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Cinnamic acid rescues behavioral deficits in a mouse model of traumatic brain injury by targeting miR-455-3p/HDAC2

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ABSTRACT

Aims: Traumatic brain injury (TBI) not only induces physiological disabilities but also leads to cognitive impairment. However, no effective therapeutic approach for TBI-related memory decline exists. In this study, we treated TBI mice with cinnamic acid (CNA) to detect whether CNA is able to rescue the memory deficits induced by TBI and to explore the potential mechanisms.

Main methods: Mice were divided into the following groups: the sham group, the TBI group, the TBI + CNA group and the CNA group. Basic physiological parameters, neurological severity score and brain water content were analyzed. The Morris water maze and inhibitory avoidance step-down task were used to determine learning and memory. Golgi staining was used to measure alterations in dendritic spines. Western blot analysis and a commercial kit were used to detect the content and activity of HDAC2. qPCR was used to detect the relative level of miR-455.

Key findings: CNA did not affect physiological function but effectively restored neurological function and brain edema. CNA alleviated the memory impairments induced by TBI in both the Morris water maze and step-down task. CNA also recovered abnormalities in the synapses of TBI mice by suppressing the activity of HDAC2. Furthermore, CNA did not alter HDAC mRNA because it promoted the expression of miR-455-3p, a miRNA that regulates HDAC2 at the posttranscriptional level.

Significance: The application of CNA effectively treats TBI-induced memory deficits by increasing miR-455-3p and by inhibiting HDAC2.

1. Introduction

Traumatic brain injury (TBI) is a critical health problem that affects over 50 million people worldwide per year [1]. TBI is usually caused by a violent blow or jolt to the head or body, including those that occur in road accidents, falls, firearms, and sports injuries [2]. Moreover, TBI results in wide-ranging physical and psychological symptoms that can produce lifelong deficits to varying degrees. Some signs or symptoms, such as brain edema and emotional changes, appear immediately after the traumatic event, while others, such as cognitive impairment, appear days or weeks later [3].

Cognitive impairment is one of the most prominent symptoms with long-term effects after TBI [4]. Numerous cognitive impairments have been found in TBI patients, and the disruption of learning and memory, attention, executive function and processing speed are the most common impairments [5]. Many studies have suggested that a history of TBI is positively correlated with an elevated risk of developing cognitive deficits [5–9]. Additionally, mild TBI was also recognized as an independent risk factor for the development of dementia [10]. By using autopsy-confirmed Alzheimer's disease (AD) cases, a history of TBI with reported loss of consciousness was found to be a risk factor for earlier AD onset [11]. Unlike the prevailing view that only a small portion of individuals who suffer a one-time mild TBI have long-term cognitive impairment, a recent comprehensive epidemiological study revealed that over 50% of individuals who experience a mild TBI have long-term consequences [12]. Fortunately, the cognitive impairment generally occur within many hours to weeks after the TBI incident and involve consequent pathological changes [13]. Thus, treating TBI patients with pharmacological, nutritional or genetic intervention in the proper window is a promising therapeutic approach to rescue cognitive

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deficits.

Cinnamic acid (CNA) is one of the key chemical elements of *Cinnamomum cassia* (L.) J. Presl, or Chinese cinnamon, and has been verified to be neuroprotective, as previously reported [14]. Chinese cinnamon is produced primarily in the southern provinces of Guangxi, Guangdong, and Yunnan. CNA is recognized as one of the 50 fundamental herbs in traditional Chinese medicine. The extraction from the bark of *C. cassia* Blume provided protection against glutamate-induced neuronal death in cultured cerebellar granule cells [15]. CNA is also present in many other plants, such as *Panax ginseng* C.A. Mey. and other fruits. Because CNA possesses anti-inflammatory and antioxidative properties [16], it has been widely used for the treatment of multiple disorders for a long time. In diabetic models, CNA is able to not only regulate the metabolic process of glucose by regulating glycogenesis and gluconeogenesis [17] but also increase insulin secretion in isolated islets [18]. Pretreatment with CNA alleviated myocardial ischemic injury and increased NO levels in the serum to exert cardioprotective effects in myocardial ischemia [19]. Moreover, CNA has also been reported to be neuroprotective. CNA attenuates the cerebral amyloid-beta plaque burden and improves memory in a mouse of AD [20]. The oral administration of CNA promotes the survival of dopaminergic neurons, preserves tyrosine hydroxylase-positive fibers in the striatum and maintains locomotor activity in MPTP-treated mice, a well-known model for Parkinson's disease. Moreover, the application of CNA recovers memory deficits in streptozotocin-induced diabetic mice [21]. Thus, we raised our question of whether CNA administration is also neuroprotective against TBI-induced memory impairment.

The current study was designed to examine the possible therapeutic effect of CNA on memory impairments in TBI mice and to explore the potential molecular mechanisms. We demonstrated that CNA treatment for 30 days could rescue memory deficits in TBI mice by reducing HDAC2 activity to recover synaptic spine maturation. Our results provide a mode of action of CNA against TBI-related injury and open a new option to treat TBI patients with this naturally available molecule.

2. Materials and methods

2.1. Animals and experimental arrangement

All animal care and experimental protocols were performed in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Ethics Committee of the Medical School of Zhengzhou University. A total of 56 male C57/BL6 mice (90–120 days old, 22–28 g) were used in this study. All mice were housed at 22 ± 2 °C and 45–75% relative humidity with a 12-h light–dark cycle (lights on at 7:30 a.m.), and water and food were provided ad libitum for 14 days before the experiment. All experimental procedures were performed as shown in Fig. 1. Forty mice that underwent TBI or sham surgery followed by CNA or vehicle treatment were subjected to behavioral tests and molecular studies (Fig. 1A). An additional 16 mice with the same treatments were only subjected to the examination of brain water content (BWC) (Fig. 1B).

2.2. Physiological and metabolic parameter detection

The physiological condition of all mice, including body weight (both pretreatment and posttreatment), respiratory frequency, heart rate and body temperature, were monitored according to a previous report by our colleague [22].

2.3. TBI and drug treatments

TBI was induced according to an experimental closed head injury (CHI) protocol as previously reported and adapted for use in mice [23]. Briefly, the mice were first anesthetized with isoflurane inhalation, which was confirmed by the lack of both the corneal reflex and toe pinch reflex. Then, to expose the skull, a midline longitudinal incision was performed. The mice were fixed on a stereotaxic instrument (RWD Life Science, Shengzhen, China). A Teflon-tipped cone (2-mm diameter) was placed 1–2 mm lateral to the midline in the mid-coronal plane. The head was held in place, and a 95-g weight was allowed a free-fall on the cone from a preestablished height (a velocity of 3.1 m/s, and an impact duration of 400 ms), resulting in focal injury to the left hemisphere. Sham controls were subjected to surgery without head attack. Trans-cinnamic acid (99% pure) was purchased from Sigma Aldrich (C80857). After TBI, the mice were treated with trans-cinnamic acid (100 mg/kg body weight per day) or vehicle (0.5% methylcellulose) via gavage for 30 consecutive days. The dose of CNA was chosen because of 1) a preliminary study that showed 100 mg/kg is enough to rescue fear memory impairment, while 200 mg/kg did not further induce a rescue effect (Supplementary Fig. 1) and 2) a previous study in AD mouse model [20].

2.4. Neurological function evaluation

Neurological severity score (NSS) was used to evaluate neurological function by using a 10-point scale, as previously reported [24]. Mice were subjected to 10 different tasks, and one point was given if the mice failed at one task. Thus, a score of 0 points indicates unimpaired neurological function, and a score of 10 points indicates the most severe neurological dysfunction.

