Research article

The effect of chronic alcohol exposure on spatial memory and BDNF–TrkB- PLCγ1 signaling in the hippocampus of male and female mice

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A R T I C L E   I N F O

A B S T R A C T

Alcohol is a commonly used drug worldwide, and abuse of alcohol has become a serious public health problem. Alcohol consumption over time can cause cognitive deficits and memory impairment, which is thought to be associated with changes in the hippocampus. Given previously known effects of brain-derived neurotrophic factor (BDNF) in regulating synaptic plasticity and learning and memory, we investigated the effect of chronic alcohol consumption on spatial memory impairment in both sexes and changes in BDNF signaling in the hippocampus. After 4 weeks of intermittent access to 20% alcohol, memory impairment in both male and female mice was evaluated using the Morris water maze and the expression of BDNF, TrkB, phosphorylation of PLCγ1 (p-PLCγ1) and PLCγ1 in the hippocampus was examined using Western blot. As expected, females spent longer escape latencies during the training phase, and both sexes spent shorter time in the target quadrant. Furthermore, after 4 weeks 20% alcohol exposure, we found significantly decreased expression levels of BDNF in the hippocampus of female mice but increased levels in male mice. TrkB and PLCγ1 expression showed no significant change in the hippocampus of both sexes. These findings suggest that chronic alcohol exposure may induce spatial memory impairment in both sexes and opposite changes in expression of BDNF and p-PLCγ1 in the hippocampus of males and females.

1. Introduction

Alcohol is a commonly used psychoactive substance with rewarding properties. Individuals initially drink alcohol primarily because of its pleasurable effects, but excessive drinking over time can result in the development of alcohol dependence and alcohol use disorder [1]. Alcohol dependence is a chronic relapsing disorder that is characterized by compulsive alcohol drinking with a concomitant loss of control over alcohol intake and negative emotional state when absent [2–4]. It has been reported that 53.6% of men and 32.3% of women aged 15 or older in the world consumed alcohol in 2016, and more than 200 health conditions are associated with harmful use of alcohol, including learning and memory impairment, cognitive impairment and depression [5].

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and performs a variety of adaptive functions in neuronal survival, differentiation, synaptic plasticity and learning and memory [6,7]. BDNF binds mainly to the tropomyosin-related

kinase B (TrkB) receptor and activates different downstream signaling pathways via mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K) and phospholipase Cγ1 (PLCγ1) [8–10]. These pathways are involved in regulating neuronal plasticity and play an important role in response to drug abuse and addiction [11–13]. It has been reported that lower plasma BDNF concentrations in alcohol use disorder patients are correlated with memory deficits [14]. Selective loss of TrkB-PLCγ1 in the nucleus accumbens shell increases cocaine self-administration behaviors accompanied by a decrease in spine density [15]. Experimental studies have shown that BDNF contributes to LTP induction in the mPFC of cocaine-treated rats by activating TrkB and increasing dendritic length in the hippocampus [16,17].

These findings indicate that BDNF may be involved in the regulation of synaptic plasticity and neurogenesis relevant to alcohol-induced memory impairment.

The hippocampus is part of the limbic system crucial in learning and memory processing [18,19]. Multiple studies have found that chronic alcohol exposure can cause structural and functional damage in the hippocampus. In humans, imaging studies reveal reductions in the volume of the hippocampus in patients with alcohol dependence [20,21]. Chronic alcohol exposure can cause spatial memory impairment and cognitive flexibility impairment in rodents, as well as changes in hippocampal dendritic spines and neuron numbers [22–27]. Furthermore, alcohol exposure inhibits hippocampal long-term potentiation (LTP) and impairs hippocampal neurogenesis [19,28–30]. Noteworthy, clinical study in older alcohol-dependent inpatients found that women performed better than men on mental flexibility, while men performed better than women on the visual processing [31]. However, most studies were carried out in male rodents and changes and responses in females are still poorly understood. Therefore, studying sex differences in responses to chronic alcohol exposure is important to fully understand the mechanisms of memory impairment induced by alcohol.

In this study, we investigated changes in spatial memory impairment in male and female mice following intermittent access to 20% alcohol and observed alterations in the expression of BDNF and its receptor TrkB and their downstream molecule PLCγ1 in the hippocampus of both sexes. Given women seem to be more susceptible to alcohol and alcohol-related impairments [32,33], we hypothesized that sex difference would be found in spatial memory impairment and protein levels of BDNF, TrkB and PLCγ1 in the hippocampus after 4-week 20% alcohol exposure.

2. Materials and methods

2.1. Animals

Seven-week-old male and female C57BL/6 mice were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). Upon arrival, male and female mice were housed with the same sex, four per cage with food and water ad libitum. Mice were given 7 days to habituate to the housing conditions (ambient temperature of 21–24 °C and relative humidity of 40–70%) with a 12-h light/dark cycle. All experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of Xuzhou Medical University.

2.2. Alcohol drinking paradigm

Ethyl alcohol, 95% (Shanghai Ling Feng Chemical Reagent Co., Ltd., Shanghai, China) mixed in tap water was used to prepare alcohol solutions (v/v). Water and alcohol solution were presented in 100 ml graduated plastic cylinders with stainless steel sipper tubes containing stainless steel ball-bearing sippers. Mice were moved to individual housing with two bottles of water at least 48 h before exposure to the alcohol solution. Male and female mice were randomly divided into a water group (male, n = 14; female, n = 14) and an alcohol group (male, n = 15; female, n = 14), respectively. Mice were given intermittent access to 20% alcohol according to a previously described drinking procedure [34]. For the alcohol groups, during the first week (week 1), mice had a two-bottle choice between a 3% alcohol solution and water on Monday, a 6% alcohol solution and water on Wednesday, and a 10% alcohol solution and water on Friday. The mice received two bottles of water on the remaining days. During the following four weeks (Week 2- Week 5), mice were given one bottle of 20% alcohol and one bottle of water for 24 h on Monday, Wednesday, and Friday of each week. The mice received two bottles of water on the remaining days. For the water groups, mice were given two bottles containing water throughout the drinking procedure. The bottles were removed and replaced by other bottles every 24 h, and the placements were counterbalanced to avoid side preferences. Before and after alcohol/water and water drinking, bottles were weighed and recorded to measure daily consumption. An empty cage with two bottles in the same environment was used to account for leakage. Mice were weighed before alcohol drinking to calculate alcohol consumption in grams of alcohol per kilogram of body weight. Alcohol preference was calculated as the volume of alcohol intake (ml) divided by the total volume of fluid intake (ml).

