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Effect of α-asarone on ethanol-induced learning and memory impairment in mice and its underlying mechanism

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ABSTRACT

Aims: Learning and memory impairment is a common symptom in the early stages of various types of dementia. It is likely to reduce the incidence of dementia with correct intervention. α-Asarone is the main bioactive substance isolated from Acorus tatarinowii Schott and has been proven to improve memory dysfunction; however, at present, the specific underlying mechanism is poorly understood. The aim of the present study was to investigate the effect of α-asarone on ethanol-impaired cognitive ability and explore the underlying mechanism in mice.

Main methods: A mouse model of impaired learning and memory was created by ethanol (2.0 g/kg, i.g.). α-Asarone (7.5, 15 or 30 mg/kg, i.p.) was delivered 10 min prior to ethanol administration. The behavioral effect of α-asarone was evaluated using the novel object recognition test. Glutamate (Glu) and γ-aminobutyric acid (GABA) levels in the hippocampus were determined by ELISA, and the protein expression levels of hippocampal GluR2, NMDAR2B, SYNI, GLT-1 and CaMKII were detected by western blotting.

Key findings: Pretreatment with α-asarone significantly improved the behavioral performance, regulated the imbalance of Glu and GABA in the hippocampus and the abnormal expression of related proteins. A possible underlying mechanism is regulation of the calcium signaling cascade to correct functioning of related proteins, and thus, maintain the level of Glu.

Significance: Our results show that the improvement in learning and memory elicited by α-asarone may providing a possible novel candidate for the prevention of learning and memory impairment in the early stages of dementia.

1. Introduction

A decline in learning and memory ability is considered a transitional stage, with mild but detectable functional impairment, that occurs between normal aging and dementia [1] including Alzheimer's disease (AD) and vascular dementia [2,3]. Owing to the continuous failure of AD drugs in clinical trials, the International Alzheimer's Association has proposed advancement of early stage AD treatment, which suggests that attempts to improve the symptoms of learning and memory disorder will re-emerge as a research hotspot for anti-dementia drugs [4,5].

Excessive ethanol intake can block memory recall in the brain, which is a well-established model of learning and memory impairment for the evaluation of potential therapeutics [6]. Ethanol produces a direct toxic effect on nerve cells, causing a series of changes in neurological functions, such as impairment of learning, memory and cognitive ability. These effects are similar to the clinical manifestations of early stage AD, the severity of which is insufficient to impair daily function. At present, it is believed that the specific mechanism of cognitive impairment caused by ethanol may be related to aberrant neurotransmission [7].

It is well known that the balance between glutamate (Glu) and γ-aminobutyric acid (GABA) levels plays an important role in maintaining the cognitive function of the hippocampus [8], and an imbalance in these neurotransmitters has been found in the early stage of AD. Both

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increases and decreases in Glu levels can cause cognitive impairment [9,10]; thus, cognitive impairment in animal models cannot be measured solely by the level of each neurotransmitter but instead is determined by the steady-state balance between them.

Rolipram, a phosphodiesterase-4 inhibitor, has been shown to enhance the survival of newborn hippocampal neurons, improve cognitive, learning and memory abilities and exert anxiolytic and antidepressant effects [11,12]. It can affect the excitatory and inhibitory amino acid neurotransmission systems in the brain by selectively regulating the Ca^{2+}/calmodulin-dependent cyclic adenosine monophosphate (AMP)-specific phosphodiesterase [13]. Studies have shown that rolipram can balance Glu levels by regulating related proteins [14]; therefore, rolipram was used as a positive control in the present.

To date, even drugs that significantly improve AD, such as donepezil and galantamine [15], have been shown to be associated with a greater risk of adverse events as compared with the beneficial effects, which has prompted exploration of other treatments. In recent years, Chinese herbal medicine has become increasingly popular in the prevention and treatment of dementia; most nootropics, including Acorus tatarinowii Schott [16–19], Ginseng [20,21], Polygala tenuifolia [22] and Salvia miltiorrhiza [23] have protective effects on neurons. Among these, Acorus tatarinowii Schott is the basic herb in many traditional Chinese medicines and can improve cognitive ability. The main bioactive phytochemicals in Acorus tatarinowii Schott are α- and β-asarone [24]. Recent research indicates that α-asarone has low toxicity and a broad therapeutic window for the treatment of learning and memory disorders [25–27]. The present study focused on the effect and underlying mechanism of α-asarone on ethanol-induced learning and memory impairment in mice.