2.5. Measurement of BWC

As described above, 16 mice that underwent TBI and were subsequently treated with CNA treatment were used to measure BWC by using a previously reported protocol [25]. After CNA or vehicle treatment, the mice were sacrificed. The brains were quickly dissected on ice with the help of a dissection microscope. The wet weight (BW) of brains was first weighed and recorded. Then, the brains were laid in a desiccating oven at 110 °C. Twenty-four hours later, the brains were weighed again, and the dry weight (BD) was recorded. The BWC was then calculated with the following formula: $BWC\ (\%) = (BW - BD) \times 100/BW$.

2.6. Morris water maze test

The Morris water maze was employed to evaluate hippocampal-dependent spatial learning and memory, as previously reported [26]. The water maze was composed of a circular pool with a diameter of 120 cm and a height of 40 cm. Four quadrants were established, and the pool was filled with water at 20–22 °C. The escape platform was painted dark gray. Before the experiment, the mice were placed in the experiment room for 60 min for habituation. All mice underwent a visible platform task by searching for a visible platform 1 cm above the water surface for 3 consecutive days. Then, the platform was hidden under the water, and a 6-day training protocol followed by an additional probe trial at day 9 was performed. In the visible platform test, a flag was placed above the platform, and the water was clear. In the hidden platform task, the platform was submerged 0.5 cm below the opaque water surface and placed in the center of a quadrant (target quadrant). Four training trials were performed every day with a 1 h intertrial interval. For one trial, the mice were trained to find the platform within 1 min and were allowed to stay on the platform for up to 30 s. The performance of every mouse was captured by an overhead camera each day. Escape latency and escape traces to reach the platform were recorded to evaluate spatial learning and memory. On the 9th day, the probe trial was performed after removing the platform. The mice were dropped into the quadrant opposite from the target quadrant and forced to swim for 60 s. The number of times the mouse crossed the platform region, the total time in the target quadrant, the latency for the first

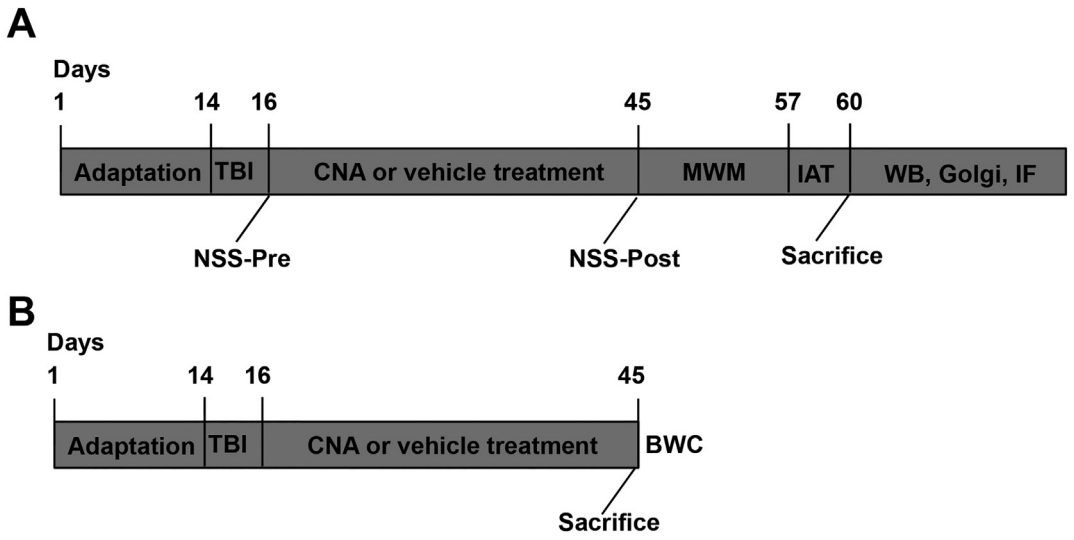

Fig. 1. A diagram of the procedure of our study.
A: A total of 40 mice were treated with TBI or underwent sham surgery after 2 weeks of adaptation. Then, the mice were treated with CNA or vehicle for 30 days and were used for behavioral tests, western blot analysis and Golgi staining.
B: An additional 16 mice were administered the same treatments as above but only subjected to BWC examination after CNA or vehicle treatment.
MWM, Morris water maze; IATm inhibitory avoidance task; WB, western blot; IF, immunofluorescence; BWC, brain water content.

crossing and the speed were recorded.

2.7. Inhibitory avoidance test

The apparatus used for inhibitory avoidance was a Plexiglas box with a size of 21 × 22.5 × 22.5 cm and a floor comprising a series of parallel stainless-steel bars (Φ = 0.4 cm) spaced 0.6 cm apart. An acrylic platform (10 cm^2) was located on the corner. In the training session, the mice were first gently placed on the platform. Immediately after stepping down on the grid, the mice received a 2-s, 0.3-mA, scrambled foot shock [27]. In the test sessions, the foot shock was not delivered, and step-down latency (the first time that the mice stepped down on the grid, maximum 180 s) was used to evaluate memory retention. The tests were independently performed at 1.5 h and 24 h after the training stage to measure short- and long-term memory.

2.8. Western blot analysis

The mice were deeply anesthetized using 1.7% isoflurane (YBH40052005; Abbott Laboratories, Shanghai, China) delivered in 2 L/min oxygen through a securely fitted mask and then sacrificed. The hippocampi were removed on ice and homogenized in RIPA buffer (R0010, Solarbio Life Sciences, Beijing, China). After centrifugation at 10000 ×*g* for half an hour, the supernatant fractions were collected for western blotting. A commercial BCA kit (Pierce, Rockford, IL, USA) was used to measure the concentration of protein in the samples. Then, β-mercaptoethanol (final concentration 10%) and bromophenol blue (final concentration 0.05%) were added to the samples. After boiling in a water bath (95 °C) for 10 min, the samples were separated by 10% SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. Then, 5% fat-free milk was used to block nonspecific reactions for 1 h, and the membrane was incubated overnight at 4 °C with antibodies against HDAC2 (ab32117), DM1A (ab7281), Ac-H4K12 (ab61238), Ac-H4K5 (ab51997) and Ac-tubulin (ab24610) for 24 h. The membranes were then rinsed three times with 1XPBS buffer and incubated with secondary antibodies for another 2 h at room temperature (RT). The bands were visualized by using an Odyssey Infrared Imaging System. Protein bands were quantitatively analyzed by Kodak Digital Science 1D software (Eastman Kodak Company, New Haven, CT, USA) [28].

2.9. HDAC2 activity assay

Hippocampal homogenates were prepared, and nuclear extracts were collected by using a commercial nuclear extraction kit (ab113474, Abcam, Shanghai, China). Then, the HDAC2 activity of the different groups was measured by a commercial kit (Epigentek Group, Brooklyn, NY) according to the manufacturer's instructions [29].

2.10. Golgi staining and spine analysis

Golgi staining was performed according to a previous report [26]. Four mice per group were deeply anesthetized with 1.5% isoflurane and then perfused with physiological saline containing 0.5% sodium nitrite, followed by 4% formaldehyde solution, and then dye solution (5% potassium dichromate, 5% chloral hydrate and 4% formaldehyde). The whole brains were carefully dissociated and immersed in the above dyeing solution for 3 days in darkness with gentle shaking and then transferred to a separate silver solution containing 1% silver nitrate with gentle shaking for another 3 days in darkness. The brain tissues were cut into 30-m slices with a vibrating microtome (Leica, Wetzlar, Germany). Dendritic spines were measured according to a previous protocol based on spine-head width and spine length [30].