2.3. Morris water maze

The Morris water maze (MWM) was used to assess spatial memory in mice after chronic alcohol exposure. The apparatus consisted of a circular water pool (120 cm in diameter, 40 cm in height), a circular platform (12.0 cm in diameter) and a video-tracking system (ANY-maze, Stoelting Co., USA). The pool was filled with water (20 ± 1 °C) and divided into four equal quadrants. The platform was submerged 1 cm below the water surface and located in the center of one of the quadrants during training trials and was removed during the probe test. The video-tracking system was used to record and analyze animal movements in the water pool. The apparatus was placed in a quiet room provided with several distal cues. The MWM procedure was performed according to a previous study [35].

The MWM task began on the third day after cessation of alcohol intake. Mice in the water groups (male, n = 6; female, n = 6) and

alcohol groups (male, n = 7; female, n = 6) had access ad libitum to two bottles of water during the MWM task. The MWM task was performed in two phases, the training phase, and the probe test. During the training phase, each mouse received 4 trials per day for seven consecutive days. For daily trials, the platform was located in the center of the southwest quadrant. Mice were put into the pool from different start positions and needed to find the platform and stay there for 10 s within 60 s. If mice failed to find the platform, they were guided to the platform and left there for 15 s. The probe test was performed 24 h after the last training, and each mouse was placed into the water and swam freely for 60 s without the platform. The average swimming speed, latency to the platform, platform site crossings and time spent in the target quadrant were recorded via the video-tracking system.
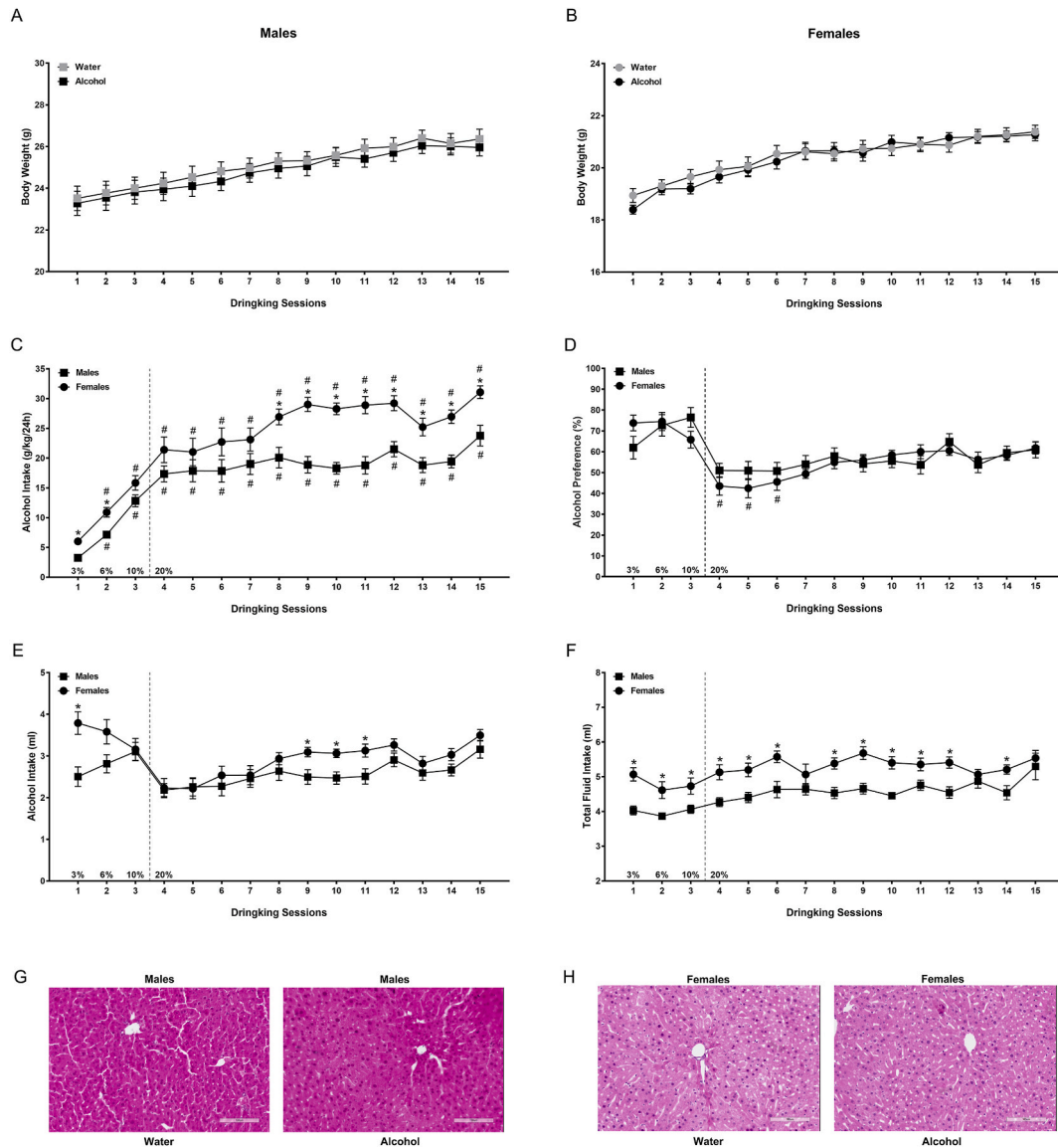

