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ORIGINAL ARTICLE

Chronic administration of Tat-GluR23Y ameliorates cognitive dysfunction targeting CREB signaling in rats with amyloid beta neurotoxicity

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Abstract

Alzheimer's disease (AD) is behaviorally characterized by memory impairments, and pathologically by amyloid β1–42 (Aβ1–42) plaques and tangles. Aβ binds to excitatory synapses and disrupts their transmission due to dysregulation of the glutamate receptors. Here we hypothesized that chronic inhibition of the endocytosis of AMPA receptors together with GluN2B subunit of NMDA receptors might improve cognition deficit induced by Aβ(1–42) neurotoxicity. Forty male Wistar rats were used in this study and divided into 5 groups: Saline + Saline, Aβ+Saline, Aβ+Ifen (Ifenprodil, 3 nmol /2 weeks), Aβ+GluR23Y (Tat-GluR23Y 3 μmol/kg/2 weeks) and Aβ+Ifen+GluR23Y (same doses and durations). Aβ(1–42) neurotoxicity was induced by intracerebroventricular (ICV) injection of Aβ1–42 (2 μg/μl/side), and then animals received the related treatments for 14 days. Cognitive performance of rats and hippocampal level of cAMP-response element-binding (CREB) were evaluated using Morris Water Maze (MWM), and western blotting respectively. Obtained data from the acquisition trials were analyzed by two way Anova and Student T test. Also one way Analysis of variance (ANOVA) with post hoc Tuckey were used to clarify between groups differences in probe test. The Group receiving Aβ, showed significant cognition deficit (long latency to platform and short total time spent in target quadrant (TTS), parallel with lower level of hippocampal CREB, versus vehicle group. While, Aβ+ GluR23Y exhibited the shortest latency to platform and the longest TTS during the probe test, parallel with the higher hippocampal level of CREB compared with other groups. The present study provides evidence that chronic administration of Tat-GluR23Y; an inhibitor of GluA2-AMPARs endocytosis, successfully restores spatial memory impaired by amyloid beta neurotoxicity targeting CREB signaling pathway.

Keywords Alzheimer's disease · AMPA · NMDA, cognitive function · CREB

Introduction

Alzheimer's disease (AD) is the most common form of dementia, affecting almost 30 million people worldwide (Stanciu et al. 2020). Early neurodegenerative symptom includes significant deficit in cognitive functions relating to the hippocampus and neocortex (Yamin 2009).

Amyloid beta (Aβ), has been known as a pathological hallmark for AD, which is produced from proteolysis of the amyloid precursor protein (O'Brien and Wong 2011). Several mechanisms have been proposed to explain the Aβ-mediated cognitive dysfunction. One is the capability of Aβ to disturb glutamate transmission and intracellular Ca^{2+} signaling in the presynaptic terminals (Talantova et al. 2013). Glutamatergic disturbances, particularly activation of N-methyl-D-aspartate receptor (NMDARs) and down regulation of amino3-hydroxy-5-methylisoxazole-4-propionic acid receptors (AMPARs) by Aβ accumulation occur at the early stages of the disease (Kamenetz et al. 2003). AMPARs are responsible for the fast synaptic transmission and plasticity by increasing the long term potentiation (LTP) (Pirotte et al. 2013). AMPARs undergo rapid recycling in the postsynaptic

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compartment, and therefore, the number of receptors on the plasma membrane reflects the balance between exocytosis and endocytosis, particularly when Aβ binds with them (Guntupalli et al. 2016), and induces endocytosis (Chen et al. 2010; Hettinger et al. 2018).

On the other hand, NMDARs are one of the most important receptors involving in memory and learning (Paoletti et al. 2013). The hippocampus contains NMDARs that exhibit a peculiar dependency on membrane voltage in becoming active only on depolarization. Depolarization causes an influx of Na+ and/or Ca2+ ions through the activated NMDARs, triggering long-term changes in synaptic efficacy. Blockade of hippocampal NMDARs could, therefore, impair learning mediated by hippocampal circuitry, but not all types of learning (Morriss in 1989). He showed that chronic intraventricular infusion of the NMDA receptor antagonist D,L2-amino-5-phosphonopentanoic acid (D,L-AP5) caused an impairment of spatial but not visual discrimination learning in rats (Morris 1989).

NMDARs are composed of two GluN1 and two GluN2 (A, B, C, and D) subunits (Huang et al. 2017). GluN2A seems to be located mainly at synaptic sites and implicates in protective pathways increasing capability of learning and memory, whereas GluN2B is located mainly at the extra-synaptic sites and increases neuronal vulnerability (Huang et al. 2017). When Aβ binds to NMDA receptors, increases Ca^{2+} toxic signaling (Alberdi et al. 2010; Tu et al. 2014; Zhang et al. 2016) and leads to oxidative stress, mitochondrial dysfunction, neuronal inflammation and cell death (Butterfield and Pocernich 2003; Danysz and Parsons 2012; Gao et al. 2007). These deleterious effects are related to GluN2B-dependent translocation to the nucleus of a signaling protein termed Jacob and subsequent activation of the cAMP response element binding (CREB) shut-off pathway (Melgarejo da Rosa et al. 2016). Phosphorylated Jacob is associated with neuroprotection after synaptic NMDAR stimulation, while, non-phosphorylated Jacob is associated with decreased CREB activity, and synaptic density after extrasynaptic NMDAR stimulation (Zhang et al. 2016). Therefore, it seems that an optimum level of NMDARs activity, and higher ratios of subunits of GLuN2A/GluN2B are required for appropriate learning and memory.

Memory is the retention of experience-dependent representations over time. It involves filtering, encoding, storing and retrieving new information, and hippocampus plays a crucial role in memory (Scoville and Milner 2000). Evidence from a broad range of species (from Drosophila and Aplysia to humans) indicates that CREB plays an important role in long term memory (LTM) formation and synaptic plasticity (Yin and Tully 1996). CREB is widely expressed in the brain regions essential for encoding learning and memory, i.e. the hippocampus and cortex (Saura and Valero 2011). Also altered CREB signaling has been implicated in cognitive disorders including AD, due to accumulation of Aβ (Chen et al. 2012; Vitolo et al. 2002).

