Title: L-α-aminoadipic Acid-Induced Astrocytes Inhibition in the Hippocampal CA1 Region Leads to Anxiety-like Behavior and Memory Impairment

Running Title: Astrocyte Inhibition, Anxiety and Memory

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Abstract:

Background: Glutamate plays a major role in synaptic plasticity that is important for learning and memory. Astrocyte is important neuroglia, involves in the neuroinflammation and plays a key role in maintaining glutamine/glutamate homeostasis. As astrocytes provide a vital support to neurons in pathological conditions. Hence, in this study, we aimed to evaluate the effect of hippocampal astrocytes ablation induced by microinjection of L-α-aminoadipic acid (L-α-AAA) on memory, anxiety and the density of GFAP-ir astrocytes in the hippocampus.

Methods: Adult male Wistar rats were randomly assigned to control, vehicle, and experimental group. L-α-AAA was injected into the hippocampal CA1 subfield for 3 days. Memory was evaluated by inhibitory passive avoidance test and anxiety-related behavior was assessed by elevated plus maze apparatus. Hippocampal sections were immunostained for GFAP and the density of GFAP-ir astrocytes were counted.

Results: Microinjection of L-α-AAA into the CA1 subfield of the hippocampus significantly decreased the step-through latency time in passive avoidance test and significantly decreased time spent in open arm and increased time spent in closed arm in elevated plus maze test. Also, administration of L-α-AAA significantly declined the density of GFAP-ir astrocytes in the hippocampus.

Conclusion: Inhibition of astrocytes impaired the memory and increased the anxiety-like behavior in male rats. Hence, the current study confirmed hippocampal astrocyte's key role in memory and anxiety-like behavior which can be considered in future therapeutic strategies.

Keywords: Astrocyte, GFAP, L-α-Aminoadipic acid, Anxiety, Memory, Hippocampus.

1. Introduction

Astrocytes, the most abundant neuroglial in the central nervous system (CNS), have vital roles in brain homeostasis, neuroprotection, synaptic plasticity, uptake and release neurotransmitter, and innate immunity (Acosta et al., 2017; Dallérac & Rouach, 2016; Navarrete & Araque, 2014; Olsen et al., 2018; Shang et al., 2015; Soung & Klein, 2020; Zorec et al., 2015). Astrocytes show a modulatory role on anti-inflammatory process as well regulate the function of microglia during brain injury through secreting cytokines (Lima et al., 2014; Tarasov et al., 2020). Moreover, they maintain the glutamate and GABA neurotransmitter reservoirs through expressing pyruvate carboxylase, the enzyme required for synthesis of these two amino acids (Schousboe et al., 2013). Inflammatory cytokines control astrocytes-released glutamate. Hence, glia-to-neuron signaling may be affected by cytokine mediators in pathological conditions (Vesce et al., 2007). Astrocytes respond to neuronal activity through expressing several receptors and altering homeostasis consequently, create a neuron-astrocyte cross-talk. Abnormal conditions could affect the neuron-astrocyte integrity, disturb cognitive functions and final behavior output. In addition to normal roles of astrocytes, recent researches have been focusing on the importance of them cognitive functions (Hosseini et al., 2020; Santello et al., 2019; Suzuki et al., 2011). Previous studies found astrocytes plasticity in rat hippocampus after spatial working memory (M Jahanshahi et al., 2008; Mehrdad Jahanshahi et al., 2008). Indeed, enhanced expression of glial fibrillary acidic protein (GFAP) as well as density of astrocytes after learning showed recruitment of astrocytes in cognition (Dallérac & Rouach, 2016). The GFAP is an intermediate filament and is mainly found in the cytoskeleton structure of mature astrocytes (Bronzuoli et al., 2018; Guillamón Vivancos et al., 2015). The mutations and anormal expression of GFAP have been observed in neurodegeneration, neuroinflammation and psychiatric disorders (Li et al., 2020). Therefore, specific inhibition of astrocytes may help to investigate the contribution of reactive astrocytes to some of the neurodegenerative diseases. L-α-Aminoadipic (L-α-AAA) is a homolog of the

excitatory amino acid glutamate, generally used to exert astrocyte-specific toxicity in vitro and in vivo studies (Guidetti & Schwarcz, 2003). Microinjection of L-α-AAA inhibits the glutamine synthetase that plays an essential role in the learning and consolidation of memories (Guidetti & Schwarcz, 2003; Robinson et al., 2015). It decreased long-term potentiation (LTP) magnitude that underlies memory impairment. It also reported that altered glutamate neurotransmission occurred in anxiety-like behavior related to traumatic brain injury in the amygdala (Beitchman et al., 2020). It is not clear how ablation of astrocytes changes the memory and behavior. However, a combination of alternations in trophic support for neurons and glutamate cycle, that is normally controlled by astrocytes, has been proposed (David et al., 2019). The effect of hippocampal astrocytes on anxiety and memory has not been extensively investigated (Leitão, 2018). In this study, we aimed to evaluate the effect of astrocyte inhibition with L-α-AAA on inhibitory passive avoidance memory, anxiety-like behavior, and the density of GFAP-ir astrocytes in rat hippocampus.

2. Materials and Methods

2.1. Animals

21 Adult male rats of Wistar strain (180–220 g) were obtained from the animal house of Golestan University of Medical Sciences. The rats were placed in the cages at 22±2ºC, 12 hours light/dark cycle with access to food and water ad libitum. All procedures were in accordance to the principles of the ethical board of Golestan University of Medical Sciences (Gorgan, Iran).

Stereotaxic procedure and L-α-AAA microinjection into the hippocampal CA1 subfield

Intrahippocampal injection was performed according to our previous studies (Azami et al., 2010; Jahanshahi et al., 2018; Moghadami et al., 2016) with minor modifications. Briefly, the rats were anaesthetized for stereotaxic surgery (David Kopf Instruments, USA) using an intraperitoneal injection of ketamine (100 mg/ml) and xylazine (20 mg/ml). Stainless guide cannulas (21-gauge)

were bilaterally implanted at the dorsal CA1 hippocampal area, the coordinates were anterior-posterior: −3 mm from bregma; medial-lateral: ± 2 mm from midline; and dorsal-ventral:−2 mm from the skull surface (Paxinos & Watson, 2007). At the end of the procedure, each cannula was temporarily closed with a stainless-steel wire to preserve it from occlusion. Seven days following surgery, 1 μL/rat (0.5 μL in each side) of L-α-AAA and/or vehicle were injected into the CA1 hippocampal area by a Hamilton micro-syringe over one minute. The needle was kept in cannula to prevent backflow. L-α-Aminoadipic acid (Sigma-Aldrich, China) was dissolved in normal saline containing 6% 2-Hydroxypropyl-β-cyclodextrin (Sigma-Aldrich, China) with PH=7. One day before and after last injection of drugs, learning and memory was assessed by passive avoidance memory task. Elevated plus maze test were evaluated one day before and thirty minutes after last injection of drugs.

