Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2025**; 30(4): 36597
https://doi.org/10.31083/FBL36597

Original Research

Aerobic Exercise Ameliorates Alzheimer's Disease-Like Pathology by Regulating Hepatic Phagocytosis of Aβ

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Academic Editor: Xudong Huang
Submitted: 25 December 2024 Revised: 19 February 2025 Accepted: 21 March 2025 Published: 22 April 2025

Abstract

Background: Alzheimer's disease (AD) is a neurodegenerative disease which significantly and negatively affects families and society. Aerobic exercise serves as a non-pharmacological strategy, potentially safeguarding against cognitive decline and lowering the risk of AD. However, how aerobic exercise ameliorates AD remains unknown. This study investigated the effects of two types of aerobic exercise, including aerobic interval training (AIT) and aerobic continuous training (ACT), on cognitive and exploratory function, brain histopathology, and hepatic amyloid beta (Aβ) clearance in amyloid precursor protein/presenilin-1 double transgenic (APP/PS1) transgenic mice. **Methods**: Twenty-four six-month-old male APP/PS1 transgenic mice (body weight: 20–22 g) were used to establish the AD model. APP/PS1 transgenic mice were randomly assigned to one of the three groups: rest (AD group, n = 8), aerobic interval training (AIT group, n = 8), and aerobic continuous training (ACT group, n = 8). The exploration ability and anxiety of AD mice were measured using the open-field test. Learning and memory of AD mice were detected using the novel object recognition test, Y-maze test, and Morris water maze test. Neuronal damage was analyzed using hematoxylin and eosin staining and Nissl staining. Aβ deposition in the brain was detected using a thioflavin-S fluorescence assay and immunofluorescence. The mechanisms underlying hepatic Aβ clearance were investigated using an immunofluorescence assay and western blotting. Data were analyzed using one-way ANOVA with Tukey's post hoc test, and $p < 0.05$ was deemed statistically significant. **Results**: The results revealed that both AIT and ACT improved the recognition memory and exploration ability of mice after 8 weeks of intervention. Additionally, both forms of aerobic exercise significantly mitigated neuronal damage and Aβ deposition in the brain and improved the hepatic clearance of Aβ. **Conclusions**: Our findings indicated that AIT and ACT can improve cognitive deficits in APP/PS1 mice, potentially by increasing the hepatic phagocytic capacity of Aβ. Hepatic clearance of Aβ may serve as a supplementary mechanism by which aerobic exercise can improve AD.

Keywords: Alzheimer's disease; aerobic exercise; Aβ clearance; hepatic phagocytosis

1. Introduction

Alzheimer's disease (AD) is the leading cause of dementia, characterized clinically by memory loss, cognitive dysfunction, language impairment, and other neuropsychiatric symptoms [1–4]. Epidemiologic studies have revealed that the prevalence and mortality rate of AD are increasing rapidly, which imposes medical and financial burdens on societies worldwide [5]. The buildup and clustering of amyloid beta (Aβ) are the key features of AD, wherein the synthesis and removal of Aβ in the central nervous system maintains the dynamic balance [6]. The reduction in the buildup of Aβ aggregates and plaques, or improvement in the brain's capacity to eliminate Aβ is considered a beneficial treatment approach for AD. Two anti-Aβ monoclonal antibodies, aducanumab and lecanemab, have shown satisfactory efficacy in recent clinical trials [7–9]. These successful clinical experiments of Aβ antibodies indicated that the clearance of Aβ from the brain is an effective therapeutic strategy for preventing and treating AD. Therefore, increasing the clearance rate of Aβ from the brain is crucial to advance the treatment of AD.

Accumulating evidence has shown the preventive and healing benefits of physical exercise [10,11]. Based on epidemiological studies, regular physical exercise can improve cognitive function in individuals with AD [12,13]. Additionally, participation in aerobic exercise can enhance cognitive abilities among elderly individuals with cognitive impairment and dementia, and animal studies have suggested it may decrease Aβ deposition and enhance cognitive functions [14,15]. Although numerous studies, including ours, showed aerobic exercise benefits AD, no study has investigated its impact on enhancing peripheral (non-central nervous system) clearance of Aβ.

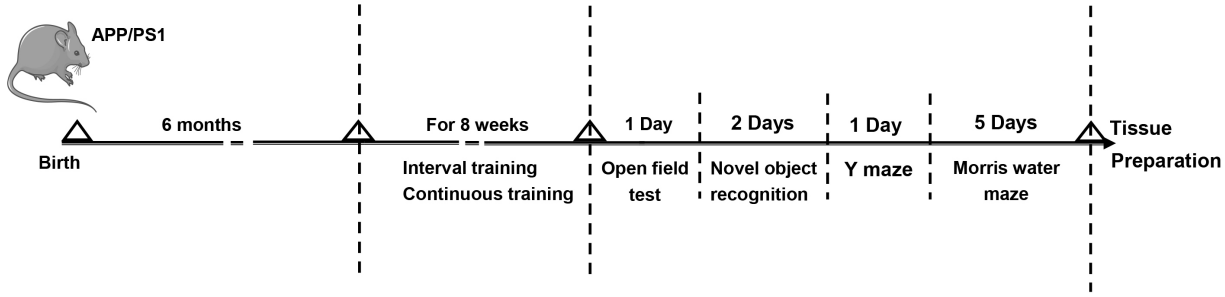

Fig. 1. The experimental workflow of animal treatments. APP/PS1, amyloid precursor protein/presenilin-1 double transgenic.

Recent studies have demonstrated that Aβ can traverse the blood-brain barrier and subsequently enter the bloodstream, where it can be cleared peripherally [16–18]. The liver serves as the primary organ tasked with the elimination of detrimental compounds from the circulatory system [19,20]. Moreover, liver dysfunction is associated with cognitive decline among humans [21,22]. A recent study indicated that the liver clears Aβ from the blood, thereby lowering the tissue levels of Aβ in the brain. This finding suggests a potential association between decreased hepatic Aβ clearance and the pathogenesis of AD [23]. Thus, enhanced hepatic Aβ clearance may be a novel and effective treatment for AD.

Hence, this study aimed to investigate the effect of various types of aerobic exercise on cognitive and exploratory functions, brain histopathology, and hepatic clearance of Aβ.

2. Materials and Methods

2.1 Animals and Experimental Design

Six-month-old male amyloid precursor protein/presenilin-1 double transgenic (APP/PS1) transgenic mice (n = 24) were obtained from Beijing Huafukang Biotechnology Co.Ltd (Beijing HFK Bio-Technology, Beijing, China). All mice were housed at the Experimental Animal Center of Xi'an Medical University, and kept under uniform conditions: 22–25 °C, 50%–60% humidity, and a 12-hour light/dark cycle. They had unlimited access to both food and water. Mice were randomly assigned to three groups: rest (AD group, n = 8), aerobic interval training (AIT group, n = 8), and aerobic continuous training (ACT group, n = 8). The animal study was conducted following the guidelines established by the National Institutes of Health regarding the care and use of laboratory animals to minimize both the number of animals and their discomfort. This study was approved by the Ethics Committee of Xi'an Medical University (XYLS2021225). The experimental design is illustrated in Fig. 1. The whole process took 125 days, of which exercise training and behavioral study encompassed 65 days, while tissue preparation, tissue section staining, immunofluorescence, and western blotting took 60 days.

2.2 Exercise Training

Exercise training was conducted as described previously, with a slightly modified protocol [24,25].

2.2.1 Aerobic Continuous Training (ACT)

The ACT training program consisted of an initial week of adaptive training for mice, involving daily 30-minute treadmill sessions at a speed of 8–12 meters per minute. After a one-week adjustment period, the mice underwent consistent training once daily, five days per week for 8 weeks. Each training session lasted approximately 60 minutes at a pace of 13 meters per minute (60%–70% VO_2, max).