It should be noticed that synaptic and extrasynaptic NMDA receptors have directly opposing effects on CREB function and neuronal fate. Synaptic complexes promote nuclear signaling to CREB, induce Brain-derived neurotrophic factor (BDNF) gene expression, and activate anti-apoptotic pathways, but extra-synaptic complexes antagonize nuclear signaling to CREB, reduce BDNF expression, and are involved cell death (Vanhoutte and Bading 2003). Thus, the biological consequences of NMDARs activation in hippocampal neurons are specified by the location of the NMDARs signaling complex activated (Hardingham et al. 2002).

Considering the role of extra synaptic GluN2B subunit of NMDARs (Rammes et al. 2017), and GluA2 dependent AMPARs endocytosis in neurotoxicity (Guntupalli et al. 2016), we hypothesized that inhibition of both GluN2B subunit of NMDARs by Ifenprodil and endocytosis of AMPARs using GluR23Y might be a potential target for the treatment of amyloid beta neurotoxicity. Ifenprodil is a non-competitive inhibitor of GluN2B subunit of NMDARs specifically acts on the glycine-binding NMDARs subunit 1 (GluN1 and 2), and also subunit 2B; GluN2B), thereby preventing NMDAR signaling (Bhatt et al. 2013; Vyklicky et al. 2014). Tat-GluR23Y is a synthetic peptide contains tyrosine residues that inhibits AMPARs endocytosis, and thereby prevents long-term depression (LTD) (Yang et al. 2011).

Materials and methods

Animals

Overall 40 adult male Wistar rats weighing 200–250 g, aged 3 months were used in this study. Animals were provided from the Laboratory Animals breeding house (School of Pharmacy, Guilan University of Medical Sciences). They were kept in a standard animal caring room with 12/12 h light/dark cycle (lights on 7:00 h) and temperature of 22+2°c with free access to food and water. Then animals were divided into 5 groups of Saline + Saline, Aβ+Saline, Aβ+Ifen, Aβ+GluR23Y, and finally Aβ+Ifen+GluR23Y.

Experimental methods were conducted in accordance with the EU animal experiments ethical guidelines (2010/63/EU), and also Guilan University of Medical Sciences ethics committee (approval cod no: IR.GUMS.REC.1397.297).

Surgery

Animals were handled and adapted to the experimental room for 5 days. Then each rat deeply was anesthetized individually, with 75 mg/ kg of ketamine (TRITTAU, Germany) and 5 mg/kg of xylazine (SciENcelab, Hostonafter fixing). After the fixing animal head in the stereotaxic apparatus, the guide cannula was placed in its support with straight position, and

made an anterior-posterior incision of about 2.5 cm on the midline of the scalp. The cannulas (27 gauge, 8 mm needle, stainless steel) were placed bilaterally in the ventricles according to the coordination's of: AP=−0.8 mm, DV=−3.6 mm, ML= ±1.6 mm (Paxinos and Watson 2013). Two sterile screws were placed into the holes which were made next to the Bregma and Lambda by sterilized hand drill, then dental cement was generously applied around the cannulas and screwed to fix them. After the cement has completely dried, the cannula support was removed and a sterile pin (stylet, 22 Gauge) was inserted into the each cannula to prevent obstruction. Finally animal was placed in recovery cage on a warm pad.

Drugs & treatments

Design of the experiment is shown in Fig. 1. As it shows, the protocol was a chronic treatment of animal model of cognitive deficit with Ifen and GLU23Y.

Aβ1–42 (Sigma-Aldrich, USA, 2 μg/μl) was dissolved in 0.9% saline and incubated for 3 days to aggregate in room temperature to form fibril (He et al. 2019). Each microliter of solution contained 2μg Amyloid beta, and each rat received 8 ul (4ul each ventricle) using Hamilton micro syringe pump for 5 min (Shen et al. 2014). Then, rats were assigned into Saline + Saline, Aβ+Saline, Aβ+Ifen (3 nmol/rat/ICV/ 4times/2 weeks), Aβ+GluR23Yand Aβ+Ifen+GluR23Y. The last group was received the same doses and frequency of injections as it was for the previous groups. The AMPA receptor endocytosis inhibitor, GluR23Y (ANASPEC, San Jose, California, USA) was dissolved in 0.9% sterile saline (Yu et al. 2018). Selective GluN2B subunit-containing NMDARs antagonist, Ifenprodil (sigma, USA) was dissolved in saline and 5% dimethyl sulfoxide (DMSO).

In the present study we had to optimize the dose of Ifen to 3 nmol due to the toxic effects of higher doses in our pilot study.

Animal received GluR23Y (3 μmol/kg/4 times/two weeks) (Yu et al. 2018) after Aβ injection (Fig. 1). For combined treatment, GluR23Ywas injected 15 min earlier than Ifen (3 nmol /rat/ ICV/ 4 times/two weeks with 3-days interval)

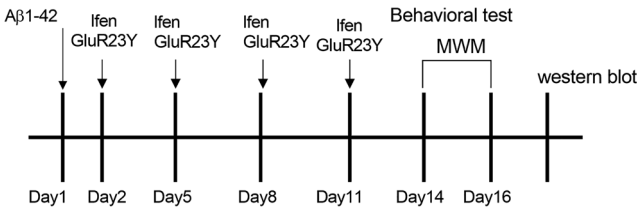

Fig. 1 Experiment design showing time course of ICV infusion of amyloid beta, Ifen,GluR23Y and Ifen+GluR23Yinjection (4 times for 2 weeks, with 3-days interval), and also behavioral experiment and western blotting

(Sánchez-Blázquez et al. 2014). Control groups received the same volume of vehicle.