Fig. 1. The alcohol intake and alcohol preference of male and female mice given intermittent access to 20% alcohol. Mice received two bottles of 3%, 6%, 10% alcohol solution and water on sessions 1–3 (week 1) and two bottles of 20% alcohol solution and water on sessions 4–15 (week 2–5). (A) Body weights of male mice during 5 weeks of intermittent access to alcohol. (B) Body weights of female mice during 5 weeks of intermittent access to alcohol. (C) Alcohol consumption in male and female mice for 5 weeks of intermittent access to alcohol. (D) Alcohol preferences of male and female mice during 5 weeks of intermittent access to alcohol. (E) The volume of alcohol (ml) in male and female mice for 5 weeks of intermittent access to alcohol. (F) The volume of total fluid (ml) in male and female mice for 5 weeks of intermittent access to alcohol. (G) Representative images of H&E staining in liver tissues of male mice (20 × , scale bar = 100 μm). (H) Representative images of H&E staining in liver tissues of female mice (20 × , scale bar = 100 μm). Water represents the water group, and alcohol represents the alcohol group. Data are shown as the mean ± SEM. *$p <$ 0.05 represents statistically significant differences between males and females. #$p <$ 0.05 represents statistically significant differences between session 1 and other sessions.

2.4. Western blotting

At the end of the alcohol drinking paradigm, mice in the water groups (male, n = 8; female, n = 8) and alcohol groups (male, n = 8; female, n = 8) were anesthetized with isoflurane, brains removed and placed on ice for dissection of the hippocampus [36,37]. Tissues were immediately frozen in liquid nitrogen and stored at −80 °C. The tissues were homogenized in RIPA lysis buffer with protease inhibitor cocktail tablets and phosphatase inhibitor cocktail tablets from Roche. The homogenates were incubated on ice for 20 min and then centrifuged at 12,000×*g* for 20 min at 4 °C. The protein concentration of the supernatants were quantified by a BCA kit (Health of Biopharmaceutical Co., Ltd, China).

Protein samples were diluted to 4:1 in 5 × reducing sample buffer (Pioneer Biotechnology, China) and denatured at 95 °C for 5 min. Equivalent amounts of protein for each sample were loaded on 10% or 12% SDS−PAGE. After electrophoresis, proteins were blotted onto PVDF membranes (Millipore, Bedford, MA, USA), and the membranes were blocked with 5% nonfat milk for 1 h at room temperature. The membranes were incubated with primary antibodies overnight at 4 °C. The membranes were incubated with secondary antibody for 1 h at room temperature after washing three times for 10 min with TBS-T and washing again after incubation. Subsequently, signals were visualized with Immobilon Western Chemiluminescent HRP Substrate (Millipore, Bedford, MA, USA), captured and analyzed by an Analytic Jena ChemStudio system with AJ Visionworks 8.8 software (Analytik Jena, Germany). The primary antibodies we used were as follows: BDNF (1:500; Abcam), TrkB (1:000; Cell Signaling Technology), phospho-PLCγ1 (Tyr783) (p-PLCγ1) (1:000; Cell Signaling Technology), PLCγ1 (1:000; Cell Signaling Technology), and β-actin (1:000; BioWorld).

2.5. Immunostaining

Mice were sacrificed under anesthesia after water and alcohol drinking and transcardially perfused with saline and 4% paraformaldehyde. Two individual mice in each group were tested by immunohistochemistry. Brains and livers were postfixed in 4% paraformaldehyde for 24 h at 4 °C and processed for histological examination with paraffin. The livers were cut into 5 μm sections and stained with hematoxylin and eosin (H&E). For the immunohistochemistry, the paraffin-embedded brains were cut into 3 μm sections and performed with a rabbit two-step detection kit (ZSGB-BIO Company, China) as described previously [38]. After deparaffinization and rehydration, sections were heated in a microwave for 10 min in 10 mM citric buffer. Then, sections were incubated with endogenous peroxidase blocker for 10 min in room temperature and primary antibodies overnight at 4 °C. After washing three times with PBS, tissue sections were incubated reaction enhancement solution (ZSGBBIO, China) at 37 °C for 30 min, washed, and incubated with secondary antibody solution (ZSGB-BIO Company, China) at 37 °C for 30 min. Immunostaining was detected by DAB kit (ZSGB-BIO Company, China) and followed by counterstaining with hematoxylin. Sections were visualized and photographed by Grundium Ocus Scanner (Grundium, Finland) and the Aperio ImageScope software. The primary antibodies were as follows: BDNF (1:100, Affinity), TrkB (1:100, Affinity), phospho-PLCγ1 (1:100, Affinity) and PLCγ1 (1:100, Cell Signaling Technology).

2.6. Statistical analysis

The results of the alcohol drinking paradigm were analyzed with two-way repeated-measures ANOVA followed by Sidak's multiple comparison test. The results of the MWM task were analyzed with two-way repeated-measures ANOVA followed by Bonferroni's multiple comparison test and independent *t*-test or one sample *t*-test when appropriated. The results of western blotting were analyzed by independent *t*-test. All data are presented as the mean ± standard error of the mean. Data were analyzed by SPSS 22.0 software (IBM, USA), and $p < 0.05$ was considered statistically significant.