Experimental design

The rats were randomly assigned into three groups (n=7); the control, with no intervention, vehicle, with stereotaxic surgery, received 6% 2-Hydroxypropyl-β-cyclodextrin (vehicle) once per day for three consecutive days and evaluated for behavioral tests, experimental group (L-α-AAA), with stereotaxic surgery, received L-α-Aminoadipic acid at dose of 25 μg/μl once daily for three consecutive days and evaluated for behavioral test.

All drugs were injected to the dorsal CA1 hippocampal area. Rats subjected to inhibitory passive avoidance memory and anxiety assessments were habituated to the condition of the testing room for 1 h before performing the behavioral tests. The two behavioral tests were not performed on the same rats and behavioral tests were done blind to treatment.

Behavioral tests

***Passive avoidance memory test*:**

The passive avoidance memory test was performed as described in our previous studies (Mahakizadeh et al., 2015; Seifhosseini et al., 2011). The apparatus (the shuttle box) composed of two light and dark compartments (20 × 20 × 30 cm^3) with identical size that were divided by a manually operated gate (7 × 9 cm^2). Floor of the dark compartment had a stainless-steel shock grid floor. Electric shocks were given using a stimulator (50 Hz, 1.5 mA intensity, 3 seconds). It has three steps, habituation, training and prob.

-Habituation

Each rat was gently placed into the light compartment and after 5 seconds the gate was opened and the rat was permitted to go into the dark compartment. Rats were excluded from the experiments if they delayed for more than 120 seconds to cross to the dark compartment.

-Training

Training was done 30 minutes after habituation. The rat was placed in the light compartment, after 5 seconds the gate was opened. When the rat entered the dark compartment with all the four feet, the gate was closed and the rat was received an electrical foot shock (50 Hz, 1.5 mA intensity, 3 seconds). After 20 seconds, the rat was returned into the cage. 120 seconds later, the rat was re-tested in the similar method as in the prior trials; successful learning was defined as the rat remained in light compartment for 120 s.

-Prob

one day after the last drug injection, the rat was placed in the light compartment, then the step through latency time to enter the dark compartment was recorded. The cut off time for retention trial was 300 seconds.

Elevated plus maze test:

The elevated plus maze (EPM) test was used to assess anxiety-related behavior in rats (Karimi et al., 2014; Nikmahzar et al., 2016). Briefly, this plus-shaped apparatus was located in 50 cm height. It made up of two open arms (50 × 10 cm) and two closed arms (50 × 10 × 40 cm) opposite to each other, linked by a central square zone (10×10 cm). The test was performed by placing the rat on the center of EPM facing an open arm and was permitted to explore the maze for 5 minutes. During the step, the number of entries into both arms and the time spent in each arm was recorded. Arm entries was recorded when the rat entered all four paws into the EPM arm. The maze was cleaned with 70% ethanol between each step for each rat. Finally, the percentage of open and closed arms time, the percentage of open and closed arm entries, open arm latency, and pure index of locomotor activity was measured. Total arm entries into the open and closed arms were measured as a pure index of locomotor activity.

Tissue preparation

Twenty-four hours after the behavioral test, rats were deeply anesthetized with chloroform and trans-cardially perfused by injecting the 0.9% saline and 4% paraformaldehyde (Scharlau, Spain). The brains were collected and kept in fixative solution (4% paraformaldehyde) for the next seven days. Afterwards, the automated tissue processor (Did Sabz, Iran), was used for the histological processing of brain samples and finally were embedded in paraffin blocks. paraffin blocks were cut using a rotary microtome to thickness of 6 µm coronal sections from hippocampus at 20 µm interval between each two successive sections (Moghadami et al., 2016). The sections were immunostained for glial fibrillary acidic protein (GFAP), a specific marker for astrocytes.

Immunohistochemistry staining for GFAP-ir astrocytes

GFAP-ir astrocytes were evaluated by immunohistochemistry staining (Nikmahzar et al., 2019; Shaabani et al., 2011). In this regards, the sections were immersed in xylene to deparaffinized and in graded ethanol to rehydrated. The antigen was retrieved in retrieval solution (pH 9, Tashkhis Baft Arajen, Iran) for 20 minutes at 90-95 °C using a laboratory water bath. Next, the slides were cooled at room temperature and rinsed with washing buffer (phosphate buffered saline (PBS)/Tween 20 in 0.1 % Triton X-100). In order to quench endogenous peroxidase activity, brain sections were incubated in 0.3% hydrogen peroxide and methanol for 10 minutes at room temperature. After rinsing in washing buffer, brain sections were incubated with avidin/biotin blocking solution (Dako, Denmark) for 30 minutes at room temperature and rinsed in washing buffer. In order to block nonspecific reactivity, brain sections were incubated with 1% bovine serum albumin (BSA) blocking solution for 60 minutes at 37 °C temperature. Then, brain sections were incubated in primary anti-rabbit polyclonal GFAP antibody (ab16997, 1:100, Abcam Inc., USA) for 120 minutes at 37 °C. After washing in buffer, the sections were incubated in secondary biotinylated goat anti rabbit IgG antibody (ab64256, Abcam Inc., USA) for 60 minutes at 37 °C and then rinsed with washing buffer. Afterwards, the brain sections were probed with Streptavidin HRP protein (1:5000, Abcam Inc., USA) for 30 minutes at room temperature. Finally, the brain sections were covered with the diaminobenzidine solution (Dako, Denmark) and rinsed gently with distilled water. After background staining with Meyer's Hematoxylin for 3-4 seconds, the brain sections were dehydrated in graded ethanol, cleared in xylene and mounted with entelan glue (Merck, Germany).