2. Materials and methods

2.1. Animals and drugs

Healthy male C57BL/6 J mice, aged 6–7 weeks old, were obtained from the Animal Testing Center of The Fourth Military Medical University. Experimental animals were maintained under a normal light cycle (12 h/12 h, lights on at 6:00 A.M.) with a liberal supply of water and food. The investigation conformed to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and was approved by the Shanxi University of Chinese Medicine Institutional Animal Care and Use Committees (No. 2017-041101 EA).

Animals were acclimated under standard conditions for 1 week prior to the behavioral test and subsequently divided into 6 groups (n = 11 per group): normal (saline, i.p. + saline, i.g.), ethanol (saline, i.p. + 2 g/kg ethanol, i.g.), α-asarone (7.5, 15 or 30 mg/kg, i.p. + 2 g/kg ethanol, i.g.) and rolipram (1 mg/kg, i.p. + 2 g/kg ethanol, i.g.). All groups were given 10 mL solution per kg body weight. Different amounts of α-asarone were dissolved in normal saline containing 10% DMSO and 10% Tween® 80 immediately prior to dosing. Rolipram was prepared in normal saline containing 10% DMSO. α-Asarone was obtained from the National Institutes for Food and Drug Control. Rolipram and DMSO were purchased from Sigma. Tween® 80 was provided by Solarbio.

2.2. Behavioral test

2.2.1. Open field test

Mice were placed in an empty box (40 cm × 40 cm × 40 cm), the bottom of which was divided into 9 equal squares. The horizontal (number of times crossing the 9 squares) and vertical (number of times lifting the front claw) locomotor activities were calculated during a period of 5 min.

2.2.2. The novel object recognition test

The novel object recognition test was divided into 3 main procedures: habituation, training and testing. *1.* Mice were fed and mildly touched for 1 week in a comfortable cage prior to being placed in the center of an empty box. Free exploration of the arena was allowed for 10 min per day. The apparatus and objects were thoroughly cleaned using 70% ethanol following removal of each mouse; *2.* Two identical objects were placed in opposite quadrants of the box; *3.* After 24 h, one object used during the second procedure (i.e., the familiar object) and one novel object were placed in opposite quadrants of the arena at the same location. The method of behavioral measurement was the time spent exploring each object.

The object recognition index (%) was computed using the following formula: the time spent exploring the novel object/the total time spent exploring both objects *100.

2.3. Determination of the glu to GABA ratio

The tissue samples were weighed after being cut and PBS pH 7.4 was immediately added and the samples cryopreserved in liquid nitrogen. Glu and GABA ELISA kits were used to detect the levels of the respective neurotransmitters.

2.4. Western blotting

The hippocampus were harvested immediately after the behavioral tests and lysed using a protein extraction reagent containing 1% PMSF and 1% protease inhibitors. The proteins were subjected to SDS-PAGE, transferred to PVDF membrane and blocked with 5% bovine serum albumin (BSA). The membrane was subsequently incubated overnight at 4 °C with the corresponding primary antibody (1:1000) in western blotting dilution buffer. Following washing in Tris-buffered saline containing 0.1% Tween® 20, the membrane was incubated for 1 h at room temperature with an anti-rabbit secondary antibody (1:2000) in western blotting dilution buffer. The membrane was subsequently washed with Tris-buffered saline containing 0.1% Tween® 20, and an ECL protein detection kit was used to visualize the hippocampal expression levels of GluR2, NMDA2B, SYNI, GLT-1 and CaMKII. The immunoblots were exposed using Investigator ProImage.

2.5. Statistical analysis

Data were analyzed by one-way ANOVA using the GraphPad Prism 5.0 software package. All values are expressed as the mean ± SD, with $P < 0.05$ being considered statistically significant.

3. Results

3.1. Performance in the behavioral test

3.1.1. Locomotor activity in the open field test

The locomotor activity of mice with learning and memory impairment was evaluated by the open field test. There was no significant difference in the horizontal (Fig. 1A) or vertical (Fig. 1B) locomotor activity between the ethanol and normal groups or the 7.5, 15 and 30 mg/kg α-asarone groups ($F_{(5,\ 48)} = 0.6536$, $P > 0.05$; $F_{(5,\ 49)} = 1.995$, $P > 0.05$). These results indicate that neither ethanol nor α-asarone affected the locomotor activity of mice.

