Life Sciences 238 (2019) 116969

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

# Effect of ceftriaxone on paired-pulse response and long-term potentiation of hippocampal dentate gyrus neurons in rats with Alzheimer-like disease

Nasrin Hamidi[a,1], Abdollah Nozad[a,1], Hamid Sheikhkanloui Milan[a], Ali-Akbar Salari[b], Mohammad Amani[a,c,*]

[a] Department of Physiology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran
[b] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Department of Pathology & Cell Biology and the Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University, New York, NY, 10032, USA

ARTICLE INFO

*Keywords:*
Ceftriaxone
Okadaic acid
Synaptic plasticity
Dentate gyrus
Alzheimer's disease

ABSTRACT

*Aims:* Glutamatergic dysfunction is posed as a main stage in neurodegenerative disorders such as Alzheimer's disease (AD). Glutamate-mediated excitotoxicity contributes to cognitive dysfunction and cell death in AD. Ceftriaxone (CFT), a well-known upregulator of GLT-1, selectively induces the expression of glutamate transporter-1 (GLT-1) in different brain regions and therefore can be posed as a potential candidate for elimination of glutamate-induced excitotoxicity which is an early prominent event in AD brains. This study was designed to investigate the electrophysiological and behavioral effects of the β-lactam antibiotic ceftriaxone in okadaic acid (OKA)-induced model of AD.

*Materials and methods:* Male Wistar rats divided into four control, ceftriaxone (CFT), OKA, and OKA plus ceftriaxone (OKA + CFT) groups. OKA was injected intracerebroventricularly (i.c.v., 200 ng/5 μl) into lateral ventricles and after two weeks the evoked field potential recorded from hippocampal perforant path-DG synapses in order to evaluate the effect of ceftriaxone treatment (200 mg/kg/day, i.p.) on long-term potentiation (LTP) and paired-pulse responses.

*Key findings:* Results of this study revealed that ceftriaxone treatment significantly ameliorates the OKA-induced attenuation of field excitatory post-synaptic potential (fEPSP) slope and population spike (PS) amplitude following high-frequency stimulation and paired-pulse paradigm indicating its beneficial effects on both short-term and long-term plasticity in these neurons. Ceftriaxone also has an improving effect on OKA-induced impairment in short- and long-term memories evaluated by alternation behavior and passive avoidance tasks in rats.

*Significance:* Therefore, this study suggests that GLT-1 might be a promising therapeutic target for treatment of neurodegenerative disorders such as AD in the future.

## 1. Introduction

Glutamatergic systems play critical roles in cognition, and dysfunction of glutamatergic neurons can underlie many psychological and neurodegenerative disorders [1,2]. Glutamate mediates fast excitatory neurotransmission in part through activation of N-methyl-D-aspartate (NMDA) receptors and $Ca^{2+}$ influx, which in turn initiate a cascade of events that ultimately lead to synaptic plasticity and memory formation [3]. Previous studies highlight the involvement of glutamatergic systems in early phases of neurodegenerative disorders such as Alzheimer's disease (AD) [4]. In AD, increased release of glutamate from presynaptic neurons and astrocytes, and decreased glutamate removal from the synaptic space give rise to over-stimulation of glutamate receptors and increases in the production of β-amyloid (Aβ) peptide, cell death and finally deterioration of learning and memory [5]. Increased Aβ levels can also induce further glutamate release from presynaptic neurons which exacerbates the excitotoxicity and results in the formation of neurofibrillary tangles (NFTs) [6]. Extracellular deposits of Aβ and intracellular NFTs are two main hallmarks of AD responsible for many pathological changes including impairment in hippocampal long-term potentiation (LTP) and facilitation of long-term depression (LTD), the two forms of synaptic plasticity associated with learning and memory [7,8].

A large body of work has shown the glutamate-mediated

* Corresponding author. Department of Physiology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, 56189-53141, Iran.
*E-mail address:* m.amani@outlook.com (M. Amani).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.lfs.2019.116969
Received 20 September 2019; Received in revised form 10 October 2019; Accepted 14 October 2019
Available online 16 October 2019
0024-3205/ 

excitotoxicity and neurodegeneration are major contributors to cognitive dysfunction and cell death in AD. There is evidence that impairments in mechanisms of glutamate uptake contribute to excitotoxic processes described above. Specifically, a decrease in glutamate uptake function has been correlated with down-regulation of glutamate transporters in different regions of the AD brain [9]. Five glutamate transporters (GLTs), also called excitatory amino acid transporters (EAAT1-5), have been identified each of which can act to regulate levels of released glutamate in synapses [10]. Glutamate transporter-1 (GLT-1), also known as EAAT2, is the predominant glutamate transporter in neocortex and hippocampus being responsible for 80–90% of glutamate uptake in these regions [11]. This transporter is primarily expressed in perisynaptic processes of astrocytes and is believed to have a pivotal role in the elimination of excessive glutamate from synaptic spaces. Reduced expression of GLT-1 protein has been reported to be an early and prominent event in AD brains and likely contributes to increases in the production of Aβ [12].

It has been well stablished that ceftriaxone (CFT), a β-lactam antibiotic, selectively induces the up-regulation of glutamate transporter-1 (GLT-1) in different brain regions such as frontal cortex, hippocampus, amygdala and thalamus [13,14]. Accordingly, a logical therapeutic strategy to combat the onset and development of AD pathology would be to target glutamate uptake functions and thereby maintain synaptic glutamate at non-pathological levels. The present study was designed to test this approach and, specifically, to investigate whether administration of ceftriaxone could offset the emergence of behavioral and synaptic plasticity impairments in an animal model of AD. Specifically, we examined the effects of ceftriaxone in the okadaic acid (OKA)-induced AD model. In this model, intracerebroventricular (i.c.v.) OKA treatment leads to neuroinflammation, oxidative stress, cholinergic dysfunction, neuroinflammation, mitochondrial dysfunction, glutamate-mediated excitotoxicity, and impairments in learning and memory. Phosphorylation of tau protein and GSK3β and thereby formation of NFTs and extracellular β-amyloid deposits as well as the consecutive neuronal loss provide an AD like neuropathology in this model [15,16]. In this electrophysiological study, we have evaluated the long-term synaptic plasticity of neurons by measuring LTP, a long-lasting increase in synaptic strength, in hippocampal DG neurons. LTP has been proposed as a model responsible for consolidation of long-term memory with proposed molecular mechanisms which can provide the level of stability needed to maintain memories for months or longer. Therefore, we explored the effect of ceftriaxone on paired-pulse paradigm and long-term potentiation of hippocampal dentate gyrus (DG) neurons, which are involved in spatial pattern separation, together with the evaluation of short- and long-term memory in OKA-treated rats.

## 2. Materials and methods

### 2.1. Animals and ethics

Male Wistar rats weighing 300–350 g maintained under standard housing conditions at an ambient temperature of 21–25 °C with food and water ad libitum. All experimental procedures were approved by the Ethics Committee of Ardabil University of Medical Sciences (GN-9423) and were performed in accordance with relevant guidelines and regulations.

### 2.2. Treatments

Animals were divided into four control (vehicle), ceftriaxone (CFT), OKA, and OKA plus ceftriaxone (OKA + CFT) groups (n = 10 in each group). OKA (Sigma-Aldrich, St.Louis, MO, USA) dissolved in sterile artificial cerebrospinal fluid (aCSF: 147 mM NaCl, 2.9 mM KCl, 1.6 mM MgCl2, 1.7 mM CaCl2 and 2.2 mM dextrose) according to the manufacturer's instructions and injected once (200 ng/5 μl) into lateral ventricles. The control group only received the same volume of aCSF as a vehicle [17]. Ceftriaxone was dissolved in 0.9% sodium chloride (saline) and administered intraperitoneally (i.p.) at the dose of 200 mg/kg/day beginning 1 h before i.c.v. administaration of OKA up to two weeks (Fig. 1).