Behavioral test

Morris water maze

As procedure of the experiment (Fig. 1) demonstrates, spatial learning and memory of animals was assessed using Morris water maze (MWM) 14 days after the treatment in 4 blocks of 16 trials (Pourmir et al. 2016). MWM is a test of spatial learning that relies on cues on the surrounded walls of the experiment room, to navigate from start locations around the perimeter of an open swimming arena to locate a submerged escape platform. Spatial learning was assessed across repeated trials and reference memory was determined by preference for the platform area when the platform was absent. The apparatus used here, consisted of a circular water tank (148 cm diameter and 60 cm high) with a rectangular platform (10 cm) at a fixed position in the target quadrant, 1.5 cm below the water level (temperature of 26 °C). The water tank was divided conceptually into four quadrants named (NW,NE,SW,SE), and the platform was always located in the NW, 25 cm from the wall of the pool. Acquisition of spatial learning consisted of 4 blocks, each of 4 trials. Animals individually performed MWM task in order to find the hidden platform based on spatial cues on the walls during 90s. The time to locate the platform was recorded. The starting positions of the animals were randomly determined which prevent any sequence of 2 trials to be repeated by the same animal during any other day. To assess reference memory, 24 h after the last trial, a probe trial was conducted on the day three. At this time, there was no escape platform in the maze at all. Each rat was placed in the maze from opposite start position of the target quadrant (SW) and allowed to explore the pool. During the probe trial, latency to the platform location, and total time spent in the target quadrant and also velocity of swimming were recorded using camera and "Ethovision 12 Noldus" tracking system (Netherland) based on our previous protocol (Jalilzad et al. 2019). Besides acquisition and probe tests, also, visible test was carried out at the end to examine the animal's vision; to be sure that animal could see platform and cues normally.

Western blot

Following the behavioral test, whole hippocampal area was separated and homogenized using a lysis buffer, and the homogenized mixture was centrifuged for 15 min at 12,000 rpm. The obtained supernatants were separated on a 15% SDS-PAGE and transferred into polyvinylidinedi fluoride (PVDF) membrane using transfer buffer (glycine, 25 mM Tris-base, 0. 037% SDS, and 20% methanol) by Bio-Rad Mini protein II system. The PVDF membrane was blocked with 5% skim

milk in SALINET (Saline and 0.05% Tween 20) for1 hours at 37 °C and then washed three times with SALINET. Then PVDF was incubated for 24 h at room temperature with the primary antibody (anti-CREB antibody ab31387, ABCAM, USA) with dilution of antibody 1/1000. After washing several times with TBS (Tris-base saline), it was incubated for 1–2 h at room temperature with secondary antibody Goat Anti-Rabbit IgG anti body, ab6721, ABCAM, USA,with dilution of 1/3000.

Then washing procedure was repeated and finally the PVDF was incubated with substrate solution DAB (3, 3′-diaminobenzidine tetrahydrochloride, Sigma) for 1–2 h at room temperature to give the band.

Statistics

Normality of data was estimated by Shapiro, then T test, repeated measure, two-way ANOVA (for acquisition part), and one-way ANOVA (for probe data) with Tukey's post test were used for comparing between group differences. Level of significance was $P < 0.05$ in all statistical evaluations. Data were analyzed in SPSS version19, and expressed as Means ± sem.

Results

Behavioral analysis

Inducing Aβ neurotoxicity

In order to induce amyloid beta neurotoxicity and cognition deficit, data obtained from MWM of two groups (Aβ+saline, saline+saline) were analyzed by T-test.

Results of working memory showed that Aβ+saline had longer escape latency compared with Saline+saline in Block 1[F(1,14) = 8.82, p=0.01], Block3 [F(1,14) = 8.63, p=0.01], Block4 [F(1.14) = 11.72, p= 0.001] which confirms deficits in memory induced by Aβ (Fig. 2a).

Results of probe test (Reference memory) showed that total time spent in target quadrant (TTS) significantly was decreased (p = 0.001), and latency time was increased in Aβ treating group compared with control (p=0.001). This confirms deficit in spatial memory retrieval induced by amyloid beta injection (Fig. 2b, c).

Working memory analysis

ANOVA repeated measure test showed significant within group differences in latency to platform in the acquisition phase of all groups indicating that all animals learned MWM task successfully ($P < 0.05$).

A two-way ANOVA (groups × blocks) on escape latency (Fig. 3a) revealed a significant group effect (F(4,35)=20.38, p=0.001) and significant blocks effect (F(3,156)=111.99,p=0.001). The interaction between groups was not significant (F(12,156)=0.9, p=0.5).

Post hoc comparisons on group effect revealed the shortest latency in the saline+saline group in comparison with Aβ +saline (p=0.001, Fig. 3a). Also Aβ+GluR23Y and Aβ+ Ifen+GluR23Y exhibited lower latencies compared with Aβ +saline (p=0.001, p=0.003, Fig.3a).

Post hoc Tukey comparisons revealed no significant difference in escape latency to platform (p=0.9) between Aβ+Ifen and Aβ+saline (Fig. 3a). A significant difference in escape latency was obtained between Aβ+Ifen compared with Aβ+ GluR23Y (p=0.001, Fig. 3a).

Also post hoc comparisons after two way Anova on blocks, revealed significant difference between B1 and B2 (p=0.001) between B2 and B3 (p=0.002) and also between B3 and B4 (p=0.001, Fig.3a).

Reference memory

One way ANOVA revealed significant between-group differences in total time spent in the target quadrant (TTS) during the probe test which platform has been removed [F (4, 35) =8.89, p=0.001] and the latency time in probe test of spatial memory [F (4, 35) =7.35, p=0.001].

First Latency in the probe test, was decreased in Aβ+ GluR23Ycompared with Aβ receiving group [F (4,35) =7.35,p = 0.001], (Fig. 3c). Also longer TTS [F(4,35)=8.89, p=0.001] for the group of Aβ+GluR23Y in probe test, showing facilitating effects of GluR23Y on retrieval of memory (Fig. 3b, e).

There was no significant difference in either escape latency [F(4,35)=7.35, p=0.07] or TTS [F(4,35)=8.89, p= 0.982] in the group receiving ICV injection of Ifen compared with Aβ+ saline respectively (Fig. 3c, b, e).

