Protective Effects of Oral Vitamin C on Memory and Learning Impairment and Attenuation of Synaptic Plasticity induced by Intracerebroventricular Injection of Beta-amyloid Peptide in Male Rats

Shima Sattari
Islamic Azad University
Gholamhasan Vaezi
Islamic Azad University
Siamak Shahidi
Hamadan University of Medical Sciences
Vida Hojati
Islamic Azad University
Alireza Komaki (alirezakomaki@gmail.com)
Hamadan University of Medical Sciences

Research Article

Keywords: Learning and Memory, Alzheimer's disease, Vitamin C, Long-Term Potentiation, Amyloid Beta, Antioxidant

Posted Date: August 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-587881/v1

Abstract

Background: In neurodegenerative diseases, like Alzheimer's disease (AD), learning and memory, and neural plasticity are impaired. Vitamin C (Vit C), as an antioxidant, protects tissues against oxidative stress. In this study, the neuroprotection and the effectiveness of Vit C in the prevention and treatment of AD in a rat model of AD induced by intracerebroventricular (ICV) injection of amyloid-beta (Aβ) were studied using behavioral tests and electrophysiological methods.

Methods: 50 male Wistar rats were randomly assigned to five groups (n=10 rats /group): Control, Sham, AD, Vit C, and AD+Vit C. After treatment (2 months), animals were assessed by learning and memory tests, including the new object recognition (NOR), passive avoidance learning (PAL), and Morris water maze (MWM) tests. Then, using the electrophysiological method, synaptic plasticity in the hippocampus was assessed and Vit C effects on long-term potentiation (LTP) were examined.

Results: ICV injection of Aβ to the AD group reduced memory in the NOR, PAL, and MWM behavioral tests. Also, Vit C reduced learning and memory impairment due to Aβ injection in rats. Aβ caused the inhibition of LTP in dentate gyrus (DG) granule cells by reducing the slope of the excitatory postsynaptic potential (EPSP) and the amplitude of the population spike (PS) in the AD group.

Conclusions: These results showed that Vit C reduced the impairment in synaptic plasticity induced by Aβ in DG of the hippocampus. Vit C supplementation through its antioxidant properties can improve the impaired synaptic plasticity and memory induced by Aβ and can be applied as a neuroprotective agent.

Background

As one of most, Alzheimer's disease (AD) is the commonest type of dementia and is a progressive, neurodegenerative disorder [1–3]. It has been recognized as the main cause of brain deterioration among the elderly, and its pathologic mechanism is still unknown [4]. AD as a globally important health problem is associated with cognitive deficits and memory loss [5–10]. In AD patients, brain atrophy may occur, neurons or synapses may be lost, tau proteins forming neurofibrillary tangles may be hyperphosphorylated within neuron cells [5, 8, 11, 12], and extracellular amyloid-beta (Aβ) protein may be accumulated in neuritic plaques. Other observations in such patients include DNA damage, chronic oxidative stress, mitochondrial dysfunction, and impaired energy metabolism [13]. On the other hand, oxidative stress can increase cognitive impairments, such as AD [14]. In the pathogenesis of several diseases, an imbalanced production of antioxidants and reactive oxygen species (ROS), known as oxidative stress, causes many neurological disorders such as Parkinson's disease, AD, and aging processes [15]. Accordingly, it has been considered that causal mechanisms of AD pathology have been underlain by inflammation and oxidative stress [12]. It is still unclear, however, whether oxidative damage directly contributes to the formation of Aβ plaques [16]. Aβ, which leads to AD development, reduces the power of antioxidants and learning in the Morris Water Maze (MWM) test [17]. Foods rich in antioxidants are preventive against neurodegenerative disorders, like AD [18–21].

Vitamin C (Vit C) is a vital nutrient for health and is used as an antioxidant against oxidative stress, as well [22]. It is a significant micronutrient and the lack of this vitamin disturbs the metabolic functioning of the body. It is widely used as a food additive to prevent oxidation. The biological role of Vit C, as a reducing agent, is an electron donator to numerous reactions that can be enzymatic or non-enzymatic [22, 23]. Ascorbate can be maintained in decreasing states aided by the glutathione presence in cells and extracellular fluids [24]. Antioxidant therapy may gain success for cognitive decline, according to biological studies. The cognitive functioning of aged mice improved significantly where their diets were supplemented with Vit C [25].

Long-term potentiation (LTP) of synaptic transmission in the hippocampus is the primary experimental model for studying the synaptic basis of learning and memory in vertebrates [26, 27]. In the present study, we examined the protective and therapeutic effects of Vit C, as an antioxidant, on the Aβ-induced learning and memory disruption, and LTP impairments in a rat model of AD.

Methods

Animals

Fifty male Wistar rats (200 ± 50 g; obtained from the Laboratory Animal Center of Hamadan University of Medical Sciences, Hamadan, Iran) were randomly assigned to five groups and kept in a 12h light-dark cycle (lights on: 07:00–19:00 h) at a controlled temperature of 23 ±1 °C. They had free access to food and water. The used experiments were approved by the Veterinary Ethics Committee (Code of Ethics Committee: Grant Number: IR.UMSHA.REC.1394.397), the Hamadan University of Medical Science and were conducted following the Guidelines of the National Institutes of Health (NIH Publication 80–23, 1996). Also, the study was carried out in compliance with the ARRIVE guidelines.

Amyloid-beta- 1–42 (Aβ1–42)

Aβ powder (Sigma–Aldrich, USA) was dissolved in normal saline (PH 7.2) at 1 μg/μL, and we incubated the solution at 37°C for a week before its application. The drug (5μl) was injected via intracerebroventricular (ICV) injection to induce the AD [28].

Preparation of Vit C

Vit C (Sigma–Aldrich.-USA) was dissolved in distilled water, and 200 mg/kg of it was administered to the rats orally for 60 days.

Experimental design

Animals were habituated to the lab environment for seven days before the study and were randomly divided into five groups (n = 10 rats/group): 1- Control: receiving no intervention and treatment; 2- Sham: receiving 5 µL of Aβ solvent via ICV injection; 3- AD: receiving 5 µL of Aβ1-42 via ICV injection, 4- Vit C: receiving 200 mg/kg of Vit C by oral gavage daily for 2 months, and 5- AD+Vit C: receiving Vit C by oral gavage daily for 30 days followed by 5 µl of Aβ1-42 and Vit C (200 mg/kg) daily for 30 days (fig.1).

Aβ injections and surgery

The rats were anesthetized by ketamine and xylazine (100 and 10 mg/kg, respectively) and transferred to a stereotaxic apparatus (Stoelting Co., USA). Aβ injection was conducted using a 5-µL microsyringe (Hamilton Laboratory Products, USA). The bregma and lambda were determined after determining the distance between the two points, according to the atlas of Paxinos, and the coordinates of the brain ventricular region were adjusted [29]. Aβ solution (5 ml) was injected into the ventricle at both sides of the brain at 5 µL / 20 min. For a better deposition of Aβ injection after 5 min, 2 min of rest was considered, and then injection was continued. After surgery, animals were kept in a warm box for one hour before returning to their cages. Animals were given two weeks to create the AD model. Then, 5–7 days of recovery time were considered after the surgery [30, 31], and then the treatment began.

Behavioral study

Locomotor activity test

The present study was based on the measurement of motor activity measured by the video tracking system. In this regard, the animals were first subjected to the treatment period. Then, they were transferred to the video tracking box for half an hour, so that their activities could be analyzed. The output of this analysis included the calculation of two criteria of the physical locomotion of the animal, namely the distance (m) and speed (cm/sec) of movement.

Object recognition test

The tendency of rodents to search for new and old objects can be examined by examining the objects. In this study, rats were placed in a wooden chamber with a length and width of about 40 × 50 cm and a height of 40 cm, with opaque paint [32, 33]. The light of the chamber was also adjusted in a way that no shadow would form. The rats were tested in three phases. First, compatibility with empty compartment; second, familiarization phase with two identical objects; and third, the test phase (test day). The rats were trained and tested for two consecutive days [24, 34]. During 5 min, the animal's exploration time was measured and recorded for the old object (cube) and the new object (circle) using a timer. The exploratory behavior was defined monitored by the rat's nose pointed toward and within a 2-cm radius of the object.