3.1.2. Cognitive performance in the novel object recognition test

The cognitive ability of mice with respect to a familiar object was evaluated by the novel object recognition test (Fig. 1C). The recognition index in the ethanol group was significantly decreased as compared with that in the normal group ($F_{(5,\ 46)} = 6.739$, $^{*}P < 0.05$) and was markedly increased in the α-asarone groups as compared with that in the ethanol group ($^{\#}P < 0.05$), with the exception of the 7.5 mg/kg α-

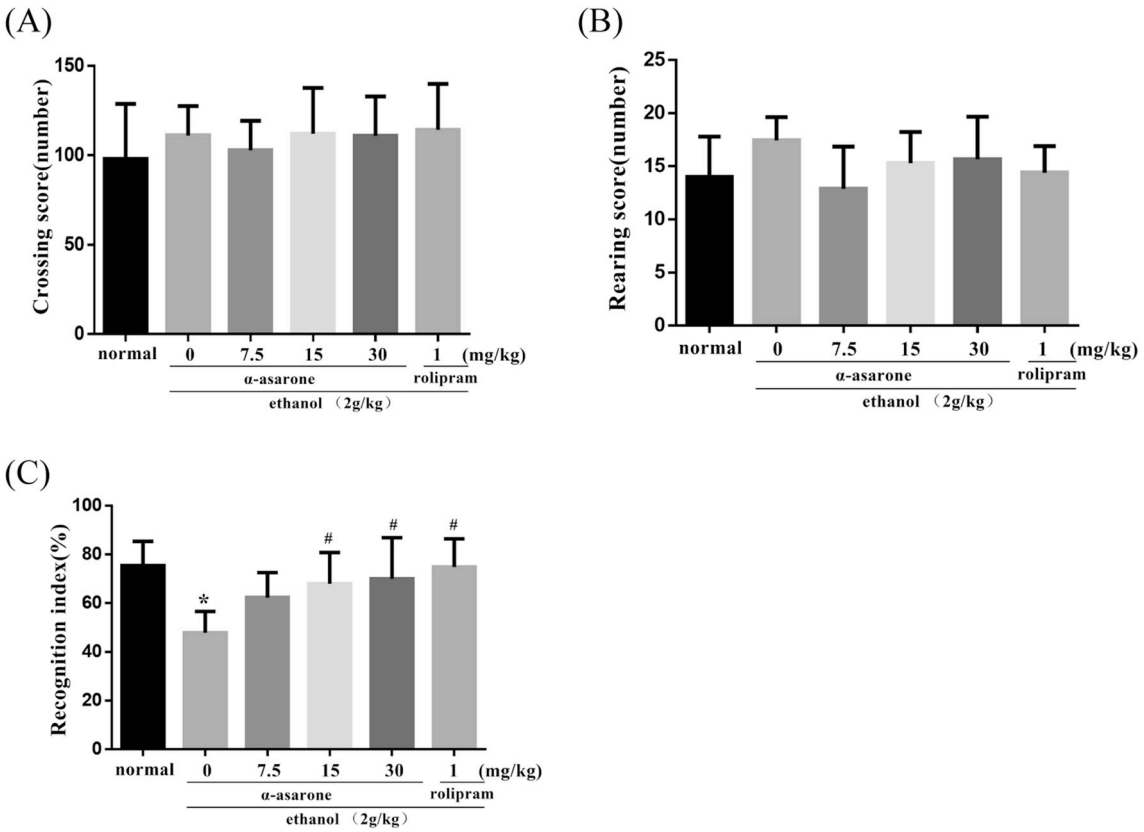

Fig. 1. Effect of α-asarone on the recognition index of the behavioral test in mice with ethanol-induced learning and memory impairment. The normal group was treated with saline (10 mL/kg, i.g.) and the remaining groups were treated with α-asarone (0, 7.5, 15 or 30 mg/kg, i.p.) or rolipram (1 mg/kg, i.p.). After 10 min, ethanol (2 g/kg, i.p.) was used to create learning and memory impairment. Subsequently, the locomotor activity and cognitive ability were evaluated by the open field and novel object recognition tests. Each column represents the mean ± SD of 11 animals per group. There was no significant difference in the crossing score (A) or the rearing score (B) among the normal, ethanol, and 7.5, 15 and 30 mg/kg α-asarone groups ($P > 0.05$). The recognition index (C) in the ethanol group was significantly decreased as compared with that in the normal group ($^{*}P < 0.05$), and markedly increased in the 15 and 30 mg/kg α-asarone groups as compared with that in the ethanol group ($^{\#}P < 0.05$), with the exception of the 7.5 mg/kg α-asarone group ($P > 0.05$).

asarone group ($P > 0.05$). These results indicate that the memory ability of mice with ethanol-induced learning and memory impairment was improved by the administration of α-asarone.