In probe test, the group receiving co treatments of Aβ+ GluR23Y+Ifen also showed short latency time F (4,35) =7.35, p=0.02], and long TTS [F(4,35)=8.89, p=0.04] compared with Aβ+saline in the retrieval of memory (Fig. 3b, c, d, e).

No significant difference in either escape latency [F(4,35)=7.35,p=0.3] or TTS [F(4,35)=8.89, p=0.298] was found between Aβ+GluR23Y+Ifen and Aβ+GluR23Y during probe trial (Fig . 3c, b). However TTS was significantly increased in the Aβ + GluR23Y compared with Aβ + Ifen group [F(4,35)=8.89, p=0.001], and first latency time was significantly reduced in the Aβ+ GluR23Y group compared to the Aβ+Ifen group [F(4,35)=7.35,p=0.001] (Fig. 3b, c).

Our data demonstrated no significant difference in swimming speed [F (4,35)=0.67, p=0.6] (Fig. 3d).

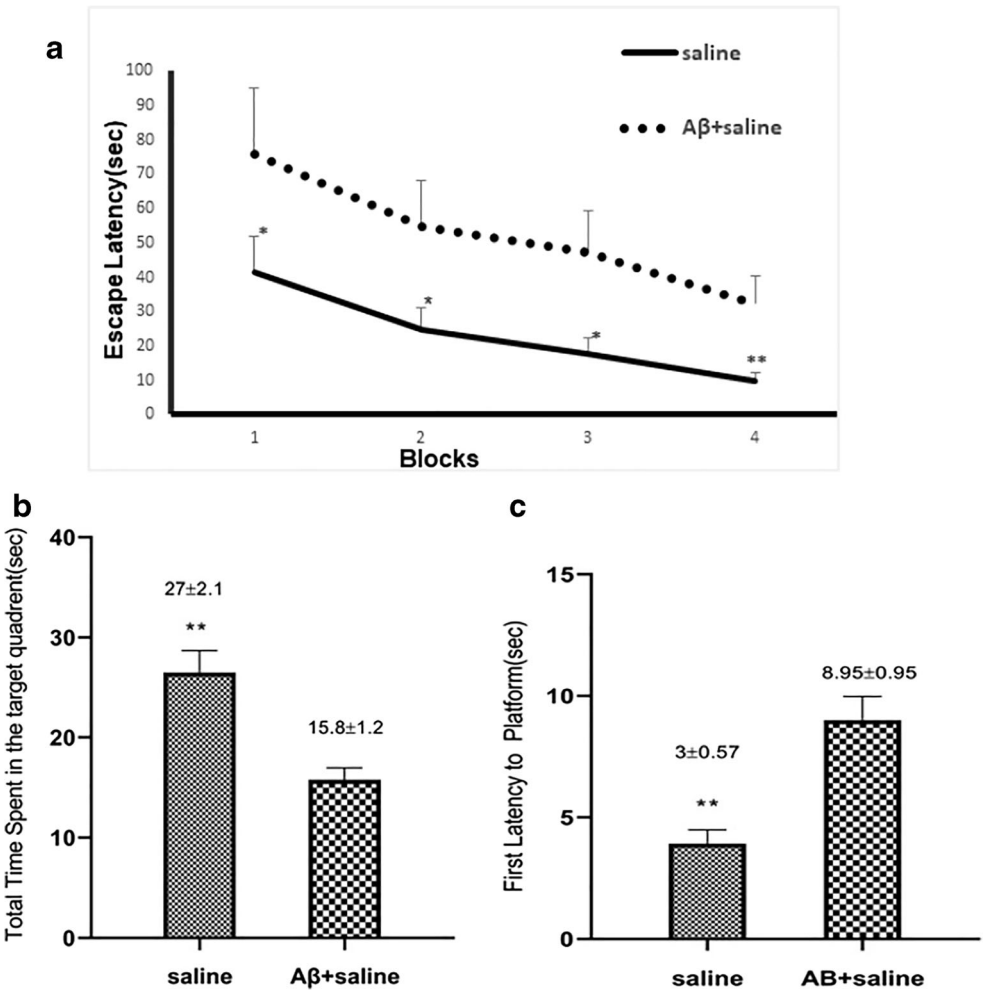

Fig. 2 **a** Comparison of the escape latency to platform in 4 blocks in the acquisition phase of spatial memory in MWM two groups Aβ+saline, saline+saline. **b** Total time spent in the target quadrant in probe test of spatial memory in MWM. **c** latency time in probe test of spatial memory in MWM. $*P < 0.05$, $**P < 0.01$, compared with Aβ+saline group. T-test and repeated measure Anova were used to calculate *p*-values. Data values are expressed as (mean ± sem, n=8 rats per group)

Molecular analysis

Molecular analysis by ANOVA one way, revealed significant between-group differences in CREB level [F(4,10)=10.59, p=0.001]. CREB level was significantly lower in the Aβ+saline than saline+saline (p=0.001, Fig. 4a, b).

The groups of Aβ+GluR23Y [F(4,10)=10.59, p=0.001] and Aβ+ GluR23Y+ Ifen [F(4,10)=10.59, p=0.02] exhibited higher level of CREB compared with Aβ+saline respectively.

There was no significant difference in hippocampal expression of CREB between Aβ+Ifen and Aβ+saline [F(4,10)=10.59, p=0.51], also between Aβ+GluR23Y+Ifen and Aβ+GluR23Y [F(4,10)=10.59, p=0.36] (Fig. 4a, b).

The group of Aβ+ GluR23Y had significantly much more level of CREB than Aβ+Ifen [F(4,35)=10.59,p=0.004] (Fig. 4a, b).

Discussion

The present study revealed cognitive deficits concomitant with a reduction in hippocampal CREB level in the animals receiving ICV injection of Aβ peptide. Amyloid beta, previously has been known to binds to excitatory synapses, particularly to GluA2-AMPA receptors (AMPAR) leading to endocytosis, inducing long term depression (LTD) and then memory impairment (Whitcomb et al. 2015). Over endocytosis of subunits of AMPAR from the postsynaptic membranes, or failure of insertion of them into postsynaptic membranes, is the main mechanism for oligomeric Aβ-induced AMPAR dysfunction (Zhang et al. 2017).