2.2.2 Aerobic Interval Training (AIT)

The AIT training program is described below: The mice exercised at 8–12 meters/min for 30 minutes per day in the first week of training. The mice first underwent running at a speed of 13 meters/min (60%–70% VO_2, max) for 5 minutes as a motor preparatory activity after a week. Then, the mice started running at 20 meters/min (85%–90% VO_2, max) for 2 minutes, with subsequent increases in speed by 10 meters/min (85%–90% VO_2, max) for 4 minutes. The above motor states were alternated for 60 minutes. The mice underwent interval training once daily, five days per week for 8 weeks.

2.3 Behavioral Study

Following the exercise training, all mice underwent behavioral assessments.

2.3.1 Open Field Test (OFT)

The open field apparatus includes a plexiglas box (50 cm × 50 cm × 50 cm) with opaque walls and floor and a behavior tracking system. Each mouse was placed in the box from one side of the stage, with their movements and positions being automatically recorded. The total distance (cm), the movement speed (cm/s), and the time and frequency of the entries in the center were measured using the software EthovisionXT 15.0 (Noldus Information Technology BV, Wageningen, Gelderland, the Netherlands).

2.3.2 Novel Object Recognition (NOR) Test

The experimental period was separated into 2 phases: exploration and test phase. Specific operations were as follows. Mice acclimated to the testing environment for 2 hours before the behavioral assessment. In the exploration session, two identical objects were placed in the box for mice to explore for five minutes. In the testing session, one object was replaced with a new object with a distinct shape and color. Following 24 hours of exploration, mice explored the two objects again for 5 minutes. A video-tracking system EthoVision XT 15.0 (Noldus Information Technology BV, Wageningen, Gelderland, the Netherlands) recorded and analyzed the duration of mice's exploration of familiar and novel objects, calculating the recognition index as the ratio of the time spent on the novel object to the total exploration time.

2.3.3 Y-Maze Test

Mice explored a Y-shaped maze for 10 minutes. Data were analyzed using EthovisionXT-15 video-tracking software by Noldus. The accuracy of spontaneous alternation was measured by recording the number of correct consecutive entries into each arm. Spontaneous alternation percentage = the number of successful spontaneous alternations/(total arm entries – 2) $\times$ 100%.

2.3.4 Morris Water Maze (MWM) Test

The MWM test included four days of learning and memory training trials followed by exploration trials on the fifth day. In the course of the training session, mice were placed in the water from various quadrants while oriented toward the wall of the pool, and the duration taken to find the platform was meticulously recorded. Then the mice were added to the water to explore the target platform for a minute after the platform was removed on the fifth day. The video tracking software SMART 3.0 (Shenzhen RWD Life Technology Co., Ltd., Shenzhen, Guangdong, China) was employed to measure the latency to target, time in target quadrant, and the platform crossings.

2.4 Tissue Preparation

Following 8 weeks of exercise training and behavioral assessments, all mice were sacrificed. Each set of three mice underwent deep anesthesia before intracardiac perfusion of physiological saline and a 4% paraformaldehyde solution. In our study, mice were deeply anesthetized with 5% isofluorane (R510-22-10, Shenzhen RWD Life Technology Co., Ltd., Shenzhen, Guangdong, China). The dosage was carefully calibrated to 5% to ensure effective anesthesia. We used an anesthesia machine air pump (R510-29, Shenzhen RWD Life Technology Co., Ltd., Shenzhen, Guangdong, China) to administer the isofluorane. The air pump was set to deliver a steady flow of gas, which was adjusted based on the physiological responses of the mice to maintain the appropriate anesthetic depth. Brain and liver tissues were preserved in 4% paraformaldehyde for one week, followed by paraffin embedding. The resulting paraffin sections were analyzed utilizing hematoxylin and eosin (HE) staining, Nissl staining, Thioflavin-S fluorescence assay, and immunohistochemical staining. Subsequently, the leftover liver tissues from each group of mice were quickly snap-frozen and subsequently preserved at –80 °C for Western blotting.

2.5 HE Staining

HE staining was conducted following the instructions of the manufacturer, utilizing the HE staining kit (G1076, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China). Briefly, after deparaffinization and hydration of the paraffin sections, they were kept in a pretreatment solution for 1 minute. Subsequently, the sections underwent staining with hematoxylin for 5 minutes, followed by exposure to eosin for 15 seconds. Following dehydration and sealing, the sections were observed using a Nikon ECLIPSE microscope (E100, Nikon Corporation, Tokyo, Japan), and images were captured using a Nikon DS-U3 image acquisition system 1.00 (Nikon Corporation, Tokyo, Japan). The degree of hippocampal neuronal damage in each group of mice was assessed using the scoring criteria established by Shi *et al*. [26] and Pulsinelli and Brierley [27].

2.6 Nissl Staining

Utilizing Nissl staining, the Nissl bodies in neurons were detected to determine neuronal survival. The Nissl Staining Solution (G1036, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China) was used for Nissl staining. After the deparaffinization and hydration of paraffin sections, Nissl solution was introduced for a 5-minute duration, mildly differentiated with 0.1% glacial acetic acid (10000218, Sinopharm Group Chemical Reagent Co., Ltd., Shanghai, China). The process was concluded by washing with tap water. Following transparency and sealing, the sections underwent Nikon ECLIPSE microscope (E100, Nikon Corporation, Tokyo, Japan) and Nikon DS-U3 image acquisition system 1.00 (Nikon Corporation, Tokyo, Japan). Cells whose nucleus and nucleolus could be clearly observed for analysis were selected.

2.7 Thioflavin-S Fluorescence Assay

Thioflavin S has been extensively employed to identify the presence and localization of amyloid plaques [28]. Brain sections embedded in paraffin, each measuring 4 µm in thickness, underwent treatment with a 0.3% thioflavin-S solution (S19293, Shanghai Ye Yuan Biotechnology Co., Ltd., Shanghai, China) at ambient temperature for 8 minutes. After cleaning with 80% ethanol (100092183, Sinopharm Group Chemical Reagent Co., Ltd., Shanghai, China) and rinsing with deionized water, the slides were exposed to DAPI staining solution (G1012, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China) for 10

minutes in the dark. To finish, anti-fluorescence quenching sealing tablets (G1401, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China) were used to seal the slides. Images were acquired using a 3DHISTECH digital scanner (Pannoramic MIDI, 3DHISTECH Ltd., Budapest, Hungary). Amyloid deposition in the mouse brain was assessed by identifying thioflavin-S-positive regions.

2.8 Immunofluorescence

The deposition of Aβ in the brain tissue was measured by single immunofluorescence staining. Paraffin brain sections underwent dewaxing, rehydration, and antigen retrieval. The sections were blocked for 30 minutes with 3% bovine serum albumin (BSA) solution (GC305010, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China) and incubated overnight at 4 °C with the anti-beta amyloid 1-40 rabbit antibody (GB111197, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China, 1:100 dilution). The Cy3 conjugated goat anti-rabbit IgG (H+L) antibody (GB21303, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China, 1:300 dilution) was incubated for an additional 50 minutes. Thereafter, cell nuclei were stained with DAPI (G1012, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China). Subsequently, the slides were affixed with anti-fluorescence quenching sealing tablets (G1401, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China). Double fluorescence labeling was conducted to measure Aβ expression in hepatic tissue. Hepatic sections were processed similarly to single-staining up to the primary antibody reaction. The first primary albumin polyclonal antibody (16475-1-AP, Proteintech Group, Inc., Rosemont, IL, USA, 1:200 dilution) and corresponding secondary HRP conjugated Goat Anti-Rabbit IgG (H+L) antibody (GB23303, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China, 1:500 dilution) were applied, followed by Tyramide Signal Amplification (TSA) dye (G1231, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China). Next, the second primary anti-beta amyloid 1-40 rabbit antibody (GB111197, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China, 1:100 dilution) was added and incubated at 4 °C overnight, followed by a 1-hour incubation with the secondary Cy3 conjugated goat anti-rabbit IgG (H+L) antibody (GB21303, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China, 1:300 dilution). DAPI (G1012, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China) staining was conducted for 10 minutes, and the slides were sealed using an anti-fluorescence quenching agent. The images were captured using a 3DHISTECH digital scanner (Pannoramic MIDI, 3DHISTECH Ltd., Budapest, Hungary). Aβ plaque density was quantified using Image J software (Image J Fiji, https://imagej.net/Fiji).