3.2. Changes in the equilibrium state of the hippocampal neurotransmitters

Table 1 shows the Glu to GABA ratio in the mouse hippocampus. This ratio was significantly elevated in the ethanol group as compared with that in the normal group (33.42 ± 0.8972 vs. 30.79 ± 0.2102, $^{*}P < 0.05$) and significantly lower in the α-asarone groups (31.99 ± 0.4986 vs. 33.42 ± 0.8972; 30.97 ± 0.1757 vs. 33.42 ± 0.8972; 30.83 ± 0.1723 vs. 33.42 ± 0.8972, $^{\#}P < 0.05$). These results indicate that the equilibrium between Glu and GABA in the hippocampus of mice administered ethanol was unbalanced and α-asarone was able to maintain this equilibrium.

Table 1
The hippocampal Glu:GABA ratio in mice with ethanol-induced learning and memory impairment (x ± SD, n = 6). Brain tissue samples from each group were treated according to specific ELISA kit requirements. The levels of Glu and GABA were determined and the Glu to GABA ratio was calculated. This ratio was significantly elevated in the ethanol group as compared with that in the normal group ($^{*}P < 0.05$) and was significantly lower in the α-asarone groups ($^{\#}P < 0.05$).

Group	Dose (10 mL/kg)	Glu:GABA
Normal	Saline (i.p.) + Saline (i.g.)	30.79 ± 0.2102
Ethanol	Saline (i.p.) + Ethanol (2 g/kg, i.g.)	33.42 ± 0.8972*
α-Asarone	7.5 mg/kg (i.p.) + Ethanol (2 g/kg, i.g.)	31.99 ± 0.4986#
α-Asarone	15 mg/kg (i.p.) + Ethanol (2 g/kg, i.g.)	30.97 ± 0.1757#
α-Asarone	30 mg/kg (i.p.) + Ethanol (2 g/kg, i.g.)	30.83 ± 0.1723#
Rolipram	1 mg/kg (i.p.) + Ethanol (2 g/kg, i.g.)	30.71 ± 0.8137#

3.3. Expression of related proteins

3.3.1. Expression of glutamate receptors and phosphorylated synaptophysin I

The expression levels of GluR2 and NMDA receptors and phosphorylated synaptophysin I (pSYNI) were evaluated by western blotting (Figs. 2 and 3). The expression levels of GluR2 (Fig. 2A), NMDAR2B (Fig. 2B) and pSYNI (Fig. 3) were significantly higher in the ethanol group as compared with those in the normal group ($^{*}P < 0.05$) and obviously lower in the α-asarone groups ($^{\#}P < 0.05$), with the exception of GluR2 and NMDAR2B in the 7.5 mg/kg α-asarone group ($P > 0.05$). These results indicate that ethanol caused an increase in Glu release and accumulation, resulting in excitotoxicity, and α-asarone was able to reduce Glu levels, thus reducing this excitotoxicity.

3.3.2. Expression of glutamate transporter type 1

Fig. 4 shows the expression level of glial glutamate transporter type 1 (GLT-1) by western blotting. The expression level of GLT-1 was significantly lower in the ethanol group as compared with that in the normal group ($^{*}P < 0.05$) and obviously higher in the α-asarone groups ($^{\#}P < 0.05$). These results indicate that α-asarone was able to repair the aberrant expression of glutamate transporters that was changed by ethanol.