The exploration ratio was calculated by dividing the time to search the new object over the total time exploring the old and the new objects [35].

Spatial memory assessment

A hippocampal-dependent spatial learning test for rodents [36], the MWM task, was used for examination of the spatial memory. The apparatus has a circular pool painted black with a diameter of 155 and a height of 60 cm, which was filled with water to a depth of 35 cm (22 ± 1 °C) and divided into four quadrants of the same size. A hidden (10 × 10 cm) Plexiglas platform was located 2 cm below the water surface at the eastern (target) quadrant center. The swim path of the rats was recorded using a video tracking system equipped with a CCD camera (Panasonic, Japan) to be analyzed later using video tracking. Visual cues were provided by large posters on the room wall. To be adapted to the environment, the rats were allowed to swim for 60 s in the tank without a platform an entire day prior to the start of the hidden platform training.

The previous procedure [36-38] was served as the basis for the training session, briefly involving one block of 4 daily trials for four days in a row. Each trial was started by placing the animals in one of the four quadrants. They were allowed to swim in the pool for 90 s in order to find the hidden platform and kept at one of the four quadrants. Animals that failed to find the platform within that period were provided with manual guidance from the researcher toward the platform. A 10-minute rest period was considered for rats between two consecutive trials.

For the assessment of the memory acquisition in the MWM test, swimming distance, and escape latency (the time required to reach the platform) were utilized. For calculation of the rats' motor activity, swimming speed was employed. For the measurement of the path, by which the rats remembered the platform location (memory retention), the time spent in the target quadrant was utilized.

A day following the fourth session, the probe test was performed, where the platform was eliminated, and rats were given a 60 s free-swimming period prior to elimination. The animals were released in water, exactly opposite the location of the platform. A video tracking system (using Maze Router homemade software) was used for recording behavior [39]. For later analysis, swimming speed and escape latency were recorded. After elevating the platform above the surface of the water, it was covered by aluminum foil in bright color and located elsewhere 30 min following the probe trial, and the rats were allowed to swim for 60 s and find the visible platform so that their visual ability could be examined. The experiments were conducted within 10 to 12 min.

Test of passive avoidance learning (PAL)

A passive avoidance apparatus (Shuttle box) consists of a light and a dark compartment. The rat was located in the light part facing away from the guillotine door that was elevated after 5 s. The door was

shut once the rat entered the dark chamber, followed by the application of a 50-Hz square wave with a 1.2 mA constant current shock for 1.5 s. The rat was given a foot shock once it entered the dark compartment again. Once the rat stayed for 120 s on a row in the lighted part, training was stopped. The rat was located in the lighted chamber again in the retention test that was conducted 24 h following the acquisition trial, and the time spent in the dark part (TDC) and step-through latency (STLr) was recorded as a criterion for retention performance [40, 41]. The behavioral tests were conducted between 8 am and 11 am. Once the rat stayed in the lighted part for 120 s on a row, training was stopped. The number of entries into the dark part (*i.e.* the number of trials, NTa) was recorded. Then, 24 h following the PAL acquisition trial, the retention test was conducted. Similar to the PAL training, the rats were located in the lighted part, and the guillotine door was elevated 5 s later. The STLr and TDC were recorded for up to 300 s. The retention test was stopped once the rat did not enter the dark part within 300 s.

Surgery and electrophysiological recording

Initially, the animal was anesthetized by intraperitoneal urethane injection (1.5 g / kg). After anesthetization, the animal was placed in a stereotactic device and a warm-up pad was used to maintain its body temperature in a range of 36.5±5.5ºC. Small holes were created on the right or left side of the skull. Two stimulating electrodes and a 125-micron microelectrode made from Teflon-coated stainless steel were placed in the perforant path (AP = 8.1 mm = 3.6 mm, ML = 3.35 mm, and DV = 3-3.3 mm from the skull surface) and teeth scattering area (AP = 3.8 mm vs. Bergema, 2.4 mm = ML, 2.7-2.7 mm / 2 mm from the skull surface) of the right or left hemisphere (fig.2). The stabilized electrode was connected to the microwave electrolyte amplifier and the stimulating electrode to the stimulating system. In response to the stimulation of the perforant path, field evoked potentials were recorded from the teeth scattering area. The perforant path was stimulated by monophasic square pulses for 0.1 ms and at 10-second intervals. An excitation command was transmitted through the computer to the stimulator, and from there through the stimulus isolator and stabilizer, and the final stimulation was transmitted through the bipolar electrodes to the perforant path. The perforant path was stimulated with different intensities until the field potential peaked in response to a single wave of the stimuli. To this end, it was necessary to replace the stimulation electrode several times in order to obtain the best points for stimulation and recording. Among the intensities used, the severity of 50% of the maximum response was selected, and to generate LTP, the stimuli were filled. High-frequency stimulation (HFS) was applied to the perforant area with the same intensity, and the percentage of excitatory postsynaptic potential (EPSP) and the number of spikes (PS) were compared. In LTP recordings, the slope of EPSP and the amplitude of the PS were analyzed for 1 h. The exciting potential in the dentate gyrus (DG) region has two components: PS and fEPSP [42]. The benchmark in this study was to measure the slope of fEPSP and the amplitude of PS. The PS amplitude was calculated as the mean voltage difference between the two peaks. FEPSP slope, the voltage variation at 80% of the middle point of the first positive wave (point A) to the peak of the first positive wave (point B) divided by the difference in time between those two points was calculated. Data were recorded and analyzed by a computer. At the end of the study, the animals were sacrificed using a high dose of ketamine.

Statistical analysis

Using GraphPad Prism (v. 5.0; GraphPad Software, Inc., La Jolla, CA) we conducted the Statistical analyses. One-way analysis of variance (ANOVA) followed by a Tukey test for multiple comparisons were used to analyze the data. All results were expressed as mean ±S.E.M. At all stages, a $P < 0.05$ was considered significant.

Results

Motor activity

The results of one-way ANOVA showed that there was no significant difference in speed ($F=2.566$, $P>0.05$) and displacement ($F=3.009$, $P>0.05$) between different groups. According to Fig. 3a and b, there was no significant change in the motor activity in the AD group and groups receiving Vit C.

Memory test for finding objects

The results of ANOVA and Tukey tests showed that there was a significant difference in the exploration time around a new object between the AD group and control and sham groups ($F=8.824$, $p <0.01$). Moreover, the exploration time around the new object in the group receiving Vit C showed a significant increase compared with the AD group ($p <0.001$). The exploration time around the new object in the AD group that received Vit C showed a significant increase compared with the AD group ($p <0.001$) (Fig. 4).

Passive avoidance task (shuttle box)

Step-through latency in the acquisition trial (STLa) was recorded when the animal placed all four paws in the dark compartment and before receiving the electrical shock. According to the results of one-way ANOVA the mean latency to enter the dark part did not differ among the groups ($F=2.54$, $P > 0.05$) (Fig.5.a). Also, according to the one-way ANOVA results, no significant difference was detected in the number of shocks received to reach the learning standard between the groups ($F=0.6300$, $P > 0.05$) (Fig.5.b). The one-way ANOVA and Tukey test were used to compare the time of the first entry to the dark part in the retention phase (STLr). AD group showed a significant reduction in STLr compared with the control ($F= 8.824$, $P <0.001$) and sham ($P <0.01$) groups. The STLr significantly increased in the group receiving Vit C ($P <0.01$) and the AD group was treated with Vit C ($P <0.01$) than the AD group (Fig. 5c). These findings indicated that Vit C improved the memory disorder caused by AD induction.

In addition, the results of the ANOVA and Tukey test indicated a statistically significant difference in the total TDC recorded in the absence of electric foot shocks, as an indicator of inhibitory avoidance behavior, between the experimental groups. The results indicated an increase in TDC in the AD group compared with the control and the sham groups ($F=9.201$, $P <0.001$). Also, TDC in the AD + Vit C group

was significantly reduced than the AD group (P <0.001). According to these results, learning in the AD group was impaired. On the other hand, Vit C improved memory impairment caused by AD induction (Fig. 5d).