2.9 Western Blotting

The liver tissues from each group of mice were lysed and homogenized using a cryogenic grinder (Wuhan Servicebio Technology, Wuhan, China) at 4 °C. Tissue proteins were extracted by centrifuging at 12,000 g and 4 °C for 10 minutes using an Eppendorf centrifuge (5804R, Eppendorf AG, Hamburg, Germany). The concentration of proteins was subsequently quantified utilizing a BCA protein assay kit (P0010, Beyotime Biotechnology, Nantong, Jiangsu, China). The expression of low-density lipoprotein receptor-related protein 1 (LRP-1) and Cathepsin-D was measured via Western blotting, considering glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal control. Protein samples were separated using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA). The membranes were blocked using 5% skim milk (GC310001, Wuhan Servicebio Technology Co., Ltd., Wuhan, Hubei, China) at ambient temperature for two hours, followed by an overnight incubation with primary antibodies at 4 °C. The membranes were then incubated for 1 hour with a HRP-goat anti-rabbit recombinant secondary antibody (H+L) (RGAR001, Proteintech Group, Inc., Rosemont, IL, USA, 1:5000 dilution). The main antibodies employed in this study were rabbit polyclonal anti-LRP1 antibody (26106-1-AP, Proteintech Group, Inc., Rosemont, IL, USA), rabbit polyclonal anti-Cathepsin D antibody (21327-1-AP, Proteintech Group, Inc., Rosemont, IL, USA), and rabbit polyclonal anti-GAPDH antibody (10494-1-AP, Proteintech Group, Inc., Rosemont, IL, USA), at a dilution of 1:1000. Image J was employed to measure band densities. Protein expression levels were measured by comparing the density of each protein to that of GAPDH, showing variations in the expression of the target proteins.

2.10 Statistical Analysis

Data were analyzed using SPSS 24.0 (IBM Corp., Armonk, NY, USA) and are presented as mean $\pm$ standard error of mean (SEM). Statistical comparisons were conducted using a one-way ANOVA with Tukey's post hoc test, with $p < 0.05$ considered statistically significant.

3. Results

3.1 Aerobic Exercise Enhanced Exploration and Reduced Anxiety in APP/PS1 Transgenic Mice

The open-field experiment assessed mice spontaneous locomotor activity, anxiety levels, and exploratory activity [29]. Fig. 2A illustrates the open-field activity trajectories for each group of mice. Compared to the AD group, the AIT and ACT groups exhibited a significant increase in both moving distance (Fig. 2B) and mean moving speed (Fig. 2C). Mice in the AIT and ACT groups spent significantly more time in the central zone (Fig. 2D) and crossed the central area (Fig. 2E) more frequently than those in the

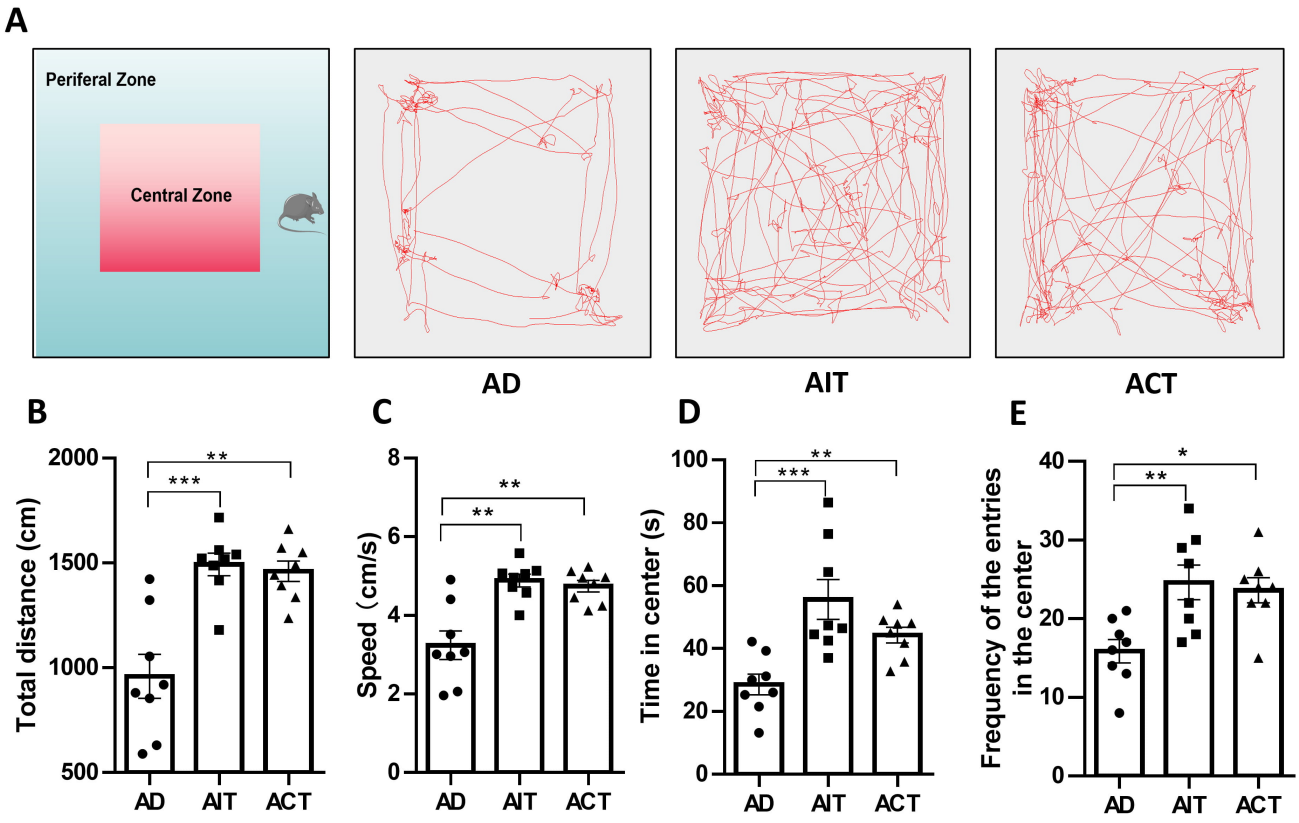

Fig. 2. Effects of AIT and ACT on exploratory behavior and anxiety in APP/PS1 mice. (A) Representative images of the movement track of mice, (B) total distance, (C) speed, (D) time in the center, (E) frequency of the entries in the center. Data are presented as mean ± SEM (n = 8 mice per group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus AD. One-way ANOVA followed by Tukey's post-hoc test (for (B–E)) was conducted to analyze data. AD, Alzheimer's disease; AD, APP/PS1 control group; AIT, aerobic interval training group; ACT, aerobic continuous training group; APP/PS1 mice, amyloid precursor protein/presenilin-1 double transgenic mice.

AD group. The results showed that both AIT and ACT exercise increased the spontaneous activity and exploration abilities of AD mice and decreased their anxiety levels.

3.2 Aerobic Exercise Improved Learning and Memory in APP/PS1 Transgenic Mice

We conducted NOR tests to evaluate mice object recognition memory by measuring exploration time of novel and familiar objects [30] (Fig. 3A). These findings indicated that both AIT and ACT significantly enhanced the capacity of mice to distinguish new from familiar objects, measured by the recognition index (Fig. 3B).