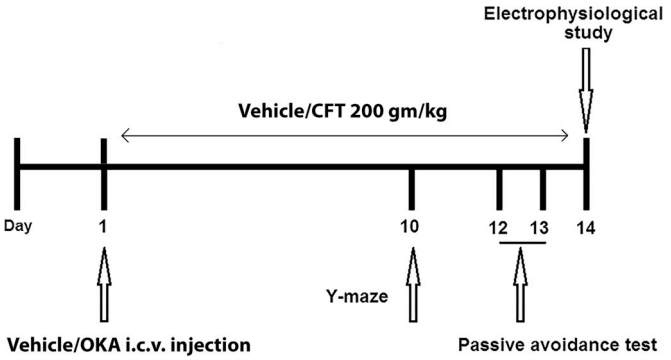


**Fig. 1.** Design of experiments. Rats were administered with OKA on the 1st day and treatment with ceftriaxone or vehicle began 1 h before a single bilateral i.c.v. microinjection of OKA and continued daily for two weeks. The animal then underwent short-term spatial memory evaluation on the 10th day using Y-maze test. Passive avoidance task also conducted for evaluation of long-term spatial memory performed on days 12 and 13 and the electrophysiological studies started one day after the last behavioral test at 14th day of OKA injection. After that, the animal was sacrificed for histological assessments.

In order to i.c.v. injection of OKA or its related vehicle, animals were intraperitoneally anesthetized with chloral hydrate (350 mg/kg) and placed in a stereotaxic instrument (Stoelting Co., Illinois, USA). A longitudinal incision was made through the scalp and two small holes drilled for inserting injection cannula into the lateral ventricles (AP = −0.8, ML = 1.6 and DV = 3.5 mm below dura) [18]. I.c.v. infusion of OKA or the vehicle carried out during 5 min using a Hamilton microsyringe at a rate of 1 μl/min and the injection needle was left in the place for another 5 min before being slowly withdrawn to avoid backflow [17].

### 2.3. Electrophysiology

Two weeks after OKA or vehicle administration and following urethane anesthesia (1.8 g/kg, i.p.), animal's head was fixed in a stereotaxic apparatus. Details of electrophysiological methods have been described in our previous studies [17,19]. Briefly, the skull was exposed and a stimulating and recording electrodes (stainless steel, 0.125 mm diameter, Advent, UK) were lowered slowly (0.2 mm/min) into perforant pathway (AP = −8.1 mm; ML = 4.3 mm; DV = 3.2 mm) and DG (AP = −3.8 mm; ML = 2.3 mm; DV = 2.7–3.2 mm) until obtaining the maximal response [18]. The signals were filtered (1 Hz–3 kHz band pass) and passed to a computer through an analogue to digital interface (eLab 2, Sciencebeam, Iran). Input/output (I/O) function of the neurons obtained using different stimulus currents (0.1–1 mA) at the frequency of 0.1 Hz and paired-pulse responses also were evaluated with inter-stimulus intervals of 10, 20, 30, 50, 70, 100 and 120 ms [20]. Baseline activity was recorded using single stimuli delivered at 0.01 Hz with stimulus intensity that produced 40% of the maximum fEPSP and LTP was induced using 400 Hz high-frequency stimulation (HFS) protocol (10 bursts of 20 stimuli and 10 s inter-burst intervals with stimulus duration of 200 μs and intensity increased by 80% of baseline level). The PS amplitude was measured as the average of the difference in voltage between the peak of the first positive wave and the peak of the first negative deflection and the difference in voltage between the peak of the second positive wave and the peak of the first negative deflection. The fEPSP slope was measured as the maximum slope of first positive wave in order to measure synaptic efficacy [17]. All of the data (60 min) after HFS used for statistical comparisons.

### 2.4. Behavioral studies

#### 2.4.1. Y-maze task

Short-term spatial memory was evaluated by monitoring spontaneous alternation behavior in a black Plexiglas Y-maze (each arm 40 * 30 * 15 cm). The experiment details were the same as that described previously as follows: the rat was placed at one of the arms and the movements through the maze monitored visually for a 8-min session. Complete entries into the three arms on overlapping triplet sets were considered as a successful alternation and the alternation behavior calculated as the percentage of actual alternations [21].

#### 2.4.2. Passive avoidance learning

The detailed methods for passive avoidance learning test have been described in our earlier study [17]. Briefly, after habituation in a light/dark chamber, rats were put in light chamber for training trial. Immediately after entering to the dark chamber, a 1.5 mA, 50 Hz foot shock was delivered for 2 s leaving the rat in the dark chamber for more 20 s. The trial was repeated after 2 min and thereafter the rat was placed in the light chamber. Staying in the light chamber for 2 min recorded as a successful learning of the task, otherwise the trial was repeated until successive avoidance learning. Twenty-four hours later the animal was placed in the bright chamber for 5 min. During this time the step-through latency (STL) was evaluated as a recalling criteria after 24 h [17,22].

### 2.5. Histology

Animals were perfused transcardially with a cold 4% paraformaldehyde solution and after decapitation, the brain removed and fixed in the same paraformaldehyde solution for 24 h at room temperature. Subsequently, coronal sections with the thickness of 5 μm were cut through the brain using a microtome and mounted on poly-L-lysine–coated glass slides and then the sections were used for evaluating the injection site.

### 2.6. Statistical analysis

Statistical analysis of electrophysiological and behavioral data conducted using independent sample *t*-test, two-way mixed ANOVA and one-way ANOVA followed by Tukey *post-hoc* test for comparison of means between different groups. Kruskal Wallis non-parametric test followed by Mann-Whitney U performed for comparison of step-through latencies between groups in passive avoidance test. A p-value of less than 0.05 was regarded as significant. Data are expressed as means ± S.E.M. IBM SPSS Statistics software (version 20) was used for all analyses.

## 3. Results

### 3.1. Electrophysiology

In this study, the input-output (I/O) responses were assessed by delivering variety of stimulus current (0.1–1 μA) for evaluation of synaptic potency prior to LTP induction. The responses show the dependence of neural action potentials to excitatory input. Two-way mixed ANOVA indicated that the stimulus-response curves recorded from DG before the paired-pulse or high-frequency stimulations were not significantly different in fEPSP slope ($F(3,24) = 0.32$, $p = 0.80$) and PS amplitude ($F(3,24) = 0.57$, $p = 0.63$) between groups (Fig. 2). Regarding these results, the possible changes in recorded paired-pulse activity or LTP would be independent of neural transmission or basal synaptic strength.

After a steady baseline recording, LTP was induced using HFS stimulation protocol and a 10% increase in fEPSP slope and 25% in PS amplitude were recognized as LTP induction criteria. Comparison of