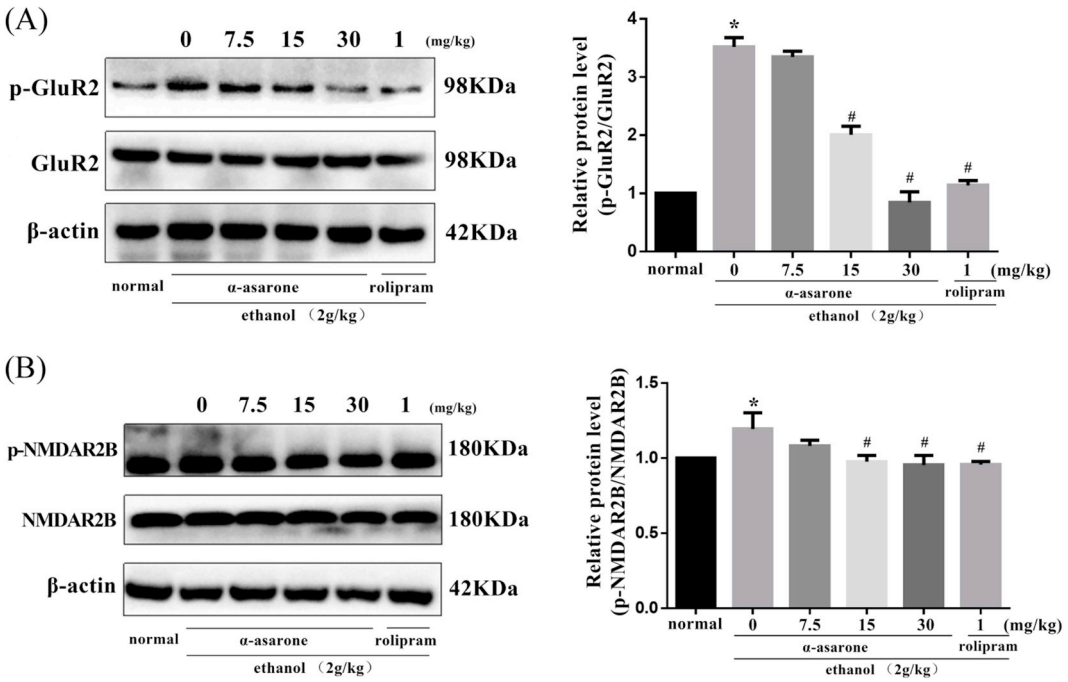

Fig. 2. Effect of α-asarone on the hippocampal expression level of the glutamate-related receptors, GluR2 and NMDAR2B, in mice with ethanol-induced learning and memory impairment. All data are expressed as the mean ± SD of 3 independent experiments. (A) The expression level of GluR2 was significantly higher in the ethanol group as compared with that in the normal group (*$P < 0.05$) and obviously lower in the α-asarone groups (2.01 ± 0.15 vs. 3.52 ± 0.16, 0.84 ± 0.18 vs. 3.52 ± 0.16, $^{\#}P < 0.05$), with the exception of the 7.5 mg/kg α-asarone group (3.34 ± 0.10 vs. 3.52 ± 0.16, $P > 0.05$). (B) The expression level of NMDAR2B was significantly higher in the ethanol group as compared with that in the normal group (*$P < 0.05$). α-Asarone significantly reduced the expression level of NMDAR2B (0.98 ± 0.04 vs. 1.19 ± 0.11, 0.95 ± 0.06 vs. 1.19 ± 0.11, $^{\#}P < 0.05$), with the exception of the 7.5 mg/kg α-asarone group (1.08 ± 0.04 vs. 1.19 ± 0.11, $P > 0.05$).

3.3.3. Expression of phosphorylated calcium/calmodulin-dependent protein kinase II

The expression level of phosphorylated calcium/calmodulin-dependent protein kinase II (pCaMKII) by western blotting is shown in Fig. 5. The expression of pCaMKII was significantly higher in the ethanol group as compared with that in the normal group (*$P < 0.05$) and obviously lower in the α-asarone groups ($^{\#}P < 0.05$), with the exception of the 7.5 mg/kg α-asarone group ($P > 0.05$). These results indicate that the excessive release of Glu caused by ethanol was due to increased pCaMKII expression, and α-asarone was able to reduce this release by downregulating the expression of pCaMKII.