Subsequently, we assessed spatial learning and memory in mice using the Y-maze spontaneous alternation test [31] (Fig. 3C). The Y-maze test indicated a significantly higher spontaneous alternation rate in the ACT group compared to the AD group (Fig. 3D).

The MWM test evaluates the learning and memory in mice [32]. During the MWM experiment, we analyzed the time and trajectory of mice in each group as they searched for a concealed platform. The results revealed that compared to the AD group, mice in the AIT and ACT groups exhibited more organized swimming patterns, with the ACT group exhibiting decreased escape latency (Fig. 3E,F). After removing the platform, mice were given 60 seconds to navigate the maze. These findings indicated that mice in the AIT and ACT groups spent more time in the target quadrant than the AD group, with the ACT group showing a higher frequency of platform crossings (Fig. 3G,H). Overall, these findings indicated that aerobic exercise, whether through AIT or ACT, can improve learning and memory functions in APP/PS1 mice.

3.3 Aerobic Exercise Improved Brain Damage in APP/PS1 Transgenic Mice

We studied pathological alterations in the brain with HE and Nissl staining to assess AIT and ACT effects on neuronal injury. Representative images of HE staining in the AD group (Fig. 4A) showed disordered neuronal arrangement, increased intercellular space, cellular vacuolization, nuclear pyknosis, and disappearance of the nucleolus, suggesting neuronal injuries in the hippocampus and brain cortex. Conversely, mice undergoing AIT and ACT showed a marked improvement in pathological alterations in the hippocampus and cortex, with a significantly lower score of neuronal damage. The results showed

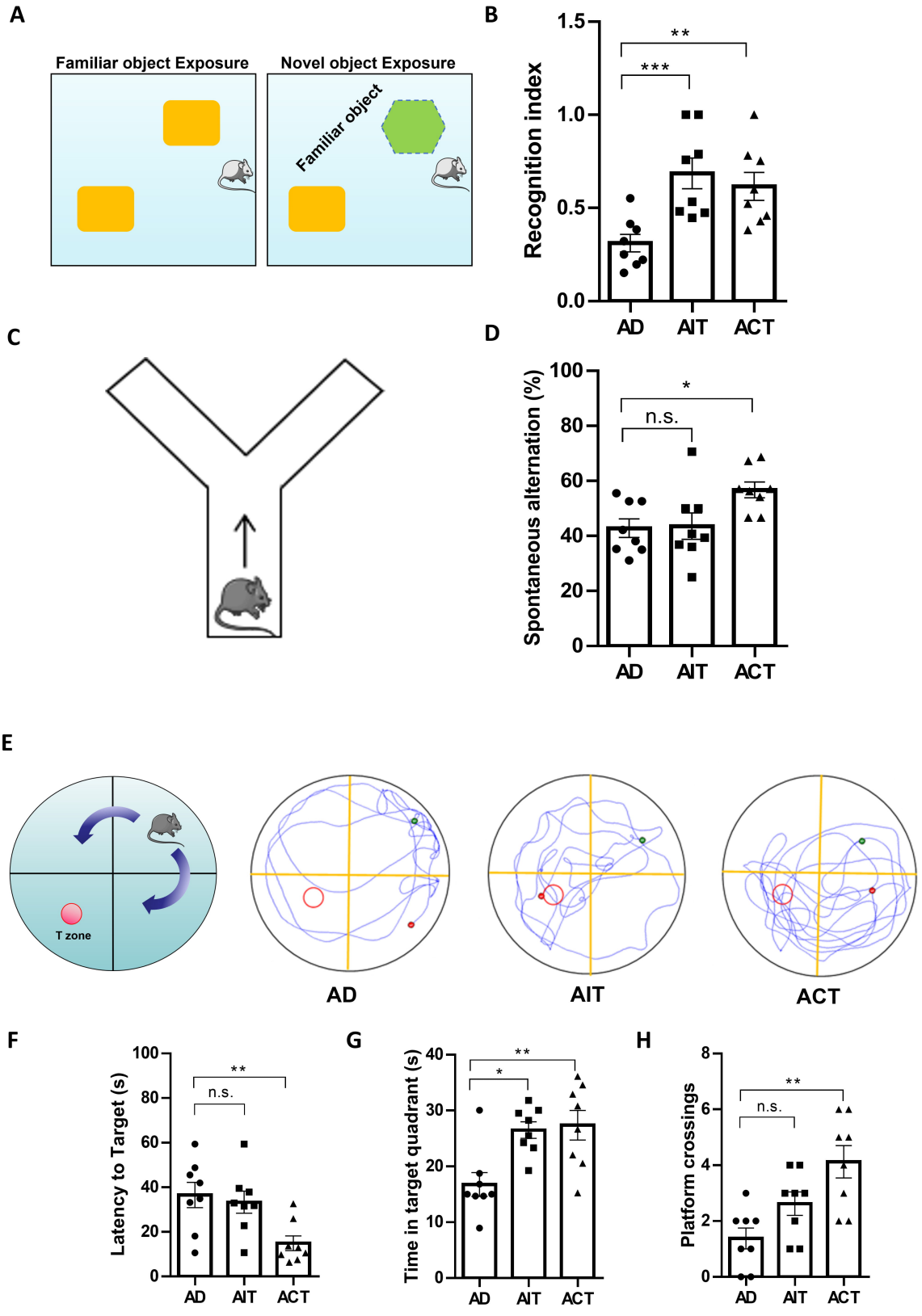

Fig. 3. Effects of AIT and ACT on cognitive impairments in APP/PS1 mice. (A) Schematic diagram of the novel object recognition (NOR) test, (B) recognition index, (C) schematic diagram of the Y maze test, (D) spontaneous alternation, (E) representative images of the movement track of mice, (F) latency to target, (G) time in target quadrant, (H) platform crossings. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus AD; n.s., not significant versus AD, $p > 0.05$. One-way ANOVA followed by Tukey's post-hoc test (for (B,D,F–H)) was used to analyze data.