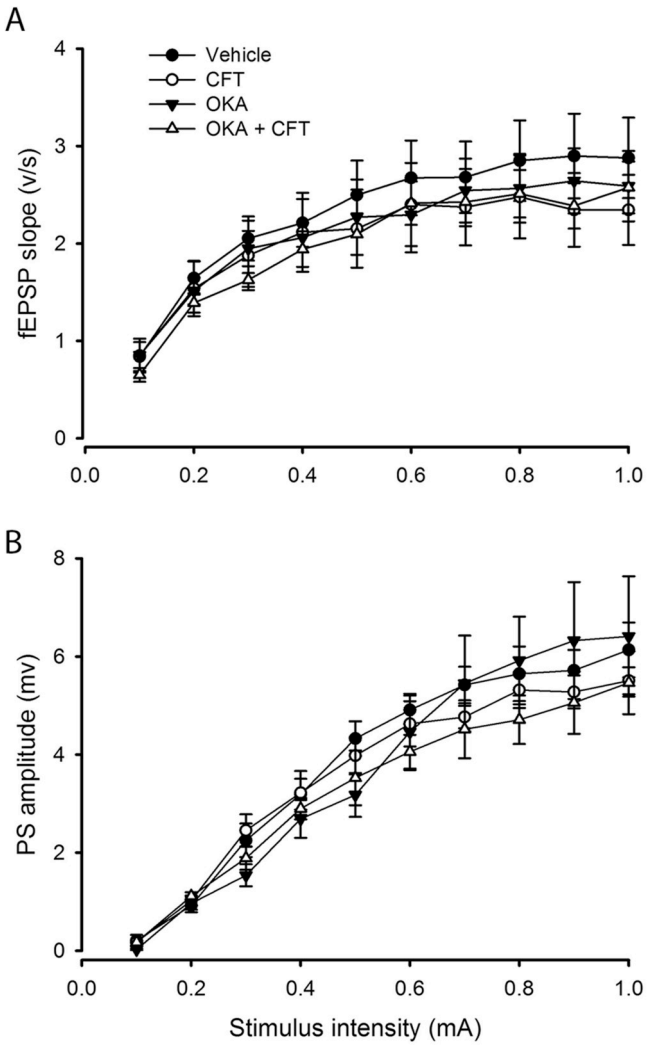


**Fig. 2.** Input-output curves (mean ± SEM) of (A) the fEPSP slope and (B) the PS amplitude in the dentate gyrus. Input/output function of the neurons obtained using different stimulus currents (0.1–1 mA) at the frequency of 0.1 Hz. There was no statistically significant difference between groups, n = 7 in each group.

data recorded before HFS did not show any significant difference between the groups in terms of EPSP slope or PS amplitude and also injection of OKA did not have significant effect on baseline recordings from DG neurons. Independent samples *t*-test also showed no significant difference in fEPSP slope ($t(10) = 0.98$, $p = 0.35$) and PS amplitude ($t(12) = 0.83$, $p = 0.42$) after HFS between the control and ceftriaxone groups (Fig. 3). Although, a small decrease in fEPSP slope and PS amplitude was seen in the ceftriaxone group in comparison with the control group but they were not statistically significant. On the other hand, one-way ANOVA showed a significant difference in fEPSP slope ($F(3,22) = 9.19$; $p < 0.001$) and PS amplitude ($F(3,22) = 6.45$; $p < 0.01$) between the control, OKA, and OKA + CFT groups. In the current study, i.c.v. injection of OKA led to a robust decrease in fEPSP slope ($p < 0.001$) and PS amplitude ($p < 0.01$) comparing with the control group and administration of ceftriaxone showed an improving effect on recorded evoked potentials in a way that there was no significant difference in fEPSP slope ($p = 0.24$) and PS amplitude ($p = 0.37$) between the OKA + CFT and control groups. A significant increase in fEPSP slope was shown in OKA + CFT group comparing to the OKA group ($p < 0.05$) (Fig. 4).

We evaluated the short-term plasticity and probability of presynaptic involvement in induction of long-term potentiation in DG

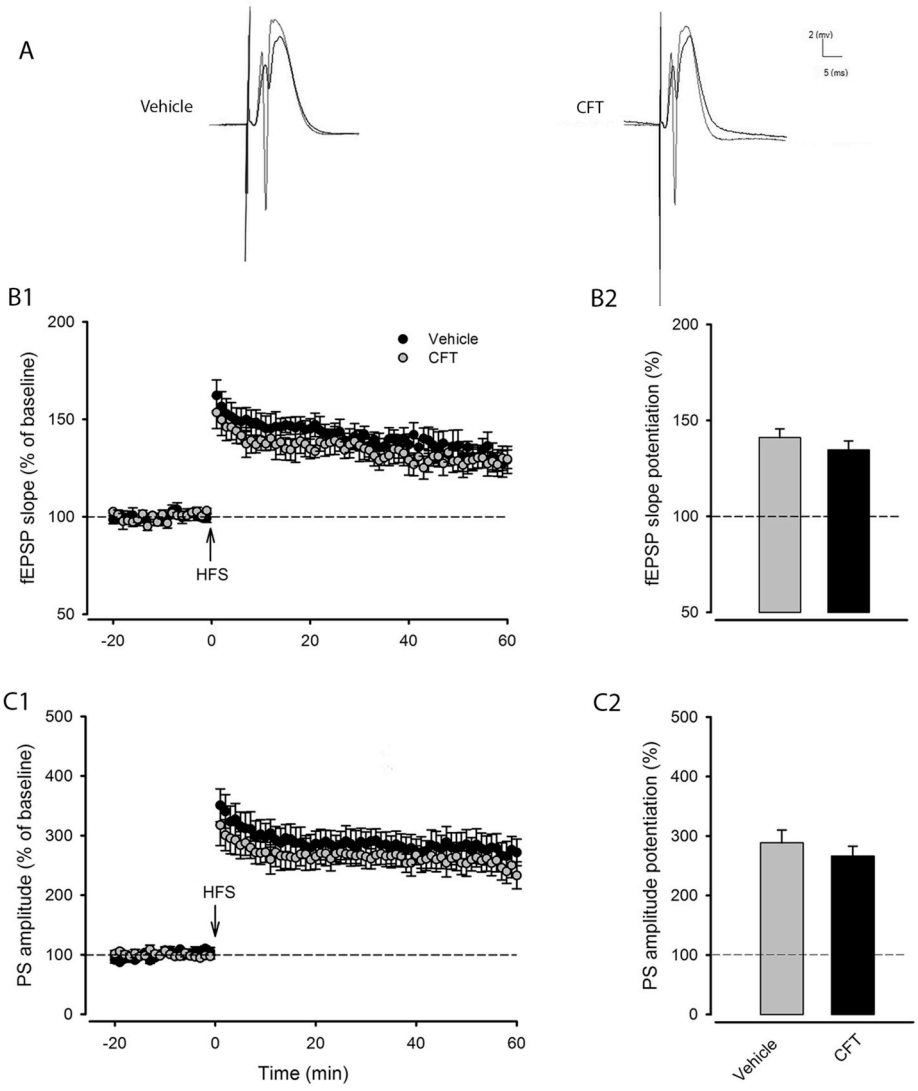


**Fig. 3.** Effect of ceftriaxone treatment on HFS-induced LTP of hippocampal dentate gyrus neurons. (A) Representative superimposed fEPSPs and PSs during baseline (black) and 5 min after (gray) HFS. (B1) The effect of ceftriaxone treatment on LTP of hippocampal dentate gyrus neurons using 400 Hz tetanic stimulation at the fEPSP slope and (C1) PS amplitude. (B2, C2) Summary bar charts show mean values of fEPSP slope or PS amplitude (%) measured 60 min following HFS. The horizontal dashed line represents the baseline value and the data are plotted as the average percentage changes from baseline responses. Results showed no significant difference in fEPSP slope ($p = 0.35$) or PS amplitude ($p = 0.42$) after between groups ($n = 7$ in each group).

neurons by delivering paired-pulse stimulation at performant path-DG synapses. As in Fig. 5, paired-pulse stimulation applied with intervals of 10, 20, 30, 50, 70, 100 and 120 ms. One-way ANOVA showed a significant difference in fEPSP slope index at inter-pulse intervals of 50 ms ($F(3,24) = 4.43$, $p < 0.05$) and 70 ms ($F(3,24) = 4.34$, $p < 0.05$), and in paired-pulse index at inter-pulse intervals of 50 ms ($F(3,23) = 5.23$, $p < 0.01$) and 70 ms ($F(3,23) = 4.93$, $p < 0.01$) between different groups. Intracerebroventricular infusion of OKA significantly decreased the fEPSP slope index at inter-pulse intervals of 50 and 70 ms ($p < 0.05$) and paired-pulse index at inter-pulse intervals of 50 ms ($p < 0.01$) and 70 ms ($p < 0.05$). In this experiment, treatment with ceftriaxone had also an ameliorating effect on paired-pulse responses recorded from OKA-injected rats in a way that there was no significant difference in fEPSP slope and paired-pulse indices between the OKA + CFT and control groups at all inter-pulse intervals.