Effects of Vit C on MWM performance

There was a significant difference in the time spent to reach the hidden platform between different groups. The results of two-way ANOVA in four training days showed that the AD group spent more time to find the hidden platform (escape latency) than the control group (first day: P <0.001, second day: P <0.0001, and third day: P <0.0001) and also they needed more time in the fourth day (P <0.0001). This difference was found to follow an increasing trend.

On the first day, there was no significant difference in the time spent to reach the hidden platform between the group receiving Vit C, AD + Vit C group and the control group. However, a significant difference was observed on the second, third, and fourth days between AD group with the control, Vit C, and AD + Vit C groups (P <0.01, P <0.001, and P <0.001, respectively). In the all four days, there was also a significant difference between the AD and AD + Vit C groups (first day: P <0.01 and second, third and fourth day: P <0.0001) (Fig.6a).

As shown in Fig.6b, a significant difference was seen in the traveled distance on the first day between the AD, control, and sham groups (P <0.0001). In this day and second day, there was also a significant difference between the AD and AD + Vit C groups (first day: P <0.0001; and second day: p <0.001, respectively).

The mean time spent in the target quadrant in the probe trial was investigated and the results of one-way ANOVA showed that the time spent in the target quadrant in the AD group was significantly lower than the control and sham groups (F=134.8, P <0.001). Moreover, a significant increase was observed in the AD + Vit C group compared with the AD group (P <0.001) (Fig.6c).

Electrophysiology Test

An example of the responses recorded in the various groups before the applying of the tannic stimulation and after it is shown in Fig. 7.

The studied groups were found with variation in the PS amplitude at different time points. In order to investigate the significant difference between the experimental groups at each time point, a two-way ANOVA followed by the Tukey test were applied when the p-value was significant. This curve showed that after HFS, there was a significant decrease in the amplitude of PS in the AD group compared with control group after 5, 30, and 60 min (p <0.0001). Moreover, an adequate intake of Vit C increased the amplitude of PS at 5, 30, and 60 min in the AD + Vit C group compared with the AD group (P <0.0001) (Fig. 8a).

Fig. 8b shows the changes in the slope of the EPSP between different groups. These findings revealed that after HFS, there was a significant difference in the EPSP slope between the AD group and control and sham groups at 5, 30, and 60 min ($p < 0.0001$). Also, a significant difference was observed in this indicator between the AD + Vit C group compared with the AD group at 5, 30, and 60 min ($P < 0.0001$).

Discussion

According to the obtained results, Vit C reduced learning and memory impairment caused by the ICV injection of Aβ in rats. Injection of Aβ caused memory impairment in the AD model. Learning and memory were assessed using behavioral tests, including NOR, PAL, and MVM test. After the ICV injection of Aβ to induce the AD model, it inhibited LTP in granular cells of DG by reducing the EPSP slope and the amplitude of PS in the AD group compared with the control and sham groups. Vit C in the treated group led to an improvement in learning and memory through improved LTP in dentate granule cells by increasing the EPSP slope and the PS amplitude compared with the AD group. Studies on AD have shown severe oxidative damages regarding two pathological characteristics of AD, namely amyloid plaques and neurofibrillary destruction [43]. It has been shown that Aβ accumulation is one of the important reasons for producing oxidative stress and subsequent damage [44]. On the other hand, the brain is a sensitive organ to oxidative stress due to the combination of the lipid membrane and low levels of antioxidant enzymes [45]. Other empirical studies have demonstrated that intra-hippocampal Aβ injection can reduce antioxidant potency [46]. In AD, memory and learning processes are affected by brain tissue damage. Many empirical studies have indicated a decline in the memory and learning in AD models, tested by behavioral tests, such as MWM and PAL tests [46–50]. Thorough aging, we can see a disruption to neurotransmitter systems, such as neuronal plasticity, and also cognitive impairment [51]. Also, neuronal plasticity is impaired in the brain in neurodegenerative disorders, like AD [52, 53], in which the LTP process is inhibited. In many previous studies, the LTP process has been controlled in AD models [53, 54]. In these studies, there has been a significant reduction in the fEPSP slope and the PS amplitude after HFS [53]. This finding is consistent with our study. Studying the compounds or drugs with positive, preventive, and therapeutic effects on AD requires a clear understanding of the pathological mechanism of this disease. According to the above-mentioned observations, exploring the mechanism of action of Vit C in the pathology of AD has been considered. A powerful reducing agent, Vit C (*i.e.* ascorbic acid) can influence many oxidation reactions that occur in biological systems [52]. The high concentrations of ascorbic acid in the brain are very regulated and can be absorbed by the choroidal network actively [55]. Ascorbic acid may function as an important member of an intracellular antioxidant network in protecting the neurons. Convincing evidence suggests that ascorbic acid acts as an antioxidant required to neurological action [56, 57]. The beneficial effect of vitamins on memory has been identified, and the positive effect of Vit C on memory has been widely reported during the past two decades [58–60]. The addition of Vit C to cells decreases the cell death caused by oxidative stress, and it helps the cell to protect the genome by reducing ROS [61, 62].

Ascorbic acid reduces the number of damaged cells in the cornea amniotic region (CA1 and CA3) and the DG of the hippocampus. This vitamin is a potent antioxidant that protects tissues against oxidative

damage. Ascorbic acid can produce small antioxidants, such as alpha-tocopherol, glutathione, acyclovir, and beta-carotene, by producing electrons for free radicals and radical production of ascorbyls prevents their oxidation [63]. Small molecular antioxidants, such as Vit C, can directly react with oxidizing radicals [64]. It has also been shown that oxidative stress disrupts the memory mechanism [65]. According to our findings, it can be concluded that an adequate intake of Vit C supplementation can improve the damage to the synaptic plasticity induced by ICV injection of Aβ. The protective role of this vitamin can be attributed to its antioxidant effects, which reduces the potential for oxidative damage induced by Aβ. In other words, this study suggested the beneficial effect of this vitamin on the improvement of damage in the synaptic formulation induced by ICV injection of Aβ in DG in vitro measured by LTP. These results also indicated the important protective effect of this vitamin against amyloid plaques resulting from Aβ injection in the DG of the hippocampus.

The oxidative metabolism rate of neurons is 10 times higher than that of glial cells [66, 67]; therefore, they are sensitive to the deficiency of ascorbic acid. As suggested, since there are homeostatic mechanisms maintaining high ascorbic acid concentrations in neurons and cerebrospinal fluid, ascorbic acid has a neuroprotective function [66, 68, 69]. The ascorbic acid efflux and recycling mechanisms that are performed by astrocytes will be addressed in the following sections. As a powerful reductant, ascorbic acid is regarded as a significant neuroprotective agent [70–72], which sustains superoxide dismutase and catalase activities and scavenges ROS production [73]. It has been suggested that oxidative stress is associated with AD pathogenesis. During the generation of the amyloid plaque, ROS are produced that then increase neuronal injury. Other resources of ROS in the brain of AD cases have also been announced [62]. Ascorbic acid provides neurons with protection against glutamate excitotoxicity, as well, and this is associated with neurodegenerative processes [74–77]. Nevertheless, the ascorbic acid functions in the brain are broader than those of Aβ [78]. The relationship between Vit C and brain pathologies is of great importance, especially considering that the oxidized type of Vit C, dehydroascorbic acid (DHA), has been widely disregarded. Although Vit C deficiency in the brain has been linked to enhanced oxidative stress, as an ideal stimulating condition for triggering some pathologies, like AD, DHA has been suggested as a molecule to trigger neuronal death [68]. It is suggested that the central nervous system (CNS) diseases are possibly linked to decreased astrocytic Vit C recycling, leading to the accumulation of DHA in neurons, resulting in cell death [69].

Conclusion

In summary, according to the results of the behavioral tests of this experiment, Vit C consumption significantly reduced the learning and memory impairment in mice receiving Aβ1–42. Also, in the electrophysiological test, there was a significant increase in the value of LTP in the AD group receiving Vit C compared with the AD group. Therefore, Vit C could improve the learning and memory and synaptic plasticity impairment of AD. Based on these findings, Vit C can be used for both the prevention and treatment of AD by improving memory and learning impairment.

