Astragaloside IV suppressed hippocampal GABAergic synaptic transmission and enhanced memory through EGR-1 mediated BDNF/TrkB signaling pathway in mice

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Research

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Abstract

**Background:** Astragaloside IV (ASIV) is one of the saponins isolated from *Astragalus membranaceus*, a widely used traditional Chinese medicine and a health product sold all over the world. However, so far, the effect of ASIV on GABAergic synaptic transmission has not been elucidated yet. In the present study, the effect of ASIV on memory and hippocampal GABAergic synaptic transmission was investigated in wild type and early growth response protein 1 (EGR-1) knockout mice.

**Methods:** Behavioral tests including radial-arm maze test and shuttle-box test, liquid chromatography-tandem mass spectrometry, western blotting analysis, quantitative PCR, electrophysiological recording, and electron microscopy were used in this study.

**Results:** ASIV was shown to enhance the learning and memory of mice in behavioral tests, such as radial-arm maze test and shuttle-box test. It significantly reduced the concentration of GABA, the expression of glutamate decarboxylase 2 (GAD65) as well as the ratio of inhibitory synapses in mouse hippocampus, which was accompanied with a suppression of hippocampal spontaneous inhibitory postsynaptic currents. ASIV administration decreased the expression of EGR-1, brain-derived neurotrophic factor (BDNF) and tyrosine receptor kinase B (TrkB) in the hippocampus. Furthermore, blockage of BDNF/TrkB signaling with K-252a abrogated the effect of ASIV on GAD65 expression. When EGR-1 was knocked out, the promotive effects of ASIV on learning and memory, as well as the inhibitory effects on GABAergic synaptic transmission and GAD65, BDNF and TrkB expression, were abolished. In addition, ASIV was found to down-regulate the pre-existing EGR-1 baseline to better adapt to the learning stimuli.

**Conclusions:** Together, these results demonstrated a novel role of ASIV in enhancing memory and reducing hippocampal GABAergic synaptic transmission through EGR-1 mediated BDNF/TrkB signaling pathway in mice.

Introduction

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in mammals and is widely distributed in the central nervous system (CNS) of mammals [1]. The hippocampus is a brain area essential for learning and memory [2]. Hippocampal GABA receptors and GABAergic synaptic transmission are implicated in memory formation and consolidation [3, 4]. GABA receptors mediate inhibitory nerve transfer, preventing neurons from overexciting in the adult brain [5]. Inhibitory GABAA_R activity regulates GABAergic synaptic plasticity through extracellular signal-regulated kinases and brain-derived neurotrophic factor (BDNF) signaling [6]. BDNF is a member of the neurotrophic family, which is found mainly in the hippocampus, amygdala, cortex, and cerebellum. It is synthesized by neurons and glial cells, and is involved in the survival, differentiation and regeneration of neurons by binding to high affinity receptor, tyrosine receptor kinase B (TrkB) [7, 8].

Early growth response protein 1 (EGR-1), also known as Zif268, Zenk, Krox-24, NGF1-A, TIS8, and Krox-24, belongs to the early growth response (EGR) gene family and is also described as an induced transcription
factor [9]. It plays a vital role in the maintenance of synaptic plasticity [10-12], which is well known to be essential for many cognitive functions. And tremendous evidence demonstrated that EGR-1 actively participates in different memory forms as well as different memory processes, from learning memory consolidation [13, 14] and system consolidation [15, 16] to reconsolidation [17, 18].

Astragaloside IV (ASIV) is a saponin molecule found in Astragalus membranaceus (Fisch.) Bge, an herbal medicine proverbially used in China and a health product widely sold in Europe. Multiple pharmacological activities of ASIV have been disclosed, such as anti-oxidation [19], anti-apoptosis [20], anti-inflammation [21], and immuno-regulation [22, 23]. In addition, ASIV also shows prominent neuroprotective effects in multifarious CNS injuries, including cerebral ischemic-reperfusion injury [24-26], Parkinson's disease [27] and Alzheimer's disease [28, 29]. However, so far, the study of the effect of ASIV on synaptic transmission and memory is lacking. In this study, we firstly reported that ASIV could enhance memory and modulate hippocampal GABAergic synaptic transmission in mice. Furthermore, our study disclosed that the effect of ASIV was achieved probably by decreasing GABAergic inhibition via EGR-1 mediated BDNF/TrkB signaling pathway. The novel findings may contribute to the potential application of ASIV in neurological diseases with impaired memory.