3. Results

3.1. The alcohol drinking paradigm

We assessed alcohol consumption and alcohol preference in male and female mice with intermittent access to 20% alcohol paradigm. Throughout the experiment, the body weights of the male and female alcohol groups were not different from those of their respective water groups (Fig. 1A, $p > 0.05$; Fig. 1B, $p > 0.05$). As shown in Fig. 1C, male mice initially consumed 17.4 g/kg/24 h, and female mice consumed 21.4 g/kg/24 h of 20% alcohol. Then, male mice increased alcohol consumption in the next 2 weeks to reach a stable baseline of 20.1 ± 2.1 g/kg/24 h, and female mice increased alcohol consumption to a stable baseline of 28.2 ± 2.0 g/kg/24 h. A two-way repeated-measures ANOVA revealed significant effects of sex [Fig. 1C; $F_{1,27} = 25.357$; $p < 0.05$], session [Fig. 1C; $F_{14,378} = 54.028$, $p < 0.05$], and an interaction between sex and session [Fig. 1C; $F_{14,378} = 2.445$, $p < 0.05$]. Post hoc analyses showed that female mice consumed significantly more alcohol than male mice during all drinking sessions except sessions 3 to 7. And compared to the session 1, the consumption of alcohol in male and female mice were significantly increased at all drinking sessions. The alcohol preferences of male and female mice are shown in Fig. 1D. Alcohol preference in female mice started at 43% and male mice 51% of 20% alcohol and stayed above 55% after two weeks. Unlike the results of alcohol consumption, there were no significant differences in alcohol preferences between male and female mice. A two-way repeated-measures ANOVA revealed a significant effect of session on alcohol preference [Fig. 1D; $F_{14,378} = 12.091$; $p < 0.05$] but no effects of sex [Fig. 1D; $F_{1,27} = 0.144$, $p > 0.05$] or interaction between both factors [Fig. 1D; $F_{14,378} = 1.599$, $p > 0.05$]. Post hoc analyses indicated that the preference for alcohol in female mice did not significantly differ from that in male mice during all drinking sessions (Fig. 1D; $p > 0.05$). Further two-way repeated-measures ANOVA were performed for the volume of alcohol consumption (ml) and total fluid consumption (ml) during all drinking sessions. Unlike the

alcohol consumption (g/kg/24 h), there was no significant change in the volume of alcohol (ml) between male and female alcohol group [Fig. 1E; effect of sex, $F_{1,27} = 2.574$; p > 0.05; effect of session, $F_{14,378} = 7.714$, p < 0.05; $F_{14,378} = 3.773$, p < 0.05]. The volume of total fluid (ml) was significantly different between male and female alcohol group, where female mice consumed greater volume of fluid (ml) than male mice [Fig. 1F; effect of sex, $F_{1,27} = 12.329$; p < 0.05; effect of session, $F_{14,378} = 4.418$, p < 0.05; effect of interaction, $F_{14,378} = 1.757$, p > 0.05]. Moreover, the alcohol groups in both sexes showed no significant histological changes in liver compared with the water groups (Fig. 1G and H).

3.2. The effect of chronic intermittent alcohol exposure on spatial memory acquisition and retention in male and female mice

The performance of male and female mice during the training phase of the MWM task is shown in Fig. 2. For males, latencies in the water group and alcohol group progressively decreased during the training phase, whereas the alcohol group took longer to find the platform than the water group (Fig. 2A). A two-way repeated-measures ANOVA indicated a significant effect of training day [Fig. 2A; $F_{6,66} = 10.698$, $p < 0.05$] but no effects of group [Fig. 2A; $F_{1,11} = 2.870$, $p > 0.05$] or interaction between group and training day [Fig. 2A; $F_{6,60} = 0.669$, $p > 0.05$]. For females, latencies in both groups gradually decreased during the training phase, but mice in the alcohol group spent more time each day finding the platform (Fig. 2B). Two-way repeated-measures ANOVA revealed significant effects of training day [Fig. 2B; $F_{6,60} = 8.877$, $p < 0.05$] and group [Fig. 2B; $F_{1,10} = 5.766$, $p < 0.05$] but no effect of interaction between both factors [Fig. 2B; $F_{6,60} = 1.064$, $p > 0.05$]. As shown in Fig. 2C and D, there was no significant difference in swimming speed between the water group and alcohol group in either sex.