Declarations

Acknowledgements

The authors are grateful to the staff of the Neurophysiology Research Center, Hamadan University of Medical Sciences for supporting this study.

Authors’ contributions

A.K. and G.V. contributed to the study design and supervision, statistical analysis of data, writing and critical revision of the manuscript; S.Sa. conducted experiments, data acquisition and drafting the manuscript; S.Sh. was responsible for administrative, technical and material support and critical revision of the manuscript for important intellectual content; V.H. contributed technical support and manuscript drafting. All authors read and approved the final manuscript.

Funding

This research was carried out at the Neurophysiology Research Center (Grant number: 9411206356), Hamadan University of Medical Sciences. This Center supported us in study design, in the collection, analysis and interpretation of data.

Availability of data and materials

The data used in our study are available from the authors on reasonable request.

Ethics approval and consent to participate

All experimental procedures using rats were conducted in accordance with the animal care and use guidelines approved by the institutional ethics committee at Hamadan University of Medical Sciences (Code of Ethics Committee: IR.UMSHA.REC.1394.397) and were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. Also, the study was carried out in compliance with the ARRIVE guidelines.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Author details

[1] Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran.

[2] Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.

References

1. Reinke AA, Gestwicki JE. Insight into Amyloid Structure Using Chemical Probes. Chemical Biology & Drug Design. 2011;77:399–411.
2. Mattson MP. Will caloric restriction and folate protect against AD and PD? Neurology. 2003;60:690.
3. Asadbegi M, Yaghmaei P, Salehi I, Komaki A, Ebrahim-Habibi A. Investigation of thymol effect on learning and memory impairment induced by intrahippocampal injection of amyloid beta peptide in high fat diet-fed rats. Metabolic brain disease. 2017;32:827–39.
4. Cheng X-l, Xiong X-b, Xiang M-q. Effect of arborvitae seed on cognitive function and α7nAChR protein expression of hippocampus in model rats with Alzheimer's Disease. Cell biochemistry and biophysics. 2013;67:181–4.
5. Cai Z, Yan Y, Wang Y. Minocycline alleviates beta-amyloid protein and tau pathology via restraining neuroinflammation induced by diabetic metabolic disorder. Clinical interventions in aging. 2013;8:1089–95.
6. Kim D-G, Krenz A, Toussaint LE, Maurer KJ, Robinson S-A, Yan A, et al. Non-alcoholic fatty liver disease induces signs of Alzheimer's disease (AD) in wild-type mice and accelerates pathological signs of AD in an AD model. Journal of Neuroinflammation. 2016;13:1.
7. Knight EM, Martins IVA, Gümüsgöz S, Allan SM, Lawrence CB. High-fat diet-induced memory impairment in triple-transgenic Alzheimer's disease (3xTgAD) mice is independent of changes in amyloid and tau pathology. Neurobiology of Aging. 2014;35:1821–32.
8. Peng D, Pan X, Cui J, Ren Y, Zhang J. Hyperphosphorylation of Tau Protein in Hippocampus of Central Insulin-Resistant Rats is Associated with Cognitive Impairment. Cellular Physiology and Biochemistry. 2013;32:1417–25.
9. Shea TB, Remington R. Nutritional supplementation for Alzheimer's disease? Current Opinion in Psychiatry. 2015;28:141–7.
10. Vinters HV. Emerging Concepts in Alzheimer's Disease. Annual Review of Pathology: Mechanisms of Disease. 2015;10:291–319.
11. Henderson VW. Alzheimer's disease: Review of hormone therapy trials and implications for treatment and prevention after menopause. The Journal of Steroid Biochemistry and Molecular Biology. 2014;142:99–106.

12. Morris MC, Tangney CC. Dietary fat composition and dementia risk. Neurobiology of aging. 2014;35:S59-S64.
13. de la Monte SM, Wands JR. Alzheimer's Disease is Type 3 Diabetes—Evidence Reviewed. Journal of Diabetes Science and Technology. 2008;2:1101–13.
14. Aboul-Enein H, Al-Duraibi I, Stefan R, Radoi C, Avramescu A, editors. Analysis of L-and D-ascorbic acid in fruits and fruit drinks by HPLC. Seminars in food analysis; 1999.
15. Aman S, Moin S, Owais M, Siddiqui M. Antioxidant activity of thymol: protective role in AAPH-induced hemolysis in diabetic erythrocytes. IJPSI. 2013;2:55–60.
16. Hernandez-Zimbron L, Rivas-Arancibia S. Oxidative stress caused by ozone exposure induces β-amyloid 1–42 overproduction and mitochondrial accumulation by activating the amyloidogenic pathway. Neuroscience. 2015;304:340–8.
17. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee J-H, et al. Vitamin C as an antioxidant: evaluation of its role in disease prevention. Journal of the American college of Nutrition. 2003;22:18–35.
18. Asadbegi M, Komaki A, Salehi I, Yaghmaei P, Ebrahim-Habibi A, Shahidi S, et al. Effects of thymol on amyloid-β-induced impairments in hippocampal synaptic plasticity in rats fed a high-fat diet. Brain research bulletin. 2018;137:338–50.
19. Esmaeili A, Khodadadi A. Antioxidant activity of a solution of thymol in ethanol. Zahedan Journal of Research in Medical Sciences. 2012;14:14–8.
20. Gerber M, Boutron-Ruault M-C, Hercberg S, Riboli E, Scalbert A, Siess M-H. Food and cancer: state of the art about the protective effect of fruits and vegetables. Bulletin du cancer. 2002;89:293–312.
21. Mirzaei F, Khazaei M, Komaki A, Amiri I, Jalili C. Virgin coconut oil (VCO) by normalizing NLRP3 inflammasome showed potential neuroprotective effects in Amyloid-β induced toxicity and high-fat diet fed rat. Food and Chemical Toxicology. 2018;118:68–83.
22. Gropper SS, Smith JL. Advanced nutrition and human metabolism. Cengage Learning; 2012.
23. Meister A. Glutathione-ascorbic acid antioxidant system in animals. Journal of Biological Chemistry-Paper Edition. 1994;269:9397–400.
24. Gustafson D, Rothenberg E, Blennow K, Steen B, Skoog I. An 18-year follow-up of overweight and risk of Alzheimer disease. Archives of internal medicine. 2003;163:1524–8.
25. Arzi A, Hemmati AA, Razian A. Effect of Vitamins C and E on cognitive function in mouse. Pharmacological Research. 2004;49:249–52.
26. Komaki H, Faraji N, Komaki A, Shahidi S, Etaee F, Raoufi S, et al. Investigation of protective effects of coenzyme Q10 on impaired synaptic plasticity in a male rat model of Alzheimer's disease. Brain research bulletin. 2019;147:14–21.
27. Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 1993;361:31–9.
28. Goryacheva AV, Kruglov SV, Pshennikova MG, Smirin BV, Malyshev IY, Barskov IV, et al. Adaptation to intermittent hypoxia restricts nitric oxide overproduction and prevents beta-amyloid toxicity in rat