Imaging and counting GFAP-ir astrocytes

Pictures were captured using a digital camera (Model: DP73, Olympus, Japan), connected to a light microscope (Model: BX 53, Olympus, Japan) with 40× magnification for CA1, CA3 and DG regions of the hippocampus. The number of GFAP-ir astrocytes was counted in a 30000 μm^2 area

at three regions of the hippocampus by the cellSens standard 1.14 software (Olympus, Japan). Imaging and counting were performed blinded to treatment.

Statistical analysis

Statistical analysis was performed by the SPSS software (version 16.0. Armonk, NY, USA). All data were expressed as mean ± SD and the Shapiro-Wilk test was used for data normality. Behavioral assessments and the histological data were analyzed by one-way analysis of variance (ANOVA) with LSD post-hoc test. $P<0.05$ was regarded significant difference.

Results:

L-α-AAA administration into the hippocampal CA1 area reduced the passive avoidance memory function

Figure 1 showed that the step through latency time in the training step was 120 seconds for all groups. Injection of L-α-AAA into the hippocampal CA1 area for three consecutive days significantly decreased the step through latency time when compared to vehicle group ($F_{2,15} = 6.213$; $P = 0.011$, Fig. 1). While there was not a significant difference in step-through latency time between the control and vehicle groups (Fig. 1).

L-α-AAA administration into the hippocampal CA1 area increased anxiety-like behavior

The data of elevated plus maze revealed that L-α-AAA significantly decreased the percentage of time spent in the open arm ($F_{2,12} = 7.940$; $P = 0.006$) and significantly increased the percentage of time spent in the closed arm ($F_{2,12} = 7.280$; $P = 0.009$) compared to control rats (Fig. 2A and B). L-α-AAA significantly reduced the percentage of time spent in the open arm at 30 minutes after last injection compared to one day before injection of L-α-AAA ($P < 0.05$, Fig. 2A). Three consecutive daily doses of L-α-AAA acid significantly decreased the percentage of open arm entries ($F_{2,12} = 34.932$; $P = 0.000$) and increased the percentage of closed arm entries ($F_{2,12} = 34.932$; $P = 0.000$) compared to control rats (Fig. 2C and D). It also significantly decreased the percentage of open arm entries and increased the percentage of closed arm entries at 30 minutes

after last injection compared to one day before injection of L-α-AAA ($P < 0.05$, Fig. 2C and D). A significant increase in the open arm latency ($F_{2,12} = 22.107$; $P = 0.000$) was observed following administration of L-α-AAA compared to control rats (Fig. 2E). The data showed a significant reduction in the pure index of locomotor activity in L-α-AAA group ($F_{2,12} = 14.647$; $P = 0.001$) compared to control rats (Fig. 2F). Moreover, there was significant difference in anxiety parameters in the elevated plus maze between control and vehicle treated group rats (Fig. 2).

L-α-AAA administration reduced the number of GFAP-ir astrocytes in the hippocampus

Three consecutive daily doses of vehicle administration into the hippocampal CA1 area significantly increased the number of GFAP-ir astrocytes in the CA1, CA3 and DG subfields of hippocampus in comparison with control rats ($P<0.001$, Fig. 3B i, ii and iii). L-α-AAA injected rats exhibited a significant decline in the number of GFAP-ir astrocytes in the CA1, CA3 and DG subfields of hippocampus three days after administration when compared to vehicle rats ($P<0.001$, Fig. 3B i, ii and iii). The number of GFAP-ir astrocytes significantly decreased after administration of L-α-AAA when compared to control rats in the CA1 ($F_{2,55} = 29.742$; $P = 0.000$), CA3 ($F_{2,55} = 39.497$; $P = 0.000$) and DG ($F_{2,55} = 61.143$; $P = 0.000$) subfields of hippocampus (Fig. 3B i, ii and iii).

Discussion:

The present study revealed that L-α-AAA microinjection into the CA1 subfield of the hippocampus could impair the passive avoidance memory and could increase the anxiety-like behavior in male rats. The number of GFAP-ir astrocytes in the hippocampus decreased after astrocyte ablation.

The substances such as fluoroacetate, fluorocitrate, methionine sulfoximine, and ethacrynic acid are metabolic inhibitors in astrocytes and in association to memory impairment (Dallérac & Rouach, 2016; Fonnum et al., 1997). It has proven that the gliotoxin, L-α-AAA, impaired medial

prefrontal cortex-depended cognitive functions, the working memory and reversal learning (Lima et al., 2014). Pereira and et al. reported that L-α-Aminoadipic significantly decreased recognition memory, a hippocampal memory, in mice (Pereira et al., 2021). In agreement with these findings, our data showed that inhibition of astrocytes by injection of L-α-AAA diminished passive avoidance memory, a hippocampus-related memory.

Inside the brain glutamine synthetase, a regulator enzyme of the glutamate/glutamine cycle in neurons, is produced by astrocytes (Son et al., 2019). The enzyme produces glutamine, the glutamate neurotransmitter, and involves in learning and memory. Studies revealed that inhibition of the enzyme caused memory impairment (Kant et al., 2014; Kulijewicz Nawrot et al., 2013; Lima et al., 2014; Robinson et al., 2015). L-α-AAA as glutamate analog, inhibits glutamine synthetase (David et al., 2018; Guidetti & Schwarcz, 2003; Pereira et al., 2021). Hence, the cause of memory impairment that we observed in our study may be the inhibition of glutamine synthetase by L-α-AAA.

Glutamatergic system plays a major role in the anxiety disorders (Kaur & Singh, 2017). Banasr et al. reported that L-α-AAA infusion (100 µg/µL) in the rat prefrontal cortex induced anxiety in the novelty suppressed feeding test (NSFT) (Banasr & Duman, 2008). In accordance to this report, we observed that L-α-AAA microinjection into the CA1 subfield of the hippocampus could increase the anxiety-like behavior in the elevated plus maze.