2.11. Immunofluorescence

The immunofluorescence experiment was performed according to a previous report [31]. Frozen brain sections (40 μm) were rinsed with PBS (pH 7.4) for 5 min three times and then incubated with 0.5% Triton in PBS for half an hour. After blocking with 0.5% BSA for 1 h, the slices were incubated with anti-synaptophysin (17785-1-AP, Proteintech, Wuhan, China) overnight at 4 °C. After washing with PBS containing 0.2% Triton X-100, the samples were incubated at RT for 1 h with Alexa Fluor 488 goat anti-rabbit IgG (1:500; Molecular Probes, Eugene, OR, USA). After staining with DAPI for 5 min at RT, the slices were photographed using a confocal laser scanning microscope (Fluoview FV1000, OLYMPUS, Tokyo, Japan).

2.12. qPCR for miRNA detection

For miR-455 detection, miRNA in the hippocampus was extracted

by an miRcute miRNA Isolation Kit (TIANGEN, Beijing, China) followed by an miRcute miRNA qPCR detection kit (TIANGEN, Beijing, China) for qRT-PCR. The relative expression level of miR-455 was calculated by the comparative Ct method ($2^{-\Delta\Delta Ct}$) after normalization to U6 [26,32]. All primers were purchased from Ribo Bio (Ribo, Guangzhou, China).

2.13. Statistics

Statistical analyses were performed in SPSS 11.0. Datasets were analyzed by one-way or two-way ANOVA followed by Tukey's post hoc test or Student's *t*-test. Data are represented as the mean ± SD or mean ± SEM. A level of $p < 0.05$ was considered statistically significant.

3. Results

3.1. CNA treatment does not alter physiological parameters

The general conditions of the mice were first evaluated by measuring body weight (both pretreatment and posttreatment), respiratory frequency, heart rate and body temperature before the behavioral test and biochemical/histochemical examinations. The body weights of all the mice increased by approximately 5–10% over the 30 days of treatment. However, we did not find any significant differences among the four groups. Additionally, no difference was found in respiratory frequency, heart rate and body temperature among the four groups (Table 1). These results indicated that CNA treatment does not affect baseline physiological function.

3.2. Effect of CNA on brain damage following TBI

After TBI, the mice displayed severe neurological impairment as indicated by increased NSS (Fig. 2A) and apparent edema as indicated by the increase in BWC to 83.41 ± 2.46% of wet brain weight (Fig. 2B). Treatment with CNA for 30 days dramatically reduced NSS and BWC. Moreover, no difference was found between the sham mice and the mice treated with CNA alone. These data suggested that TBI in our study could induce brain damage and that the application of CNA could rescue TBI-induced brain damage.

3.3. CNA treatment attenuates spatial memory deficits in TBI mice

Then, we tested whether the application of CNA could rescue the memory deficits induced by TBI. In the first 3-day visible platform test (Fig. 3A), we found that the escape latencies of mice with different treatments did not show any statistically significant difference (repeated one-way ANOVA, $p > 0.05$). Thus, neither TBI nor CNA affected the visual ability of the mice.

Then, the mice were subjected to the hidden platform task. As shown in Fig. 3B and C, the TBI mice displayed apparent deficits in learning to find the invisible platform. On day 7, the search strategy of TBI mice was still random (Fig. 3B). Moreover, TBI treatment also induced a longer latency to find the platform from day 3 (Fig. 3C). These data strongly suggest that spatial learning is severely impaired in TBI mice. Simultaneously, treatment with CNA effectively attenuated spatial learning ability, as illustrated by changing to a linear search strategy and shortening the latency from day 3 (Fig. 3B–C).

After the training session, the probe trial task was used to measure the spatial memory retention of mice. We found that TBI mice displayed an obviously shorter time in the target quadrant and fewer platform-crossings than did the sham mice (one-way ANOVA, $p < 0.05$), while CNA treatment increased the total time spent in the target quadrant and the number of times to the mouse crossed the platform zone (Fig. 3C, D). Additionally, CNA also shortened the latency for the first time to reach the platform in TBI mice (Fig. 3F). No difference was found between the CNA-treated mice and the sham mice. We also found that the swimming speed of mice with different treatments was not any different throughout the task (Fig. 3G). These data suggested that CNA treatment could restore the spatial learning and memory retention impairment caused by TBI and that this rescue was not due to altered locomotor activity.

Table 1
CNA does not alter basic physiological functions.

	Sham	TBI	TBI + CNA	CNA
n	10	10	10	10
BW-Pre	26 ± 1.45	25.26 ± 1.57	25.92 ± 1.52	26.46 ± 0.89
BW-Post	27.01 ± 1.28	26.03 ± 1.77	27.05 ± 1.66	27.85 ± 1.08
RF	172.5 ± 13.44	182 ± 9.42	181.8 ± 10.92	177.9 ± 10.14
HR	579.3 ± 15	575.4 ± 17	568.2 ± 14.01	580.2 ± 17.59
Temp	38.08 ± 0.71	38.04 ± 0.45	37.78 ± 0.56	38.22 ± 0.54

All the data are expressed as the mean ± S.E.M. BW-Pre, body weight before the treatment; BW-Post, body weight after the treatment; RF, respiratory frequency; HR, heart rate; Temp, body temperature; TBI, traumatic brain injury; CNA, cinnamic acid.

3.4. CNA treatment rescues TBI-induced fear memory deficits

To further examine whether CNA treatment could rescue memory deficits, we used inhibitory avoidance step-down tasks at 24 h after the Morris water maze. In this task, we evaluate short-term fear memory and long-term fear memory at 1.5 h and 24 h after the training session. As expected, the TBI mice showed shorter latency in both the short-term and long-term fear memory tests (Fig. 4). The administration of CNA was able to extend the latency to the normal level observed in the sham mice. No difference was detected between the CNA- and vehicle-treated sham mice. These data further demonstrated the protective effects of CNA treatment on the memory impairments caused by TBI.

3.5. CNA treatment restores the synapse loss in TBI mice

Synapses are the basis of the physiological function of the brain, especially learning and memory [33]. The disruption of presynaptic vesicle release, postsynaptic spine maturation or synaptic plasticity has been implicated in many neurological disorders associated with memory impairments [34]. We then evaluated the dendritic spines in the CA1 neurons of the hippocampus in the four groups. We found that in the TBI mice, the density of dendritic spines and the percentage of mature type spines (mushroom, stubby) were reduced, while the percentage of immature type spines (Filo) was increased (Fig. 5A–C). These abnormalities were fully restored by treatment with CNA. No difference was found in the mice treated with CNA alone. Thus, we suggest that CNA is able to ameliorate the dendritic spine abnormalities induced by TBI. To further understand the protective effect of CNA on synapse impairments in TBI, we detected the protein level of synaptophysin, a synaptic marker, by western blot analysis and immunofluorescence in the hippocampus. We found that the intensity of synaptophysin in TBI mice was significantly decreased, while supplementation with CNA was able to rescue the synaptophysin level in the hippocampus. These data further confirmed the synaptic protective role of CNA in TBI mice.