### 3.2. Behavioral studies

In the current research, we evaluated the alternation behavior using the Y-maze task in order to assess the short-term spatial memory of the animals. Results showed that the total number of entries into the different arms did not change significantly. One-way ANOVA indicated a significant difference in the alternation score between different groups ($F(3,28) = 4.45$, $p < 0.05$). Tukey HSD posthoc test showed a significantly decreased alternation score in OKA-treated rats comparing to the control group ($p < 0.05$). Our results also indicated that treatment with ceftriaxone improved alternation score in OKA-treated rats as there was no significant difference in alternation score between OKA + CFT group and the control group indicating the ameliorating effect of ceftriaxone on short-term spatial memory in an OKA-induced model of AD (Fig. 6A).

The non-parametric Kruskal Wallis analysis of variance followed by Mann-Whitney *U* test (two-tailed) used for evaluation of difference in STL in passive avoidance retention test. Results of Kruskal Wallis test revealed a significant difference in STL between the groups ($H(3) = 9.54$, $p < 0.05$) with a mean rank of 27.78 for control, 27.44 for ceftriaxone, 14.11 for OKA and 22.00 for OKA + CFT groups. Mann-Whitney *U* test showed that OKA-injected rats had a STL value lower than those of the control group ($Z = 2.36$, $p < 0.05$) and application of ceftriaxone had an improving effect on passive avoidance learning in OKA-treated rats in a way that there was no significant difference in STL between OKA + CFT group and the control group (Fig. 6B).

## 4. Discusstion

This study aimed to investigate whether treatment with ceftriaxone, a well-known upregulator of GLT-1, could ameliorate an AD-like impairment in behavior and synaptic plasticity in OKA-induced AD in rats. Application of β-lactam antibiotic ceftriaxone at a dose known to stimulate the expression of glial GLT-1 [23,24] significantly offset effects

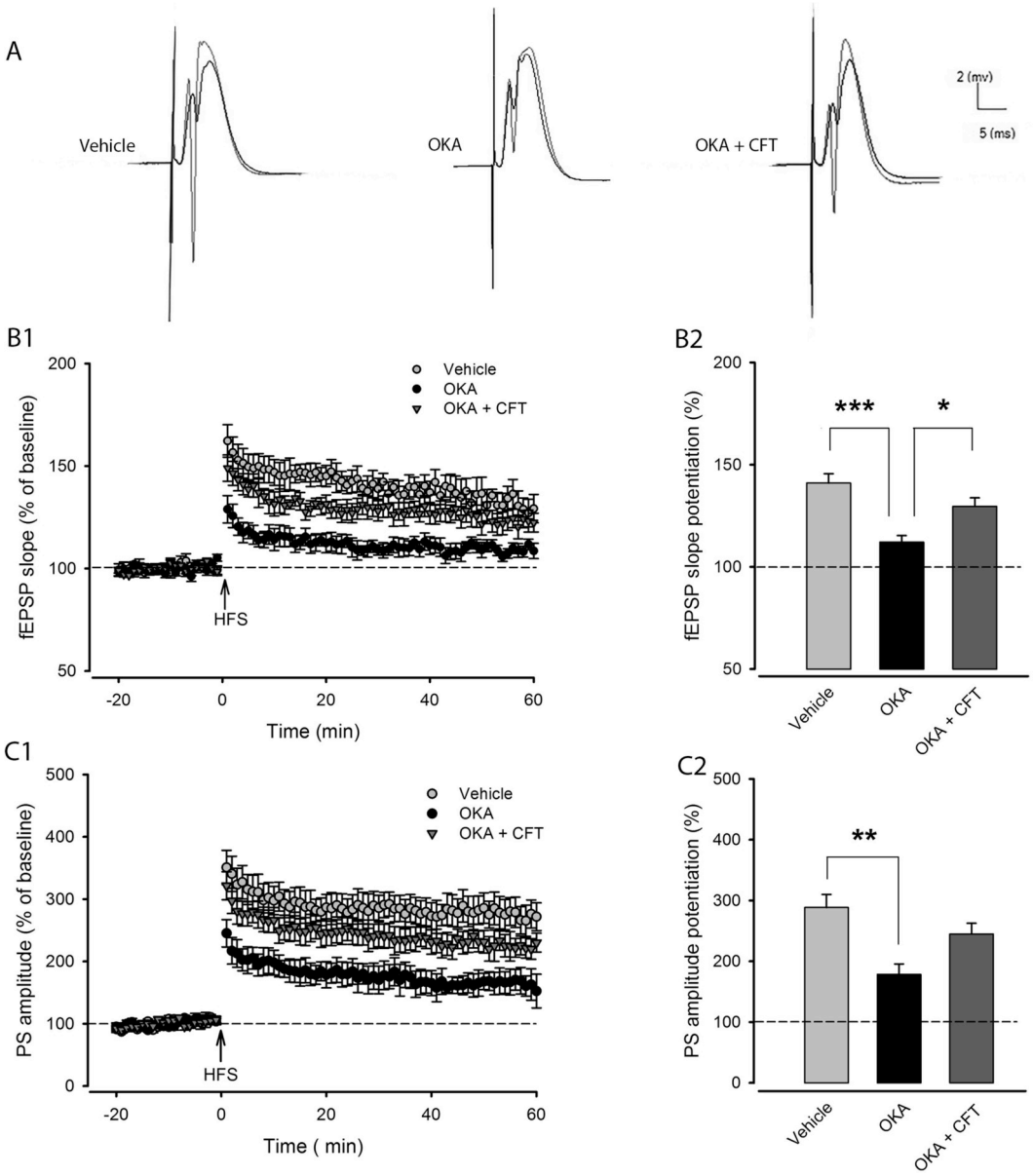


**Fig. 4.** The effect of ceftriaxone treatment on LTP of dentate gyrus neurons in OKA-injected rats. (A) Representative superimposed fEPSPs and PSs during baseline (black) and 5 min after (gray) LTP induction. (B1) Effect ceftriaxone treatment on LTP of hippocampal dentate gyrus neurons using 400 Hz tetanic stimulation at the fEPSP slope and (C1) PS amplitude. (B2, C2) Summary bar charts show mean values of fEPSP slope or PS amplitude (%) measured 60 min following HFS. The horizontal dashed line represents the baseline value and the data are plotted as the average percentage change from baseline responses. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$; $n = 7$ in each group).

of OKA on behavior and electrophysiological activity of DG neurons. More specifically, i.c.v. injection of OKA alone significantly attenuated the high-frequency stimulation-induced increase in fEPSP slope and PS amplitude recorded from hippocampal DG. Administration of ceftriaxone beginning with the day of OKA treatment and continuing daily for 2 weeks thereafter had a remarkable protective effect on LTP and memory following application of OKA. Ceftriaxone eliminated the effects of OKA on both perforant path-DG evoked fEPSP slope and PS amplitude and measures of short and long-term memory.

There have been only a few *in vitro* studies on the attenuating action of OKA on LTP in the hippocampus [25]. In this study, administration of OKA did not change the baseline activity of the neurons but had a significant ameliorating effect on potentiation of fEPSPs and PSs recorded from the DG granule cells. It has been previously reported that inhibition of PP1 and PP2A enzymes by their selective inhibitor OKA alters LTP induction in hippocampal slices and this action was attributed to the role of these protein phosphatases in the phosphorylation status of signaling mediators [26]. Decreases in PP1 and PP2 activity also have been observed in AD brain [27]. Other studies have shown deleterious effects of OKA on a cognitive performance together with numerous pathological changes (oxidative stress, mitochondrial dysfunction, neuroinflammation, cholinergic dysfunction, neuro-excitotoxicity) that are also evident in neurodegenerative diseases such as AD [28,29]. These observations have encouraged the use of the OKA treatment as a powerful means for studying AD pathology.