Previous studies have displayed that the number of hippocampal astrocytes increased after different vehicle microinjection (such as dimethyl sulfoxide and normal saline) in rat hippocampus (Emamian et al., 2010; Jahanshahi et al., 2012). Also, it is reported that 2-hydroxypropyl-β-cyclodextrin injection enhanced astrocytic activity (GFAP intensity) in the cerebellum (Jeong et al., 2019). The current study found that intra-CA1 injection of vehicle (6% 2-Hydroxypropyl-β-cyclodextrin) increased the density of GFAP-ir astrocytes in the hippocampus in comparison with control rats. While, L-α-AAA reduced the density of GFAP-ir astrocytes in the hippocampus after

three consecutive daily doses. It is claimed that L-α-AAA downregulates the mRNA expression of GFAP in astrocytes moreover, induces cell death in cultured astrocytes (David et al., 2018; Nishimura et al., 2000). After L-α-AAA injection into the substantia nigra, locus coeruleus and amygdala, it reduced the amount of GFAP-ir astrocytes (Chang et al., 1993; Khurgel et al., 1996). Microinjection of 6.4 nmol L-α-AAA within 3 days caused a transient loss of GFAP-ir astrocytes in the rat hippocampus (Rodríguez et al., 2004). Also, the density of GFAP-ir astrocytes was decreased after microinjection of L-α-AAA (50 and 100 µg/µl) to the rodent pre-limbic and medial prefrontal cortex (Banasr & Duman, 2008; David et al., 2018; Domin et al., 2014; Lee et al., 2013). In another study, L-α-AAA injection (20 µg/µl) leaded to the ablation of GFAP-ir astrocytes in the rat medial prefrontal cortex (Lima et al., 2014). One day after injection of L-α-AAA at the dose of 25 nmol, GFAP mRNA expression was declined in the rat anterior cingulate cortex (Chen et al., 2012). Injection of L-α-AAA (50 µg/µl, 2 injections) to corpus callosum reduced the density of GFAP-ir astrocytes and expression of GFAP in demyelination model mice (Madadi et al., 2019). Alterations of hippocampal astrocytes may be linked to cognitive deficits (David et al., 2019). Moreover, it is reported that the density of GFAP-ir astrocytes was decreased in the pre-limbic cortex and CA3 area of the hippocampus for up to 72 h after administration of L-α-AAA in rodents (David et al., 2019). A more recent study found that ICV injected L-α-Aminoadipate not only reduced the immunoreactivity of GFAP but also declined the S100β, the other marker of astrocytes, in CA1 and CA3 regions. Taken together, these findings support the gliotoxicity of L-α-AAA on hippocampal astrocytes, leading to memory deficiency and anxiety-like behavior. Further, noticing the important role of astrocytes in anxiety-like behaviors will emerge new ideas about anxiety and the brain.

Conclusion:

This study focused on the role of astrocytes in memory function and anxiety-like behaviors and we concluded that L-α-AAA-related inhibited astrocytes impaired passive avoidance memory and increased anxiety-like behavior which can be considered in future therapeutic strategies.

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Highlights

L-α-AAA declined passive avoidance memory function.

L-α-AAA induced anxiety-like behavior when microinjected in CA1 region in rats.

L-α-AAA as an astrocyte-specific toxicity reduced GFAP-ir astrocytes in hippocampus.

Plain Language Summary

Astrocyte are the most prevalent glia inside the brain so understanding their role in disorders should been considered. L-α-AAA causes an astrocyte-specific toxicity and inhibits astrocytes. This research found that L-α-AAA leaded to decline in GFAP (a marker for astrocytes)-immunoreactive astrocytes in hippocampus which followed by impaired passive avoidance memory and induced anxiety-like behavior. This finding confirmed the astrocytes' role in memory and anxiety-like behavior, which are important to future research to reveal the clear mechanism and roles that astrocytes play in hippocampal related memory and behavior.

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Competing Interests:

There are no competing interests to declare.

Data Availability:

All data generated or analyzed during this study are included in this article.

Ethics approval:

All the experiments were in line with the Ethical Committee of Golestan University of Medical Sciences, Gorgan, Iran (Ethics Committee ir.goums.rec. 1394.206).

Consent to participate:

Unusable for animal studies.

Consent to publish:

Unusable for animal studies. All authors agreed to publish the article in this journal.

References:

Acosta, C., Anderson, H. D., & Anderson, C. M. (2017). Astrocyte dysfunction in Alzheimer disease. *J Neurosci Res*, *95*(12), 2430-2447. https://doi.org/https://doi.org/10.1002/jnr.24075

Azami, N. S., Piri, M., Oryan, S., Jahanshahi, M., Babapour, V., & Zarrindast, M. R. (2010). Involvement of dorsal hippocampal α-adrenergic receptors in the effect of scopolamine on memory retrieval in inhibitory avoidance task. *Neurobiol Learn Mem*, *93*(4), 455-462. https://doi.org/https://doi.org/10.1016/j.nlm.2010.01.003

Banasr, M., & Duman, R. S. (2008). Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. *Biol Psychiatry*, *64*(10), 863-870. https://doi.org/https://doi.org/10.1016/j.biopsych.2008.06.008

Beitchman, J. A., Griffiths, D. R., Hur, Y., Ogle, S. B., Bromberg, C. E., Morrison, H. W., . . . Thomas, T. C. (2020). Experimental traumatic brain injury induces chronic glutamatergic dysfunction in amygdala circuitry known to regulate anxiety-like behavior. *Frontiers in neuroscience*, *13*, 1434.

Bronzuoli, M. R., Facchinetti, R., Steardo, L., Romano, A., Stecca, C., Passarella, S., . . . Scuderi, C. (2018). Palmitoylethanolamide Dampens Reactive Astrogliosis and Improves Neuronal Trophic Support in a Triple Transgenic Model of Alzheimer's Disease: In Vitro and In Vivo Evidence. *Oxid Med Cell Longev*, *2018*, 4720532. https://doi.org/https://doi.org/10.1155/2018/4720532

Chang, F. W., Wang, S. D., Lu, K. T., & Lee, E. H. (1993). Differential interactive effects of gliotoxin and MPTP in the substantia nigra and the locus coeruleus in BALB/c mice. *Brain Res Bull*, *31*(3-4), 253-266. https://doi.org/https://doi.org/10.1016/0361-9230(93)90215-w

Chen, F. L., Dong, Y. L., Zhang, Z. J., Cao, D. L., Xu, J., Hui, J., . . . Gao, Y. J. (2012). Activation of astrocytes in the anterior cingulate cortex contributes to the affective component of pain in an inflammatory pain model. *Brain Res Bull*, *87*(1), 60-66. https://doi.org/https://doi.org/10.1016/j.brainresbull.2011.09.022