3.6. CNA treatment suppresses the activity of HDAC2 by elevating miR-455-3p

Numerous studies have suggested that HDAC2 plays a critical role in mediating dendritic abnormalities and learning/memory impairments in AD [30,35]. We then examined whether the protective effects of CNA are mediated by HDAC2 suppression. We first analyzed the protein level of HDAC2 by western blot analysis and found that HDAC2 protein is dramatically increased in the hippocampus of TBI mice. CNA treatment

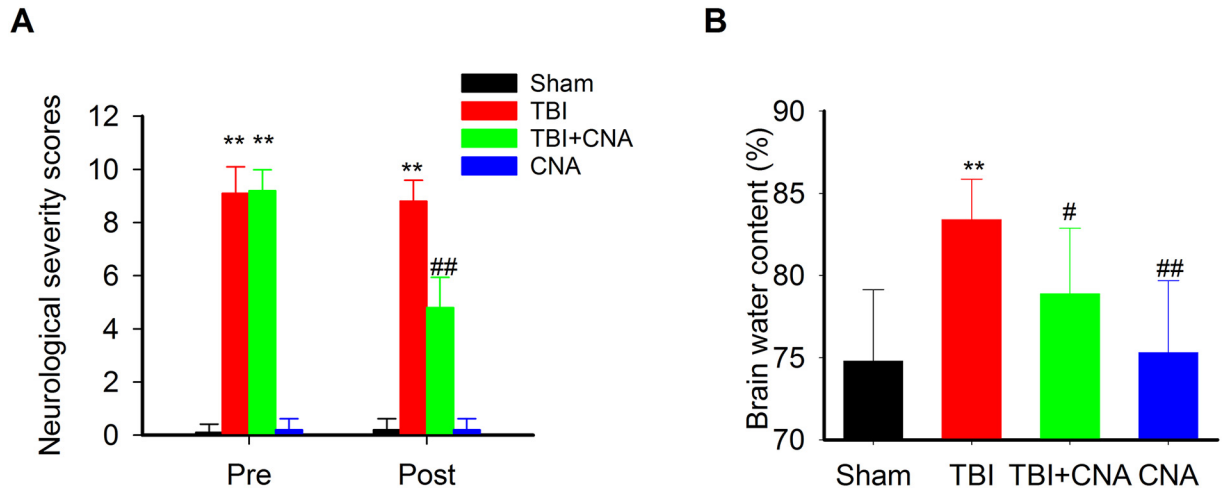

Fig. 2. CNA treatment recovers neurological function and rescues brain edema.
A: NSS was analyzed at both pre- and post-CNA or vehicle treatment. (n = 10).
B: BWC was calculated as described in the Materials and methods section. (n = 4).
**p < 0.01 vs. sham; #p < 0.05, ##p < 0.01 vs. TBI.

effectively suppressed HDAC2 protein expression (Fig. 6A–B). By using a commercial kit, we analyzed the activity (relative protein level) of HDAC2. We found that in TBI mice, the HDAC2 level increased to approximately 6380 ng/mg protein, while CNA treatment decreased the HDAC2 level to 3100 ng/mg protein. Interestingly, CNA-treated sham mice also showed an apparent reduction in HDAC2 activity (Fig. 6C). We also analyzed the lysine acetylation of known substrates of HDAC2. We found that TBI indeed resulted in a decrease in the acetylation of H4K12 and H4K5. The application of CNA was able to rescue the acetylation of those substrates (Fig. 6D–E). Consistent with Fig. 6C, the acetylation of H4K12 and H4K5 were also elevated in CNA-treated sham mice compared with vehicle-treated sham mice. Thus, CNA is able to inhibit the activity of HDAC2 and alter the HDAC2 protein, and suppression of HDAC2 may be involved in the neuroprotective effect of CNA on TBI.

Finally, we wanted to know why CNA treatment was able to suppress HDAC2 activity. We first analyzed the mRNA level of HDAC2 but did not find any difference among the groups (Fig. 7A). This result indicated that transcriptional regulation might not be involved in the HDAC2 suppression induced by CNA. miRNAs have been reported to play important roles in posttranscriptional regulation. We then tested whether some miRNAs participate in HDAC2 inhibition. By using Targetscan, we found that only miR-455-3p could bind to the 3′UTR of HDAC2 and that the binding site is highly conserved in mammals (Fig. 7B). We then examined the levels of miR-455-3p in the hippocampus of different groups and found that it was decreased in the TBI mice. CNA treatment increased the level of miR-455-3p in TBI mice but not in control mice (Fig. 7C). Thus, CNA could inhibit HDAC2 levels by enhancing the expression of miR-455-3p.

4. Discussion

In this study, we demonstrated that 30 days of CNA treatment after TBI is able to rescue the neurological dysfunction, brain edema, learning/memory impairments, and dendritic spine abnormalities caused by TBI. We also reported that CNA could suppress HDAC2 activity, which is activated in TBI.

The neuroprotective effect of CNA has been widely recognized in many previous reports, but the underlying molecular mechanisms in addition to its antioxidant and anti-inflammatory properties are still unknown. In a recent study, CNA application remarkably reduced the Aβ plaque burden in the cortex and improved memory in AD (5XFAD) mice. CNA specifically binds to PPARα in the ligand-binding domain and then activates PPARα, which in turn results in the transcriptional activation of TFEB and stimulates the biogenesis of lysosomes [20]. In addition, the activation of PPARα is also required for CNA to protect dopaminergic neurons [36]. In a previous report, CNA was able to upregulate the acetylation of histone proteins in a dose-dependent manner both in vitro and in vivo by acting as an HDAC inhibitor [37]. Based on this finding, a series of derivatives from CNA were designed as HDAC inhibitors with antitumor activity [38,39]. In another study, the inhibition of HDAC2 leads to the activation of PPARα by a grape seed procyanidin extract [40]. In our study, we demonstrated that CNA treatment significantly reduced the activity of HDAC2 in the hippocampus. This finding is consistent with the above studies. An interesting finding is that CNA treatment alone does not alter HDAC2 expression but decreases HDAC2 activity; however, this decrease in HDAC2 activity did not improve learning and memory ability. This finding seems to be contradictory to the previous study [35]. We proposed that another compensatory mechanism may mediate this discrepancy.

As an important epigenetic regulator, HDAC2 plays an important role in the regulation of transcriptional events, developmental and cell cycling events, and normal brain function, including learning and memory. HDAC2, but not HDAC1, was identified as a negative factor of associative and spatial memory because the overexpression of only HDAC2 in mice led to impaired memory performance [13]. The critical role of HDAC2 in the regulation of memory can also be observed in many neurological disorders with memory impairments. For example, elevated HDAC2 levels can be found in both AD patient brains and in multiple lines of AD mouse models [41,42]. In AD mouse models, the application of HDAC inhibitors or knockdown by shRNA could effectively restore impaired cognitive function [41,42]. Furthermore, HDAC2 activation also participates in the formation of senile plaques and neurofibrillary tangles, two important pathological hallmarks of AD [30,43]. In this study, we found that CNA treatment significantly rescued memory impairments in TBI mice and suppressed HDAC2 activity. Given the important role of HDAC2 in learning and memory, we concluded that HDAC2 inhibition might mediate the neuroprotective effect of CNA on TBI. Indeed, numerous studies have verified the protective roles of HDAC inhibitors in TBI-induced injury [44]. For example, the HDAC inhibitor ITF2357 was shown to decrease neuronal degeneration and lesion volume in an experimental TBI mouse model [45]. Another inhibitor, LB-205, was able to preserve NGF-mediated cell survival in TBI rats [46]. Scriptaid could prevent white matter injury by modulating microglia/macrophage polarization in TBI [47]. Interestingly, we found that the mRNA level of HDAC2 was not changed