Meanwhile, reduction in the level of hippocampal CREB in the presence of Aβ also contributes to synaptic dysfunction, long-term deficits in long term potentiation (LTP) and neural survival (Paramanik and Thakur 2013; Varga et al. 2015).

Moreover, our data for a first time to our knowledge, showed that cognition deficit and CREB reduction, were successfully restored by inhibiting AMPA receptors endocytosis using GluR23Y. Surprisingly, chronic administration of GluR23Y alone, possessed much more cognitive-enhancing properties in acquisition and retrieval of spatial memory, than co treatment by Ifenprodil. Given the behavioral and molecular data, it can be proposed that memory improvement was

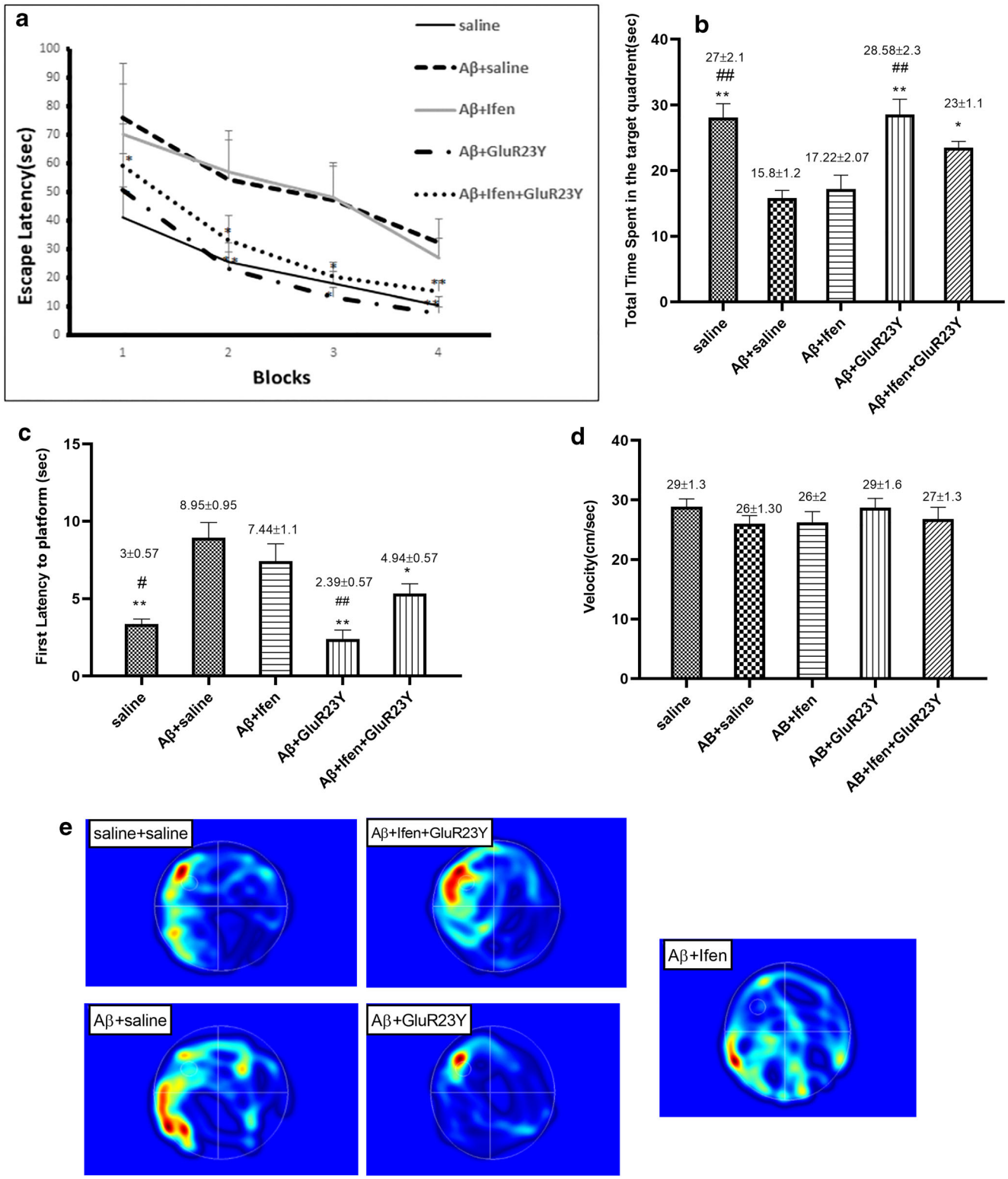

Fig. 3 Effect of GluR23Y, Ifen and Ifen+GluR23Y on (**a**) on the escape latency to platform in 4 blocks in the acquisition phase of spatial memory in MWM. Two-way ANOVA followed by Tukey'spost-hoc tests were used to calculate p values. **b** Total time spent in the target quadrant in probe test of spatial memory in MWM. **c** The latency time in probe test of spatial memory in MWM. **d** Comparison of swimming speed (Velocity) in MWM among groups. **e** Heat maps from Ethovision version 12, Noldus represent MWM tracking of groups during retrieval of memory. Increasing color intensity (arbitrary scale) represents increased time spent. Group receiving GluR23Y, showed longer TTS in the target quadrant than other groups. One-way ANOVA followed by Tukey'spost-hoc tests revealed significant between groups differences: $*P < 0.05$, $**P < 0.01$, compared with Aβ+saline group, #P < 0.05, ##P < 0.01, vs. Aβ+Ifen. Values are expressed as mean ± sem, n=8 rats per group