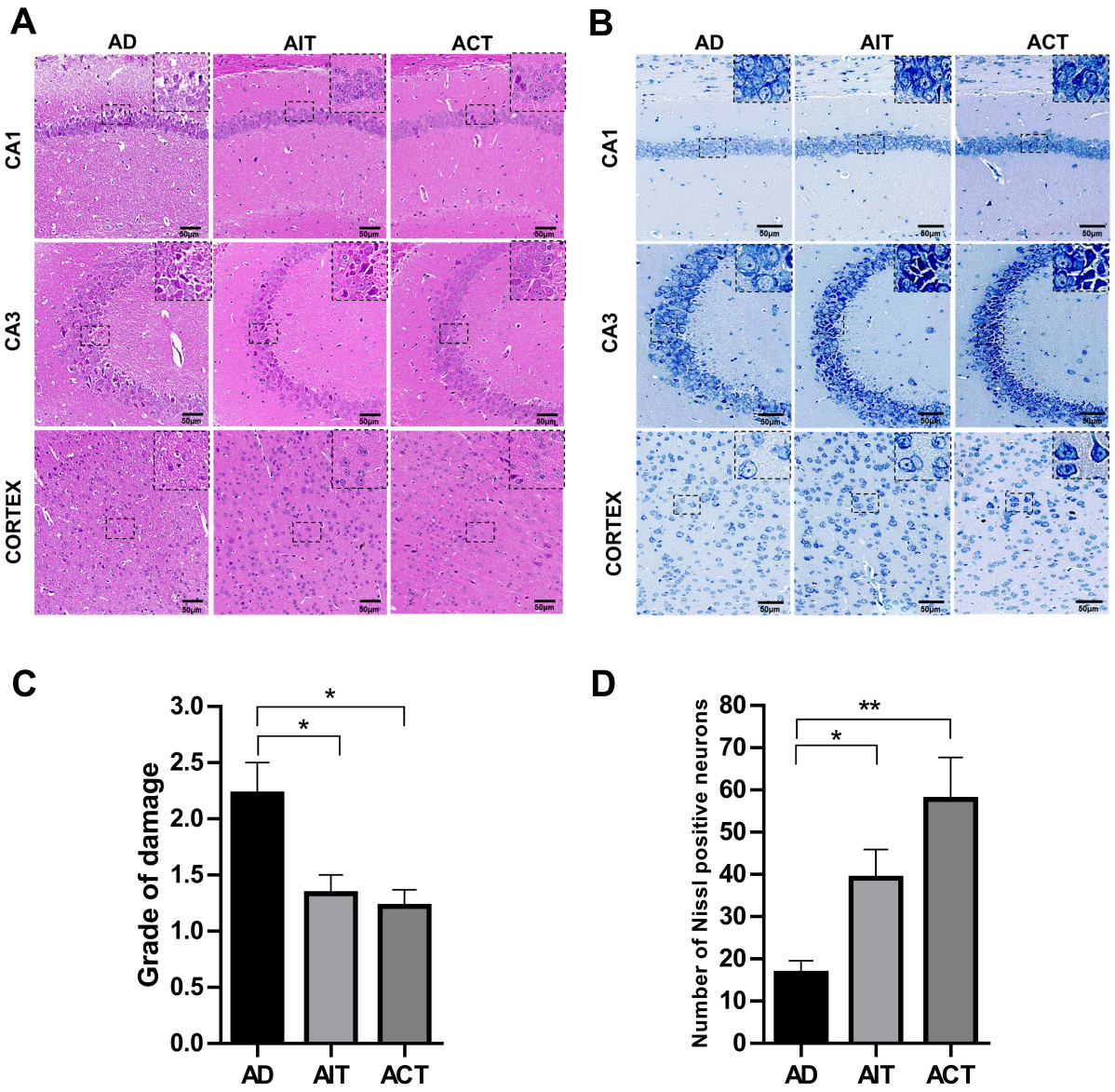

Fig. 4. Effects of AIT and ACT on the histopathological changes of APP/PS1 mice. (A) Representative HE images of different regions in the brain. (B) Representative images of Nissl-stained brain sections. Scale bars: 50 µm. (C) Statistical analysis of the grade of neuronal damage of HE staining. (D) Statistical analysis of Nissl positive cells. Representative images were captured from 3 mice per group. All data are expressed as mean ± SEM. * $p < 0.05$, ** $p < 0.01$ compared to AD. One-way ANOVA followed by Tukey's post-hoc test (for (C,D)) was conducted to analyze data. HE, hematoxylin and eosin; CA1, hippocampal subregion CA1; CA3, hippocampal subregion CA3.

neatly organized neuronal cells with pale red cytoplasm, blue nucleus and clear nucleolus (Fig. 4A,C). Nissl staining showed significantly more Nissl-positive neurons in the hippocampus and cortex of the AIT and ACT groups than in the AD group (Fig. 4B,D). These findings demonstrated that both AIT and ACT protected against neuronal damage in APP/PS1 mice.

3.4 Aerobic Exercise Reduced Aβ Deposition in the Brain of APP/PS1 Mice

We subsequently measured Aβ deposition in the brain through thioflavin-S and antibody staining. Representative thioflavin-S staining and antibody staining images are presented in Fig. 5A–C. Aβ plaques were significantly reduced in the hippocampus and cortex of AIT and ACT treated APP/PS1 mice (Fig. 5D–F). Consequently, both AIT and ACT downregulated Aβ plaques in the brain of APP/PS1 mice.

3.5 Aerobic Exercise Increased the Uptake of Aβ by Hepatocytes in APP/PS1 Mice

Hepatocytes, the primary cells of the liver, are essential for the hepatic clearance of Aβ. We used immunofluorescence to evaluate AIT and ACT exercise ef-

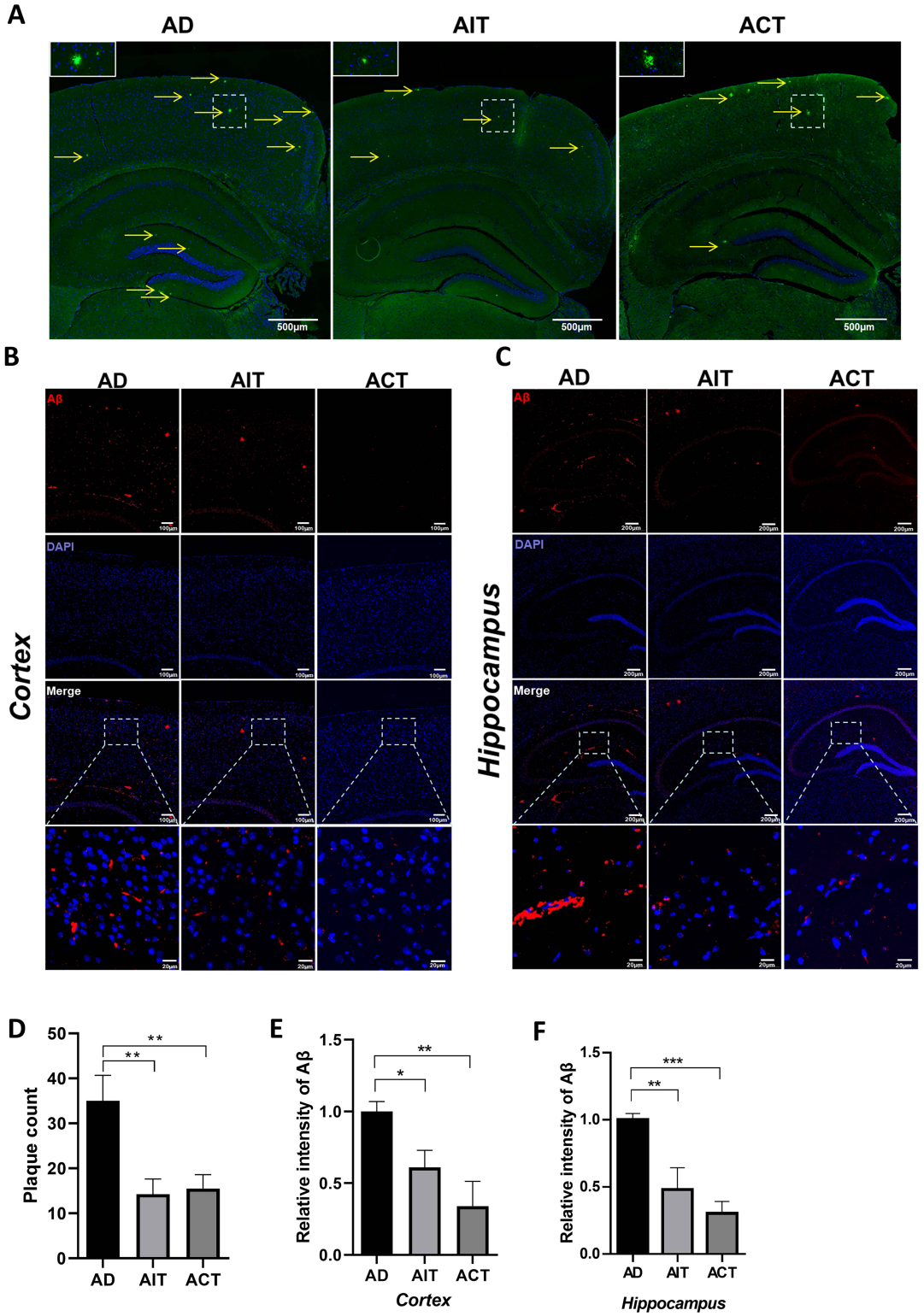

Fig. 5. Effects of AIT and ACT on Aβ deposition in the brain of APP/PS1 mice. (A) Representative immunofluorescence images of thioflavin S in the cortex and hippocampus. Scale bars: 500 μm. Arrows indentify Thioflavin-S binding amyloid plaques. (B) Representative immunofluorescence micrographs of Aβ (red) deposition in the cortex of mice. Scale bars: 100 μm or 20 μm. (C) Representative immunofluorescence micrographs of Aβ (red) deposition in the hippocampus of mice. Scale bars: 200 μm or 20 μm. (D–F) Quantification of Aβ deposition area based on immunofluorescence staining assessed by ImageJ. Data are expressed as mean ± SEM (n = 3 mice per group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus AD. One-way ANOVA followed by Tukey's post-hoc test (for (D–F)) was conducted to analyze data. Aβ, amyloid beta.