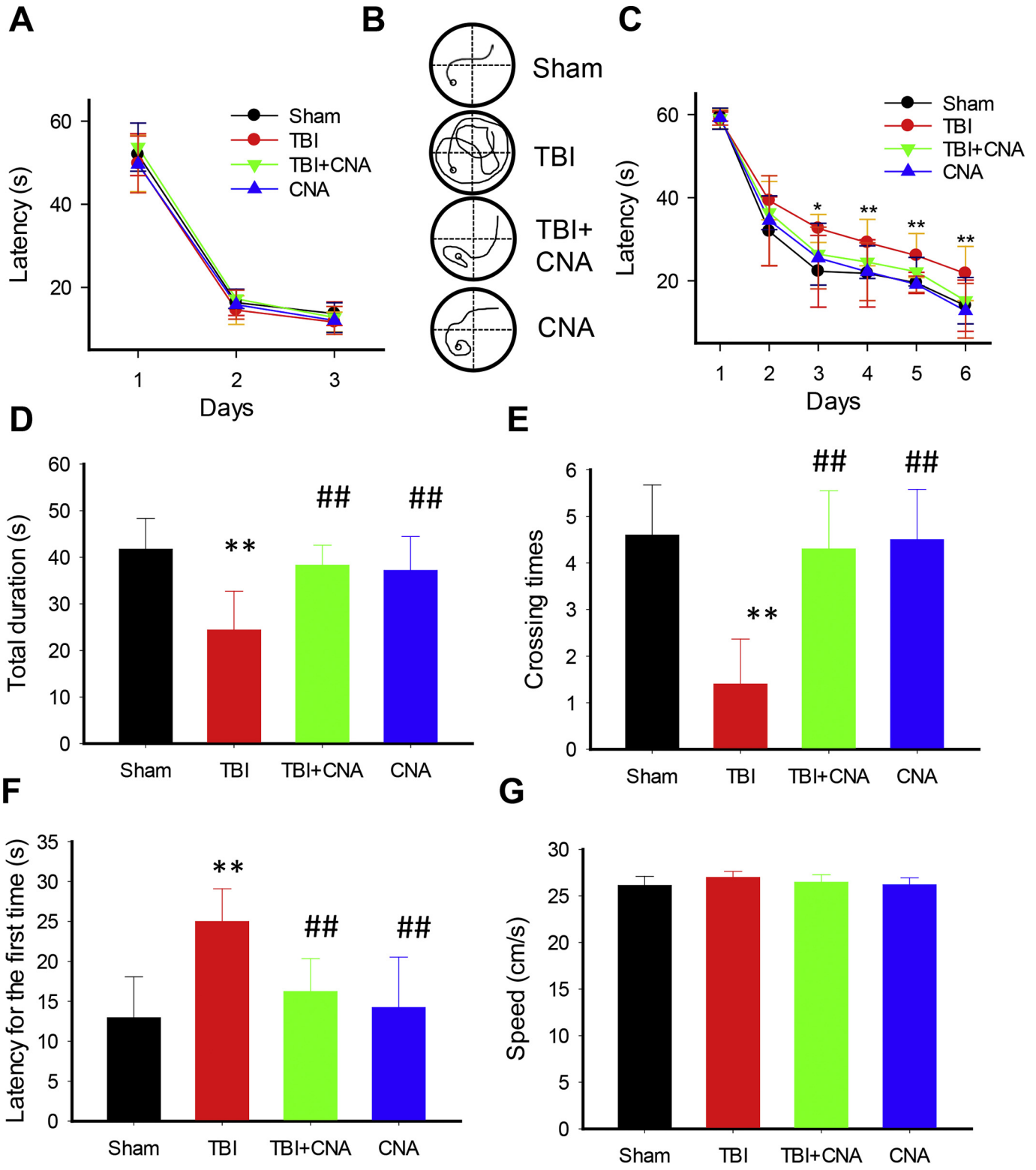

Fig. 3. CNA treatment alleviates spatial learning and memory impairments in TBI mice.
A. In the visible platform test, the latency over three days is recorded. All the groups showed comparable latency.
B–C. In the invisible platform test, the tracks on day 7 (B) and all the latencies from day 1–6 (C) were recorded. *p < 0.05, **p < 0.01 vs. sham.
D–F. In the probe trial, the total time spend in the target quadrant (D), the number of platform region crossings (E), and the latency for the first crossing time (F) were recorded.
G: The swimming speed of all the mice.
All values are expressed as the mean ± SEM (n = 10). **p < 0.01 vs. the sham group; ##p < 0.01 vs. the TBI group.

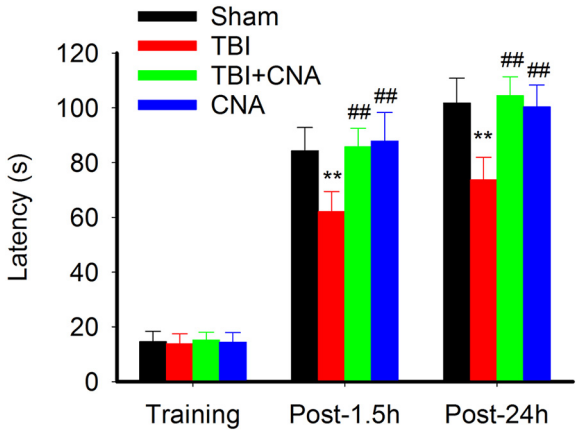

Fig. 4. CNA treatment ameliorates fear memory impairments in TBI mice.
The inhibitory avoidance step-down task was used to evaluate fear memory. During the training session, all the mice displayed similar freezing times. The short-term fear memory and long-term fear memory tests were carried out at 1.5 h and 24 h, respectively. The step-down latency is expressed as the mean ± SEM (n = 10). **p < 0.01 vs. the sham group; ##p < 0.01 vs. the TBI group.

in different groups, indicating the involvement of posttranscriptional regulation. We also identified that CNA treatment could elevate the level of miR-455-3p, a miRNA that regulates HDAC2 by binding to the 3′UTR region of HDAC2. As mir-455-3p has been suggested to be a potential biomarker for AD [48], our current study might also imply a role for it in the memory decline that occurs in TBI.

We also found that CNA treatment recovered dendritic spine abnormalities in TBI mice. Dendritic spines are elaborate structural units that act as postsynaptic compartments with a specific morphology that allows for rapid and local neuronal signal transmission [49]. The morphology of spines is highly dynamic, and spines are usually classified into the following four types: filopodia, thin, mushroom and stubby [50]. Mushroom and stubby spines are thought to be tightly correlated with memory performance [51]. CNA treatment significantly increases the percentage of mushroom and stubby spines but reduces the percentage of filopodia spines. This is consistent with the protective role of CNA in memory impairment. HDAC2 inhibition plays an important role in mediating the increase in the dendritic spine density of CA1 pyramidal neurons induced by BDNF. Our study demonstrated that CNA, as an HDAC2 inhibitor, could rescue TBI-induced memory impairment and dendritic abnormalities, which can be considered a potential clinical drug for TBI.

Overall, CNA treatment for 30 days can rescue TBI-induced memory deficits and dendritic spine impairments by inhibiting HDAC2 activity.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.116819.

Author contribution

S.G., Y.Z., and Z.Z. researched data, contributed to the discussion, and wrote the manuscript. S.G., G.Z., and X.Z. designed the study. S.G., Y.Z., and Z.Z. did the animal study, biochemical study. S.G., G.Z., and X.Z. contributed to the discussion, and reviewed and edited the manuscript.

Data availability

The data used to support the findings of this study are available from the corresponding authors upon request.