4. Discussion

The aim of the present study was to explore the mechanism of α-asarone with respect to the improvement in learning and memory impairment. The novel object recognition test is the most widely used behavioral test for the appraisal of learning and memory ability [28–31]. This test allows mice to stay in an almost natural environment and requires significantly less time to perform than other commonly used memory tests such as the water maze and passive avoidance tests, both of which may cause stress to the mice. Lueptowet al. (2017) reported that this method is advantageous, since there is no need for numerous training sessions or positive or negative reinforcement to motivate behavior. The novel object recognition test typically evaluates the ability of a rodent to remember objects as measured by the total exploration time of familiar and novel objects [32–34]. Akkerman et al. (2012) reported that the novel object recognition test has two analytical parameters: the difference score (DS), calculated by the absolute difference between the exploration time of the novel object and the familiar object; and the investigation ratio (IR), which is based on the difference between each object, both of which are feasible. We

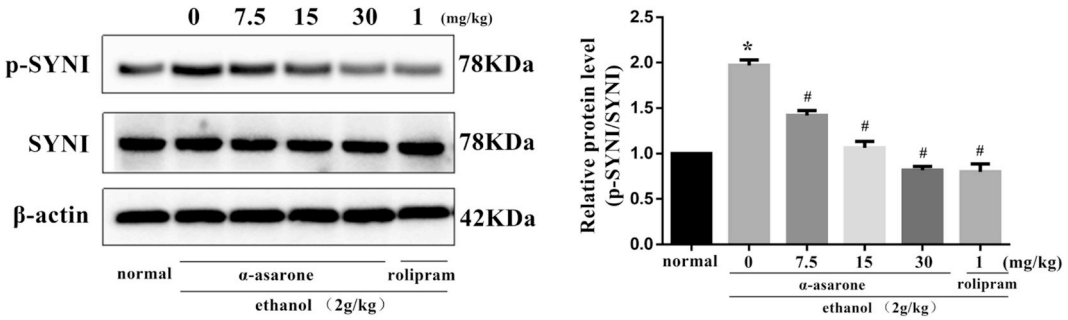

Fig. 3. Effect of α-asarone on the hippocampal expression level of phosphorylated synaptophysin I (pSYNI) in mice with ethanol-induced learning and memory impairment. All data are expressed as the mean ± SD of 3 independent experiments. The expression level of pSYNI was significantly higher in the ethanol group as compared with that in the normal group (*$P < 0.05$) and obviously lower in the α-asarone groups (1.42 ± 0.05 vs. 1.97 ± 0.06, 1.06 ± 0.07 vs. 1.97 ± 0.06, 0.82 ± 0.04 vs 1.97 ± 0.06, $^{\#}P < 0.05$).

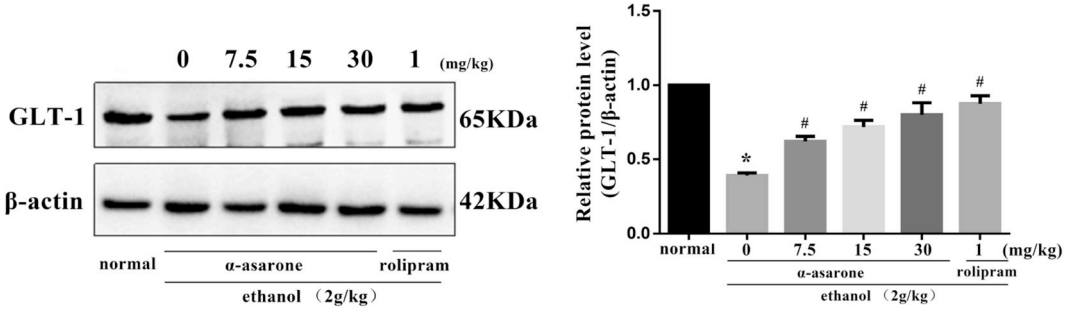

Fig. 4. Effect of α-asarone on the hippocampal expression level of the glial glutamate transporter type 1 (GLT-1) in mice with ethanol-induced learning and memory impairment. All data are expressed as the mean ± SD of 3 independent experiments. The expression level of GLT-1 was significantly lower in the ethanol group as compared with that in the normal group ($^{*}P < 0.05$) and obviously higher in the α-asarone groups (0.62 ± 0.03 vs. 0.39 ± 0.02, 0.72 ± 0.04 vs. 0.39 ± 0.02, 0.80 ± 0.08 vs. 0.39 ± 0.02, $^{\#}P < 0.05$).

calculated the percentage of time exploring the novel object as compared with the total time exploring both objects, namely the recognition index. In previous experiments, since the mice in the normal group had memory of the familiar object and were driven by natural impulses, they constantly explored the novel object, making the recognition index higher. As expected, the index in the ethanol group was lower. This phenomenon was reproduced in the present study. The recognition index in the ethanol-administered mice treated with rolipram was significantly increased in the behavioral tests; thus, we can be certain that this study system can be used to evaluate the efficacy of α-asarone. According to the behavioral tests, we believe that α-asarone is effective in improving the learning and memory disorder caused by ethanol.