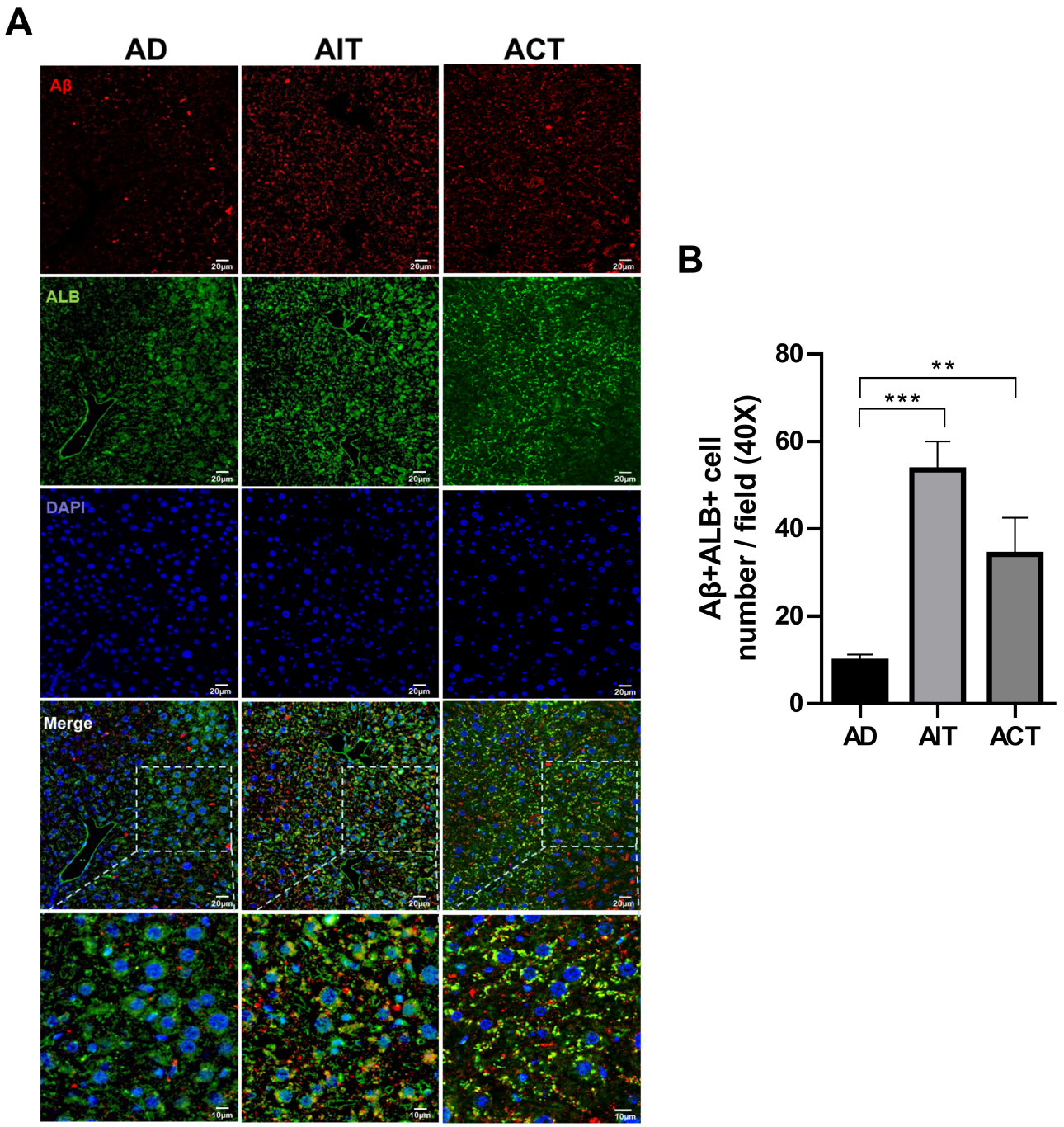

Fig. 6. Effects of AIT and ACT on Aβ uptake by hepatocytes in APP/PS1 mice. (A) Representative immunofluorescence micrographs of Aβ (red), ALB-positive hepatocytes (green), and DAPI (blue). Hepatocytes retaining Aβ are shown in yellow in the merged images. Scale bars: 20 µm or 10 µm. (B) Quantitative analysis of the number of both Aβ-positive and ALB-positive hepatocytes (Representative images were captured from 6 slices of 3 mice per group). All data are expressed as mean ± SEM. ** $p < 0.01$, *** $p < 0.001$ versus AD. One-way ANOVA followed by Tukey's post-hoc test (for (B)) was conducted to analyze data. ALB, Albumin.

fects on Aβ uptake in hepatocytes. The results of fluorescence assay revealed that APP/PS1 mice undergoing AIT and ACT had a significantly increased abundance of Aβ^+ Albumin (ALB)$^+$ hepatocytes compared to the AD group (Fig. 6A,B). These findings indicate that aerobic exercise enhanced the uptake capacity of Aβ in hepatocytes.

3.6 Aerobic Exercise Enhanced the Expression of Molecules Associated With Aβ Degradation in the Liver of APP/PS1 Mice

The expression of Aβ degradation-related molecules was measured via western blotting to study the molecular mechanisms of aerobic exercise on hepatic Aβ clearance. LRP-1 has been recognized as the receptor that mediates