Materials And Methods

Animal and Drug Administration

C57BL/6 mice (Male, 14~18 g, 4~5 weeks old) were provided by the Laboratory Animal Center of Shanghai University of Traditional Chinese Medicine (SHUTCM, Shanghai). All experiments on animals were performed according to the protocol approved by Animal Care and Use Committee of SHUTCM and all animals received humane care (Ethical approval no. SZY201610005). Animals were acclimatized for 2 weeks before the formal behavior experiments. Mice were randomly assigned to ASIV/solvent groups. ASIV group mice were injected intraperitoneally (i.p.) with ASIV (25 mg/kg, 40 % 1,2-Propanediol + 5 % Ethanol + 1 % Polyethylene glycol in phosphate buffer saline solution) for two or five weeks. For BDNF / TrkB signal intervention experiments, mice were extra i.p. administered with 25 μg/kg TrkB inhibitor (K-252a) (cat: HY-N6732/CS-0014825, Invitrogen, Carlsbad, CA, USA) [30] for two weeks. EGR-1 +/- mice maintained on the C57BL/6 background was obtained from the Jackson Laboratory (Bar Harbor, ME, USA). Experiments were carried out on EGR-1 +/- (EGR-1 KO) mice and their EGR-1 +/- littermates that were derived from EGR-1 +/- × EGR-1 +/- breeding. All mice were housed at room temperature (25 ± 1°C) under a 12 h light / 12 h dark cycle, and fed with food and drank with water ad libitum. All behavioral tests were conducted in the light phase between 12:00 a.m. and 18:00 p.m. To avoid experimental deviation, all behavioral observers were blinded to the treatment of the experimental mice. All animal experiments were conducted in accordance with the guidelines for Laboratory Animal care and Use Committee of SHUTCM and National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Behavioral tests
Radial-arm Maze Test (RAMT): The apparatus for radial-arm maze (RAM) (Mobiledatum Inc, Shanghai, China) consists of eight equally spaced arms (length 30 cm, height 15 cm, width 6 cm) radiating from a central maze hub (diameter 12 cm). It was made of opaque plexiglass with manually operated doors leading from the hub of the central maze to each arm. RAMT was conducted in accordance with the procedure described previously, with minor modification [31]. As shown in Fig.1A, the mice were acclimatized to the RAM for 4 days before administrated with ASIV on day 1. Then, they were trained to explore the RAM with four arms placed with 50 mg bait (sugar: regular chow=1:1) from day 2 to day 6. At the time of a week's administration, the mice were placed in the octagonal arena at the beginning of the experiment. The experiment ended when the mice explored the maze for 5 min or visited four baited arms. At the time of two weeks' administration, the test was conducted again. The maze was wiped down with 10 % ethanol between each run to reduce olfactory cues. During these days, mice were given semi-food diet feeding. In these two tests, reference and working memory errors and the time required to complete the tasks were recorded and analyzed.

Shuttle-box test (SBT): SBT was performed following RAMT on day 22. The chamber (Mobiledatum Inc, Shanghai, China) is divided into two equal compartments connected by a gate. A light is switched on alternately in the two compartments for conditioned stimulus. The test was conducted following a procedure described by Cheng et al [32]. Briefly, each mouse was allowed to adapt to the chambers for 4 min before the formal test. At the beginning of the experiment, the mice were placed in a compartment of the shuttle-box and back to the gate. Each mouse was given 30 consecutive trials at intervals of 20 s (light 5 s; interval, 3 s; 0.2 mA electric shock, 5 s; interval, 7 s) for five consecutive days. The active avoidance response was recorded automatically if the mouse moved to another compartment during conditioned stimulus. At the day 35 of continuous ASIV administration, all animals received the same experimental protocol to assess memory consolidation.