All mice received a probe test after a 7-day training, and the performance of the probe test is shown in Fig. 3. For males, the alcohol group spent less time in the target quadrant than the water group [Fig. 3A, $t_{11} = 2.695$, $p < 0.05$]. And independent t-test revealed a significant difference in the platform site crossings between the alcohol group and water group [Fig. 3C; $t_{11} = 3.388$, $p < 0.05$]. The one sample t-test showed that the percentage of time spent in target quadrant was significantly different from the 25% chance level for the water group [Fig. 3A; $t_5 = 2.863$, $p < 0.05$], but no significant differences for the alcohol group [Fig. 3A; $t_6 = -1.644$, $p > 0.05$].
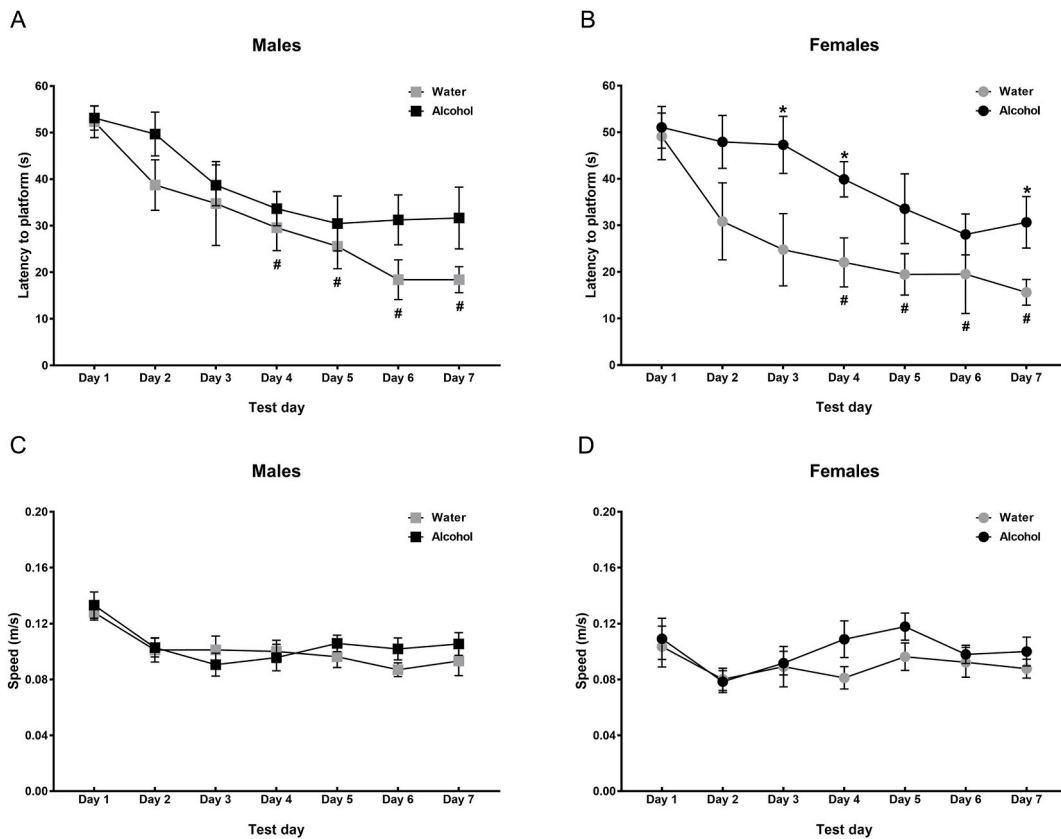

Fig. 2. The performance of male and female mice during the training phase of the Morris water maze task. (A) The latency to reach the platform in male mice during the training phase. (B) The latency to reach the platform in female mice during the training phase. (C) The average speed of male mice during the training phase. (D) The average speed of female mice during the training phase. Data of latency to the platform (A and B) and speed (C and D) are expressed as the average of four trials for each mouse on training days. Water represents the water group, and alcohol represents the alcohol group. Data are shown as the mean ± SEM. *$p < 0.05$ represents statistically significant differences between the water group and the alcohol group. #$p < 0.05$ represents statistically significant differences between Day 1 and other days.
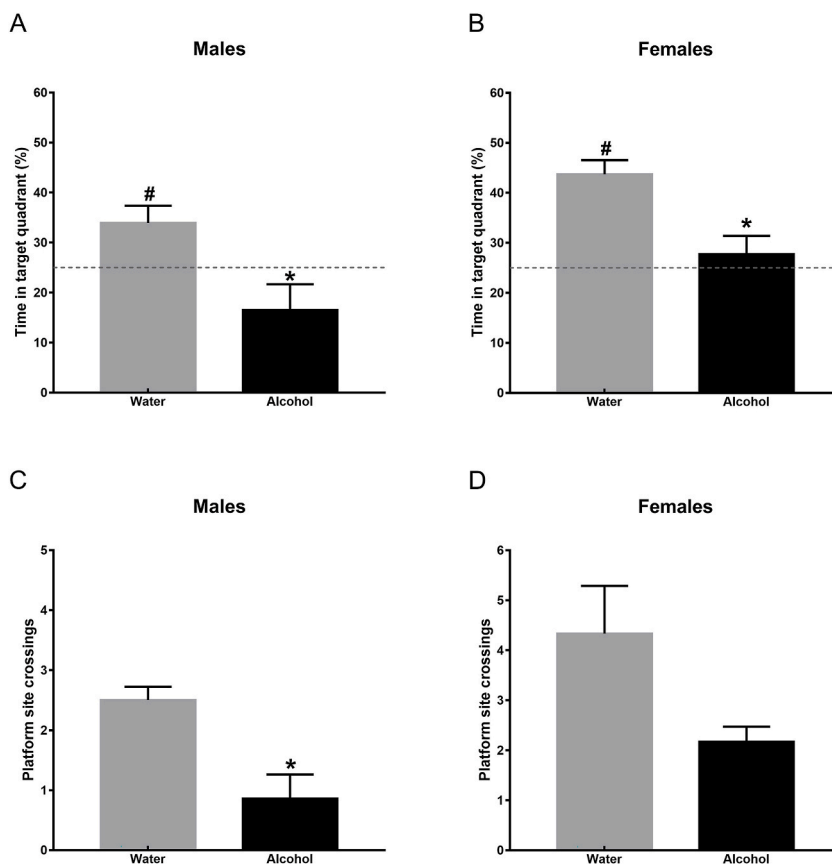
Fig. 3. The performance of male and female mice during the Morris water maze probe test. (A) The percent time that male mice spent in the target quadrant during the probe test. (B) The percent time that female mice spent in the target quadrant during the probe test. (C) The platform site crossings of male mice during the probe test. (D) The platform site crossings of female mice during the probe test. Water represents the water group, and Alcohol represents the alcohol group. Data are shown as the mean ± SEM. Dotted line represents the chance level (25%). *$p < 0.05$ represents statistically significant differences between the water group and alcohol group. #$p < 0.05$ represents statistically significant differences between the percent time in the target quadrant and the chance level.

For females, compared with water group, alcohol group spent significantly less time in the target quadrant [Fig. 3B, $t_{10} = 3.459$, $p < 0.05$] and showed less crossings of the platform position [Fig. 3D; $t_{10} = 2.161$, $p > 0.05$] in the probe test. In addition, female mice in the alcohol group spent less time in the target quadrant [Fig. 3B; t5 = 0.746, p > 0.05] while female mice in the water group spent significantly more time in the target quadrant as compared to 25% [Fig. 3B; t5 = 6.602, $p < 0.05$].