brain. Nitric Oxide. 2010;23:289–99.
29. Paxinos G, Watson C. The rat brain in stereotaxic coordinates: hard cover edition. Elsevier; 2006.
30. Azadeh E-H, Asadbegi M, Salehi I, Yaghmaei P, Komaki A. Neuroprotective role of antidiabetic drug metformin against amyloid β peptide-induced neuronal loss in hippocampal CA1 pyramidal neurons in rats fed high fat diet. Journal of Chemical and Pharmaceutical Sciences. 2016;9:3460–5.
31. Asadbegi M, Yaghmaei P, Salehi I, Ebrahim-Habibi A, Komaki A. Neuroprotective effects of metformin against Aβ-mediated inhibition of long-term potentiation in rats fed a high-fat diet. Brain research bulletin. 2016;121:178–85.
32. Winocur G, Greenwood CE. The effects of high fat diets and environmental influences on cognitive performance in rats. Behavioural brain research. 1999;101:153–61.
33. Beydoun MA, Beydoun H, Wang Y. Obesity and central obesity as risk factors for incident dementia and its subtypes: a systematic review and meta-analysis. Obesity reviews. 2008;9:204–18.
34. Ganji A, Salehi I, Nazari M, Taheri M, Komaki A. Effects of Hypericum scabrum extract on learning and memory and oxidant/antioxidant status in rats fed a long-term high-fat diet. Metabolic brain disease. 2017;32:1255–65.
35. Greenwood CE, Winocur G. Cognitive impairment in rats fed high-fat diets: a specific effect of saturated fatty-acid intake. Behavioral neuroscience. 1996;110:451.
36. Karimi SA, Salehi I, Shykhi T, Zare S, Komaki A. Effects of exposure to extremely low-frequency electromagnetic fields on spatial and passive avoidance learning and memory, anxiety-like behavior and oxidative stress in male rats. Behavioural brain research. 2019;359:630–8.
37. Zarrinkalam E, Ranjbar K, Salehi I, Kheiripour N, Komaki A. Resistance training and hawthorn extract ameliorate cognitive deficits in streptozotocin-induced diabetic rats. Biomedicine & Pharmacotherapy. 2018;97:503–10.
38. Omidi G, Karimi SA, Rezvani-Kamran A, Monsef A, Shahidi S, Komaki A. Effect of coenzyme Q10 supplementation on diabetes induced memory deficits in rats. Metabolic brain disease. 2019;34:833–40.
39. Baharlouei N, Sarihi A, Komaki A, Shahidi S, Haghparast A. Blockage of acquisition and expression of morphine-induced conditioned place preference in rats due to activation of glutamate receptors type II/III in nucleus accumbens. Pharmacology Biochemistry and Behavior. 2015;135:192–8.
40. Zarrinkalam E, Heidarianpour A, Salehi I, Ranjbar K, Komaki A. Effects of endurance, resistance, and concurrent exercise on learning and memory after morphine withdrawal in rats. Life sciences. 2016;157:19–24.
41. Shiri M, Komaki A, Oryan S, Taheri M, Komaki H, Etaee F. Effects of cannabinoid and vanilloid receptor agonists and their interaction on learning and memory in rats. Canadian journal of physiology and pharmacology. 2017;95:382–7.
42. Omidi G, Karimi SA, Shahidi S, Faraji N, Komaki A. Coenzyme Q10 supplementation reverses diabetes-related impairments in long-term potentiation induction in hippocampal dentate gyrus granular cells: An in vivo study. Brain Research. 2020;1726:146475.

43. Cookman GR, King W, Regan CM. Chronic low-level lead exposure impairs embryonic to adult conversion of the neural cell adhesion molecule. Journal of neurochemistry. 1987;49:399–403.
44. Hasan F, Cookman G, Keane G, Bannigan J, King W, Regan C. The effect of low level lead exposure on the postnatal structuring of the rat cerebellum. Neurotoxicology and teratology. 1989;11:433–40.
45. Regan CM. Lead-impaired neurodevelopment. Mechanisms and threshold values in the rodent. Neurotoxicology and teratology. 1989;11:533–7.
46. Marchetti C. Molecular targets of lead in brain neurotoxicity. Neurotoxicity research. 2003;5:221–35.
47. McCauley P, Bull R, Tonti A, Lutkenhoff S, Meister M, Doerger J, et al. The effect of prenatal and postnatal lead exposure on neonatal synaptogenesis in rat cerebral cortex. Journal of Toxicology and Environmental Health, Part A Current Issues. 1982;10:639–51.
48. Sanders T, Liu Y, Buchner V, Tchounwou PB. Neurotoxic effects and biomarkers of lead exposure: a review. Reviews on environmental health. 2009;24:15–46.
49. Newton AC. Protein kinase C: structure, function, and regulation. Journal of Biological Chemistry. 1995;270:28495–8.
50. Matthies H, Palfrey HC, Hirning LD, Miller RJ. Down regulation of protein kinase C in neuronal cells: effects on neurotransmitter release. Journal of Neuroscience. 1987;7:1198–206.
51. Akers RF, Lovinger DM, Colley PA, Linden DJ, Routtenberg A. Translocation of protein kinase C activity may mediate hippocampal long-term potentiation. Science. 1986;231:587–9.
52. Routtenberg A. A tale of two contingent protein kinase C activators: both neutral and acidic lipids regulate synaptic plasticity and information storage. Progress in brain research: Elsevier; 1991. p. 249 – 61.
53. Hwang K-Y, Lee B-K, Bressler JP, Bolla KI, Stewart WF, Schwartz BS. Protein kinase C activity and the relations between blood lead and neurobehavioral function in lead workers. Environmental health perspectives. 2002;110:133.
54. Lidsky TI, Schneider JS. Lead neurotoxicity in children: basic mechanisms and clinical correlates. Brain. 2003;126:5–19.
55. Rice ME. Ascorbate regulation and its neuroprotective role in the brain. Trends in Neurosciences. 2000;23:209–16.
56. Grundman M, Delaney P. Antioxidant strategies for Alzheimer's disease. Proceedings of the Nutrition Society. 2009;61:191–202.
57. Wilson MA, Johnston MV, Goldstein GW, Blue ME. Neonatal lead exposure impairs development of rodent barrel field cortex. Proceedings of the National Academy of Sciences. 2000;97:5540-5.
58. An L, Zhang T. Vitamins C and E reverse melamine-induced deficits in spatial cognition and hippocampal synaptic plasticity in rats. Neurotoxicology. 2014;44:132–9.
59. Shahidi S, Komaki A, Mahmoodi M, Atrvash N, Ghodrati M. Ascorbic acid supplementation could affect passive avoidance learning and memory in rat. Brain research bulletin. 2008;76:109–13.

60. Vereker E, O'Donnell E, Lynch M. The inhibitory effect of interleukin-1β on long-term potentiation is coupled with increased activity of stress-activated protein kinases. Journal of Neuroscience. 2000;20:6811–9.
61. Guaiquil VH, Vera JC, Golde DW. Mechanism of vitamin C inhibition of cell death induced by oxidative stress in glutathione-depleted HL-60 cells. Journal of Biological Chemistry. 2001;276:40955–61.
62. Lutsenko EA, Cárcamo JM, Golde DW. Vitamin C prevents DNA mutation induced by oxidative stress. Journal of Biological Chemistry. 2002;277:16895–9.
63. ROSTAMI S, MOMENI Z, BEHNAM RM, GHAYOUR N. COMPARISON OF ANTIOXIDANT EFFECT OF MELISSA OFFICINALIS LEAF AND VITAMIN C IN LEAD ACETATEINDUCED LEARNING DEFICITS IN RAT. 2010.
64. Chang B-J, Jang B-J, Son TG, Cho I-H, Quan F-S, Choe N-H, et al. Ascorbic acid ameliorates oxidative damage induced by maternal low-level lead exposure in the hippocampus of rat pups during gestation and lactation. Food and chemical toxicology. 2012;50:104–8.
65. Rowan M, Klyubin I, Wang Q, Hu N, Anwyl R. Synaptic memory mechanisms: Alzheimer's disease amyloid β-peptide-induced dysfunction. Portland Press Limited; 2007.
66. Wilson JX. Antioxidant defense of the brain: a role for astrocytes. Canadian journal of physiology and pharmacology. 1997;75:1149–63.
67. Hediger MA. New view at C. Nature medicine. 2002;8:445.
68. May JM. Vitamin C transport and its role in the central nervous system. Water Soluble Vitamins: Springer; 2012. p. 85–103.
69. Harrison FE, May JM. Vitamin C function in the brain: vital role of the ascorbate transporter SVCT2. Free Radical Biology and Medicine. 2009;46:719–30.
70. Padh H. Cellular functions of ascorbic acid. Biochemistry and Cell Biology. 1990;68:1166–73.
71. Naseer M, Ullah N, Ullah I, Koh P, Lee H, Park M, et al. Vitamin C protects against ethanol and PTZ-induced apoptotic neurodegeneration in prenatal rat hippocampal neurons. Synapse. 2011;65:562–71.
72. Niki E. Action of ascorbic acid as a scavenger of active and stable oxygen radicals. The American journal of clinical nutrition. 1991;54:1119S-24S.
73. Santos ÍMS, Tomé AdR, Saldanha GB, Ferreira PMP, Militão GCG, De Freitas RM. Oxidative stress in the hippocampus during experimental seizures can be ameliorated with the antioxidant ascorbic acid. Oxidative medicine and cellular longevity. 2009;2:214–21.
74. Qiu S, Li L, Weeber EJ, May JM. Ascorbate transport by primary cultured neurons and its role in neuronal function and protection against excitotoxicity. Journal of neuroscience research. 2007;85:1046–56.
75. Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. Science. 1993;262:689–95.