Dallérac, G., & Rouach, N. (2016). Astrocytes as new targets to improve cognitive functions. *Prog Neurobiol*, *144*, 48-67. https://doi.org/https://doi.org/10.1016/j.pneurobio.2016.01.003

David, J., Gormley, S., McIntosh, A. L., Kebede, V., Thuery, G., Varidaki, A., . . . Harkin, A. (2019). L-alpha-amino adipic acid provokes depression-like behaviour and a stress related increase in dendritic spine density in the pre-limbic cortex and hippocampus in rodents. *Behav Brain Res*, *362*, 90-102. https://doi.org/https://doi.org/10.1016/j.bbr.2019.01.015

David, J., O'Toole, E., O'Reilly, K., Thuery, G., Assmann, N., Finlay, D., & Harkin, A. (2018). Inhibitors of the NMDA-Nitric Oxide Signaling Pathway Protect Against Neuronal Atrophy and Synapse Loss Provoked by l-alpha Aminoadipic Acid-treated Astrocytes. *Neuroscience*, *392*, 38-56. https://doi.org/https://doi.org/10.1016/j.neuroscience.2018.09.023

Domin, H., Szewczyk, B., Woźniak, M., Wawrzak-Wleciał, A., & Śmiałowska, M. (2014). Antidepressant-like effect of the mGluR5 antagonist MTEP in an astroglial degeneration

model of depression. *Behav Brain Res*, *273*, 23-33. https://doi.org/https://doi.org/10.1016/j.bbr.2014.07.019

Emamian, S., Naghdi, N., Sepehri, H., Jahanshahi, M., Sadeghi, Y., & Choopani, S. (2010). Learning impairment caused by intra-CA1 microinjection of testosterone increases the number of astrocytes. *Behav Brain Res*, *208*(1), 30-37. https://doi.org/https://doi.org/10.1016/j.bbr.2009.11.004

Fonnum, F., Johnsen, A., & Hassel, B. (1997). Use of fluorocitrate and fluoroacetate in the study of brain metabolism. *Glia*, *21*(1), 106-113.

Guidetti, P., & Schwarcz, R. (2003). Determination of α-aminoadipic acid in brain, peripheral tissues, and body fluids using GC/MS with negative chemical ionization. *Mol Brain Res*, *118*(1-2), 132-139. https://doi.org/https://doi.org/10.1016/j.molbrainres.2003.08.004

Guillamón Vivancos, T., Gómez Pinedo, U., & Matías Guiu, J. (2015). Astrocytes in neurodegenerative diseases (I): function and molecular description. *Neurología (English Edition)*, *30*(2), 119-129. https://doi.org/https://doi.org/10.1016/j.nrl.2012.12.007

Hosseini, S., Michaelsen-Preusse, K., Grigoryan, G., Chhatbar, C., Kalinke, U., & Korte, M. (2020). Type I interferon receptor signaling in astrocytes regulates hippocampal synaptic plasticity and cognitive function of the healthy CNS. *Cell Reports*, *31*(7).

Jahanshahi, M., Azami, N. S., & Nickmahzar, E. (2012). Effect of Scopolamine-based Amnesia on the Number of Astrocytes in the Rat's Hippocampus. *Int J Morphol*, *30*(2), 388-393.

Jahanshahi, M., Nikmahzar, E., Elyasi, L., Babakordi, F., & Hooshmand, E. (2018). α2-Adrenoceptor-ir neurons' density changes after single dose of clonidine and yohimbine administration in the hippocampus of male rat. *Int J Neurosci*, *128*(5), 404-411. https://doi.org/https://doi.org/10.1080/00207454.2017.1389926

Jahanshahi, M., Sadeghi, Y., Hosseini, A., Naghdi, N., & Marjani, A. (2008). The effect of spatial learning on the number of astrocytes in the CA3 subfield of the rat hippocampus. *Singapore Med J*, *49*(5), 388-391.

Jahanshahi, M., Sadeghi, Y., Hosseini, A., Naghdi, N., & Piriaie, A. (2008). Working memory learning method and astrocytes number in different subfields of rat's Hippocampus. *Pak J Biol Sci*, *3*(1), 28-31. https://doi.org/https://doi.org/10.3923/pjbs.2007.3964.3966

Jeong, M. S., Bae, J. S., & Jin, H. K. (2019). Vascular endothelial growth factor improves the therapeutic effects of cyclodextrin in Niemann-Pick type C mice. *Anim Cells Syst*, *23*(5), 346-354. https://doi.org/https://doi.org/10.1080/19768354.2019.1651768

Kant, D., Tripathi, S., Qureshi, M. F., Tripathi II, S., Pandey, S., Singh, G., . . . Jha, S. K. (2014). The effect of glial glutamine synthetase inhibition on recognition and temporal memories in the rat. *Neurosci Lett*, *560*, 98-102. https://doi.org/https://doi.org/10.1016/j.neulet.2013.12.033

Karimi, S., Jahanshahi, M., & Golalipour, M. J. (2014). The effect of MDMA-induced anxiety on neuronal apoptosis in adult male rats' hippocampus. *Folia Biol*, *60*(4), 187-191.

Kaur, S., & Singh, R. (2017). Role of different neurotransmitters in anxiety: a systemic review. *International Journal of Pharmaceutical Sciences and Research*, *8*(2), 411.

Khurgel, M., Koo, A. C., & Ivy, G. O. (1996). Selective ablation of astrocytes by intracerebral injections of α-aminoadipate. *Glia*, *16*(4), 351-358. https://doi.org/https://doi.org/10.1002/(SICI)1098-1136(199604)16:4<351::AID-GLIA7>3.0.CO;2-2

Kulijewicz Nawrot, M., Syková, E., Chvátal, A., Verkhratsky, A., & Rodríguez, J. J. (2013). Astrocytes and glutamate homoeostasis in Alzheimer's disease: a decrease in glutamine synthetase, but not in glutamate transporter-1, in the prefrontal cortex. *ASN neuro*, *5*(4), AN20130017. https://doi.org/https://doi.org/10.1042/AN20130017

Lee, Y., Son, H., Kim, G., Kim, S., Lee, D. H., Roh, G. S., . . . Kim, H. J. (2013). Glutamine deficiency in the prefrontal cortex increases depressive-like behaviours in male mice. *J Psychiatry Neurosci*, *38*(3), 183-191. https://doi.org/https://doi.org/10.1503/jpn.120024

Leitão, C. I. M. (2018). *Impact of an astrocytic pathology on hippocampal memory* Universidade de Coimbra].