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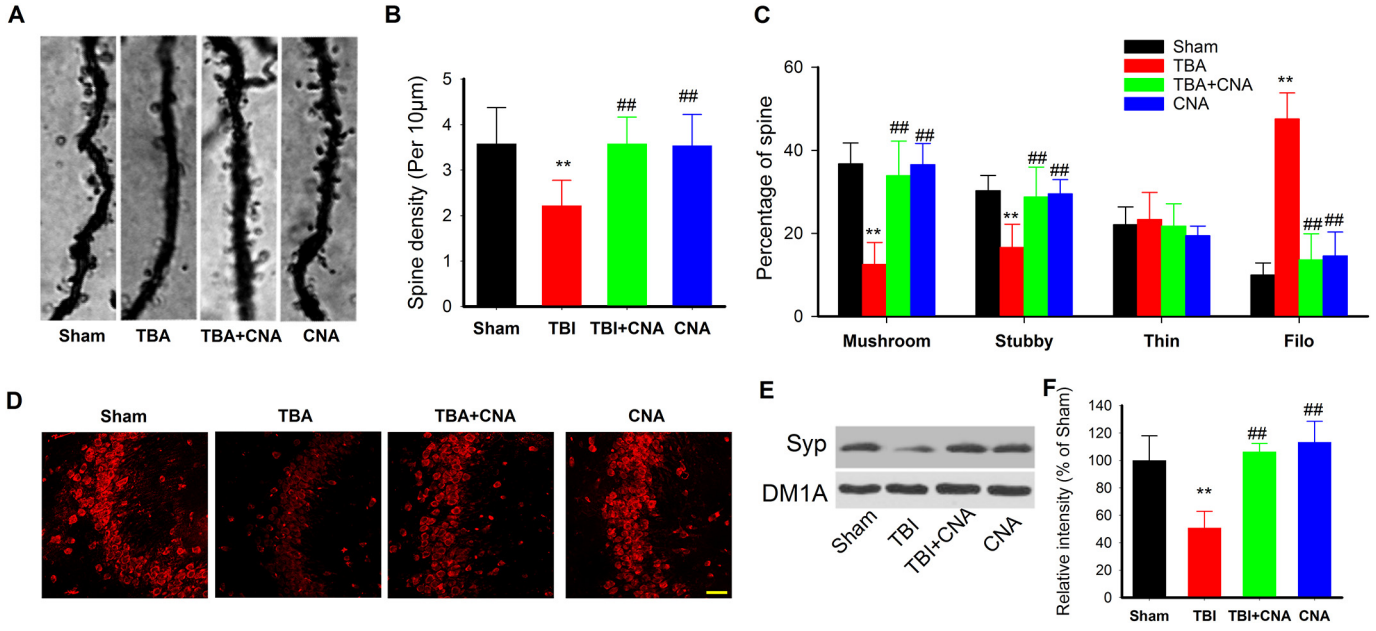

Fig. 5. CNA treatment restores synapse abnormalities.
(A–C) Golgi staining was used to evaluate the change in dendritic spines in the different groups. Representative images of dendritic spines (A), the total density of spines (B) and the percentage of different types of spines (C) were analyzed. N = 30 dendrites from 4 mice per group. **p < 0.01 vs. the sham group; ##p < 0.01 vs. the TBI group.
(D) Immunofluorescence with anti-synaptophysin to detect the expression level of synaptophysin in the CA3 of the hippocampus. Bar = 50 μm.
(E–F) Western blotting was used to detect the expression level of synaptophysin in the hippocampus. Representative images are shown in (E), and the quantified data are shown in (F). **p < 0.01 vs. the sham group; ##p < 0.01 vs. the TBI group.

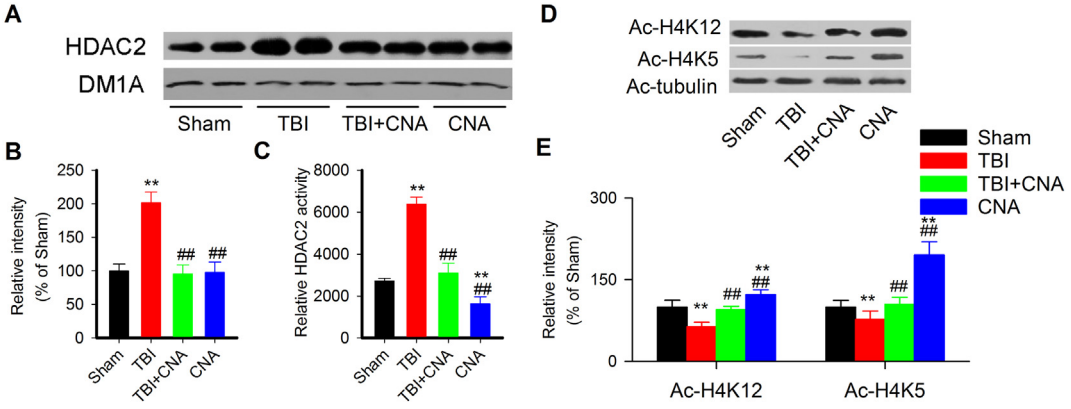

Fig. 6. CNA treatment inhibits the activity of HDAC2 in TBI mice.
(A–B) Representative blots of HDAC2 (A), and the quantitative analysis (B) was performed by using DM1A as a loading control. N = 6.
(C) The relative activity of HDAC2 was analyzed by a commercial kit. N = 6.
(D–E) Representative blots of the acetylation of H4K12, H4K5, and tubulin (D) and quantitative analysis (E). N = 4.
Data are expressed as the mean ± SEM. **p < 0.01 vs. the sham group; ##p < 0.01 vs. the TBI group.

Fig. 7. CNA treatment elevates the miR-455-3p level in TBI mice.
(A) The relative levels of HDAC2 among different groups. N = 6.
(B) The binding sites of miR-455-3p on HDAC2 are conserved in mammals.
(C) Relative levels of miR-455-3p among the different groups. N = 6.
Data are expressed as the mean ± SEM. **p < 0.01 vs. the sham group; ##p < 0.01 vs. the TBI group.

Declaration of competing interest

The authors have no conflict of interest to report.

References

[1] J.Y. Jiang, G.Y. Gao, J.F. Feng, Q. Mao, L.G. Chen, X.F. Yang, J.F. Liu, Y.H. Wang, B.H. Qiu, X.J. Huang, Traumatic brain injury in China, The Lancet. Neurology 18 (3) (2019) 286–295.

[2] L. Holm, J.D. Cassidy, L.J. Carroll, J. Borg, W.H.O.C.C. Neurotrauma Task Force on Mild Traumatic Brain Injury of the, Summary of the WHO Collaborating Centre for Neurotrauma Task Force on Mild Traumatic Brain Injury, J. Rehabil. Med. 37 (3) (2005) 137–141.

[3] J.R. De Kruijk, A. Twijnstra, P. Leffers, Diagnostic criteria and differential diagnosis of mild traumatic brain injury, Brain Inj. 15 (2) (2001) 99–106.

[4] A.R. Rabinowitz, H.S. Levin, Cognitive sequelae of traumatic brain injury, The Psychiatric Clinics of North America 37 (1) (2014) 1–11.

[5] D.C. Perry, V.E. Sturm, M.J. Peterson, C.F. Pieper, T. Bullock, B.F. Boeve, B.L. Miller, K.M. Guskiewicz, M.S. Berger, J.H. Kramer, K.A. Welsh-Bohmer, Association of traumatic brain injury with subsequent neurological and psychiatric disease: a meta-analysis, J. Neurosurg. 124 (2) (2016) 511–526.

[6] M. Rapoport, U. Wolf, N. Herrmann, A. Kiss, P. Shammi, M. Reis, A. Phillips, A. Feinstein, Traumatic brain injury, Apolipoprotein E-epsilon4, and cognition in older adults: a two-year longitudinal study, The Journal of Neuropsychiatry and Clinical Neurosciences 20 (1) (2008) 68–73.

[7] K. Dams-O'Connor, L.E. Gibbons, J.D. Bowen, S.M. McCurry, E.B. Larson, P.K. Crane, Risk for late-life re-injury, dementia and death among individuals with traumatic brain injury: a population-based study, J. Neurol. Neurosurg. Psychiatry 84 (2) (2013) 177–182.