Under normal physiological conditions, the functions of the hippocampus are intact and Glu levels are in equilibrium with GABA, which is vital to learning and memory ability [35,36]. Cui et al. (2015) suggested that changes in the levels of central amino acids in AD, especially the imbalance of Glu and GABA levels, are key factors that lead to neuron injury. Therefore, the status of the hippocampus cannot be solely determined by the levels of Glu or GABA but instead their ratio should be used to judge whether the status is normal [37]. Both Wang et al. (2014) and Cui et al. (2015) used this method in their studies; therefore, we used the Glu to GABA ratio in the present study to evaluate whether ethanol affects learning and memory ability in mice.

It has already been verified [38,39] that the abnormal metabolism and excess release of Glu, as well as high-frequency stimulation of postsynaptic receptors, induce excitotoxicity. Moreover, the majority of studies have detected the levels of hippocampal receptor proteins related to Glu [40,41]. In the present study, ionic Glu receptors were used as the research object. Ionic receptors are mainly the N-methyl-D-aspartic acid (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors. Of these, the AMPA receptors is widely distributed in hippocampal and cerebellar tissues, mediating excitatory neurotransmission following activation by Glu. Hiester et al. (2018) reported the AMPA receptors to be involved in the process of long-term potentiation and depression in the synapses of nerve cells to regulate learning and memory ability [42–44]. Similar to AMPA receptors, NMDA receptors have also been shown to play a key role in regulating dendrites, axon development and synaptic plasticity [45–47]. In a large number of studies on learning and memory function [21], the determination of protein phosphorylation has been conducted. Under pathological conditions, Glu causes excitotoxicity via the overactivation of Glu receptors. In this case, the activity of AMPA and NMDA proteins is increased and the phenomenon of overexpression occurs; therefore, we detected the levels of GluR2 and NMDAR2B proteins in the mouse hippocampus. We found that α-asarone reduced the overexpression of Glu receptors that was caused by excitotoxicity.

Based on these results, we were able to determine that α-asarone can improve the learning and memory impairment caused by ethanol-induced excitotoxicity. Liu et al. (2017) reported that the accumulation of Glu was due to excessive release and reduced transport capacity [48]. To further verify the accuracy of this information, we detected the protein expression level of phosphorylated synaptophysin I (pSYNI) at the vesicle membrane [49,50], which affects the release of Glu, as well as the glial glutamate transporter type 1 (GLT-1) [51]. SYNI is a specific protein that exists at the membrane of synaptic vesicles; it can respond to Ca^{2+} signals, and therefore regulates the release of Glu [52,53]. GLT-1 is the most important glutamate transporter in the central nervous system and plays a key role in the transmission of Glu [54]. Under the influence of ethanol toxicity, the function of nerve cells is damaged and the uptake function of transporters is also reduced, resulting in a significant increase in the extracellular Glu concentration. The results of the present study show that the expression level of pSYNI in the ethanol