76. Lipton SA, Rosenberg PA. Excitatory amino acids as a final common pathway for neurologic disorders. New England Journal of Medicine. 1994;330:613–22.
77. Choi DW. Glutamate neurotoxicity and diseases of the nervous system. Neuron. 1988;1:623–34.
78. Moreira PI, Santos MS, Oliveira CR. Alzheimer's disease: a lesson from mitochondrial dysfunction. Antioxidants & redox signaling. 2007;9:1621–30.

Figures

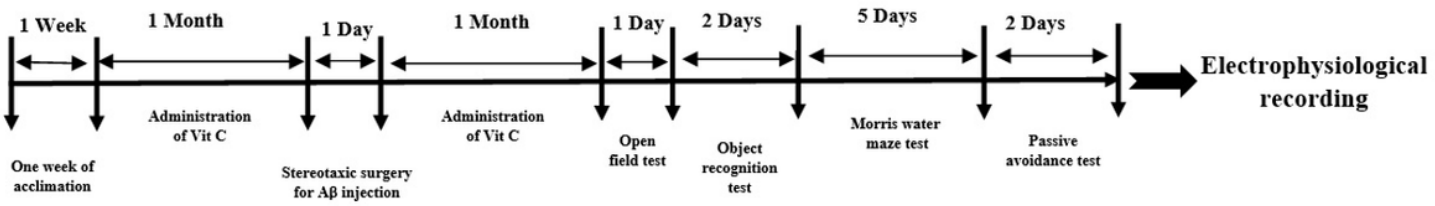

Figure 1

Experimental timeline. Timeline: Following 1 month of daily oral gavage vitamin C in experimental groups, to generate an Alzheimer's model, rats were anesthetized with xylazine (10 mg/kg) and ketamine (100 mg/kg) and transferred to a stereotaxic device. The Aβ solution (5 µl) was injected by intracerebroventricular at a rate of 1 µL/2 min. After recovery, again vitamin C was administered by a daily oral gavage for a period of 1 month. Open field and object recognition tests were used to record the motor activity and cognitive memory of the animal, respectively. For measurement of spatial (acquisition and retention) and aversive (acquisition and retention) learning and memory after the training programs, Morris water maze (MWM) and shuttle box tests were used, respectively. Then, the rats were anesthetized with an IP injection of urethane and placed in a stereotaxic device for surgical procedure and electrophysiological recording. Once a stable baseline of responses was obtained for at least 20 min, LTP was elicited employing a high-frequency stimulation protocol.

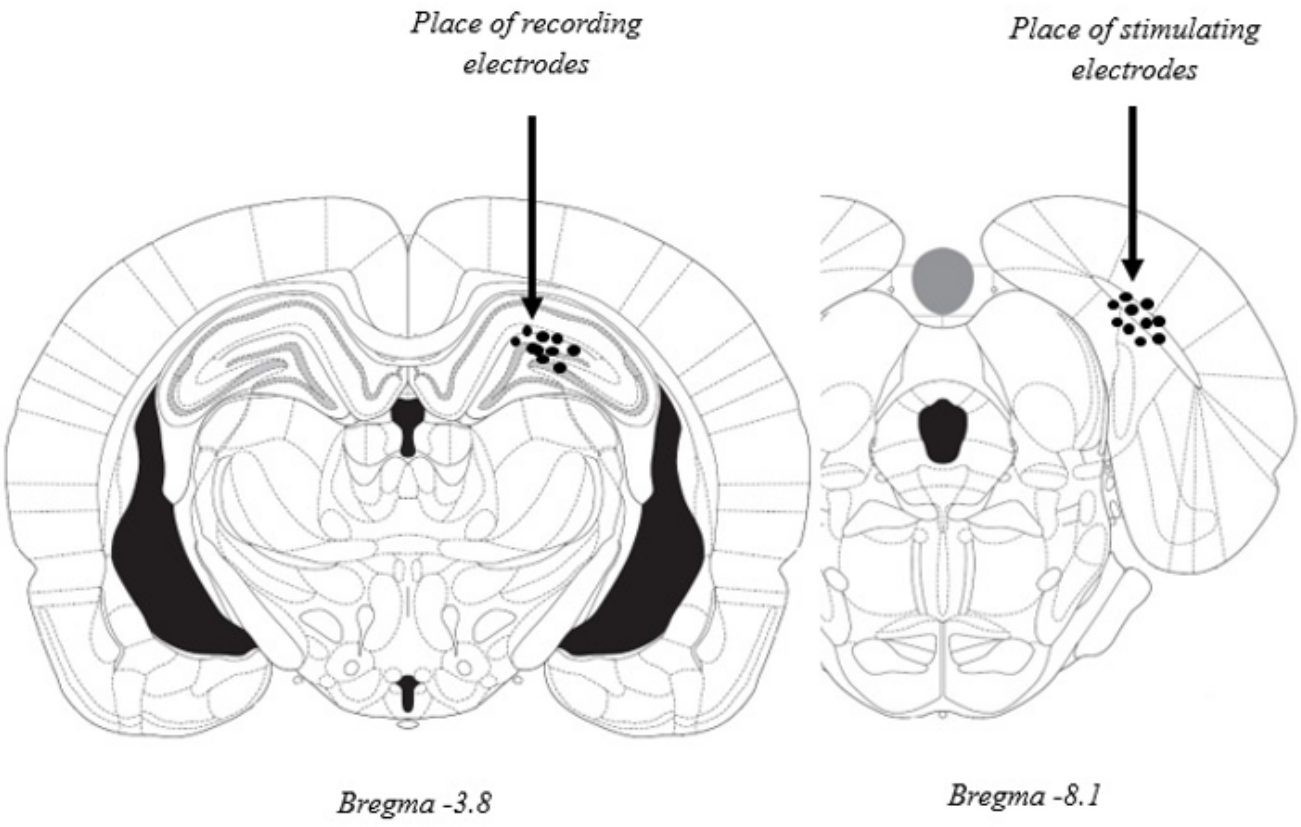

Figure 2

Places of stimulating and recording electrodes tips (black circles) in a hippocampus sagittal section. Stimulating and recording electrode traces can be seen at the right and left sides, respectively (arrows). Schematic drawing of several accepted injection sites in the experiments [29].