3.3. The effect of chronic intermittent alcohol exposure on the expression of BDNF, TrkB and PLCγ1 in the hippocampus of male and female mice

The expression levels of BDNF, TrkB and PLCγ1 in the hippocampus after chronic alcohol exposure are shown in Figs. 4 and 5. As shown in Fig. 4, in male mice, the expression levels of BDNF and p-PLCγ1 in the hippocampus of the alcohol group were significantly increased compared to those in the water group [Fig. 4B, BDNF, $t_{14} = -2.191$, $p < 0.05$; Fig. 4E, p-PLCγ1, $t_{14} = -2.614$, $p < 0.05$; Fig. 4G, p-PLCγ1/PLCγ1, $t_{14} = -3.602$, $p < 0.05$]. There was no significant difference between the alcohol group and the water group in the expression of TrkB and PLCγ1 in the hippocampus of male mice [Fig. 4C, TrkB-140, $t_{14} = -0.783$, $p > 0.05$; Fig. 4C TrkB-90, $t_{14} = -0.266$, $p > 0.05$; Fig. 4F, PLCγ1, $t_{14} = 0.227$, $p > 0.05$]. Similarly, there were strong stainings of BDNF and p-PLCγ1 in the hippocampus of the alcohol group based on immunohistochemistry analysis. For females, the expression levels of BDNF and p-PLCγ1 in the hippocampus were significantly decreased after chronic alcohol exposure [Fig. 5B, BDNF, $t_{14} = 2.214$, $p < 0.05$; Fig. 5E, p-PLCγ1, $t_{14} = 2.162$, $p < 0.05$; Fig. 5G, p-PLCγ1/PLCγ1, $t_{14} = 3.613$, $p < 0.05$]. There was no significant difference between the alcohol group and the water group in the expression of TrkB and PLCγ1 in the hippocampus of female mice [Fig. 5C, TrkB-140, $t_{14} = 0.561$, $p > 0.05$; Fig. 5C, TrkB-90, $t_{14} = 0.717$, $p > 0.05$; Fig. 5F, PLCγ1, $t_{14} = 1.788$, $p > 0.05$]. The similar decreases in the expression of BDNF and p-PLCγ1 in the hippocampus of the alcohol group compared with water group were observed on immunohistochemistry (Fig. 5H).
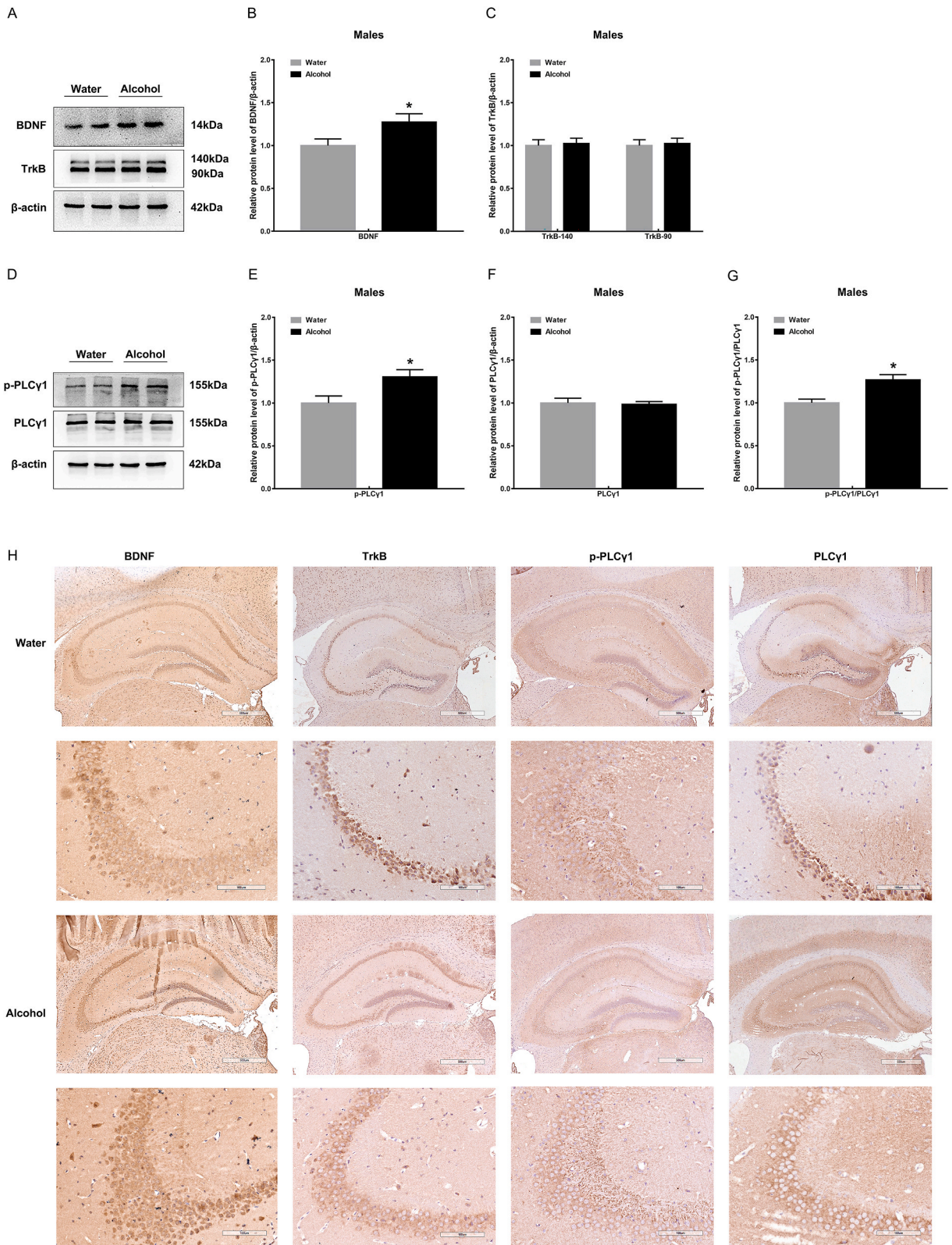
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Fig. 4. Changes in the expression of BDNF, TrkB and PLCγ1 in the hippocampus of male mice after chronic alcohol exposure. (A) Representative blots of BDNF and TrkB in the hippocampus of male mice. (B) and (C) BDNF and TrkB expression levels in the hippocampus after chronic alcohol exposure. (D) Representative blots of p-PLCγ1 and PLCγ1 in the hippocampus of male mice. (E), (F) and (G) The p-PLCγ1 and PLCγ1 expression levels in the hippocampus after chronic alcohol exposure. (H) Representative images of BDNF, TrkB p-PLCγ1 and PLCγ1 immunoreactivities in the hippocampus of male mice (low magnification at 4 ×, scale bar = 500 μm; high magnification at 20 ×, scale bar = 100 μm). Water represents the water group, and Alcohol represents the alcohol group. p-PLCγ1 represents phospho-PLCγ1, and TrkB-140 and TrkB-90 represent bands at 140 kDa and 90 kDa, respectively. The expression levels of the water group were set at 1 for quantification. Data are shown as the mean ± SEM. *$p < 0.05$ represents statistically significant differences between the water group and the alcohol group.