Glutamate NMDA receptors are highly expressed in different brain regions and are considered to be critical for normal physiological actions including the learning and memory [30]. Decreased glutamate uptake from synaptic space and increased glutamatergic release from presynaptic neurons and astrocytes can lead to hyper-excitation of glutamate receptors and $Ca^{2+}$ overload which in turn initiate a cascade of events that finally gives rise to excitotoxicity, cell death, and learning

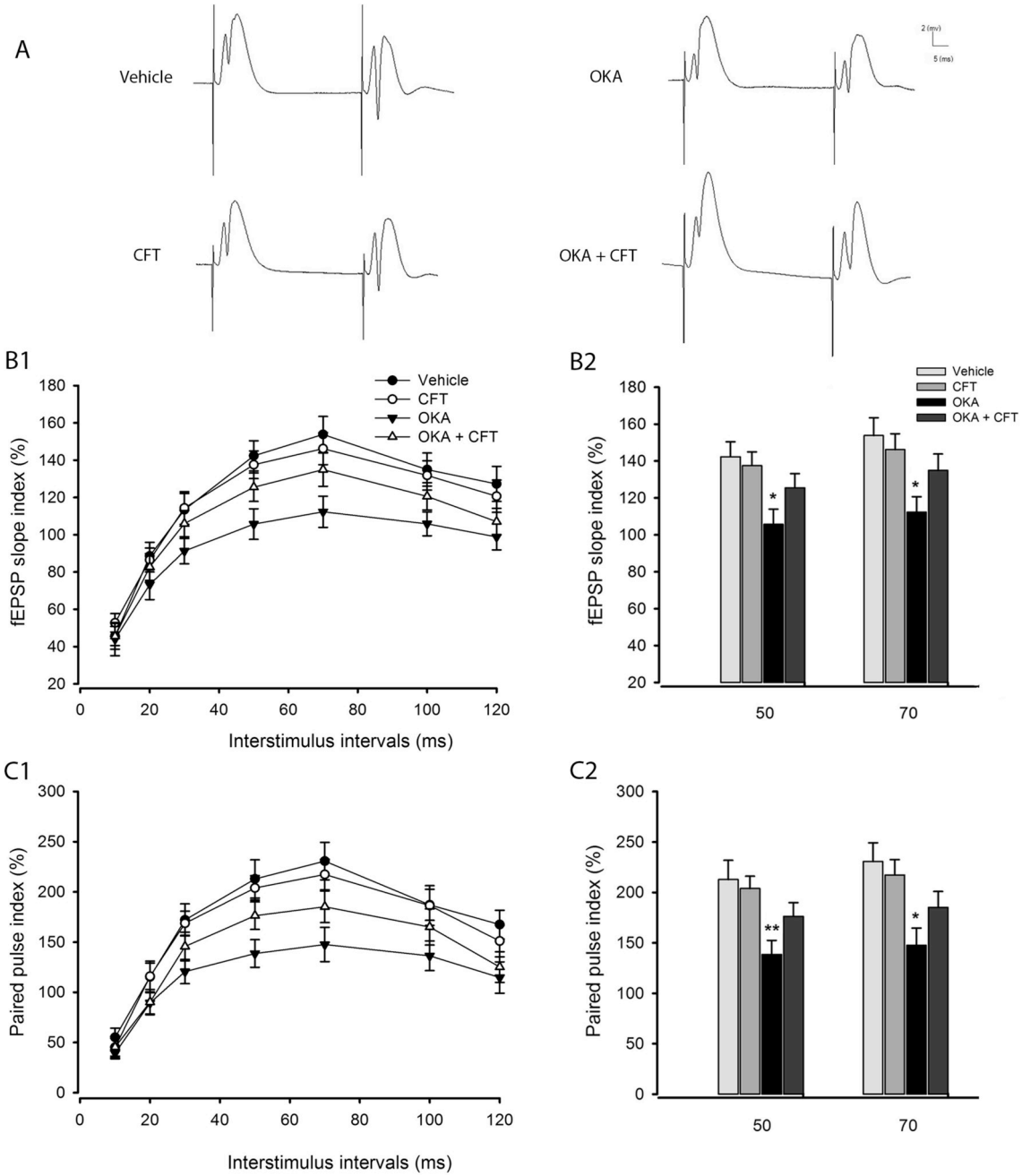


**Fig. 5.** Paired-pulse responses in different experimental groups recorded from hippocampal DG cell layer using inter-stimulus intervals of 10, 20, 30, 50, 70, 100 and 120 ms. (A) Single traces show paired-pulse facilitation in different groups at an inter-stimulus interval of 50 ms. (B1, C1) Effect of ceftriaxone treatment on paired-pulse responses recorded from dentate gyrus granule cells at fEPSP slope ratio (percentage of mean fEPSP2/fEPSP1 ± SEM) and the PS amplitude ratio (percentage of mean PS2/PS1 ± SEM). Bar charts show a significant difference in fEPSP slope (B2) and PS amplitude ratio (C2) between the groups at an inter-stimulus interval of 50 and 70 ms (*$p < 0.05$, and **$p < 0.01$; $n = 7$ in each group).

and memory impairment. intracellular $Ca^{2+}$ influx through enhanced NMDA channel activities gives rise to dysfunction of mitochondrial electron transport system, generation of reactive oxygen species (ROS) and excitotoxicity in neural cells [31]. It is reported that OKA can induce over-expression of NR1 and NR2B subunits in NMDA receptor, the effect responsible for excessive $Ca^{2+}$ influx in rats [29]. Normally, the NR2A and NR2B subunits are highly expressed in the hippocampus [32] and it has been previously reported that the NR2 subunits play a critical role in synaptic plasticity of the neurons. Interestingly, they have shown that NR2A subunits associate with the induction of LTP but NR2B subunits contribute to long-term depression (LTD) [33]. It is also demonstrated that over-expression of NR2B subunits leads to the excessive $Ca^{2+}$ influx through NMDA receptors and results in neural excitotoxicity and subsequent neurodegeneration [34]. The NR1/NR2B form of NMDA receptors is remarkably increased in AD neurons and excitotoxicity in AD requires its over-activity as well [31]. It has been reported that administration of OKA selectively increases the presynaptic release of glutamate and extracellular glutamate concentration leading to hyperactivity of NMDA receptors, elevated calcium influx, hyperphosphorylation of tau proteins, and ultimately excitotoxicity and synaptic dysfunction [35,36]. Increase in NMDA receptor NR2B subunits also has been demonstrated in the cortex and hippocampus of OKA-injected rats [29]. Therefore, in the current study, one of the possible reasons for attenuating effect of OKA on memory and LTP

