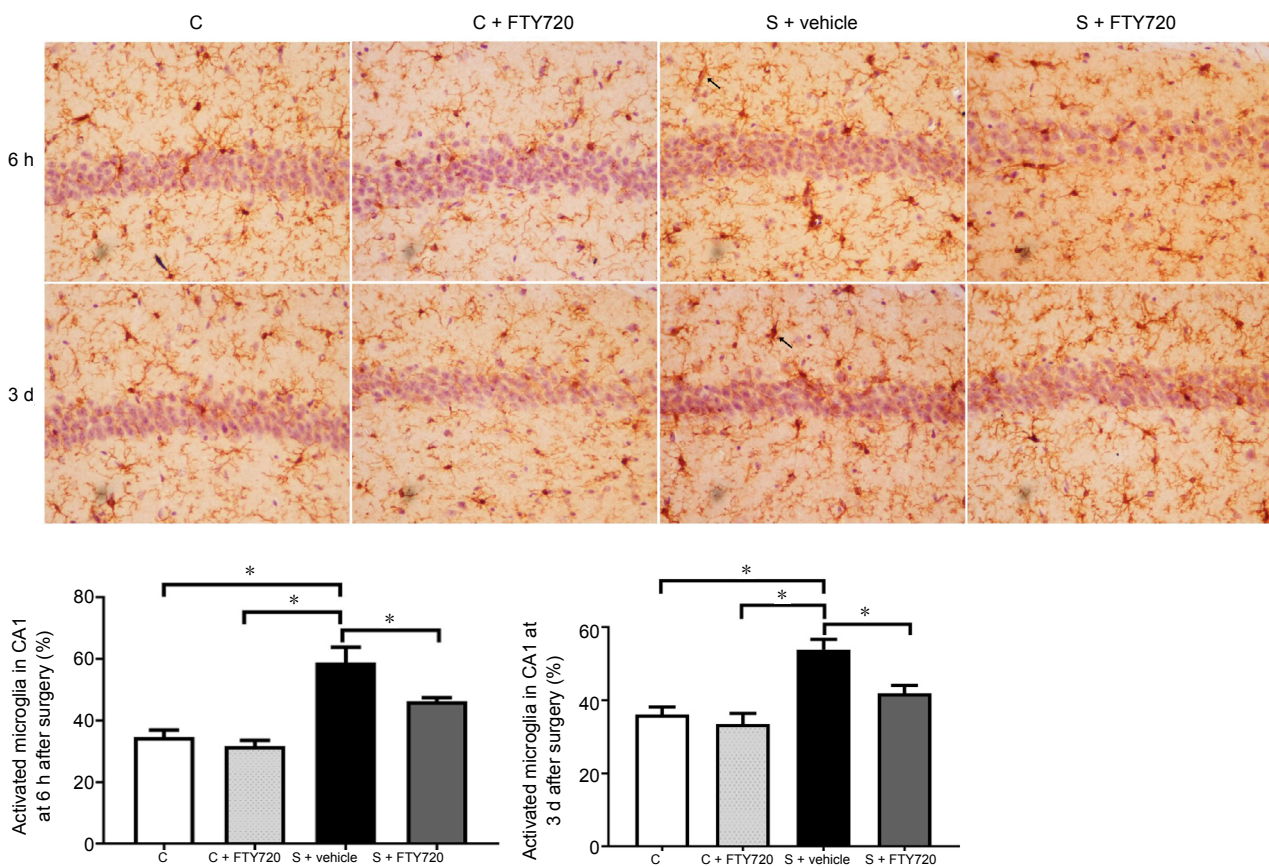


Figure 2 Perioperative fingolimod (FTY720) administration limits microglial activation after anesthesia and surgery in mice subjected to D-galactose-induced aging.

Representative images showing Iba-1 staining (yellow) in CA1 at 6 hours and 3 days after surgery. Typical Iba-1-stained activated microglia cells in CA1 had bigger cell bodies and shortened or twisted branches (indicated by black arrows). The percentage of activated microglia was statistically different among the four groups. Scale bar: 50 μ m. Data are expressed as the mean \pm SEM ($n = 4$ per time point per group; one-way analysis of variance followed by Tukey's *post hoc* tests). * $P < 0.05$. C group: Received no anesthesia, surgery, or FTY720; C + FTY720 group: received FTY720, but no anesthesia or surgery; S + vehicle group: received vehicle, anesthesia, and surgery; S + FTY720 group: received FTY720, anesthesia, and surgery.