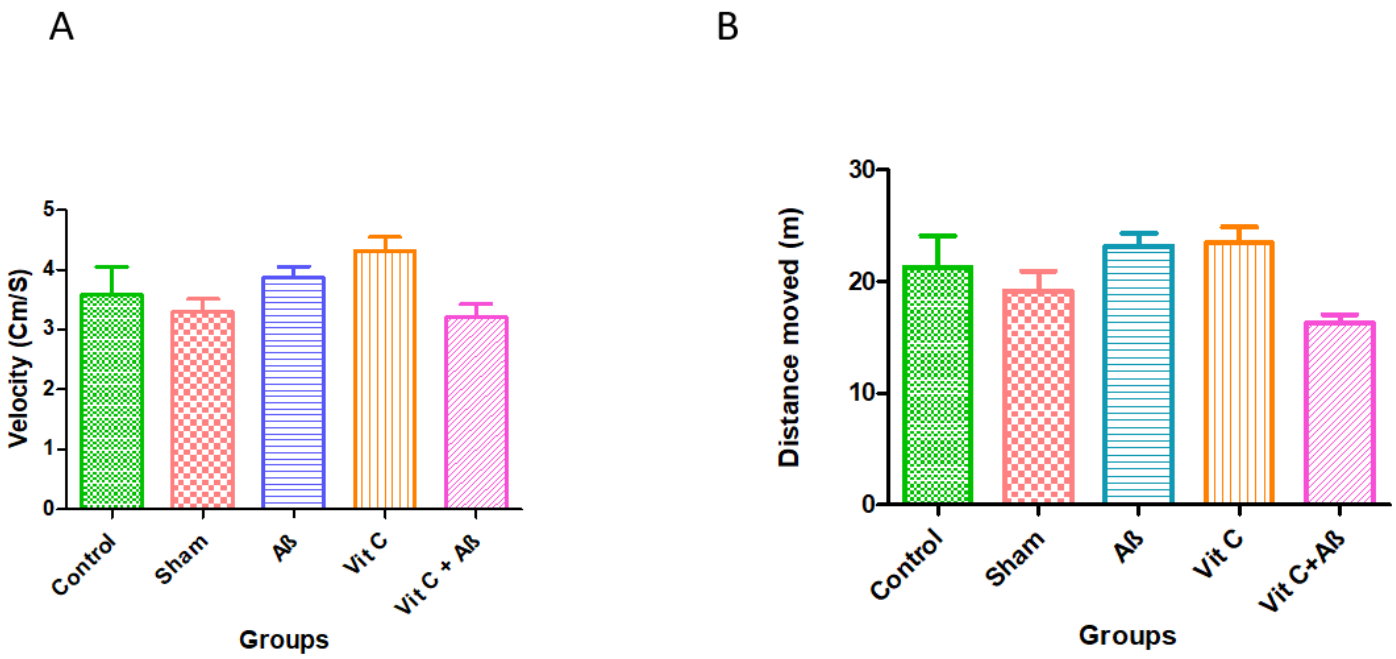

Figure 3

Comparison of the speed between different groups in an open field test (a). Comparison of the distance traveled between different groups in open field test (b). Each column and bar represents the mean ± S.E.M. p> 0.05.

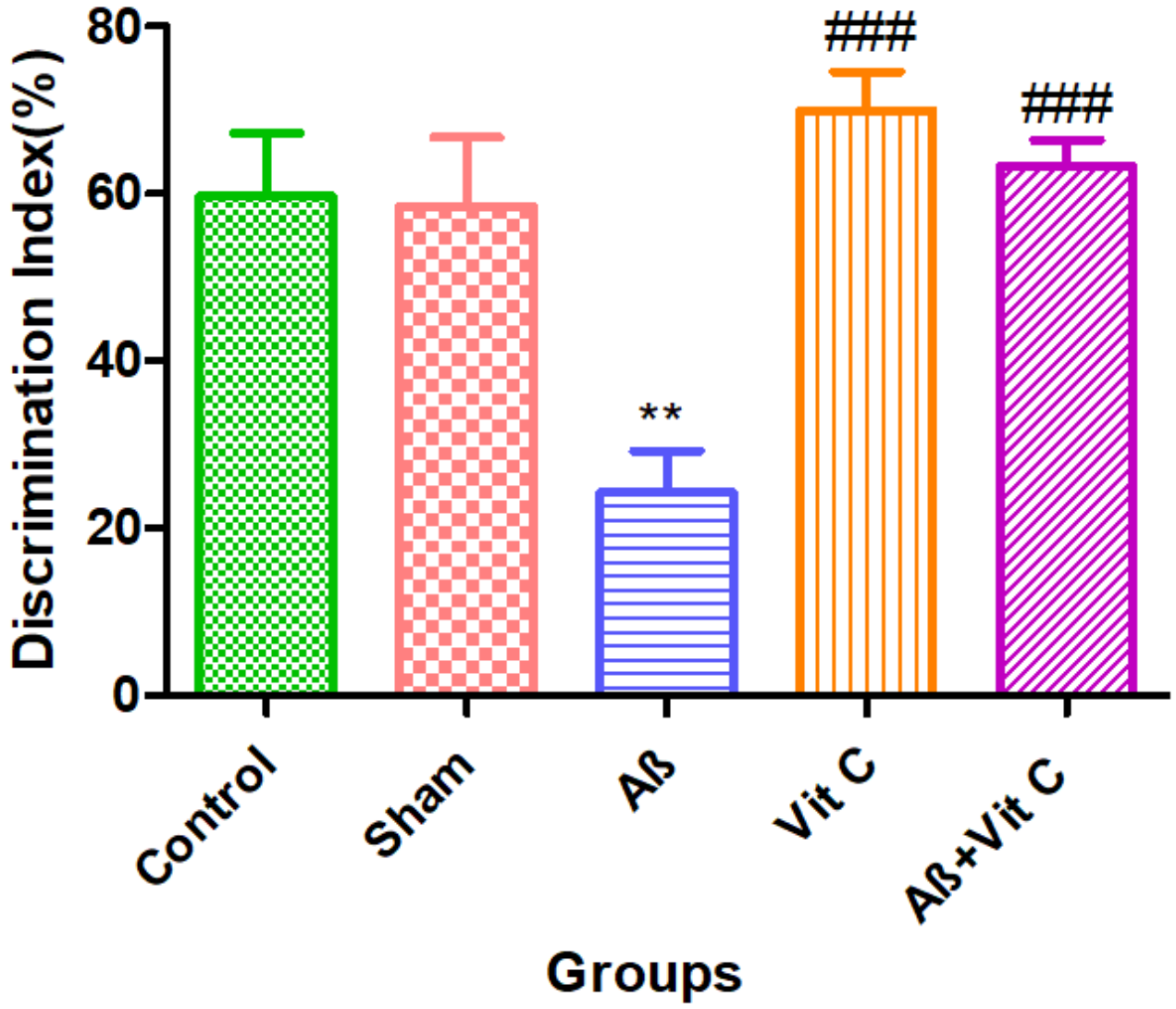

Figure 4

Comparison of the exploration time to search a new object between different groups in the novel object recognition (NOR) test. **: Alzheimer's (Aβ) group compared with the control group (p <0.01). ##: p <0.01 compared with the Aβ group.

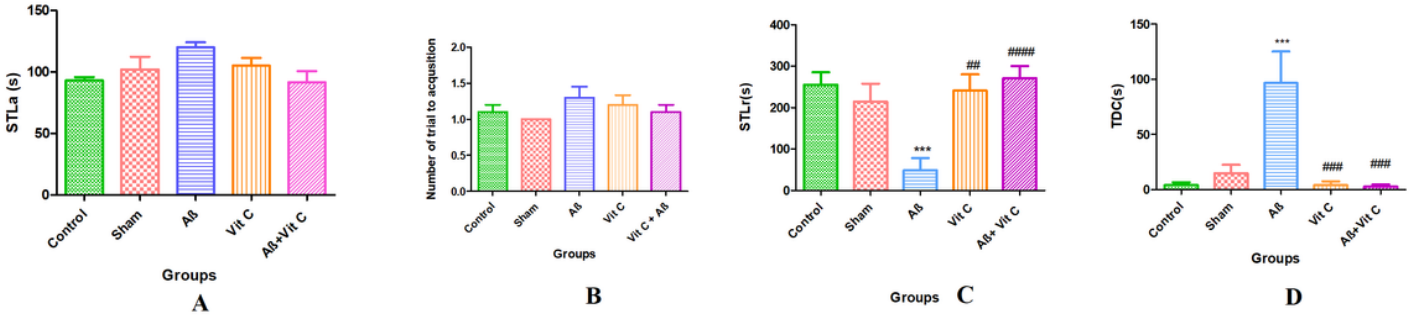

Figure 5

Comparison of the latency to enter the dark part of the device in the habituation phase (a). The number of shocks received to reach the learning stage (b). Comparison of the first time entry to the dark part in the passive avoidance retention test (STLr) (c). Comparison of the time spent in the dark compartment (TDC) (d). Each column and bar represents mean ± S.E.M. ***p<0.001 compared with the control group. ## p<0.01 and ### p<0.001 compared with the Aβ group.