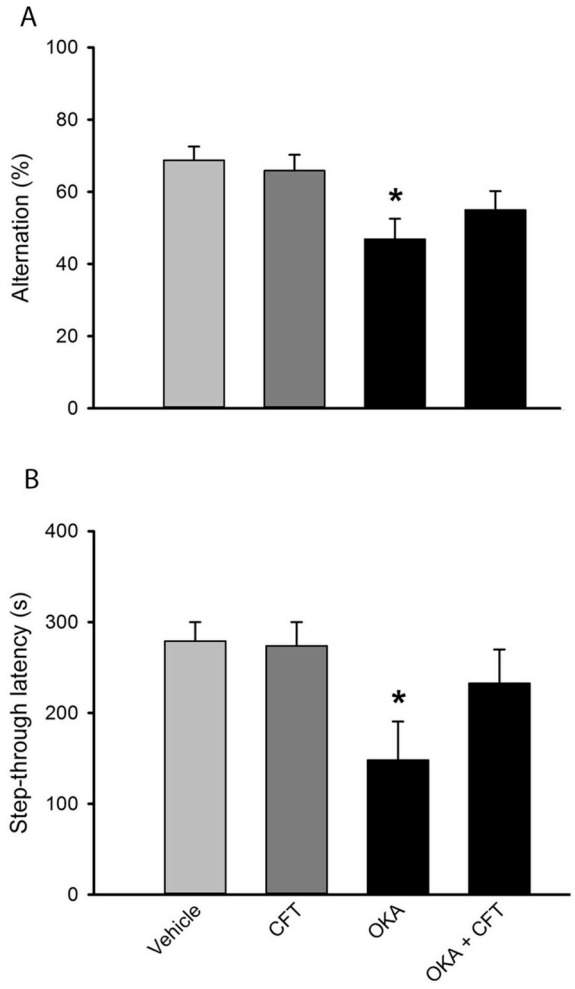


**Fig. 6.** Effect of ceftriaxone treatment on alternation behavior in Y-maze and step-through latency of rats in single-trial passive avoidance test. (A) One-way ANOVA followed by Tukey HSD posthoc test showed a significantly reduced alternation behavior in OKA-treated rats in comparison with the control (vehicle) group and treatment with ceftriaxone improved alternation score in OKA-treated rats (*$p < 0.05$ as compared with the control group, $n = 10$ in each group). (B) Kruskal Wallis followed by Mann-Whitney U posthoc tests indicated a significantly decreased STL values in OKA-injected rats compared to control group and application of ceftriaxone had an improving effect on passive avoidance learning in OKA-treated rats (*$p < 0.05$ compared to the control group, $n = 10$ in each group).

induction would be a high production of hippocampal NR1 and NR2B subunits.

The astrocytic glutamate transporters, GLAST and GLT-1, are responsible for maintaining low extracellular glutamate levels and among them, the GLT-1 is responsible for about 80–90% of all extracellular glutamate scavenging actions [37]. On the other hand, a decrease in GLT-1 expression has been reported in neurodegenerative disorders such as AD [38], and so the question arises whether upregulation of GLT-1 can have a protective effect on learning and memory and electrophysiological characteristics of hippocampal neurons in an OKA-induced model of AD. For this purpose, we used β-lactam antibiotic ceftriaxone which is well-known that selectively upregulates the expression of glial GLT-1 in the hippocampus and different cortical areas [23,24]. In this study, application of ceftriaxone significantly improved the learning and memory in Y maze and passive avoidance tasks and ameliorated the HFS induced LTP of hippocampal DG neurons in OKA-treated rats. Administration of ceftriaxone increased the recorded PS amplitude and fEPSP slope without changing the input-output function and baseline activity of these neurons. The effects can be attributed to enhanced expression of perisynaptic astrocytic GLT-1 and the consequent increase in glutamate scavenging activity in order to establish a lower extracellular glutamate concentration. These results were further supported by improving effect of ceftriaxone on learning and memory in passive avoidance tests. Maintenance of a low level of neurotransmitter glutamate at the synaptic space might be a key strategy in preventing the production of β-amyloid (Aβ) peptide, oxidative stress, cell death and deterioration of learning and memory in AD [7]. It has been previously well-established that administration of ceftriaxone up-regulates the hippocampal GLT-1 expression and also prevents the hypoxia-induced cognitive dysfunction, excitotoxicity and neural loss in rats [39]. Ceftriaxone-induced GLT-1 expression and hence a decrease in glutamate over-activity are shown to have a neuroprotective effect in MPTP-induced Parkinson's disease rat model [40].

The decrease in synaptic proteins PSD-95 has been shown in different regions of AD brain [41]. Significant changes in PSD95 levels also have been observed in different subregions of the hippocampus in 3xTg-AD mice [42]. A positive correlation also has been shown between the GLT-1 and PSD95 levels, and that application of ceftriaxone can restore PSD95 in different hippocampal subregions (DG, CA1, CA3) to normal levels [43]. Furthermore, previous studies have determined that OKA administration results in hyperphosphorylation of tau protein through an inhibitory action on PP2A and thereby gives rise to the production of NFTs and subsequent neurodegeneration [36,44]. GLT-1 indirectly affects calcium influx by scavenging excessive extracellular glutamate and, as a consequence, GLT-1 downregulation could contribute to tau hyperphosphorylation and microtubule-associated dysfunction in AD. Therefore, in this study, the beneficial effects of ceftriaxone on both electrophysiological responses of the dentate gyrus granule cells and behavioral performance might be in part due to a restoration or protection of synaptic PSD95 levels and maintenance of the normal phosphorylation state of tau protein through upregulation of glial GLT-1.

However, far too little attention has been paid to the effect of GLT-1 on learning and memory, in this study we also set out to explore the effect of ceftriaxone alone on electrophysiological and behavioral performance in rats. Interestingly, one unanticipated finding was that administration of ceftriaxone alone had no significant effect on baseline activity, PS amplitude and fEPSP slope recorded from hippocampal DG. There were also no significant changes in short- and long-term memory performed using Y-maze and passive avoidance tests. A possible explanation for this might be the role of compensatory mechanisms involved in maintaining the normal homeostasis in synaptic spaces. Although, the role of GLT-1 on hippocampal learning and memory is not well-known but contrary to our findings, one approach for exploring the effect of GLT-1 in learning and memory is that increased GLT-1 expression leads to attenuation of LTP and metabotropic glutamate receptor dependent LTD at hippocampal CA3 neurons, the results which were further supported by the detrimental effect of ceftriaxone on novel object recognition in rats [45]. This discrepancy may be attributed to using different hippocampal subregions and behavioral tasks.

In the current study, we have also evaluated the effect of ceftriaxone on paired-pulse ratio (PPR) as short-term synaptic plasticity of DG neurons in OKA-injected rats. Paired-pulse facilitation (PPF) and depression (PPD) are typically accepted as a model for evaluation of the presynaptic component of synaptic plasticity [46,47]. Mechanisms responsible for changes in paired-pulse response include facilitation of calcium channel, altered vesicle pool properties, quantal size and activation/inactivation status of neurotransmitter release sites. PPF results from an increase in probability of neurotransmitter release following the first stimulus and is mainly attributed to residual $Ca^{2+}$ in presynaptic terminals [48]. Nevertheless, recurrent inhibition mediated by GABAergic interneurons results in a decreased fEPSP slope in the second response compared to the first and therefore leads to PPD at inter-stimulus intervals of 10–40 ms [49]. In this study, we designed to

explore the presynaptic activity using paired-pulse stimulations at inter-stimulus intervals of 10, 20, 30, 50, 70, 100 and 120 ms. Application of paired stimulus at intervals of 10, 20 and 30 ms did not show any changes in recurrent inhibition since there was no significant difference in fEPSP slope and PS amplitude between the groups. On the other hand, the results showed an OKA-induced decrease in fEPSP slope and PS amplitude at inter-stimulus intervals of 50 and 70 ms suggesting the negative effects of OKA on presynaptic activity and PPF accompanied by deleterious changes observed in postsynaptic function. Recently, it has been reported that OKA decreases the mRNA expression level of synaptophysin, a presynaptic protein associated with small synaptic vesicles, in the rat hippocampus [50]. In consistent with this effect of OKA, a significant decrease in presynaptic synaptophysin also has been shown in the entorhinal cortex (EC) and other brain regions in AD [51]. In addition, it is reported that OKA impairs the mobility and transport of recently internalized vesicles within ribbon-containing synaptic terminals, a perturbation in synaptic vesicles trafficking which is also demonstrated in AD [52,53]. On the other hand, results of previous studies have demonstrated that ceftriaxone increases the expression level of synaptophysin and enhances the number of vesicles in nerve terminals in the hippocampal DG neurons. Furthermore, a positive correlation has been shown between the GLT-1 activity and presynaptic synaptophysin levels [54]. Therefore, in this study, the beneficial effect of ceftriaxone on paired-pulse activity of hippocampal DG might be in part due to the restoration of presynaptic synaptophysin and synaptic vesicles trafficking in these neurons.