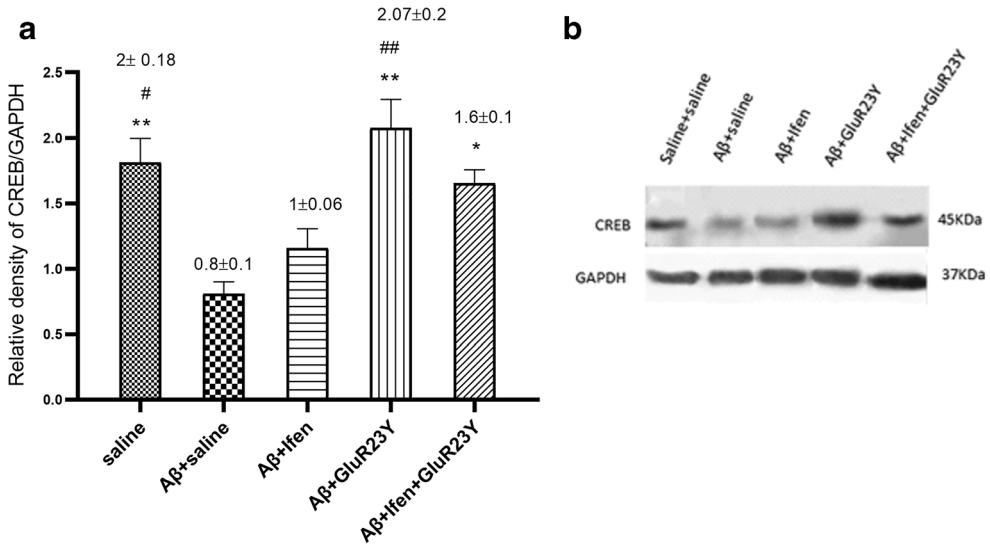

Fig. 4 Western blot analysis of CREB expression in different group. **a** Representative western blot analysis comparing the expression of CREB in different group. GADPH was used for protein loading. **b** Densitometric comparison of the average expression of CREB. One-way ANOVA followed by Tukey'spost-hoc tests were used to calculate p values. Values are expressed as mean ± sem, *P < 0.05, **P < 0.01, compared with Aβ+saline group, #P < 0.05, ##P < 0.01, vs. Aβ+Ifen

achieved by inhibiting GluA2 -dependent AMPA receptors endocytosis.

In line with our findings, previous electrophysiological studies reported that GluR23Y prevents hippocampal LTD (Dong et al. 2015; Hardt et al. 2014; Lüscher and Malenka 2012), and enhances LTP (Hsieh et al. 2006). Considering the previous findings indicating that alteration in AMPA currents during the early stage of AD are greater than NMDA currents (Liu et al. 2019), therefore inhibiting the AMPAR endocytosis at the initial of the neurotoxicity induced by Aβ, seems to be efficient to prevent cognition deficits in the amyloid neurotoxicity which is similar to the early stage of AD as previously reported by (Bayer and Wirths 2010; Zhao et al. 2010).

On the contrary, GluN2B antagonist, Ifenprodil in a dose used here, showed no significant improvement in neither of memory indices. In addition, the group receiving both treatments of GluR23Y and Ifenprodil also exhibited improvement in acquisition and retrieval of memory in MWM compared with Aβ receiving group. However mono therapy by GluR23Y was sufficient enough to restore the cognition deficit induced by Aβ.

To answer the question of why combined and chronic treatment of Ifen with GluR23Y did not show a synergistic response, compared with mono-therapy by GluR23Y, one hypothesis might be the pharmacokinetic interaction of Ifenprodil (Bhatt et al. 2013; Li et al. 2016). Ifenprodil inhibition is incomplete, noncompetitive and allosteric, which only makes channel openings duration shorter (Legendre and Westbrook 1991). Meanwhile, the interaction of Ifen with some of the other receptors in the CNS such as 5-Hydroxytryptamine (5-HT), and extracellular ligands including glycine, zinc, protons, and polyamines (Bhatt et al. 2013; Li et al. 2016; Williams 2008), might lead to undesired side effects and influence on the signaling pathways involved in the cognition performances. Meanwhile, in line with our findings, there are evidences showing that Ifen disturbs conditioned place preference and spatial learning and memory in rats (Hendricson et al. 2002; Ma et al. 2011).

As previously, Morris stated in 1986 and 1989, NMDA receptors participate in certain types of learning. He and his colleagues reported that intracerebral infusion of the NMDA receptor antagonists AP5, at a concentration sufficient to cause a total blockade of hippocampal LTP without effect on normal fast synaptic transmission, didn't cause impairment in visual discrimination learning or retention of well-established spatial information (Morris 1989; Morris et al. 1986).

Considering contradictory results in literatures, also limitation of the present study in optimizing the dose of Ifen to 3 nmol due to the toxic effects of higher doses, for future studies we suggest to examine the lower doses in co treatment with GluR23Y.

In conclusion, improvement in the acquisition and retrieval of spatial memory following the chronice treatment with GluR23Y parallel with elevation in hippocampal level of CREB, might reflect the activation of different target genes of CREB, involved in memory formation, storage and retrieval (Balamotis et al. 2012; Kim et al. 2014; Wang et al. 2018) .

CREB is a transcription factor at the core of various signaling pathways for memory formation and neuronal survival (Lakhina et al. 2015). In hippocampal neurons, CREB-dependent gene expression is causally linked to the long-lasting phase of activity-dependent neuroprotection against apoptotic and excitotoxic insults (Hardingham and Bading 2010).

In clinical importance of view, there are preclinical findings which suggest that impaired CREB phosphorylation may be a pathological component in neurodegenerative disorders, in particular Alzheimer's disease (AD). In this regard, pharmacological-induced CREB, such as GluR23Y, in brain

regions associated with cognition, i.e. cortex and hippocampus may represent a mechanistic basis for the development of novel AD therapeutics (Bitner 2012).

Therefore GluR23Y may rescue the memory deficits by offsetting the action of Aβ on AMPAR endocytosis via elevating in CREB signaling pathway.

Conclusion

Altogether, in clinical importance of view, inhibition of endocytosis of GluA2 subunit of AMPARs using GluR23Y is a useful therapeutic strategy in treating Aβ neurotoxicity targeting CREB signaling.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11011-020-00662-8.

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Author's contribution PB designed the study, and interpreted data, had a major contribution in writing and revising the manuscript. FA performed experiment, drafted the manuscript and analyzed data. AJ interpreted western blot data. All authors read and approved the final manuscript.

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Data availablity The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest The author declare that they have no conflict of interest.