4. Discussion

In this study, we examined the effect of chronic alcohol exposure on spatial memory in males and females and measured the expression of BDNF, TrkB and PLCγ1 in the hippocampus. Using the MWM and western blotting, our results showed impairment of spatial memory in both sexes and sex differences in the expression of BDNF and p-PLCγ1 in the hippocampus after chronic alcohol exposure.

Clinical and animal studies have demonstrated that chronic alcohol consumption produces neurocognitive and memory deficits in males [14,39,40], but few studies have observed changes in memory in females. In the present study, male mice given intermittent access to 20% alcohol 4 weeks showed no significant decrease in latency during the training phase, but spent shorter time in the target quadrant and had fewer platform site crossings during the probe test. These results indicated that chronic alcohol exposure caused impairment in spatial memory retention of male mice. Similar to the males, chronic alcohol exposure induced impairment of spatial memory acquisition and retention in females, as supported by significantly longer escape latencies during the training phase and shorter time spent in the target quadrant during the probe test. These findings are consistent with studies showing alcohol-induced spatial memory impairment in males [40,41] and spatial memory retention in females [42]. Our findings suggested that intermittent access to 20% alcohol for four weeks can lead to spatial memory impairment in males and females.

The impairment of spatial memory observed in the present study presumably reflects that chronic alcohol exposure causes damage to the central nervous system, especially the hippocampus. BDNF plays an important role in neuronal plasticity and learning and memory [6,7], an effect that is mediated by its associated receptor TrkB and its downstream effector PLCγ1 [12]. BDNF in hippocampus is required for the persistence of long-term memory storage [43], and PLCγ1 is thought to be essential for drug-induced dendritic spine formation [15]. After chronic alcohol exposure, our results showed significantly decreased expression of BDNF and p-PLCγ1 in the hippocampus of females that exhibited spatial memory impairment. Similarly, after 4 weeks alcohol consumption, decreased dendritic spine density, proliferation and neurogenesis were found in the hippocampus of females with cognitive impairment [29]. Moreover, intrahippocampal BDNF infusion is able to increase neurogenesis of granule cells [44] and enhance declarative memory [45]. Based on these findings, the downregulation of BDNF-TrkB-PLCγ1 signaling in the hippocampus seem to be involved in alcohol-induced impairment of spatial memory in females.

Different from females, our results showed significantly increased expression level of BDNF and p-PLCγ1 in the hippocampus of males after chronic alcohol exposure. In agreement with our results, increased dendritic spine density and neurogenesis in the dentate gyrus and enhancement of hippocampal neuronal arborization were found in alcohol-exposed male rats [23,46,47]. Since BDNF plays a potential role in promoting cell proliferation and neurogenesis, these reactive responses are thought to contribute to repair and recovery of the hippocampus of males that showed spatial memory impairment [48,49]. Thus, the upregulation of BDNF-TrkB-PLCγ1 signaling in the hippocampus of males were hypothesized to contribute to recovery after hippocampal damage induced by alcohol. In comparison to males, females with alcohol-related cognitive impairment have a slower recovery after 6 weeks of abstinence [50]. Similarly, increased cell proliferation and immature neurons number were found in female rats on the 14th day of abstinence from alcohol [49]. These observations indicate that females may suffer from more prolonged memory impairment compared to males and had a slower recovery from alcohol. Additionally, although alcohol consumption in females was not affected by the estrous cycle when rats were given either intermittent access to alcohol or continuous access to alcohol [51], the role of gonadal hormones and sex chromosome complement in regulation of BDNF expression following alcohol exposure will be pursued in future studies.

It should be noted that the concentrations and patterns of alcohol intake may also contribute to sex difference in responses to alcohol. Opposing changes to synaptic strength onto parvalbumin-expressing interneurons of the prefrontal cortex was found in females and males that given intermittent access to 20% alcohol for 4 weeks [52]. In comparison, continuous access to 10% alcohol for 3 weeks, there was no significant change in BDNF expression in the hippocampus of both sexes [53]. This difference in the concentrations of alcohol consumption suggests that the expression of BDNF need to be further detected by comparing different concentrations and pattens of alcohol consumption within males and females.