Li, D., Liu, X., Liu, T., Liu, H., Tong, L., Jia, S., & Wang, Y. F. (2020). Neurochemical regulation of the expression and function of glial fibrillary acidic protein in astrocytes. *Glia*, *68*(5), 878-897.

Lima, A., Sardinha, V. M., Oliveira, A., Reis, M., Mota, C., Silva, M., . . . Sousa, N. (2014). Astrocyte pathology in the prefrontal cortex impairs the cognitive function of rats. *Mol Psychiatry*, *19*(7), 834-841. https://doi.org/https://doi.org/10.1038/mp.2013.182

Madadi, S., Pasbakhsh, P., Tahmasebi, F., Mortezaee, K., Khanehzad, M., Boroujeni, F. B., . . . Kashani, I. R. (2019). Astrocyte ablation induced by La-aminoadipate (L-AAA) potentiates remyelination in a cuprizone demyelinating mouse model. *Metab Brain Dis*, *34*(2), 593-603. https://doi.org/https://doi.org/10.1007/s11011-019-0385-9

Mahakizadeh, S., Jahanshahi, M., Haidari, K., & Shahbazi, M. (2015). Dopamine Receptors Gene Expression in Male Rat Hippocampus after Administration of MDMA (Ecstasy). *Int J Morphol*, *33*(1), 301-308.

Moghadami, S., Jahanshahi, M., Sepehri, H., & Amini, H. (2016). Gonadectomy reduces the density of androgen receptor-immunoreactive neurons in male rat's hippocampus: testosterone replacement compensates it. *Behav Brain Funct*, *12*(1), 5. https://doi.org/https://doi.org/10.1186/s12993-016-0089-9

Navarrete, M., & Araque, A. (2014). The Cajal school and the physiological role of astrocytes: a way of thinking. *Frontiers in neuroanatomy*, *8*, 33.

Nikmahzar, E., Jahanshahi, M., Elyasi, L., Saeidi, M., Babakordi, F., & Bahlakeh, G. (2019). Human chorionic gonadotropin attenuates amyloid-β plaques induced by streptozotocin in the rat brain by affecting cytochrome c-ir neuron density. *Iran J Basic Med Sci*, *22*, 166-172. https://doi.org/https://doi.org/10.22038/ijbms.2018.31412.7569

Nikmahzar, E., Jahanshahi, M., Ghaemi, A., Naseri, G. R., Moharreri, A. R., & Lotfinia, A. A. (2016). Hippocampal serotonin-2A receptor-immunoreactive neurons density increases after testosterone therapy in the gonadectomized male mice. *Anat Cell Biol*, *49*(4), 259-272. https://doi.org/https://doi.org/10.5115/acb.2016.49.4.259

Nishimura, R. N., Santos, D., Fu, S. T., & Dwyer, B. E. (2000). Induction of cell death by L-alpha-aminoadipic acid exposure in cultured rat astrocytes: relationship to protein synthesis. *Neurotoxicology*, *21*(3), 313-320.

Olsen, M., Aguilar, X., Sehlin, D., Fang, X. T., Antoni, G., Erlandsson, A., & Syvänen, S. (2018). Astroglial Responses to Amyloid-Beta Progression in a Mouse Model of Alzheimer's Disease. *Mol Imaging Biol*, *20*(4), 605-614. https://doi.org/https://doi.org/10.1007/s11307-017-1153-z

Paxinos, G., & Watson, C. (2007). *The rat brain in stereotaxic coordinates*. Academic Press.

Pereira, M. F., Amaral, I. M., Lopes, C., Leitão, C., Madeira, D., Lopes, J. P., . . . Agostinho, P. (2021). l-α-aminoadipate causes astrocyte pathology with negative impact on mouse hippocampal synaptic plasticity and memory. *The FASEB Journal*, *35*(8), e21726.

Robinson, S. R., Lee, A., Bishop, G. M., Czerwinska, H., & Dringen, R. (2015). Inhibition of astrocytic glutamine synthetase by lead is associated with a slowed clearance of hydrogen peroxide by the glutathione system. *Front Integr Neurosci*, *9*, 61. https://doi.org/https://doi.org/10.3389/fnint.2015.00061

Rodríguez, M. J., Martínez Sánchez, M., Bernal, F., & Mahy, N. (2004). Heterogeneity between hippocampal and septal astroglia as a contributing factor to differential in vivo AMPA excitotoxicity. *J Neurosci Res*, *77*(3), 344-353. https://doi.org/https://doi.org/10.1002/jnr.20177

Santello, M., Toni, N., & Volterra, A. (2019). Astrocyte function from information processing to cognition and cognitive impairment. *Nature neuroscience*, *22*(2), 154-166.

Schousboe, A., Bak, L. K., & Waagepetersen, H. S. (2013). Astrocytic control of biosynthesis and turnover of the neurotransmitters glutamate and GABA. *Frontiers in endocrinology*, *4*, 102.

Seifhosseini, S., Jahanshahi, M., Moghimi, A., & Aazami, N. S. (2011). The effect of scopolamine on avoidance memory and hippocampal neurons in male Wistar rats. *Basic Clin Neurosci*, *3*(1), 9-15.

Shaabani, R., Jahanshahi, M., Nowrouzian, M., Sadeghi, Y., & Azami, N. (2011). Effect of morphine based CPP on the hippocampal astrocytes of male Wistar rats. *Asian J Cell Biol*, *6*(3), 89-96.

Shang, X. L., Wang, Q. B., Liu, X. P., Yao, X. Q., Cao, F. Y., Wang, Q., . . . Liu, G. P. (2015). Fluorocitrate induced the alterations of memory-related proteins and tau hyperphosphorylation in SD rats. *Neurosci Lett*, *584*, 230-235. https://doi.org/https://doi.org/10.1016/j.neulet.2014.10.036

Son, H., Kim, S., Jung, D.-h., Baek, J. H., Lee, D. H., Roh, G. S., . . . Lee, D. K. (2019). Insufficient glutamine synthetase activity during synaptogenesis causes spatial memory impairment in adult mice. *Scientific reports*, *9*(1), 1-9.