Neurotransmitter analysis

After all behavioral tests were completed, the mice were sacrificed after anesthetized with 1.5 % pentobarbitalum. The hippocampus of mice was dissected on ice, frozen rapidly in liquid nitrogen and stored at - 80 ºC until analysis. The content of GABA in mouse hippocampus was determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) method as reported previously [33].

Western blotting analysis

The hippocampus of mice was homogenized, sectioned, and analyzed by Western blotting. Totally thirty micrograms of proteins from each sample were separated by 10 % SDS-PAGE electrophoresis. After transferred to polyvinylidene fluoride membrane (GE Healthcare, Little Chalfont, Buckinghamshire, UK), the proteins were incubated with respective primary antibodies against glutamate decarboxylase 2 (GAD65) (cat: ab26113), EGR-1 (cat: ab194357), BDNF (cat: ab88901), TrkB (cat: ab101696) and GAPDH (cat: ab181602) provided by Abcam (Cambridge, MA, USA). Sequentially, the proteins were further incubated with secondary antibodies as described previously [19]. The protein bands were visualized by
ECL reagent kit (cat: RPN2232, GE Healthcare) and quantified with Gel-Pro analyzer software (Media Cybernetics, L.P., MD, Rockville, USA).

Quantitative PCR

Total RNAs from mouse hippocampus were extracted using Trizol reagent (Life Technologies, Waltham, MA, USA). Afterwards, the RNA samples were reverse-transcribed into cDNA with Revert Aid First Strand cDNA Synthesis kit (Fermentas, Waltham, MA, USA). The synthesized cDNA was used as templates for quantitative real-time PCR with Universal SYBR Green/ROX qPCR Master Mix (Roche, Basel, Switzerland) on ABI ViiA7 quantitative real-time PCR platform (Applied Biosystems, Foster City, CA, USA). Quantification of the target gene was conducted by comparative Ct method with GAPDH as a reference gene. Primers used were listed as following: for EGR-1, 5'- AGCAGCGCCTTCAATCCTCA- 3', 5'- TCTCCACCATCGCTTCTCA - 3'; for GAPDH, 5'- ATGTGTCCGTCGTGGATCTGA - 3', 5'- ATGCCTGCTTCACCACCTTCT - 3'.

Electrophysiological recording

Patch clamp recordings in brain slices: The measurements of GABAergic inhibitory postsynaptic spontaneous currents (sIPSCs) were obtained from pyramidal cells by whole cell recording patch clamp in CA1 of hippocampal slices. After anesthesia induced by sodium pentobarbital (i.p. 50 mg/kg), the mice were perfused transcardially with ice-cold high-sucrose artificial cerebrospinal fluid (ACSF) saturated with 95% O₂/5% CO₂ consisting of (in mM): 212 sucrose, 3 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 10 D-glucose and 7 MgCl₂. The brain was quickly removed from skull and coronal 300 μm brain slices containing the CA1 were prepared using a vibroslicer (VT 1200 S, Leica, Germany). Slices were then transferred into 95% O₂ and 5% CO₂ oxygenated normal ACSF (composition in mM: 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 1.3 MgSO₄, 26 NaHCO₃, 2 CaCl₂, and 20 D-glucose). After a recovery at 35°C for 1 h, slices were maintained at room temperature before further recording. Whole-cell patch clamp recordings were performed on CA1 neurons with borosilicate glass pipettes (3–5 MΩ) filled with an internal solution (composition in mM: 110 CsCl, 10 NaCl, 5 MgCl₂, 0.6 EGTA, 40 HEPES, 2 Mg-ATP and 0.2 Na₃-GTP). During recording sessions, CA1 neurons were visualized with an Olympus BX51WI microscope (Olympus, Tokyo, Japan). Patch clamp recordings were acquired with an Axopatch-700B amplifier (Axon Instruments, Foster City, CA) and the signals were fed into a computer through a Digidata-1440A interface (Axon Instruments) for data capture and analysis (pClamp 10.4, Axon Instruments). Neurons were held at a membrane potential of -70 mV and characterized by injection of rectangular voltage pulse (5 mV, 50 ms) to monitor the whole-cell membrane capacitance, membrane resistance and series resistance. The sIPSCs were recorded in the presence of D-APV (50 µM) and NBQX (20 µM; Tocris Bioscience). Neurons were excluded from the experiments if the series resistance was not stable or exceeded 20 MΩ.