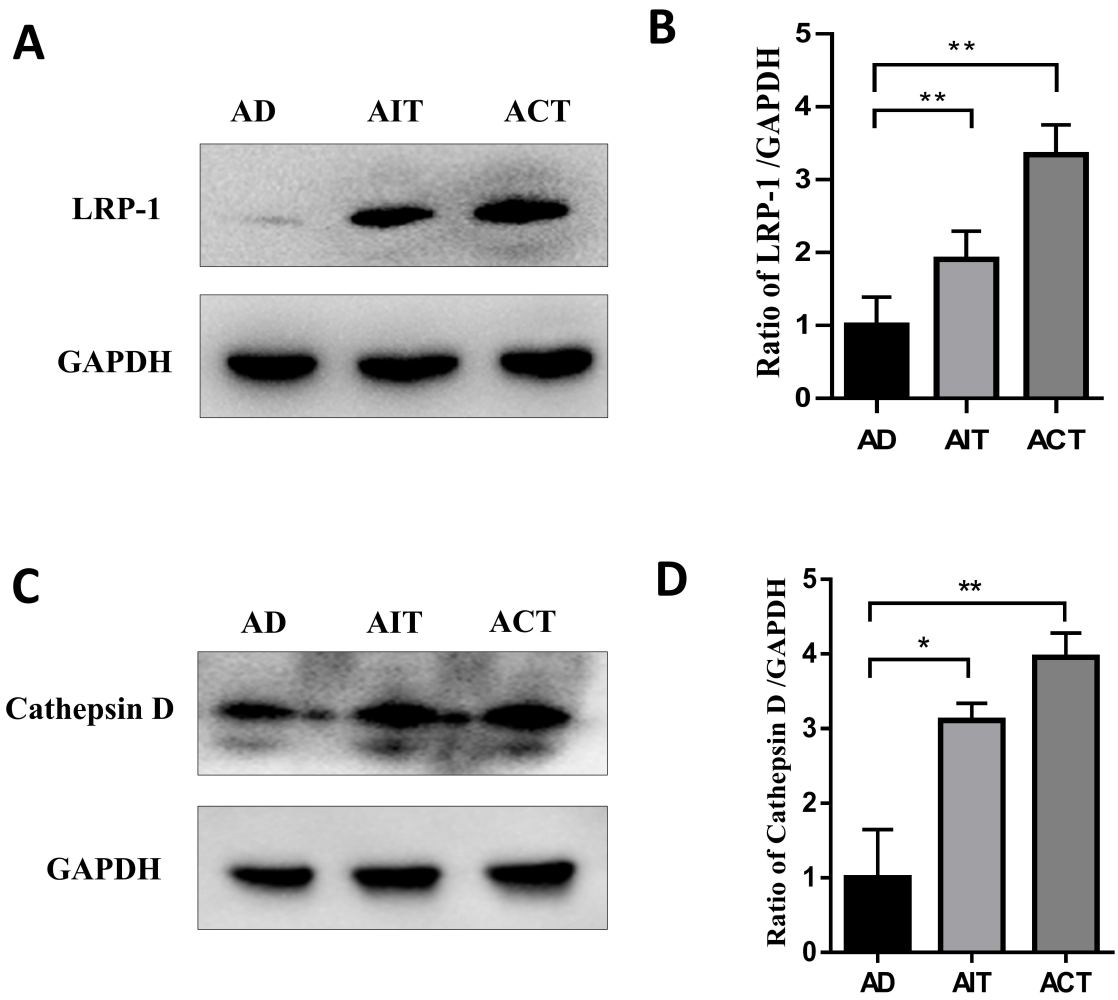

Fig. 7. The effects of AIT and ACT on Aβ metabolism-related molecules in the liver of APP/PS1 mice. (A) Western blotting for LRP-1 expression in mouse liver. (B) Quantification of LRP-1 expression in immunoblots (n = 3). (C) Western blotting of Cathepsin D expression in mouse liver. (D) Quantification of Cathepsin D expression in immunoblots (n = 3). All data are expressed as mean ± SEM. * $p < 0.05$, ** $p < 0.01$ versus AD. One-way ANOVA followed by Tukey's post-hoc test (for (B,D)) was conducted to analyze data. LRP-1, low-density lipoprotein receptor-related protein 1.

the phagocytosis of Aβ by hepatocytes [33]. LRP-1 overexpression enhances Aβ clearance in the liver, which in turn lowers the accumulation of cerebral Aβ and alleviates cognitive impairments observed in APP/PS1 transgenic mice [23]. Cathepsin D, an Aβ-degrading enzyme, plays a critical role in hepatic Aβ clearance [34]. Western blotting confirmed an increase in the hepatic expression of LRP-1 (Fig. 7A,B) and Cathepsin D (Fig. 7C,D) after AIT and ACT. These results suggest that improved ability after aerobic exercise to uptake and phagocytize Aβ in the liver may be attributed to increased expression of LRP-1 and Cathepsin D.

4. Discussion

Engaging in suitable physical exercise has been shown to effectively prevent and delay neurodegenerative diseases like AD [35,36]. Although there is no agreement on the most effective exercise program for enhancing cognitive function in individuals with AD, aerobic exercise is widely regarded as an important adjunctive treatment. Nevertheless, the exact mechanism by which aerobic exercise confers a protective effect in AD remains unclear. Most studies have focused on changes in brain cells and pathological markers, like Aβ and tau protein [37–39], but we focused on the peripheral effects of aerobic exercise, specifically how clearing peripheral Aβ can prevent the buildup of central Aβ. In summary, our study comprehensively investigated the effects of AIT and ACT on AD mice. These findings suggest that participating in AIT and ACT for eight consecutive weeks can improve spatial and cognitive memory deficits, ameliorate anxiety, mitigate histopathological alterations, and diminish Aβ deposition in the brains of AD mice. Moreover, the study highlights that there is no significant disparity between the effects of AIT and ACT. Further mechanistic experiments indicated that the positive effects

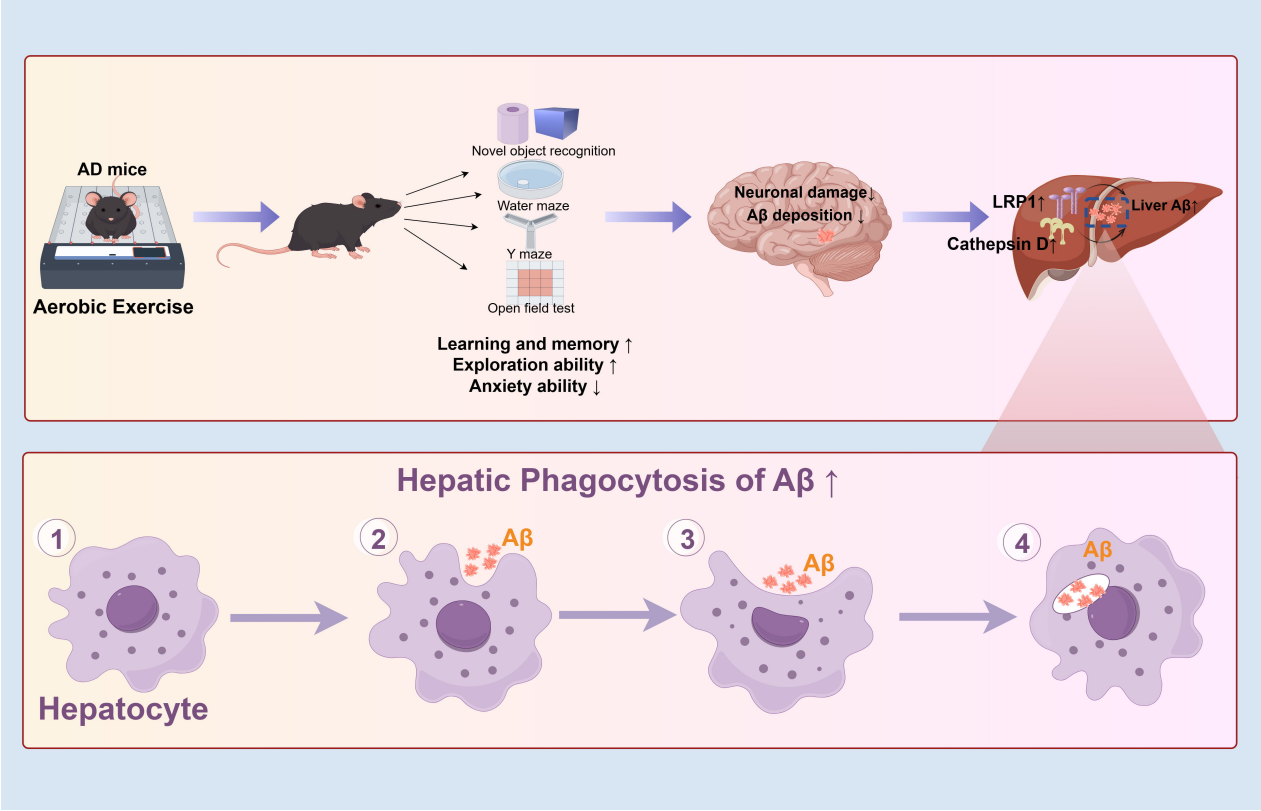

Fig. 8. Schematic diagram showing the protective effect of aerobic exercise on APP/PS1 transgenic mice and its underlying mechanism. Aerobic exercise improved learning and memory, enhanced exploration ability, and ameliorated the anxiety of APP/PS1 transgenic mice. Additionally, aerobic exercise decreased neuronal damage and Aβ deposition in the brain, potentially by enhancing the capacity of the liver to phagocytize Aβ. Arrows ↑ or ↓ represent up-regulation or down-regulation. By Figdraw (https://www.figdraw.com/#/).

of AIT and ACT in AD mice may be partly due to the hepatic clearance of circulating Aβ in the periphery (Fig. 8). These results can deepen and refine our understanding of aerobic exercise interventions in AD.