References

Alberdi E et al (2010) Amyloid β oligomers induce Ca2+ dysregulation and neuronal death through activation of ionotropic glutamate receptors. Cell Calcium 47:264–272

Balamotis MA, Tamberg N, Woo YJ, Li J, Davy B, Kohwi-Shigematsu T, Kohwi Y (2012) Satb1 ablation alters temporal expression of immediate early genes and reduces dendritic spine density during postnatal brain development. Mol Cell Biol 32:333–347

Bayer TA, Wirths O (2010) Intracellular accumulation of amyloid-Beta - a predictor for synaptic dysfunction and neuron loss in Alzheimer's disease. Front Aging Neurosci 2:8. https://doi.org/10.3389/fnagi.2010.00008

Bhatt JM, Prakash A, Suryavanshi PS, Dravid SM (2013) Effect of ifenprodil on GluN1/GluN2B N-methyl-D-aspartate receptor gating. Mol Pharmacol 83:9–21

Bitner RS (2012) Cyclic AMP response element-binding protein (CREB) phosphorylation: a mechanistic marker in the development of memory enhancing Alzheimer's disease therapeutics. Biochem Pharmacol 83:705–714

Butterfield DA, Pocernich CB (2003) The glutamatergic system and Alzheimer's disease. CNS Drugs 17:641–652

Chen X, Wagener JF, Morgan DH, Hui L, Ghribi O, Geiger JD (2010) Endolysosome mechanisms associated with Alzheimer's disease-like pathology in rabbits ingesting cholesterol-enriched diet. J Alzheimers Dis 22:1289–1303

Chen Y et al (2012) Alzheimer's β-secretase (BACE1) regulates the cAMP/PKA/CREB pathway independently of β-amyloid. J Neurosci 32:11390–11,395

Danysz W, Parsons CG (2012) Alzheimer's disease, β-amyloid, glutamate, NMDA receptors and memantine–searching for the connections. Br J Pharmacol 167:324–352

Dong Z et al (2015) Long-term potentiation decay and memory loss are mediated by AMPAR endocytosis. J Clin Invest 125:234–247

Gao X et al (2007) NMDA receptor activation induces mitochondrial dysfunction, oxidative stress and apoptosis in cultured neonatal rat cardiomyocytes. Physiol Res 56:559

Guntupalli S, Widagdo J, Anggono V (2016) Amyloid-β-induced dysregulation of AMPA receptor trafficking. Neural Plast 3204519. https://doi.org/10.1155/2016/3204519

Hardingham GE, Bading H (2010) Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. Nat Rev Neurosci 11:682–696

Hardingham GE, Fukunaga Y, Bading H (2002) Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. Nat Neurosci 5:405–414

Hardt O, Nader K, Wang Y-T (2014) GluA2-dependent AMPA receptor endocytosis and the decay of early and late long-term potentiation: possible mechanisms for forgetting of short-and long-term memories. Philos Trans R Soc B Biol Sci 369:20130141

He MT, Lee AY, Kim JH, Park CH, Shin YS, Cho EJ (2019) Protective role of Cordyceps militaris in Aβ 1–42-induced Alzheimer's disease in vivo. Food Sci Biotechnol 28:865–872

Hendricson AW, Miao CL, Lippmann MJ, Morrisett RA (2002) Ifenprodil and ethanol enhance NMDA receptor-dependent long-term depression. J Pharmacol Exp Ther 301:938–944. https://doi.org/10.1124/jpet.301.3.938

Hettinger JC, Lee H, Bu G, Holtzman DM, Cirrito JR (2018) AMPA-ergic regulation of amyloid-β levels in an Alzheimer's disease mouse model. Mol Neurodegener 13:22. https://doi.org/10.1186/s13024-018-0256-6

Hsieh H, Boehm J, Sato C, Iwatsubo T, Tomita T, Sisodia S, Malinow R (2006) AMPAR removal underlies Aβ-induced synaptic depression and dendritic spine loss. Neuron 52:831–843

Huang Y et al (2017) Modulating the balance of synaptic and extrasynaptic NMDA receptors shows positive effects against amyloid-β-induced neurotoxicity. J Alzheimers Dis 57:885–897

Jalilzad M, Jafari A, Babaei P (2019) Neuregulin1β improves both spatial and associative learning and memory in Alzheimer model of rats possibly through signaling pathways other than Erk1/2. Neuropeptides 78:101963

Kamenetz F et al (2003) APP processing and synaptic function. Neuron 37:925–937. https://doi.org/10.1016/s0896-6273(03)00124-7

Kim J, Kwon J-T, Kim H-S, Josselyn SA, Han J-H (2014) Memory recall and modifications by activating neurons with elevated CREB. Nat Neurosci 17:65

Lakhina V et al (2015) Genome-wide functional analysis of CREB/long-term memory-dependent transcription reveals distinct basal and memory gene expression programs. Neuron 85:330–345

Legendre P, Westbrook GL (1991) Ifenprodil blocks N-methyl-D-aspartate receptors by a two-component mechanism. Mol Pharmacol 40:289–298

Li L, Liu X, Qiao C, Chen G, Li T (2016) Ifenprodil Attenuates Methamphetamine-Induced Behavioral Sensitization and