Input-output (I/O) function in viva: I/O curve reflects the relationship between the amplitude of fEPSP and the intensity of stimulation, which is used to evaluate the synaptic potency [34]. After continuous i.p. injection of ASIV (25 mg/kg) for two weeks from 6 weeks old, the mice were anesthetized with 25%
urathane (1 mL/100 g) and fixed on the stereotaxic device (Narishige Ins, Tokyo, Japan). The location parameters of granular cell layer in DG area were 2.0 mm after anterior fontanel, 1.4 mm near midline, 1.5 mm deep under dura mater; and 3.8 mm after anterior fontanel, 3.0 mm near midline, 1.5 mm deep under dura mater. In the front fontanel, recording electrode and stimulating electrode (Nihon Kohden, Tokyo, Japan) were almost in a straight line. According to the above positioning parameters, drill holes in the skull with a skull drill (Minimo, Tokyo, Japan), then insert recording electrodes into the granular cell layer of DG area and insert stimulating electrodes into performant pathway. The reference electrodes were clamped on the scalp. With 0.1 mA (0.1-2.0 mA) as a step, the stimulation current was changed systematically to produce I/O curve. Three responses at each current level were averaged, and the population spike (PS) amplitude was examined. The signals were transferred through the amplifier (Axon, CA, USA), and filtered by PCLab202 software (Microsignalstar, Beijing, China).

**Electron Microscopy**

After deeply anesthetized with 20 % urethane, mice were perfused with 2.5 % glutaraldehyde in 0.1 M phosphate (pH 7.4) transcardially. The brains were dissected and post-fixed in 2.5 % glutaraldehyde for 24 h. Sequentially, they were incubated with 2 % osmium tetroxide, dehydrated with series acetone and embedded with epoxy resin. The 60 nm-thick ultrathin sections were obtained on an ultra-microtome (ultracut UCT, Leica, Wetzlar, Germany) and observed by transmission electron microscope (Tecnai G2 Spirit BioTWIN, Hongkong, China).

**Statistical analysis**

All data were analyzed through Graphpad Prism 6 (Graphpad, La Jolla, CA). Differences were evaluated by unpaired student t-test or Mann-Whitney U test or one-way analysis of variance (ANOVA). Error bars represented the standard error of the mean (S.E.M.). *P*-value less than 0.05 was regarded as a significant difference.

**Results**

**ASIV enhanced the memory of mice in both RAMT and SBT**

To explore the effect of ASIV on the memory of mice, the mice were subjected to the RAMT and SBT after ASIV administration. For RAMT, the working memory and reference memory that refer to short-term memory and long-term memory respectively were analyzed. As shown in Fig. 1B and Fig. 1C, ASIV treated mice performed better in the RAMT as their working memory error times (Fig. 1B, for 1-week, \(U = 62.00, P = 0.0782\); for 2-week, \(U = 54.50, P = 0.0358\)) and reference memory error times were reduced significantly (Fig. 1C, for 1-week, \(U = 46.00, P = 0.1276\); for 2-week, \(U = 33.00, P = 0.0174\)), compared with the control mice. Moreover, ASIV markedly increased the active avoidance times of mice in SBT (Fig. 1D, \(U = 30.50, P = 0.0012\)). These results showed that ASIV could enhance the memory of mice.