Soung, A., & Klein, R. S. (2020). Astrocytes: initiators of and responders to inflammation. *Glia in Health and Disease*, *89760*(10.5772).

Suzuki, A., Stern, S. A., Bozdagi, O., Huntley, G. W., Walker, R. H., Magistretti, P. J., & Alberini, C. M. (2011). Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell*, *144*(5), 810-823. https://doi.org/https://doi.org/10.1016/j.cell.2011.02.018

Tarasov, V. V., Svistunov, A. A., Chubarev, V. N., Sologova, S. S., Mukhortova, P., Levushkin, D., . . . Aliev, G. (2020). Alterations of astrocytes in the context of schizophrenic dementia. *Frontiers in pharmacology*, *10*, 1612.

Vesce, S., Rossi, D., Brambilla, L., & Volterra, A. (2007). Glutamate release from astrocytes in physiological conditions and in neurodegenerative disorders characterized by neuroinflammation. *International review of neurobiology*, *82*, 57-71.

Zorec, R., Horvat, A., Vardjan, N., & Verkhratsky, A. (2015). Memory formation shaped by astroglia. *Front Integr Neurosci*, *9*, 56. https://doi.org/https://doi.org/10.3389/fnint.2015.00056

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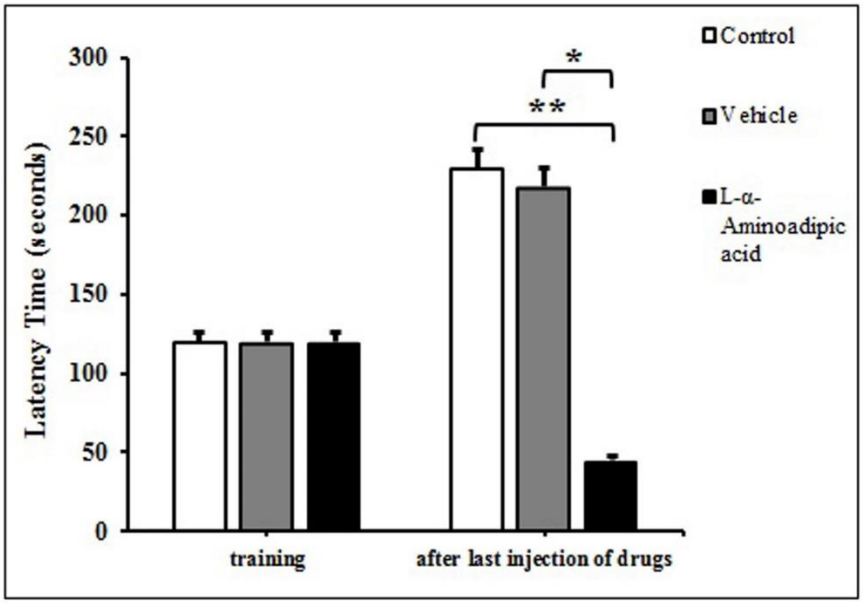

Fig. 1 L-α-AAA impaired passive avoidance memory. L-α-AAA administration into the CA1 subfield of hippocampus on the passive avoidance memory at training step and 24 h after the last drug injection revealed that L-α-AAA could decline the step through latency time in compare to control and vehicle groups. The data are expressed as Mean ± SD. * P<0.05 and** P<0.01 means significant. One-way ANOVA and LSD post-hoc test. n=7 per group. L-α-AAA; L-α-Aminoadipic acid