[8] R.C. Gardner, J.F. Burke, J. Nettiksimmons, A. Kaup, D.E. Barnes, K. Yaffe, Dementia risk after traumatic brain injury vs nonbrain trauma: the role of age and severity, JAMA Neurology 71 (12) (2014) 1490–1497.

[9] O.P. Almeida, G.J. Hankey, B.B. Yeap, J. Golledge, L. Flicker, Prevalence, associated factors, mood and cognitive outcomes of traumatic brain injury in later life: the health in men study (HIMS), International Journal of Geriatric Psychiatry 30 (12) (2015) 1215–1223.

[10] P. Nordstrom, K. Michaelsson, Y. Gustafson, A. Nordstrom, Traumatic brain injury and young onset dementia: a nationwide cohort study, Ann. Neurol. 75 (3) (2014) 374–381.

[11] J. Schaffert, C. LoBue, C.L. White, H.S. Chiang, N. Didehbani, L. Lacritz, H. Rossetti, M. Dieppa, J. Hart, C.M. Cullum, Traumatic brain injury history is associated with an earlier age of dementia onset in autopsy-confirmed Alzheimer's disease, Neuropsychology 32 (4) (2018) 410–416.

[12] K. McInnes, C.L. Friesen, D.E. MacKenzie, D.A. Westwood, S.G. Boe, Mild Traumatic Brain Injury (mTBI) and chronic cognitive impairment: a scoping review, PLoS One 12 (4) (2017) e0174847.

[13] M.W. Greve, B.J. Zink, Pathophysiology of traumatic brain injury, The Mount Sinai Journal of Medicine, New York 76 (2) (2009) 97–104.

[14] X. Liu, J. Fu, X.J. Yao, J. Yang, L. Liu, T.G. Xie, P.C. Jiang, Z.H. Jiang, G.Y. Zhu, Phenolic constituents isolated from the twigs of Cinnamomum cassia and their potential neuroprotective effects, J. Nat. Prod. 81 (6) (2018) 1333–1342.

[15] Y. Shimada, H. Goto, T. Kogure, K. Kohta, T. Shintani, T. Itoh, K. Terasawa, Extract prepared from the bark of Cinnamomum cassia Blume prevents glutamate-induced neuronal death in cultured cerebellar granule cells, Phytotherapy Research: PTR 14 (6) (2000) 466–468.

[16] K. Patra, S. Bose, S. Sarkar, J. Rakshit, S. Jana, A. Mukherjee, A. Roy, D.P. Mandal, S. Bhattacharjee, Amelioration of cyclophosphamide induced myelosuppression and oxidative stress by cinnamic acid, Chem. Biol. Interact. 195 (3) (2012) 231–239.

[17] D.-W. Huang, S.-C. Shen, Caffeic acid and cinnamic acid ameliorate glucose metabolism via modulating glycogenesis and gluconeogenesis in insulin-resistant mouse hepatocytes, J. Funct. Foods 4 (1) (2012) 358–366.

[18] R.M. Hafizur, A. Hameed, M. Shukrana, S.A. Raza, S. Chishti, N. Kabir, R.A. Siddiqui, Cinnamic acid exerts anti-diabetic activity by improving glucose tolerance in vivo and by stimulating insulin secretion in vitro, Phytomedicine: International Journal of Phytotherapy and Phytopharmacology 22 (2) (2015) 297–300.

[19] F. Song, H. Li, J. Sun, S. Wang, Protective effects of cinnamic acid and cinnamic aldehyde on isoproterenol-induced acute myocardial ischemia in rats, J. Ethnopharmacol. 150 (1) (2013) 125–130.

[20] S. Chandra, A. Roy, M. Jana, K. Pahan, Cinnamic acid activates PPARalpha to stimulate Lysosomal biogenesis and lower Amyloid plaque pathology in an