**ASIV enervated hippocampal GABAergic system in mice**
To determine the effect of ASIV on the GABAergic system, the concentration of GABA and the expression of GAD65 in hippocampi of mice were examined, respectively. As shown in Fig. 2A, hippocampal GABA level in ASIV treated mice was reduced when compared with that of the control mice (for 2-week, \( t_{(20)} = 5.003, P = 0.0000 \); for 5-week, \( t_{(25)} = 2.507, P = 0.0190 \)). Accordingly, the expression of GAD65 in hippocampus of ASIV treated mice was significantly lower than that of the control mice (Fig. 2B and C, for 2-week, \( t_{(4)} = 3.349, P = 0.0286 \); for 5-week, \( t_{(4)} = 2.779, P = 0.0499 \)). To further investigate if ASIV could affect the strength of inhibitory synaptic transmission in hippocampus, ultrathin sections obtained from blocks (1 mm × 1 mm × 1 mm) of CA3 region were subjected to electron microscopy observation. According to the reports, there are two major morphologic types of synapses, i.e. asymmetric and symmetric synapses [35, 36]. Excitatory synapses are asymmetrical synapses with significant postsynaptic density, while inhibitory synapses are symmetrical synapses with thinner postsynaptic density. And there is also another type of synapse with oblique synaptic cleft and associated membrane density that is considered to be uncharacterized synapses [37]. As displayed in Fig. 2D, excitatory and inhibitory synapses could be clearly identified in the electron microscopy image. ASIV treatment for 2 weeks and 5 weeks decreased the ratio of inhibitory synapse remarkably in CA3 region (Fig. 2E, for 2-week, \( t_{(5)} = 2.632, P = 0.0464 \); for 5-week, \( t_{(5)} = 2.769, P = 0.0394 \)). Since GABAergic system mainly participates in the synaptic inhibition, we next investigated the impact of ASIV on the GABAergic synaptic transmission on CA1 pyramidal neurons by whole-cell patch clamp recording in the presence of d-APV and NBQX, the antagonists for AMPA and NMDA receptors, respectively. As shown in Fig. 3A-B, ASIV treated mice showed reduced amplitude (\( t_{(15)} = 2.762, P = 0.015 \)) and frequency (\( t_{(15)} = 3.304, P = 0.005 \)) of the GABAergic spontaneous inhibitory postsynaptic currents (sIPSCs) compared with the control mice. Therefore, these results suggested that ASIV could enervate hippocampal GABAergic neurotransmission.

**ASIV suppressed the expression of GAD65 through regulating the BDNF/TrkB signaling pathway**

To explore the possible action mechanism of ASIV, the expressions of proteins, such as BDNF and TrkB that actively involved in the modulation of memory were detected. In our experiments, the expression of BDNF as well as its receptor, TrkB, was also decreased in mouse hippocampus after treated with ASIV for 2 weeks and 5 weeks (Fig.5A and B, for 2-week BDNF, \( t_{(4)} = 4.294, P = 0.0127 \); for 5-week BDNF, \( t_{(4)} = 2.794, P = 0.0491 \); Fig.5C and D, for 2-week TrkB \( t_{(4)} = 3.512, P = 0.0249 \); for 5-week TrkB, \( t_{(4)} = 3.203, P = 0.0328 \)). Treatment with K-252a reduced the inhibitory effect of ASIV on GAD65 expression (Fig.4 E and F, \( F_{(3, 8)} = 8.219, \) for Control vs ASIV, \( P = 0.0022 \); for K-252a vs K-252a + ASIV, \( P = 0.2465 \)). The results suggested that ASIV exerted its function on GAD65 through regulating the BDNF/TrkB signaling pathway.

**ASIV decreased the expressions of EGR-1 in mouse hippocampus**

To explore the possible action mechanism of ASIV, the expression of EGR-1, which actively involves in the modulation of memory, was detected. In Fig. 4A-C, ASIV treatment for 2 weeks and 5 weeks significantly suppressed the expressions of hippocampal EGR-1 at both mRNA (for 2-week, \( t_{(10)} = 2.730, P = 0.0212 \);
for 5-week, \( t(5) = 3.260, P = 0.0225 \) and protein levels (for 2-week, \( t(4) = 2.817, P = 0.0480 \); for 5-week, \( t(4) = 3.587, P = 0.0247 \). The results suggested that ASIV might enhance the memory of mice through EGR-1.