A primary clinical characteristic of patients with AD is the progressive deterioration of cognitive abilities, a phenomenon also observed in mouse models of APP/PS1 [40,41]. These mice also show Aβ accumulation, synaptic loss, and neuronal death [42,43]. Studies demonstrated that various exercise, such as treadmill exercise, high-intensity interval training (HIIT), and resistance physical exercise, can alleviate AD symptoms in mouse models [44–47]. We found that mice treated with AIT and ACT performed better in various cognitive tests, such as the MWM, NOR, and Y-maze tests. These findings align with the existing literature indicating that aerobic exercise can enhance cognitive performance in individuals with AD. It is essential to highlight that both AIT and ACT are equally effective in reducing cognitive dysfunction in mice. However, AIT showed no significant difference compared to the AD group in certain instances, such as the escape latency and the frequency of platform crossings observed in the MWM test, and the spontaneous alter country score in the Y maze test. This can be potentially attributed to the limited sample size and variations between individuals. We will increase the sample size in future studies to improve reliability. Furthermore, we observed that AIT and ACT decreased the hyperactive behavior of APP/PS1 mice in the OFT [48], which resembled the agitation seen among patients with AD. This finding suggests that both AIT and ACT, forms of aerobic exercise, can improve AD-related behaviors.

Behavioral dysfunction in AD has been linked to the structural and functional deficits observed in particular brain regions. Neuronal loss in various brain regions significantly contributes to the development of AD [49]. The hippocampus and cortex are particularly susceptible to Aβ [49]. The study has indicated that treadmill training can enhance memory functions associated with the hippocampus and improve the dendritic architecture of neurons. It also indicates that treadmill exercise can enhance memory associated with the amygdala and the dendritic structure of basolateral amygdalar neurons [50]. Additionally, treadmill exercise reduces Aβ accumulation and tau protein phosphorylation, possibly by modulating APP processing and Glycogen Synthase Kinase (GSK) 3-dependent signaling pathway [51]. Moreover, neuronal energy requirements rely on mi-

tochondria, and both HIIT and moderate-intensity continuous training reduce Aβ levels, mitigate mitochondrial fragmentation, and enhance the structural integrity of mitochondria [52]. Existing evidence indicates that physical exercise can serve as an effective therapeutic approach to enhance neuronal function and reduce the levels of AD biomarkers. Our study also confirmed that eight weeks of AIT and ACT can ameliorate neuronal degeneration, morphological changes, and functional impairments in the hippocampus and cortex, resulting in a significant decrease in Aβ levels in the brain of mice. These results indicate that aerobic exercise has positive effects on neurons and Aβ deposition in AD.

AD has traditionally been viewed as a brain-specific condition. However, the study has shown that nearly 50% of the Aβ in the brain can be transported to the peripheral circulation via the transport mechanisms of the blood-brain barrier and through lymphatic systems [16–18]. Recent phase III trials have demonstrated that Aβ antibodies like aducanumab and lecanemab significantly lower brain Aβ in AD patients [8,9]. This suggests that promoting the clearance of peripheral Aβ is feasible. The liver is essential for detoxification, eliminating nearly 13.9% of Aβ42 and 8.9% of Aβ40 in one pass through the organ [23,53,54]. Previous study has shown that diminished hepatic clearance of Aβ can lead to its accumulation in the brain, thereby intensifying the symptoms of AD. Conversely, hepatocytes can directly absorb Aβ from the peripheral bloodstream, thereby contributing significantly to the role of the liver in Aβ clearance [55]. LRP-1 primarily mediates Aβ phagocytosis in hepatocytes [33]. Increased hepatic clearance of Aβ by enhancing the expression of hepatic LRP-1 has been found to improve AD lesions in the brain, and enhance cognitive performance in the mouse models of AD [23]. Furthermore, the liver possesses various Aβ-degrading enzymes, including cathepsin D, which are crucial for hepatic Aβ clearance and mitigating its deleterious effects on brain injury [34]. Consistently, augmenting lysosomal cathepsin activity has been shown to alleviate Aβ toxicity [56,57] and restore autophagy-lysosomal pathway has been demonstrated to decrease Aβ accumulation and improve memory performance [58]. The study has also shown that targeting the soluble epoxide hydrolase (sEH) enzyme of the liver can help lower Aβ load and tau protein levels and mitigate the behavioral symptoms of AD [59]. These results together indicate that improving the clearance of hepatic Aβ could be a novel and promising therapeutic strategy for AD. In the study concerning the effect of exercise on liver health, it was observed that weeks of aerobic exercise before surgery can considerably ameliorate liver injury and inflammation caused by ischemia-reperfusion in mice [60]. Additionally, a separate study demonstrated that moderate-intensity exercise can enhance Kupffer cell-mediated phagocytosis [61]. Due to the correlation between exercise, liver function, and Aβ, it is worth exploring whether AIT or ACT can enhance the ability of the liver to clear Aβ. As expected, our findings indicated that the enhanced ability of the liver to phagocytize Aβ may underlie the beneficial effects of AIT and ACT exercise.

In summary, our study demonstrated that both AIT and ACT over an eight-week period can ameliorate spatial and cognitive memory impairment, reduce spontaneous activity and anxiety-like behaviors, mitigate neuronal injury, and diminish the synthesis and accumulation of Aβ in the brain of APP/PS1 mice. The potential neuroprotective effects of aerobic exercise may be mediated through increased Aβ phagocytosis by the liver in mice with AD.

5. Conclusions

Taken together, our findings demonstrated that aerobic exercise improved learning and memory, enhanced exploration ability, and reduced the anxiety of APP/PS1 transgenic mice. Moreover, aerobic exercise reduces neuronal damage and Aβ deposition in the brain potentially by enhancing Aβ phagocytic by the liver. The hepatic clearance of Aβ can serve as a supplementary mechanism by which aerobic exercise enhances AD.

Disclosure

The paper is listed as "Aerobic Exercise Ameliorate Alzheimer's Disease-Like Pathology by Regulating Hepatic Phagocytosis of Aβ" as a preprint on (SSRN) at https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4923184.

Abbreviations

AD, Alzheimer's disease; AIT, aerobic interval training; ACT, aerobic continuous training; Aβ, amyloid beta; OFT, open field test; NOR, novel object recognition; MWM, Morris water maze; HE, Hematoxylin and eosin; LRP-1, low-density lipoprotein receptor-related protein 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; APP/PS1 mice, amyloid precursor protein/presenilin-1 double transgenic mice.

Availability of Data and Materials

The data sets generated and/or analyzed during the current study are not publicly available due to Further research is needed, but are available from the corresponding author on reasonable request.

Author Contributions

QW: Data curation, Methodology, Software, Visualization, Funding acquisition, Writing—original draft. FH: Data curation, Methodology, Validation, Software, Visualization. XG: Conceptualization, Project administration, Funding acquisition, Resources, Writing—review & editing. SW: Conceptualization, Funding acquisition, Methodology, Supervision, Writing—review & editing. NJ: Conceptualization, Funding acquisition, Formal analysis,

Project administration, Writing—review & editing. All authors contributed to editorial changes in the manuscript. 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

Animal experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and the experimental protocol was approved by the Ethics Committee of Xi'an Medical University (approval number: XYLS2021225).

Acknowledgment

The authors would like to express their gratitude to EditSprings (https://www.editsprings.cn) for the expert linguistic services provided and thank Figdraw as the Fig. 8 in this article was created using Figdraw.

Funding

This study was supported by grants from the National Natural Science Foundation of China (82201599 and 81971330), Natural Science Basic Research Program of Shaanxi (2021JM-505), Innovation Capacity Support Program-Science and Technology Resources Open and Sharing Platform of Shaanxi (2024CX-GXPT-08), Scientific Research Project of Xi'an Medical University (2023QN04).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBL36597.

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