In summary, we performed a study to investigate the effect of intermittent access to 20% alcohol on spatial memory and BDNF–TrkB signaling in the hippocampus of male and female mice. Our findings indicated that chronic alcohol exposure induces spatial memory impairment in both sexes and sex difference in expression of BDNF/TrkB/PLCγ1 pathway in the hippocampus. These observations suggest that the neuronal and molecular adaptations induced by alcohol may differ between males and females. Future investigations targeting BDNF expression and adaptive plasticity in the hippocampus, in both females and males, should improve our understanding of how alcohol-related adaptations in the hippocampus may relate to sex-dependent changes.

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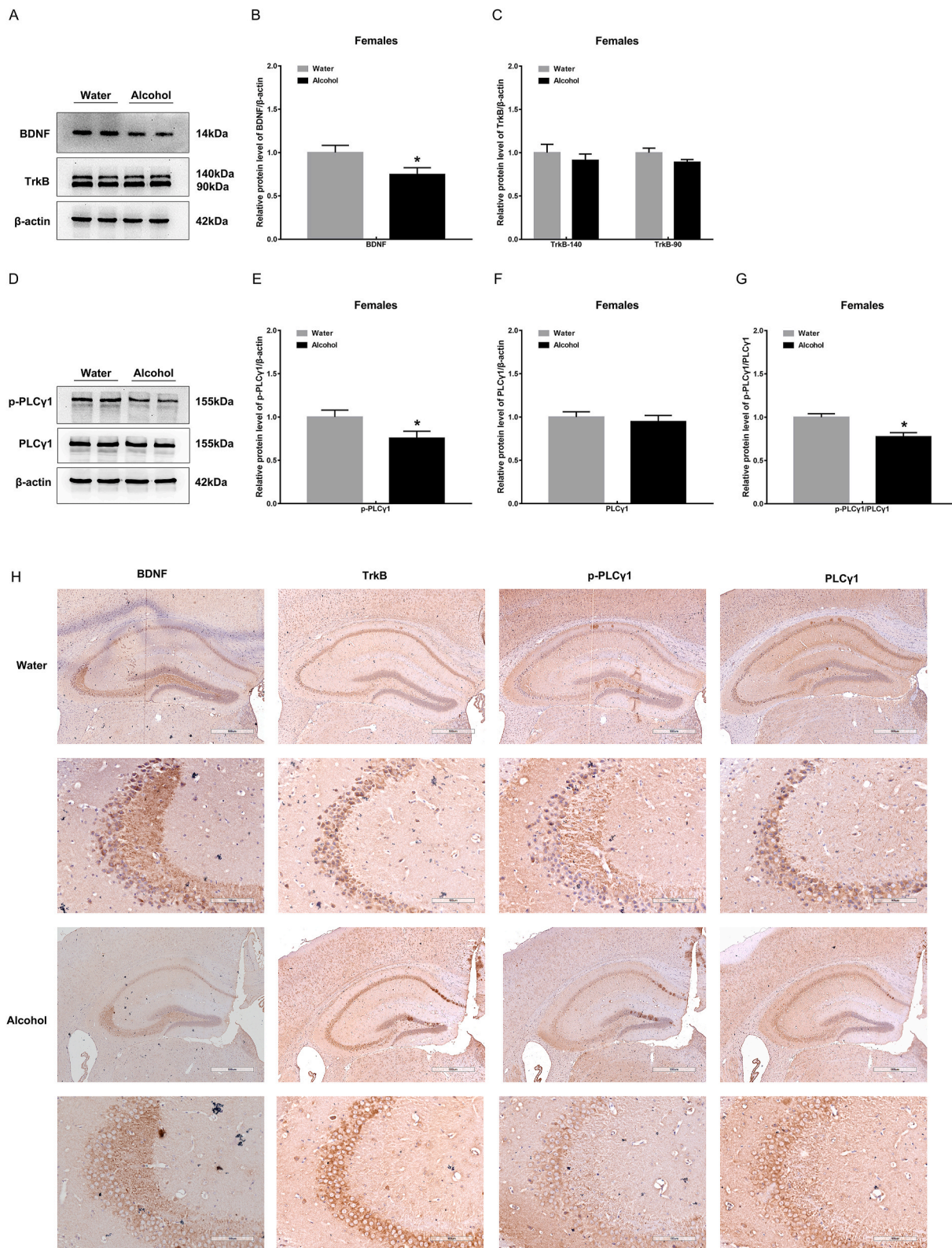
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Fig. 5. Changes in the expression of BDNF, TrkB and PLCγ1 in the hippocampus of female mice after chronic alcohol exposure. (A) Representative blots of BDNF and TrkB in the hippocampus of female mice. (B) and (C) BDNF and TrkB expression levels in the hippocampus after chronic alcohol exposure. (D) Representative blots of p-PLCγ1 and PLCγ1 in the hippocampus of female mice. (E), (F) and (G) The p-PLCγ1 and PLCγ1 expression levels in the hippocampus after chronic alcohol exposure. (H) Representative images of BDNF, TrkB p-PLCγ1 and PLCγ1 immunoreactivities in the hippocampus of female mice (low magnification at 4 ×, scale bar = 500 μm; high magnification at 20 ×, scale bar = 100 μm). Water represents the water group, and alcohol represents the alcohol group. p-PLCγ1 represents phospho-PLCγ1, and TrkB-140 and TrkB-90 represent bands at 140 kDa and 90 kDa, respectively. The expression levels of the water group were set at 1 for quantification. Data are shown as the mean ± SEM. *$p < 0.05$ represents statistically significant differences between the water group and the alcohol group.

Author contribution statement

Feng Xiong, Guokai Dong, Wenjiang Yin: Performed the experiments; Contributed reagents, materials, analysis tools or data; Analyzed and interpreted the data.

Jingyuan Ma, Zhouru Li, Xiaoming Sun, Hongxing Cai: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16660.

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