Alzheimer's disease mouse model, Neurobiol. Dis. 124 (2019) 379–395.
[21] A.A. Hemmati, S. Alboghobeish, A. Ahangarpour, Effects of cinnamic acid on memory deficits and brain oxidative stress in streptozotocin-induced diabetic mice, The Korean Journal of Physiology & Pharmacology: Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology 22 (3) (2018) 257–267.
[22] C. Chen, H. Zhang, H. Xu, R. Xue, Y. Zheng, T. Wu, Y. Lian, Harpagoside rescues the memory impairments in chronic cerebral hypoperfusion rats by inhibiting PTEN activity, Journal of Alzheimer's Disease: JAD 63 (2) (2018) 445–455.
[23] M.A. Flierl, P.F. Stahel, K.M. Beauchamp, S.J. Morgan, W.R. Smith, E. Shohami, Mouse closed head injury model induced by a weight-drop device, Nat. Protoc. 4 (9) (2009) 1328–1337.
[24] Y. Chen, S. Constantini, V. Trembovler, M. Weinstock, E. Shohami, An experimental model of closed head injury in mice: pathophysiology, histopathology, and cognitive deficits, J. Neurotrauma 13 (10) (1996) 557–568.
[25] S. Hatashita, J.T. Hoff, S.M. Salamat, Ischemic brain edema and the osmotic gradient between blood and brain, Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism 8 (4) (1988) 552–559.
[26] X. Wang, D. Liu, H.Z. Huang, Z.H. Wang, T.Y. Hou, X. Yang, P. Pang, N. Wei, Y.F. Zhou, M.J. Dupras, F. Calon, Y.T. Wang, H.Y. Man, J.G. Chen, J.Z. Wang, S.S. Hebert, Y. Lu, L.Q. Zhu, A novel microRNA-124/PTPN1 signal pathway mediates synaptic and memory deficits in Alzheimer's disease, Biol. Psychiatry 83 (5) (2018) 395–405.
[27] V.F. Prado, C. Martins-Silva, B.M. de Castro, R.F. Lima, D.M. Barros, E. Amaral, A.J. Ramsey, T.D. Sotnikova, M.R. Ramirez, H.G. Kim, J.I. Rossato, J. Koenen, H. Quan, V.R. Cota, M.F. Moraes, M.V. Gomez, C. Guatimosim, W.C. Wetsel, C. Kushmerick, G.S. Pereira, R.R. Gainetdinov, I. Izquierdo, M.G. Caron, M.A. Prado, Mice deficient for the vesicular acetylcholine transporter are myasthenic and have deficits in object and social recognition, Neuron 51 (5) (2006) 601–612.
[28] H. Tang, M. Ma, Y. Wu, M.F. Deng, F. Hu, H. Almansoub, H.Z. Huang, D.Q. Wang, L.T. Zhou, N. Wei, H. Man, Y. Lu, D. Liu, L.Q. Zhu, Activation of MT2 receptor ameliorates dendritic abnormalities in Alzheimer's disease via C/EBPalpha/miR-125b pathway, Aging Cell 18 (2) (2019) e12902.
[29] H. Sugiura, H. Kawabata, T. Ichikawa, A. Koarai, S. Yanagisawa, T. Kikuchi, Y. Minakata, K. Matsunaga, M. Nakanishi, T. Hirano, K. Akamatsu, K. Furukawa, M. Ichinose, Inhibitory effects of theophylline on the peroxynitrite-augmented release of matrix metalloproteinases by lung fibroblasts, American Journal of Physiology. Lung Cellular and Molecular Physiology 302 (8) (2012) L764–L774.
[30] D. Liu, H. Tang, X.Y. Li, M.F. Deng, N. Wei, X. Wang, Y.F. Zhou, D.Q. Wang, P. Fu, J.Z. Wang, S.S. Hebert, J.G. Chen, Y. Lu, L.Q. Zhu, Targeting the HDAC2/HNF-4A/miR-101b/AMPK pathway rescues tauopathy and dendritic abnormalities in Alzheimer's disease, Molecular Therapy: The Journal of the American Society of Gene Therapy 25 (3) (2017) 752–764.
[31] S.S. Song, B.J. Kang, L. Wen, H.J. Lee, H.R. Sim, T.H. Kim, S. Yoon, B.J. Yoon, G.J. Augustine, J.H. Baik, Optogenetics reveals a role for accumbal medium spiny neurons expressing dopamine D2 receptors in cocaine-induced behavioral sensitization, Front. Behav. Neurosci. 8 (2014) 336.
[32] Y. Su, M.F. Deng, W. Xiong, A.J. Xie, J. Guo, Z.H. Liang, B. Hu, J.G. Chen, X. Zhu, H.Y. Man, Y. Lu, D. Liu, B. Tang, L.Q. Zhu, MicroRNA-26a/death-associated protein kinase 1 signaling induces synucleinopathy and dopaminergic neuron degeneration in Parkinson's disease, Biol. Psychiatry 85 (9) (2019) 769–781.
[33] G. Leal, C.R. Bramham, C.B. Duarte, BDNF and hippocampal synaptic plasticity, Vitam. Horm. 104 (2017) 153–195.
[34] K. Lepeta, M.V. Lourenco, B.C. Schweitzer, P.V. Martino Adami, P. Banerjee, S. Catuara-Solarz, M. de La Fuente Revenga, A.M. Guillem, M. Haidar, O.M. Ijomone, B. Nadorp, L. Qi, N.D. Perera, L.K. Refsgaard, K.M. Reid, M. Sabbar, A. Sahoo, N. Schaefer, R.K. Sheean, A. Suska, R. Verma, C. Vicidomini, D. Wright, X.D. Zhang, C. Seidenbecher, Synaptopathies: synaptic dysfunction in neurological disorders - a review from students to students, J. Neurochem. 138 (6) (2016) 785–805.
[35] J.S. Guan, S.J. Haggarty, E. Giacometti, J.H. Dannenberg, N. Joseph, J. Gao, T.J. Nieland, Y. Zhou, X. Wang, R. Mazitschek, J.E. Bradner, R.A. DePinho, R. Jaenisch, L.H. Tsai, HDAC2 negatively regulates memory formation and synaptic plasticity, Nature 459 (7243) (2009) 55–60.
[36] T. Prorok, M. Jana, D. Patel, K. Pahan, Cinnamic acid protects the nigrostriatum in a mouse model of Parkinson's disease via peroxisome proliferator-activated receptoralpha, Neurochem. Res. 44 (4) (2019) 751–762.
[37] B. Zhu, B. Shang, Y. Li, Y. Zhen, Inhibition of histone deacetylases by trans-cinnamic acid and its antitumor effect against colon cancer xenografts in athymic mice, Mol. Med. Rep. 13 (5) (2016) 4159–4166.
[38] P.G. Anantharaju, D.B. Reddy, M.A. Padukudru, C.M.K. Chitturi, M.G. Vimalambike, S.V. Madhunapantula, Induction of colon and cervical cancer cell death by cinnamic acid derivatives is mediated through the inhibition of Histone Deacetylases (HDAC), PLoS One 12 (11) (2017) e0186208.
[39] S. Dallavalle, R. Cincinelli, R. Nannei, L. Merlini, G. Morini, S. Penco, C. Pisano, L. Vesci, M. Barbarino, V. Zuco, M. De Cesare, F. Zunino, Design, synthesis, and evaluation of biphenyl-4-yl-acrylohydroxamic acid derivatives as histone deacetylase (HDAC) inhibitors, Eur. J. Med. Chem. 44 (5) (2009) 1900–1912.
[40] L.E. Downing, B.S. Ferguson, K. Rodriguez, M.L. Ricketts, A grape seed procyanidin extract inhibits HDAC activity leading to increased Pparalpha phosphorylation and target-gene expression, Mol. Nutr. Food Res. 61 (2) (2017).
[41] J. Graff, D. Rei, J.S. Guan, W.Y. Wang, J. Seo, K.M. Hennig, T.J. Nieland, D.M. Fass, P.F. Kao, M. Kahn, S.C. Su, A. Samiei, N. Joseph, S.J. Haggarty, I. Delalle, L.H. Tsai, An epigenetic blockade of cognitive functions in the neurodegenerating brain, Nature 483 (7388) (2012) 222–226.
[42] M. Gonzalez-Zuniga, P.S. Contreras, L.D. Estrada, D. Chamorro, A. Villagra, S. Zanlungo, E. Seto, A.R. Alvarez, c-Abl stabilizes HDAC2 levels by tyrosine phosphorylation repressing neuronal gene expression in Alzheimer's disease, Mol. Cell 56 (1) (2014) 163–173.
[43] Y.M. Sung, T. Lee, H. Yoon, A.M. DiBattista, J.M. Song, Y. Sohn, E.I. Moffat, R.S. Turner, M. Jung, J. Kim, H.S. Hoe, Mercaptoacetamide-based class II HDAC inhibitor lowers Abeta levels and improves learning and memory in a mouse model of Alzheimer's disease, Exp. Neurol. 239 (2013) 192–201.
[44] C.L. Gibson, S.P. Murphy, Benefits of histone deacetylase inhibitors for acute brain injury: a systematic review of animal studies, J. Neurochem. 115 (4) (2010) 806–813.
[45] N.A. Shein, N. Grigoriadis, A.G. Alexandrovich, C. Simeonidou, A. Lourbopoulos, E. Polyzoidou, V. Trembovler, P. Mascagni, C.A. Dinarello, E. Shohami, Histone deacetylase inhibitor ITF2357 is neuroprotective, improves functional recovery, and induces glial apoptosis following experimental traumatic brain injury, FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology 23 (12) (2009) 4266–4275.
[46] J. Lu, J.M. Frerich, L.C. Turtzo, S. Li, J. Chiang, C. Yang, X. Wang, C. Zhang, C. Wu, Z. Sun, G. Niu, Z. Zhuang, R.O. Brady, X. Chen, Histone deacetylase inhibitors are neuroprotective and preserve NGF-mediated cell survival following traumatic brain injury, Proc. Natl. Acad. Sci. U. S. A. 110 (26) (2013) 10747–10752.
[47] G. Wang, Y. Shi, X. Jiang, R.K. Leak, X. Hu, Y. Wu, H. Pu, W.W. Li, B. Tang, Y. Wang, Y. Gao, P. Zheng, M.V. Bennett, J. Chen, HDAC inhibition prevents white matter injury by modulating microglia/macrophage polarization through the GSK3beta/PTEN/Akt axis, Proc. Natl. Acad. Sci. U. S. A. 112 (9) (2015) 2853–2858.
[48] S. Kumar, P.H. Reddy, MicroRNA-455-3p as a potential biomarker for Alzheimer's disease: an update, Front. Aging Neurosci. 10 (2018) 41.
[49] V.A. Alvarez, B.L. Sabatini, Anatomical and physiological plasticity of dendritic spines, Annu. Rev. Neurosci. 30 (2007) 79–97.
[50] A. Peters, I.R. Kaiserman-Abramof, The small pyramidal neuron of the rat cerebral cortex. The perikaryon, dendrites and spines, Am. J. Anat. 127 (4) (1970) 321–355.
[51] M. Phillips, L. Pozzo-Miller, Dendritic spine dysgenesis in autism related disorders, Neurosci. Lett. 601 (2015) 30–40.