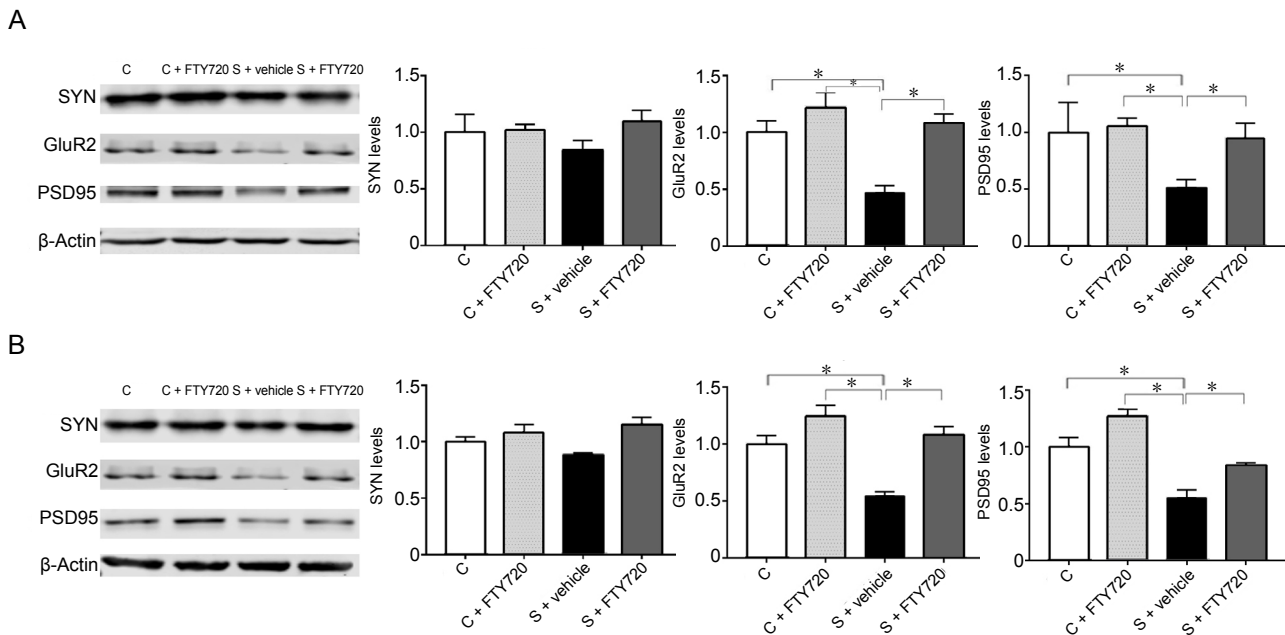


Figure 3 Perioperative fingolimod (FTY720) administration selectively increases postoperative synaptic protein expressions in mice subjected to D-galactose-induced aging.

Representative images and analysis showing the expression levels of SYN, GluR2, and PSD95 in the hippocampus, as revealed by western blot assay, at 6 hours (A) and 3 days (B) after surgery. Data are expressed as the mean \pm SEM ($n = 3$; one-way analysis of variance followed by Tukey's *post hoc* tests). * $P < 0.05$. C group: Received no anesthesia, surgery, or FTY720; C + FTY720 group: received FTY720, but no anesthesia or surgery; S + vehicle group: received vehicle, anesthesia, and surgery; S + FTY720 group: received FTY720, anesthesia, and surgery. GluR2: Glutamate receptor 2; PSD95: postsynaptic density protein 95; SYN: synaptophysin.

whether the memory-enhancing effect of FTY720 was correlated with synaptic plasticity in the hyperactivity disorder model, Miguez et al. (2015) examined the expression of PSD-95 in the hippocampus and found that FTY720 treatment prevented the expected decrease in PSD-95 protein levels, indicating a role for FTY720 in modulating structural synaptic plasticity. All of these previous studies demonstrate that FTY720 has a protective effect on the central nervous system and that it can rescue impaired cognitive function. S1P receptors are widely expressed in lymphocytes and neural cells. Furthermore, FTY720 is fat-soluble and can penetrate the blood-brain barrier. In future work, we hope to determine whether FTY720 improves the symptoms of POCD by limiting the infiltration of lymphocytes into the brain or by directly acting on neural cells.

Few studies have examined the protective effect of FTY720 on POCD. In this exploratory study, we examined the effect of FTY720 on postoperative cognitive function in a mouse model of rapid aging. We found that FTY720 could improve postoperative cognitive impairment in mice subjected to D-galactose-induced aging, which is associated with inhibitory microglial activation and the loss of synaptic proteins (PSD95, GluR2). However, additional factors might be involved in the neuroprotective mechanisms of FTY720. S1PR is also expressed in astrocytes, oligodendrocytes, and neurons. Thus, further studies are needed to investigate other possible mechanisms of the effect of FTY720 on POCD, and particularly to determine which brain cells are mainly affected.

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