### EGR-1 KO abrogated the memory beneficial effect of ASIV on mice in both RAMT and SBT

To reveal the role of EGR-1 in the memory beneficial effect of ASIV on mice, EGR-1 KO mice were treated with ASIV and subjected to the RAMT and SBT using the same timeline as illustrated in Fig. 1A. As shown in Fig. 6B and C, ASIV treatment did not reduce the working memory (for 1-week, \( U = 19.00, P = 0.2228 \); for 2-week, \( U = 43.00, P = 0.6158 \)) and reference memory errors (for 1-week, \( U = 23.00, P = 0.4815 \); for 2-week, \( U = 35.00, P = 0.2775 \)) of EGR-1 KO mice in RAMT. And ASIV treatment also did not change the active avoidance times of EGR-1 KO mice in SBT (Fig. 6D, \( U = 44.00, P = 0.6684 \)). These results confirmed that EGR-1 played an indispensable role in the memory beneficial effect of ASIV.

### EGR-1 KO abolished the inhibition of ASIV on GABAergic synaptic transmission in mice

To further corroborate the role of EGR-1 in hippocampal GABAergic system of ASIV treated mice, the sIPSCs of EGR-1 KO mice treated with ASIV for 2 weeks were recorded. As shown in Fig. 7A-B, ASIV treatment failed to alter the amplitude (\( t(17) = 0.337, P = 0.740 \)) and frequency (\( t(17) = 1.362, P = 0.191 \)) of sIPSCs. These results demonstrated that EGR-1 KO abolished the inhibition of ASIV on hippocampal GABAergic synaptic transmission.

### EGR-1 KO blocked the inhibition of ASIV on GAD65, BDNF and TrkB in mouse hippocampus

To further validation of EGR-1 in the inhibitory effect of ASIV on GABAergic synaptic transmission, the hippocampal protein expression of GAD65, BDNF and TrkB were examined in EGR-1 KO mice treated with ASIV for 5 weeks. As shown in Fig. 8, EGR-1 KO decreased GAD65 expression (Fig.8A and B, \( t(4) = 5.517, P = 0.0067 \)) but did not affect expression of BDNF (Fig.8E and F, \( t(4) = 0.3035, P = 0.7766 \)) and TrkB (Fig.8 E and F, \( t(4) = 0.6084, P = 0.5758 \)) in hippocampus. ASIV treatment did not change the expression levels of GAD65 (Fig. 8C and D, \( t(6) = 0.6829, P = 0.5202 \), BDNF (Fig. 8G and H, \( t(6) = 1.331, P = 0.2317 \)) and TrkB (Fig. 8G and H, \( t(6) = 0.2750, P = 0.7958 \)) in hippocampus of EGR-1 KO mice, suggesting that ASIV modulated GABAergic synaptic transmission through EGR-1.

### ASIV increased basic synaptic transmission and enhanced mRNA expression of EGR-1 in response to external stimuli in mice

To test the effects of ASIV on basic synaptic transmission in the CA1 region, the I/O function of ASIV treated mice was evaluated. As illustrated in Fig. 9A, the PS amplitude of ASIV-treated mice showed the increasing tendency, especially when the stimulus current was at 1.2 mA, 1.3 mA, 1.5 mA and 1.6 mA \((P < 0.1)\), suggesting that ASIV might enhance hippocampal synaptic transmission in mice.

To further understand the physiological significance of the inhibitory effect of ASIV on EGR-1, the mice-treated with ASIV for 2 weeks were subjected to a single-trial SBT together with the control mice.
Consequently, the hippocampal mRNA expression of EGR-1 was analyzed. In Fig. 9B, mRNA expression level of EGR-1 in both control and ASIV-treated mice was elevated. However, the increase amplitude of the mRNA expression level of EGR-1 in ASIV treated mice was greater than that in the vehicle-treated control mice ($t_{(11)} = 2.327, P = 0.0400$). These results suggested ASIV could increase the response of EGR-1 to external stimuli.