In conclusion, returning to the hypothesis posed at the beginning of this study it is possible to state that treatment with ceftriaxone as a well-known up-regulator of GLT-1 expression can ameliorate OKA-induced attenuation of fEPSP slope and PS amplitude after high-frequency stimulation and also paired-pulse responses recorded from hippocampal DG cell layer. These indicate the beneficial effect of ceftriaxone on both pre- and post-synaptic function in these neurons. Ceftriaxone treatment also has an improving effect on OKA-induced impairment in learning and memory. Therefore, it is suggested that GLT-1 might be a promising therapeutic target for the treatment of neurodegenerative disorders such as AD in the future.

## Declaration of competing interest

The authors have no conflicts of interest to declare.

## Acknowledgments

This study was supported by a grant from Vice Chancellor of Research of Ardabil University of Medical Sciences (GN-9423).

## References

[1] J. Lewerenz, P. Maher, Chronic glutamate toxicity in neurodegenerative diseases—what is the evidence? Front. Neurosci. 9 (2015) 469.
[2] R. Cacabelos, M. Takeda, B. Winblad, The glutamatergic system and neurodegeneration in dementia: preventive strategies in Alzheimer's disease, Int. J. Geriatr. Psychiatry 14 (1999) 3–47.
[3] R.C. Malenka, R.A. Nicoll, NMDA-receptor-dependent synaptic plasticity: multiple forms and mechanisms, Trends Neurosci. 16 (1993) 521–527.
[4] E.L. Schaeffer, W.F. Gattaz, Cholinergic and glutamatergic alterations beginning at the early stages of Alzheimer disease: participation of the phospholipase A 2 enzyme, Psychopharmacology (Berl) 198 (2008) 1–27.
[5] R. Wang, P.H. Reddy, Role of glutamate and NMDA receptors in Alzheimer's disease, J. Alzheimer's Dis. 57 (2017) 1041–1048.
[6] M. Talantova, S. Sanz-Blasco, X. Zhang, P. Xia, M.W. Akhtar, S. Okamoto, G. Dziewczapolski, T. Nakamura, G. Cao, A.E. Pratt, others, Aβ induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss, Proc. Natl. Acad. Sci. 110 (2013) E2518–E2527.
[7] T.J. Revett, G.B. Baker, J. Jhamandas, S. Kar, Glutamate system, amyloid-beta peptides and tau protein: functional interrelationships and relevance to Alzheimer disease pathology, J. Psychiatry Neurosci. 38 (2013) 6–23, https://doi.org/10.1503/jpn.110190.
[8] G.M. Shankar, S. Li, T.H. Mehta, A. Garcia-munoz, N.E. Shepardson, I. Smith, F.M. Brett, M.A. Farrell, M.J. Rowan, C.A. Lemere, C.M. Regan, D.M. Walsh, B.L. Sabatini, D.J. Selkoe, Amyloid- b protein dimers isolated directly from Alzheimer ' s brains impair synaptic plasticity and memory, Nat. Med. 14 (2008) 837–842, https://doi.org/10.1038/nm1782.
[9] X. Dong, Y. Wang, Z. Qin, Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases, Acta Pharmacol. Sin. 30 (2009) 379.
[10] J.D. Rothstein, L. Martin, A.I. Levey, M. Dykes-Hoberg, L. Jin, D. Wu, N. Nash, R.W. Kuncl, Localization of neuronal and glial glutamate transporters, Neuron 13 (1994) 713–725.
[11] A.L. Sheldon, M.B. Robinson, The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention, Neurochem. Int. 51 (2007) 333–355.
[12] N.J. Maragakis, J.D. Rothstein, Mechanisms of disease: astrocytes in neurodegenerative disease, Nat. Rev. Neurol. 2 (2006) 679.
[13] S.-G. Lee, Z.-Z. Su, L. Emdad, P. Gupta, D. Sarkar, A. Borjabad, D.J. Volsky, P.B. Fisher, Mechanism of ceftriaxone induction of excitatory amino acid transporter-2 expression and glutamate uptake in primary human astrocytes, J. Biol. Chem. 283 (2008) 13116–13123.
[14] J. Rothstein, S. Patel, M. Regan, C. Haenggeli, Y. Huang, B-Lactam antibotics offer neuroprotection by increasing glutamate transporter expression, Nature 433 (2006) 73–77.
[15] P.K. Kamat, S. Rai, S. Swarnkar, R. Shukla, C. Nath, Molecular and cellular mechanism of okadaic acid (OKA)-induced neurotoxicity: a novel tool for Alzheimer's disease therapeutic application, Mol. Neurobiol. 50 (2014) 852–865.
[16] P.K. Kamat, C. Nath, Okadaic acid: a tool to study regulatory mechanisms for neurodegeneration and regeneration in Alzheimer's disease, Neural Regen. Res. 10 (2015) 365.
[17] N. Hamidi, A. Nozad, H. Sheikhkanloui Milan, M. Amani, Okadaic acid attenuates short-term and long-term synaptic plasticity of hippocampal dentate gyrus neurons in rats, Neurobiol. Learn. Mem. 158 (2019) 24–31, https://doi.org/10.1016/j.nlm.2019.01.007.
[18] P. George, W. Charles, The Rat Brain in Stereotaxic Coordinates, Qingchuan Zhuge Transl, People's Med. Publ. House, Beijing, 2007, p. 32.
[19] S. Babri, M. Amani, G. Mohaddes, A. Alihemmati, H. Ebrahimi, Protective effects of troxerutin on β-amyloid (1-42)-induced impairments of spatial learning and memory in rats, Neurophysiology (2012), https://doi.org/10.1007/s11062-012-9309-6.
[20] S. Babri, G. Mohaddes, I. Feizi, A. Mohammadnia, A. Niapour, A. Alihemmati, M. Amani, Effect of troxerutin on synaptic plasticity of hippocampal dentate gyrus neurons in a β-amyloid model of Alzheimer' s disease: an electrophysiological study, Eur. J. Pharmacol. 732 (2014) 19–25.
[21] J. Bak, H.-I. Pyeon, J.-I. Seok, Y.-S. Choi, Effect of rotation preference on spontaneous alternation behavior on Y maze and introduction of a new analytical method, entropy of spontaneous alternation, Behav. Brain Res. 320 (2017) 219–224.
[22] M. Nategh, S. Nikseresht, F. Khodagholi, F. Motamedi, Inactivation of nucleus incertus impairs passive avoidance learning and long term potentiation of the population spike in the perforant path-dentate gyrus evoked field potentials in rats, Neurobiol. Learn. Mem. 130 (2016) 185–193.
[23] S.M. Rawls, M. Zielinski, H. Patel, S. Sacavage, D.A. Baron, D. Patel, Beta-lactam antibiotic reduces morphine analgesic tolerance in rats through GLT-1 transporter activation, Drug Alcohol Depend. 107 (2010) 261–263.
[24] G.S. Goodrich, A.Y. Kabakov, M.Q. Hameed, S.C. Dhamne, P.A. Rosenberg, A. Rotenberg, Ceftriaxone treatment after traumatic brain injury restores expression of the glutamate transporter, GLT-1, reduces regional gliosis, and reduces post-traumatic seizures in the rat, J. Neurotrauma 30 (2013) 1434–1441.
[25] Y.M. Lu, I.M. Mansuy, E.R. Kandel, J. Roder, Calcineurin-mediated LTD of GABAergic inhibition underlies the increased excitability of CA1 neurons associated with LTP, Neuron 26 (2000) 197–205.
[26] A. Jouvenceau, G. Hédou, B. Potier, M. Kollen, P. Dutar, I.M. Mansuy, Partial inhibition of PP1 alters bidirectional synaptic plasticity in the hippocampus, Eur. J. Neurosci. 24 (2006) 564–572.
[27] S.P. Braithwaite, J.B. Stock, P.J. Lombroso, A.C. Nairn, Protein phosphatases and Alzheimer's disease, Prog. Mol. Biol. Transl. Sci. 106 (2012) 343.
[28] P.K. Kamat, S. Tota, G. Saxena, R. Shukla, C. Nath, Okadaic acid (ICV) induced memory impairment in rats: a suitable experimental model to test anti-dementia activity, Brain Res. 1309 (2010) 66–74.
[29] P.K. Kamat, S. Rai, S. Swarnkar, R. Shukla, C. Nath, Mechanism of synapse redox stress in okadaic acid (ICV) induced memory impairment: role of NMDA receptor, Neurochem. Int. 76 (2014) 32–41.
[30] C. Castellano, V. Cestari, A. Ciamei, NMDA receptors and learning and memory processes, Curr. Drug Targets 2 (2001) 273–283.
[31] Y. Zhang, P. Li, J. Feng, M. Wu, Dysfunction of NMDA receptors in Alzheimer's disease, Neurol. Sci. 37 (2016) 1039–1047.
[32] A.J. Law, C.S. Weickert, M.J. Webster, M.M. Herman, J.E. Kleinman, P.J. Harrison, Expression of NMDA receptor NR1, NR2A and NR2B subunit mRNAs during development of the human hippocampal formation, Eur. J. Neurosci. 18 (2003) 1197–1205.
[33] P.V. Massey, B.E. Johnson, P.R. Moult, Y.P. Auberson, M.W. Brown, E. Molnar, G.L. Collingridge, Z.I. Bashir, Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression, J. Neurosci. 24 (2004) 7821–7828.
[34] C.J. Fox, K.I. Russell, Y.T. Wang, B.R. Christie, Contribution of NR2A and NR2B NMDA subunits to bidirectional synaptic plasticity in the hippocampus in vivo, Hippocampus 16 (2006) 907–915.
[35] N. Broetto, F. Hansen, G. Brolese, C. Batassini, F. Lirio, F. Galland, J.P.A. dos Santos, M.F. Dutra, C.-A. Gonçalves, Intracerebroventricular administration of okadaic acid