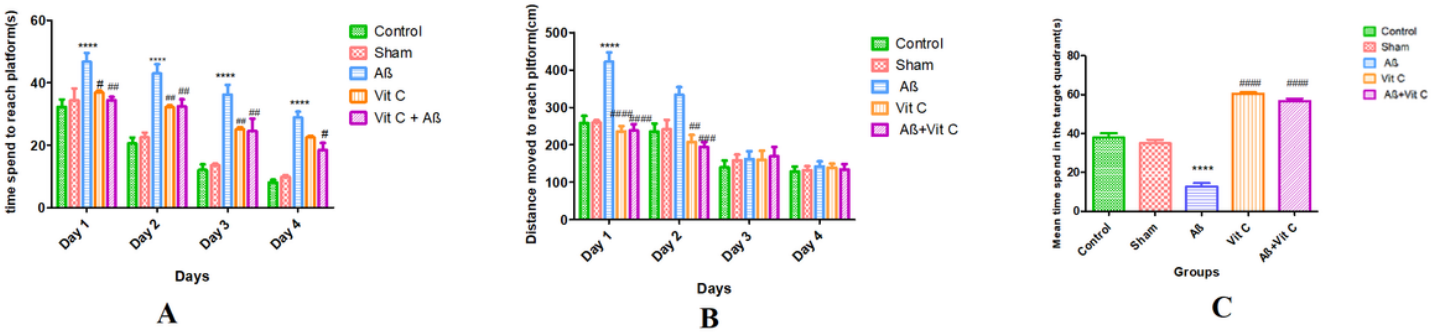

Figure 6

Comparison of the time spent to reach the hidden platform in Morris Water Maze (MWM) Test (a). Comparison of distance traveled to reach the hidden platform (b). Comparison of swimming time in the target quadrant on the fifth day of the MWM test in the probe trial (c). Data are presented as mean ± S.E.M. *p<0.05, **p<0.01, and ***p<0.001 compared with the control group. ## p<0.01, and ### p<0.0001 compared with Aβ group.

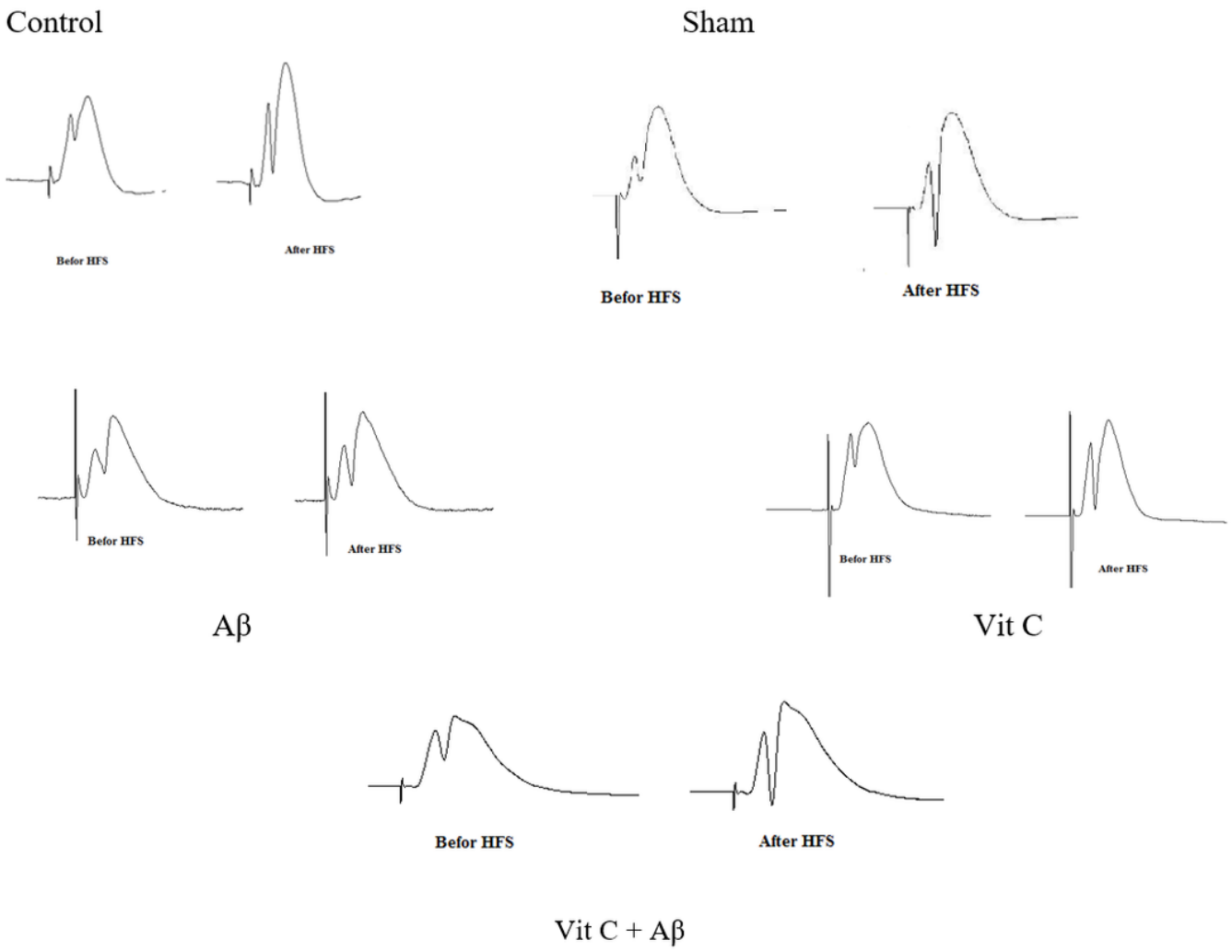

Figure 7

The population spike (PS) amplitude and excitatory postsynaptic potential (EPSP) slope of representative sample traces of field potential were recorded in the perforant pathway-dentate gyrus (PP-DG) synapses of all groups. Representative sample traces of evoked field potential in the DG were recorded prior to and after high-frequency stimulation (HFS) in all groups.

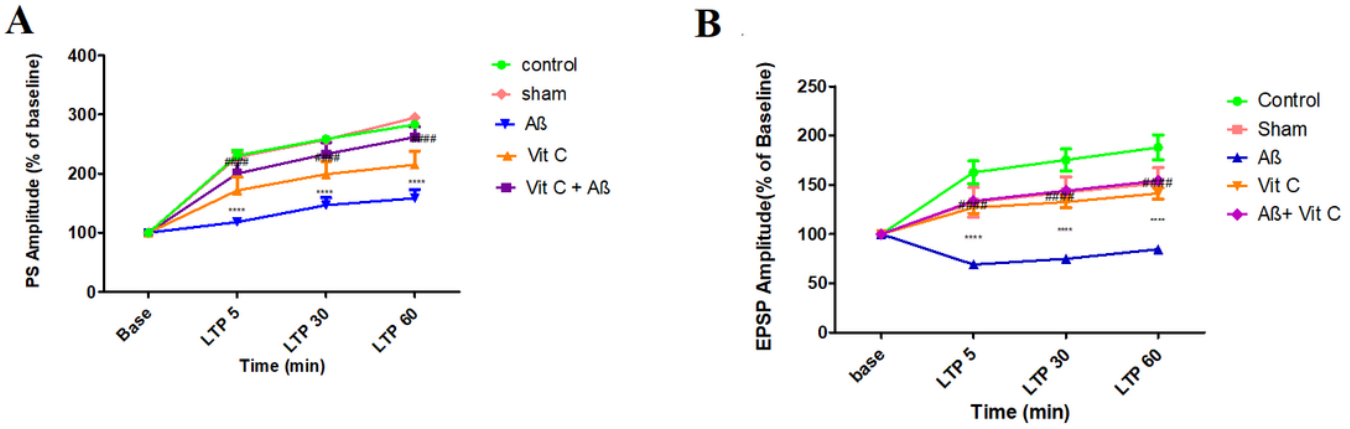

Figure 8

The population spike (PS) amplitude of granular cells following high-frequency stimulation (HFS) of the perforant pathway in experimental groups (a). The slope of the excitatory postsynaptic potential (EPSP) in granular cells following the HFS of the perforant pathway in experimental groups (b). Data are presented as mean ± S.E.M. ****p<0.0001 compared with the control group. #### p<0.0001 compared with the Aβ group.