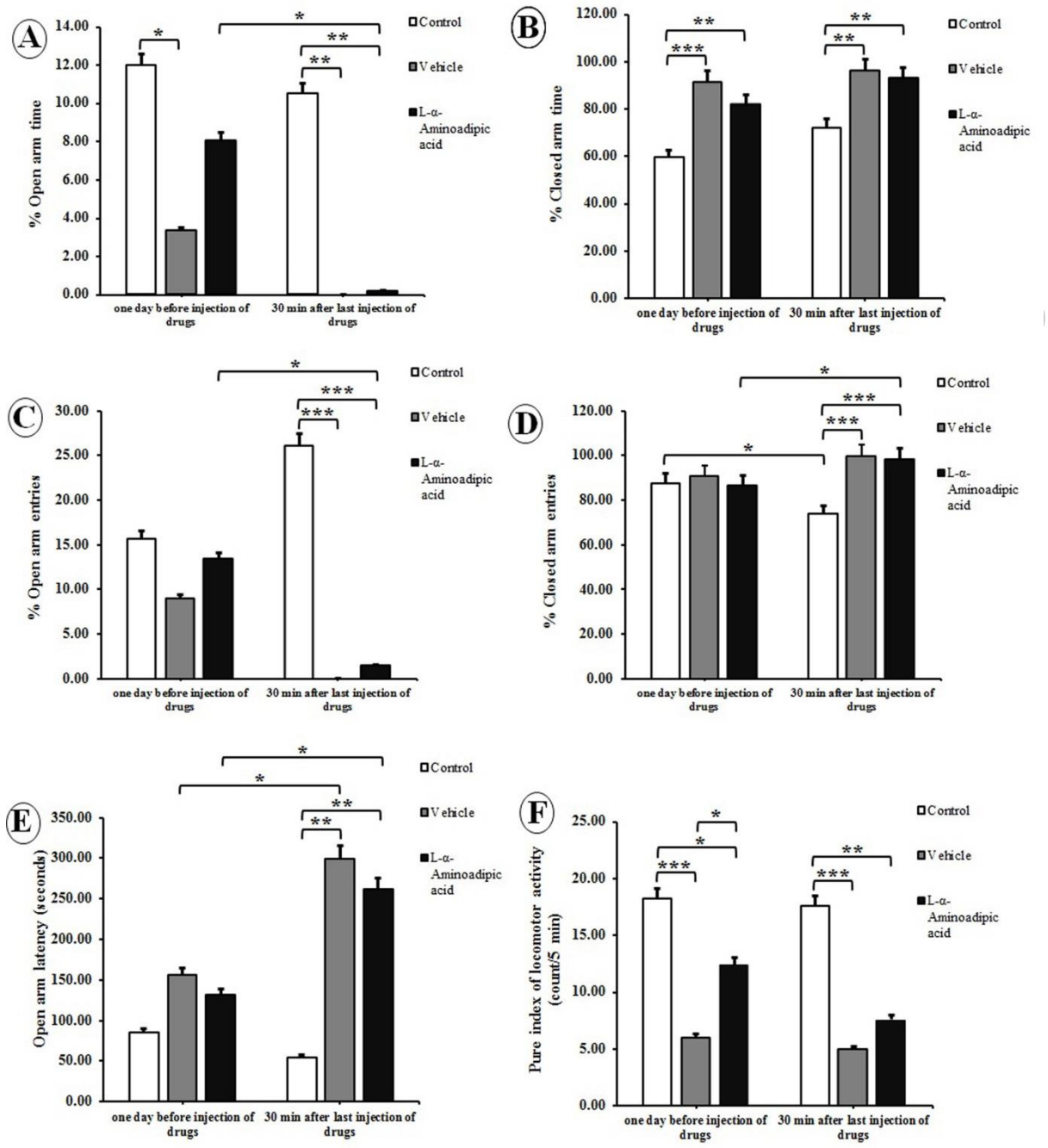

Fig. 2 L-α-AAA induced anxiety-like behavior. Anxiety-related behavior in rat at one day before injection of L-α-AAA and 30 minutes after last injection of L-α-AAA in the elevated plus maze test. Parameters for assessing anxiety included percentage of the time spent in open (A) and closed (B) arms, percentage of open (C) and closed (D) arm entries, open arm latency (E), and pure index of locomotor activity (F). The data are expressed as mean ± SD. * P<0.05, ** P<0.01 and *** P<0.001 means significant. Also, the alternations were significant when compared to the control group (A-E). One-way ANOVA and LSD post-hoc test. n=7 per group. L-α-AAA; L-α-Aminoadipic acid.

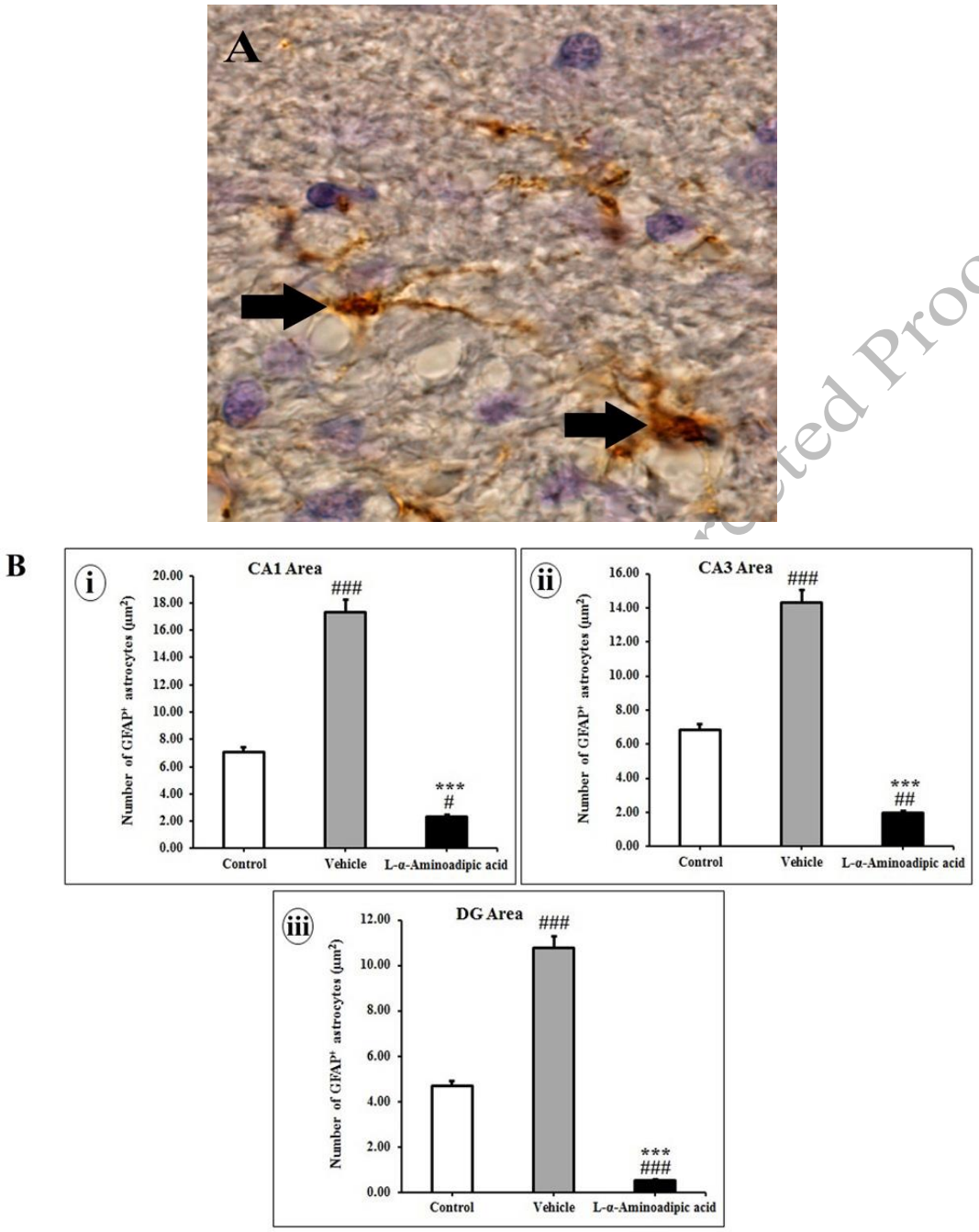

Fig. 3 L-α-AAA reduced GFAP-ir (A) Immunohistochemistry staining for GFAP expressing astrocytes in coronal sections of rat hippocampus. GFAP-ir astrocytes were observed in brown color (black arrows demarcated) in the hippocampus (×100). (B) L-α-AAA injection significantly reduced the number of GFAP-ir astrocytes in the CA1 (i), CA3 (ii) and DG (iii) subfields of hippocampus. # $P<0.05$, ## $P<0.01$ and ### $P<0.001$ as compared to the control group; *** $P<0.001$ as compared to the vehicle group. Data expressed as mean ± SD. One-way ANOVA and LSD post-hoc test. n=7 per group. L-α-AAA; L-α-Aminoadipic acid.