Activation of Ras-ERK-Δ FosB Pathway in the Caudate Putamen. Neurochem Res 41:2636–2644
Liu J, Chang L, Song Y, Li H, Wu Y (2019) The role of NMDA receptors in Alzheimer's disease. Front Neurosci. https://doi.org/10.3389/fnins.2019.00043
Lüscher C, Malenka RC (2012) NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). Cold Spring Harb Perspect Biol 4:a005710
Ma YY, Yu P, Guo CY, Cui CL (2011) Effects of ifenprodil on morphine-induced conditioned place preference and spatial learning and memory in rats. Neurochem Res 36:383–391. https://doi.org/10.1007/s11064-010-0342-9
Melgarejo da Rosa M, Yuanxiang P, Brambilla R, Kreutz MR, Karpova A (2016) Synaptic GluN2B/CaMKII-α signaling induces synaptonuclear transport of ERK and Jacob. Front Mol Neurosci 9:66
Morris R (1989) Synaptic plasticity and learning: selective impairment of learning rats and blockade of long-term potentiation in vivo by the N-methyl-D-aspartate receptor antagonist AP5. J Neurosci 9:3040–3057
Morris R, Anderson E, Lynch GS, Baudry M (1986) Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature 319:774–776
O'Brien RJ, Wong PC (2011) Amyloid precursor protein processing and Alzheimer's disease. Annu Rev Neurosci 34:185–204
Paoletti P, Bellone C, Zhou Q (2013) NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. Nat Rev Neurosci 14:383–400
Paramanik V, Thakur MK (2013) Role of CREB signaling in aging brain. Arch Ital Biol 151:33–42
Paxinos G and Watson Ch (2013) The rat brain in stereotaxic coordinates 7th edition. Academic Press
Pirotte B, Francotte P, Goffin E, de Tullio P (2013) AMPA receptor positive allosteric modulators: a patent review. Expert Opin Ther Pat 23:615–628. https://doi.org/10.1517/13543776.2013.770840
Pourmir M, Babaei P, Soltani TB (2016) Kisspeptin-13 ameliorates memory impairment induced by streptozotocin in male rats via cholinergic system. Physiol Pharmacol 20(1):38–47
Rammes G et al (2017) Involvement of GluN2B subunit containing N-methyl-d-aspartate (NMDA) receptors in mediating the acute and chronic synaptotoxic effects of oligomeric amyloid-beta (Abeta) in murine models of Alzheimer's disease (AD). Neuropharmacology 123:100–115. https://doi.org/10.1016/j.neuropharm.2017.02.003
Sánchez-Blázquez P, Rodríguez-Muñoz M, Herrero-Labrador R, Burgueño J, Zamanillo D, Garzón J (2014) The calcium-sensitive Sigma-1 receptor prevents cannabinoids from provoking glutamate NMDA receptor hypofunction: implications in antinociception and psychotic diseases. Int J Neuropsychopharmacol 17:1943–1955
Saura CA, Valero J (2011) The role of CREB signaling in Alzheimer's disease and other cognitive disorders. Rev Neurosci 22:153–169
Scoville WB, Milner B (2000) Loss of recent memory after bilateral hippocampal lesions. 1957. J Neuropsychiatry Clin Neurosci 12(1):103–113
Shen W-X, Chen J-H, Lu J-H, Peng Y-P, Qiu Y-H (2014) TGF-β1 protection against Aβ1–42-induced neuroinflammation and neurodegeneration in rats. Int J Mol Sci 15:22092–22108
Stanciu GD et al (2020) Alzheimer's disease pharmacotherapy in relation to cholinergic system involvement. Biomolecules 10(1)40
Talantova M et al (2013) Abeta induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss. Proc Natl Acad Sci U S A 110:E2518–E2527. https://doi.org/10.1073/pnas.1306832110
Tu S, Okamoto S-I, Lipton SA, Xu H (2014) Oligomeric Aβ-induced synaptic dysfunction in Alzheimer's disease. Mol Neurodegener 14(9):48. https://doi.org/10.1186/1750-1326-9-48
Vanhoutte P, Bading H (2003) Opposing roles of synaptic and extrasynaptic NMDA receptors in neuronal calcium signalling and BDNF gene regulation. Curr Opin Neurobiol 13:366–371
Varga E, Juhász G, Bozsó Z, Penke B, Fülöp L, Szegedi V (2015) Amyloid-β 1–42 disrupts synaptic plasticity by altering glutamate recycling at the synapse. J Alzheimers Dis 45:449–456
Vitolo OV, Sant'Angelo A, Costanzo V, Battaglia F, Arancio O, Shelanski M (2002) Amyloid β-peptide inhibition of the PKA/CREB pathway and long-term potentiation: reversibility by drugs that enhance cAMP signaling. Proc Natl Acad Sci 99:13217–13,221
Vyklicky V et al (2014) Structure, function, and pharmacology of NMDA receptor channels. Physiol Res 63(1):S191–203. https://doi.org/10.33549/physiolres.932678
Wang H, Xu J, Lazarovici P, Quirion R, Zheng W (2018) cAMP response element-binding protein (CREB): a possible signaling molecule link in the pathophysiology of schizophrenia. Front Mol Neurosci 11:–255
Whitcomb DJ et al (2015) Intracellular oligomeric amyloid-beta rapidly regulates GluA1 subunit of AMPA receptor in the hippocampus. Sci Rep 5:10934
Williams K (2008) Biology of the NMDA Receptor. In: Van Dongen AM (ed). CRC Press/Taylor & Francis, Boca Raton
Yamin G (2009) NMDA receptor–dependent signaling pathways that underlie amyloid β-protein disruption of LTP in the hippocampus. J Neurosci Res 87:1729–1736
Yang K, Xiong W, Yang G, Kojic L, Taghibiglou C, Wang YT, Cynader M (2011) The regulatory role of long-term depression in juvenile and adult mouse ocular dominance plasticity. Sci Rep 1:203
Yin JC, Tully T (1996) CREB and the formation of long-term memory. Curr Opin Neurobiol 6:264–268. https://doi.org/10.1016/s0959-4388(96)80082-1
Yu Y, Huang Z, Dai C, Du Y, Han H, Wang YT, Dong Z (2018) Facilitated AMPAR endocytosis causally contributes to the maternal sleep deprivation-induced impairments of synaptic plasticity and cognition in the offspring rats. Neuropharmacology 133:155–162
Zhang Y, Li P, Feng J, Wu M (2016) Dysfunction of NMDA receptors in Alzheimer's disease. Neurol Sci 37:1039–1047
Zhang J et al. (2017) Endophilin2 Interacts with GluA1 to Mediate AMPA receptor endocytosis induced by oligomeric amyloid-β Neural Plast 2017.
Zhao W-Q et al (2010) Inhibition of calcineurin-mediated endocytosis and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors prevents amyloid β oligomer-induced synaptic disruption. J Biol Chem 285:7619–7632

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