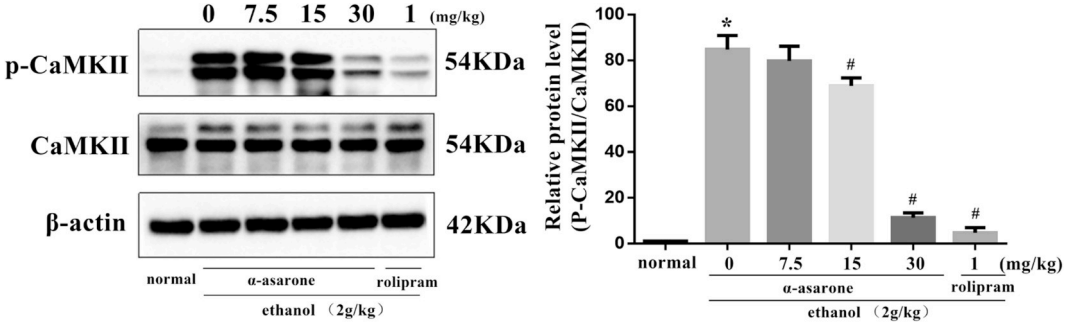

Fig. 5. Effect of α-asarone on the hippocampal expression level of phosphorylated calcium/calmodulin-dependent protein kinase II (pCaMKII) in mice with ethanol-induced learning and memory impairment. All data are expressed as the mean ± SD of 3 independent experiments. The subunits of CaMKII expressed in hippocampus are mainly CaMKII-α and -β, which may be the reason for the double band. The expression level of pCaMKII was significantly higher in the ethanol group as compared with that in the normal group ($^{*}P < 0.05$) and obviously lower in the α-asarone groups (68.86 ± 3.48 vs. 84.83 ± 6.07, 11.4 ± 2.10 vs. 84.83 ± 6.07, $^{\#}P < 0.05$), with the exception of the 7.5 mg/kg α-asarone group (79.76 ± 6.49 vs. 84.83 ± 6.07, $P > 0.05$).

group was significantly increased as compared with that in the normal group and significantly decreased following treatment with rolipram and various doses of α-asarone. With respect to GLT-1, the expression level in the ethanol group was significantly lower as compared with that in the normal group. Under the action of rolipram and various doses of α-asarone, the expression level of GLT-1 in ethanol-administered mice was significantly increased and tended toward the normal level.

Through the above studies on Glu and the expression of its related proteins, we found that α-asarone had a significant effect on the release, transport and receptor binding of Glu. One important factor that caught our attention was the overload of Ca^{2+} ions. The exact mechanism of excitotoxicity is not yet fully understood; however, the overload of Ca^{2+} may have an important correlation [55]. When excitotoxicity occurs, a large amount of Ca^{2+} flow into cells, resulting in an imbalance in intracellular Ca^{2+} homeostasis. AMPA, NMDA, SYNI and GLT-1 proteins are all related to Ca^{2+}. Among these, the NMDA receptors is the main channel through which Ca^{2+} flows into cells, and activation of Ca^{2+} signal transduction is involved in the regulation of synaptic plasticity [56]. Currently, it is considered that calcium-mediated AMPA membrane expression is an important mechanism in determining synaptic strength, and increasing evidence shows that AMPA receptors also have permeability to Ca^{2+} [57]. SYNI is mainly expressed following Ca^{2+} signaling [58]. Moreover, the internalization of GLT-1 also requires the participation of Ca^{2+} [59]. Calmodulin (CaM) is an important signaling molecule that is mainly activated by binding to Ca^{2+} and can further activate calcium/calmodulin-dependent protein kinase II (CaMKII). As an important signaling molecule downstream of the NMDA receptor, CaMKII has been widely studied [60] and is recognized to be involved in synaptic plasticity, learning and memory [61]. There is evidence that CaMKII dysfunction can lead to abnormalities in Glu; therefore, we evaluated CaMKII protein expression to verify whether Ca^{2+} overload is a mechanism of excitotoxicity. The expression level of CaMKII was significantly higher in the ethanol group as compared with that in the normal group, and obviously lower in the α-asarone groups. These results indicate that the excessive release of Glu caused by ethanol is due to an increased concentration of Ca^{2+}, which is consistent with the conclusion given by Rivero-Segura et al. (2017) that Ca^{2+} overload may be a mechanism of excitotoxicity [55].

5. Conclusion

Based on the aforementioned results, it is reasonable to suggest that α-asarone can improve the learning and memory ability caused by the excitotoxicity of ethanol by: *1.* controlling calcium overload, reducing the activity of SYNI and decreasing the Glu release; *2.* improving the functional damage of Glu transporter GLT-1 and normalizing Glu transport function; and *3.* reducing the overactivity of the Glu receptors, AMPA and NMDA, via a decrease in the Glu concentration and regulation of pCaMKII levels. Its underlying mechanism is Ca^{2+} regulation to maintain the equilibrium between Glu and GABA levels. According to these conclusions and the fact that the positive effect of 30 mg/kg α-asarone is almost the same as that of rolipram, α-asarone may be a promising compound for the treatment of learning and memory impairment, which is an early symptom of dementia.

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Declaration of competing interest

The authors declare that there are no conflicts of interest.

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