**Discussion**

Memory formation is a complex process. This is partly due to the information is assumed to be transiently stored in short-term memory while it is considered to integrate long-term memory permanently [38]. At present, a lot of knowledge about memory comes from the study of memory impairment, especially amnesia [39-41]. Other neurological diseases, such as Alzheimer's disease [42], Parkinson's disease [43, 44] and Korsakoff's syndrome [45] also accompany with impaired memory. In addition, a common temporary failure of memory retrieval is often found under some specific situations like emotion [46], aging [47], sleep [48] or other unrecognized factors [49, 50]. Therefore, drugs facilitating information process, consolidation, store or retrieval may contribute to the improvement of memory deficits under the aforementioned conditions. In our study, ASIV was found to facilitate the memory formation of mice as it promoted reference memory (long-term memory) and working memory (short-term memory) in RAMT, as well as procedural memory (long-term memory) in SBT, suggesting that ASIV might benefit memory loss occurred in neurological disorders.

Meanwhile, ASIV decreased the hippocampal GABA level as well as the expression of GAD65 with the reduced inhibitory synapse ratio. In addition, we observed a clear decrease in the spontaneous GABAergic synaptic activity of ASIV group. This observation strongly supports the idea that the decreased inhibitory synaptic transmission in the hippocampus, as shown by the reduced peak amplitudes and frequency of sIPSCs, is the most likely underlying mechanism for the enhanced performance of ASIV-treated mice in the learning and memory tasks.

In recent years, the most remarkable conclusion is that EGR-1 not only plays a key role in different forms of memory, but also is important in different processes operating on memory, from post learning memory consolidation and system consolidation to reconsolidation, updating, and extinction [51]. In the present study, EGR-1 was found to be decreased in mouse hippocampus after ASIV administration. Apart from that, our Figure 9 suggested that a single-trail of SBT could boost the expression of EGR-1 rapidly, which also implicated that ASIV could decrease the basal level of EGR-1 and in turn increases its activity-dependent capacity in response to external stimuli.

Previous studies have shown that expression of GAD65 is likely modulated by BDNF and TrkB at mRNA or protein level [52-54]. It can be imagined that the activity-dependent release of BDNF may affect the inhibition circuit by triggering GAD65 transcription, thereby increasing the synaptic level of GABA, thereby regulating the activity of neural networks [55]. Here we described that BDNF/TrkB was decreased together with the inhibition of GABAergic transmission of ASIV and this effect was blocked by TrkB inhibitor and
EGR-1 knockout. These results provide evidence that the GABAergic inhibitory effect of ASIV is through BDNF/TrkB signaling pathway mediated by EGR-1.

A limitation of this study is lacking the results of behavioral and electrophysiological results from littermates of EGR-1 KO mice, however, previous studies from other groups have shown a satisfactory degree of comparison between EGR-1 KO mice and their littermates in synaptic plasticity and memory [56]. In addition, our further experiments will determine the role of hippocampal EGR-1 in brain plasticity.

Taken together, ASIV enhanced the memory and modulate hippocampal GABAergic synaptic transmission of mice, which was achieved through decreasing hippocampal GABAergic inhibition through BDNF/TrkB signaling pathway mediated EGR-1. Our current work would also benefit for the clinical application of ASIV in the prevention and treatment of memory-impaired diseases.

**Declarations**

**Ethics approval and consent to participate**

All experiments on animals were performed according to the protocol approved by Animal Care and Use Committee of SHUTCM and all animals received humane care (Ethical approval no. SZY201610005). The animal-related procedures we studied are in accordance with the 1975 Declaration of Helsinki on animal rights (as revised in 2008).

**Consent for publication**

Not applicable.

**Availability of data and material**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Competing of interests**

The authors declare that they have no competing interests.

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**Authors' contributions**

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