induces hippocampal glucose uptake dysfunction and tau phosphorylation, Brain Res. Bull. 124 (2016) 136–143.
[36] P.K. Kamat, S. Rai, S. Swarnkar, R. Shukla, S. Ali, A.K. Najmi, C. Nath, Okadaic acid-induced Tau phosphorylation in rat brain: role of NMDA receptor, Neuroscience 238 (2013) 97–113.
[37] W. Chen, C. Aoki, V. Mahadomrongkul, C.E. Gruber, G.J. Wang, R. Blitzblau, N. Irwin, P.A. Rosenberg, Expression of a variant form of the glutamate transporter GLT1 in neuronal cultures and in neurons and astrocytes in the rat brain, J. Neurosci. 22 (2002) 2142–2152.
[38] A. Hoshi, A. Tsunoda, T. Yamamoto, M. Tada, A. Kakita, Y. Ugawa, Altered expression of glutamate transporter-1 and water channel protein aquaporin-4 in human temporal cortex with Alzheimer's disease, Neuropathol. Appl. Neurobiol. 44 (6) (2018) 628–638.
[39] S.K. Hota, K. Barhwal, K. Ray, S.B. Singh, G. Ilavazhagan, Ceftriaxone rescues hippocampal neurons from excitotoxicity and enhances memory retrieval in chronic hypobaric hypoxia, Neurobiol. Learn. Mem. 89 (2008) 522–532.
[40] S.-C. Ho, C.-C. Hsu, C.R. Pawlak, M.A. Tikhonova, T.-J. Lai, T.G. Amstislavskaya, Y.-J. Ho, Effects of ceftriaxone on the behavioral and neuronal changes in an MPTP-induced Parkinson's disease rat model, Behav. Brain Res. 268 (2014) 177–184.
[41] R. Sultana, W.A. Banks, D.A. Butterfield, Decreased levels of PSD95 and two associated proteins and increased levels of BCl2 and caspase 3 in hippocampus from subjects with amnestic mild cognitive impairment: insights into their potential roles for loss of synapses and memory, accumulation of A$β, J. Neurosci. Res. 88 (2010) 469–477.
[42] C. Carvalho, M.S. Santos, C.R. Oliveira, P.I. Moreira, Alzheimer's disease and type 2 diabetes-related alterations in brain mitochondria, autophagy and synaptic markers, Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1852 (2015) 1665–1675.
[43] J. Zumkehr, C.J. Rodriguez-Ortiz, D. Cheng, Z. Kieu, T. Wai, C. Hawkins, J. Kilian, S.L. Lim, R. Medeiros, M. Kitazawa, Ceftriaxone ameliorates tau pathology and cognitive decline via restoration of glial glutamate transporter in a mouse model of Alzheimer's disease, Neurobiol. Aging 36 (2015) 2260–2271.
[44] C.-X. Gong, T. Lidsky, J. Wegiel, L. Zuck, I. Grundke-Iqbal, K. Iqbal, Phosphorylation of microtubule-associated protein tau is regulated by protein phosphatase 2A in mammalian brain implications for neurofibrillary degeneration in Alzheimer's disease, J. Biol. Chem. 275 (2000) 5535–5544.
[45] F. Matos-Ocasio, A. Hernández-López, K.J. Thompson, Ceftriaxone, a GLT-1 transporter activator, disrupts hippocampal learning in rats, Pharmacol. Biochem. Behav. 122 (2014) 118–121.
[46] W. Gottschalk, L.D. Pozzo-Miller, A. Figurov, B. Lu, Presynaptic modulation of synaptic transmission and plasticity by brain-derived neurotrophic factor in the developing hippocampus, J. Neurosci. 18 (1998) 6830–6839.
[47] Y. Chen, J.E. Chad, H.V. Wheal, Synaptic release rather than failure in the conditioning pulse results in paired-pulse facilitation during minimal synaptic stimulation in the rat hippocampal CA1 neurones, Neurosci. Lett. 218 (1996) 204–208.
[48] D. Fioravante, W.G. Regehr, Short-term forms of presynaptic plasticity, Curr. Opin. Neurobiol. 21 (2011) 269–274.
[49] R.A. Pearce, S.D. Grunder, L.D. Faucher, Different mechanisms for use-dependent depression of two GABAA-mediated IPSCs in rat hippocampus, J. Physiol. 484 (1995) 425–435.
[50] A.-H. Xu, Y. Yang, Y.-X. Sun, C.-D. Zhang, Exogenous brain-derived neurotrophic factor attenuates cognitive impairment induced by okadaic acid in a rat model of Alzheimer's disease, Neural Regen. Res. 13 (2018) 2173.
[51] X. Yang, C. Yao, T. Tian, X. Li, H. Yan, J. Wu, H. Li, L. Pei, D. Liu, Q. Tian, others, A novel mechanism of memory loss in Alzheimer's disease mice via the degeneration of entorhinal–CA1 synapses, Mol. Psychiatry 23 (2016) 199–210.
[52] C. Guatimosim, C. Hull, H. Von Gersdorff, M.A.M. Prado, Okadaic acid disrupts synaptic vesicle trafficking in a ribbon-type synapse, J. Neurochem. 82 (2002) 1047–1057.
[53] P.J. Yao, Synaptic frailty and clathrin-mediated synaptic vesicle trafficking in Alzheimer's disease, Trends Neurosci. 27 (2004) 24–29.
[54] D. Yu, Z. Cheng, A.I. Ali, J. Wang, K. Le, E. Chibaatar, Y. Guo, Chronic unexpected mild stress destroys synaptic plasticity of neurons through a glutamate transporter, GLT-1, of astrocytes in the ischemic stroke rat, Neural Plast. 2019 (2019) 1615925.